GENETIC MARKER STUDIES IN HUMANS

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PREFACE

Results from this investigation were published during the course of my candidature. These works are presented in the Publications section. Publication prior to thesis submission was undertaken in order to avoid obsolescence of data and to safeguard the originality of investigations.

Each chapter in this thesis is preceded by a title page setting out contents, including any relevant publications. The text for each chapter was written on the assumption that the corresponding publications are read before the chapter text.

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SUMMARY

Genetic markers on human chromosomes were examined for the purposes of gene mapping and testing associations involving the protease inhibitor system. These heritable markers consisted of 20 rare chromosomal variants (fragile sites, translocations, an inversion, a centromere heteromorphism), nine blood groups and 19 electrophoretic polymorphisms of enzymes and proteins determined from individuals, kindreds and the local population. Seventy kindreds segregated with fragile sites. Some of the kindreds examined were extremely large with up to 91 persons phenotyped for the range of genetic markers routinely studied. Many of the markers examined had not been assigned to specific chromosomes when the study commenced.

No definitive gene assignments were made for any of the unassigned loci examined. These included loci for diseases (epidermolysis bullosa simplex, Köbner type and epidermolysis bullosa dystrophica, Cockayne- Touraine type) blood groups (Kell and P), enzymes (cholinesterase-2 and glutamic pyruvic transaminase) and proteins (factor 13B and transferrin). Loci for complement 3, factor 13A, immunoglobulin heavy polypeptide, immunoglobulin kappa polypeptide, Kidd blood group, Lutheran blood group and the protease inhibitor (PI) system were assigned by other investigators during the study period.

Linkage analysis from 78 kindreds and deletion mapping from 16 monosomic individuals provided:

a)   Extensive additions to the exclusion map of man for the unassigned loci mentioned above,

b)   a clear chromosomal localisation for the galactose-1-phosphate uridyltransferase locus to region (9)(p13),

c)   a hint of linkage between the cholinesterase-2 and haptoglobin

loci on chromosome 16,

d)   a hint of linkage between the epidermolysis bullosa - Köbner type locus and the Duffy blood group on chromosome 1,

e)   evidence supporting the regional localisation of the human leucocyte antigen genes (HLA) to region (6)(p21.3).

One kindred segregated for a previously undescribed protease inhibitor allele. This rare variant was designated PI*NADELAIDE and has been accepted as a new variant by the International PI Committee.

Association studies involving PI variants segregating in the kindreds examined provided:

a)   A tentative association between mildly deficient PI phenotypes and mutation to folate sensitive fragile sites, and

b)   no support for non-random segregation of PI*Z as proposed by other investigators.

Some of the kindreds with heritable fragile sites provided insight into the genetics of fragile sites themselves. Linkage studies were consistent with the location of DNA causing fragile sites being at the point of their expression. This was demonstrated in one kindred segregating for the rare fragile site at (6)(p23) and two kindreds each segregating for fragile sites at (10)(q23) and (10)(q25). Fragile sites did not appear to measurably affect recombination frequency in nearby chromosomal segments, even when two linked fragile sites were present on the one homologue (at 10q23 and 10q25). This genetic behaviour of fragile sites is probably general and has important implications in the search for tightly linked DNA fragments for use in the detection and characterisation of fragile sites.

Results were published during the study period. A total of 18 published papers are appended to this thesis.

DECLARATION

The examinable work presented in this thesis is the result of my own investigation. It has not previously been submitted for a higher degree. The breadth of these investigations often required the collaboration of other investigators with specialist expertise in cytogenetics, immunology, medical genetics, paediatric medicine, clinical biochemistry or electrophoresis. The contribution of collaborators in each publication is fully defined in the section Explanations Regarding Joint Authorship.

Publications 1 and 6 were published prior to formal acceptance of my candidature, but are included because they provide a basis for subsequent examinable work.

I consent to the thesis being made available for photocopying and loan if accepted for award of this degree.

J.C. MULLEY

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LIST OF PUBLICATIONS PRESENTED IN THIS THESIS

Publication

Number

1   Mulley, J.C. (1980) Distribution of the six α_1-antitrypsin (PI) subtypes from a sample of blood donors. Aust. J. Exper. Biol. Med. Sci. 58: 409-414.

2   Mulley, J.C. (1982a) Distribution of α_1-antitrypsin (PI) subtypes from newborns. Aust. J. Exper. Biol. Med. Sci. 60: 207-209.

3   Nicholls, C. and Mulley, J.C. (1982a) Distribution of the GC (group-specific component) subtypes in cord bloods and blood donors. Aust. J. Exper. Biol. Med. Sci. 60: 427-431.

4   Nicholls, C. and Mulley, J.C. (1982b) Distribution of six TF C (transferrin) subtypes in cord bloods and blood donors. Aust. J. Exper. Biol. Med. Sci. 60: 433-436.

5   Mulley, J.C. (1982b) Polymorphism of human galactose-1-phosphate uridyltransferase. Hum.Hered. 32: 42-45.

6   Mulley, J.C., Bryant, G.D. and Sutherland, G.R. (1980) Additions to the exclusion map of man. Ann. Genet. 23: 198-200.

7   Mulley, J.C. and Sutherland, G.R. (1982) Further exclusions by deletion mapping. Ann. Genet. 25: 152-153.

8   Sutherland, G.R., Baker, E. and Mulley, J.C. (1982) Genetic length of a human chromosomal segment measured by recombination between two fragile sites. Science 217: 373-374.

9   Mulley, J.C., Hay, J., Sheffield, L.J. and Sutherland, G.R. (1983a) Regional localisation for HLA by recombination with a fragile site at 6p23. Am. J. Hum. Genet. 35: 1284-1288.

10   Mulley, J.C., Nicholls, C. and Sutherland G.R. (1983b) A search for linkage in families with fragile sites. Hum. Genet. 65: 79-81.

11   Mulley, J.C. (1985) Genetic linkage relationships of fragile sites. In: Sutherland, G.R. and Hecht, F. (Eds.) Fragile Sites on Human Chromosomes, pp.179-200. Oxford University Press, New York.

12   Mulley, J.C., Cox, D.W. and Sutherland, G.R. (1983c) A new allele of α_1-antitrypsin: PI*NADELAIDE. Hum. Genet. 63: 73-74.

13   Mulley, J.C., Hill, G. and McPhee, A. (1983d) A transient cathodal PI phenotype in an infant with mild persistent jaundice. Dis. Mark. 1: 55-57.

14   Sutherland, G.R., Mulley, J.C. and Goldblatt, E. (1981). A large kindred with an inv(3)(p25;q23): clinical, cytogenetic and genetic marker studies. Ann. Genet. 24: 202-205.

15   Mulley, J.C., Nicholls, C.M., Propert, D.M., Turner, T. and Sutherland, G.R. (1984). Genetic linkage analysis of epidermolysis bullosa simplex, Koebner type. Am. J. Med. Genet. 19: 573-577.

16   Mulley, J.C., Turner, T., Nicholls, C.M., Propert, D.M. and Sutherland, G.R. (1985). Genetic linkage analysis of

16   Mulley, J.C., Turner, T., Nicholls, C.M., Propert, D.M. and Sutherland, G.R. (1985). Genetic linkage analysis of epidermolysis bullosa dystrophica, Cockayne-Touraine type. Clin. Genet. (in press).

17   Mulley, J.C. and Sutherland, G.R. (1983) Protease inhibitor (PI) phenotype of individuals with chomosomal fragile sites. Ann. Genet. 26: 143-146.

18.   Mulley, J.C. and Sutherland, G.R. (1981) Distribution of α_1-antitrypsin (PI) phenotypes in chromosome abnormalities. Hum. Genet. 57: 176-179.

EXPLANATIONS REGARDING JOINT AUTHORSHIP

<u>Publication</u>

<u>Number</u>

3, 4   Experimental work, data analysis and manuscript preparation was carried out by Ms. C. Nicholls under my supervision as partial fulfilment for the degree of Master of Applied Science in Medical Technology.

6   The electrophoretic investigations and manuscript preparation were carried out myself. Blood grouping and cytogenetics was carried out by Mr. G.D. Bryant and Dr. G.R. Sutherland respectively.

7, 17, 18   The electrophoretic investigations, data analysis and manuscript preparation was carried out myself. Dr. G.R. Sutherland was responsible for the cytogenetics.

8   The manuscript was prepared by Dr. G.R. Sutherland. Mrs. E. Baker assisted with the cytogenetics and the linkage analysis was carried out myself.

9   The linkage analysis and manuscript preparation was carried out myself, HLA typing was the responsibility of Dr. J. Hay, the family was ascertained by Dr. L.J. Sheffield, and the cytogenetics was done under the supervision of Dr. G.R. Sutherland.

10   The electrophoretic phenotyping, linkage analysis and manuscript preparation was carried out myself. Two of the electrophoretic markers (GC and TF) from families with the fragile site at 10q25 and all bloodgrouping was carried out by

Ms. C. Nicholls. Dr. G.R. Sutherland was responsible for the cytogenetics. Linkage relationships between GC, TF and the bloodgroups in families with the fragile site at 10q25 was studied by Ms. C. Nicholls under my supervision on partial fulfilment for the degree of Master of Applied Science in Medical Technology.

12   Identification of the new variant by polyacrylamide isoelectric focusing, family study and manuscript preparation was carried out myself. The specialised starch and agarose gel electrophoresis required for the confirmation of this new variant was carried out by Dr. D.W. Cox. Fragile site studies were carried out by Dr. G.R. Sutherland.

13   Detection of this undescribed variant, its description, and manuscript preparation was carrid out myself. Initial clinical chemistry on the patient was the responsiblity of Mr. G. Hill and the responsible paediatrician was Dr. A. McPhee.

14   The cytogenetics and manuscript preparation was the responsiblity of Dr. G.R. Sutherland. Genetic marker studies and linkage analysis was carried out myself and the clinician responsible for the patient was Dr. E. Goldblatt.

15   The electrophoretic investigations, linkage analysis and manuscript preparation was carried out myself. The bloodgrouping was carried out by Ms. C. Nicholls, the IGK and IGM typing by Dr. D.M. Propert, the epidermolysis bullosa diagnosis by Dr. T. Turner and the cytogenetics by Dr. G.R. Sutherland.

16   The genetic marker investigations, linkage analysis and manuscript preparation was carried out myself. Dr. T. Turner was responsible for the diagnosis, Ms. C. Nicholls carried out the bloodgrouping, Dr. D.M. Propert did the IGK and IGM typing and Dr. G.R. Sutherland was responsible for the collection of samples and counselling of family members where appropriate.

I was the sole author of Publications 1, 2, 5 and 11. I was the senior author of Publications 6, 7, 9, 10, 12, 13, 15, 16, 17 and 18.

ACKNOWLEDGEMENTS

CHAPTER 1

INTRODUCTION

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(a)   GENETIC MARKERS

A genetic marker is "any stable characteristic which has a simple mode of inheritance and thus can be used as a tag or label for a gene locus, for a larger genetic region, for a whole chromosome, or for an entire population of cells, or, indeed, of people" (Giblett, 1979). The inheritance of a genetic marker can be traced from generation to generation.

Genetic markers have several applications:

1)   As marker loci in gene mapping by linkage analysis,

2)   Marker loci in association studies (which can involve disease states or metric characteristics),

3)   Paternity testing,

4)   Zygosity testing,

5)   Assessment of marrow engraftment (genetic markers of bone marrow donor are detectable after a few months),

6)   Sample identification,

7)   Species identification and

8)   Prenatal and postnatal diagnosis by linkage.

The most useful genetic markers are polymorphic ones, which have two or more common alleles segregating in the population. Ford (1953) described a polymorphism as the occurrence of two or more distinct forms in a population such that the rarest could not be maintained by mutation alone. A polymorphic marker is operationally defined as one where the frequency of the common allele is less than or equal to 0.99. Such common genetic variation is usually considered benign because common alleles are rarely implicated in disease associations. The 51 heritable markers used in the studies to

be presented are listed in Table 1.1 using current marker loci terminology (Human Gene Mapping 7, 1984).

Extensive polymorphism is a characteristic feature of human populations (Harris, 1980). Each polymorphic gene is represented in an individual as a pair of alleles, one from each parent, carried by two homologous chromosomes. Each allelic form of a gene specifies a corresponding polypeptide difference through the steps of translation, processing and transcription. This variation is maintained by the evolutionary forces of natural selection or random genetic drift. The source of allelic variation is either mutation or immigration into the population.

This store of variation within populations is responsible for the harmless genetically determined differences between individuals. This variation could also contribute to differences in disease susceptibility between individuals. The analysis of individual differences detectable as enzyme, protein and blood group polymorphisms can be applied to fundamental problems in human genetics and medicine including gene mapping (Human Gene Mapping 5, 1979) and associations with diseases (Mourant et al., 1978) and chromosomal abnormalities (Kueppers et al., 1975; Fineman et al., 1976).

TABLE 1.1

HERITABLE MARKER LOCI USED IN THIS THESIS

Blood Group, Immunoglobulin and Leucocyte Markers

1.	ABO	ABO blood group
2.	Fy	Duffy blood group
3.	HLA	Human leucocyte antigens
4.	IGH	Immunoglobulin heavy chain
5.	IGK	Immunoglobulin kappa light chain
6.	Jk	Kidd blood group
7.	K	Kell blood group
8.	Lu	Lutheran blood group
9.	MNS	MNS blood group
10.	P	P blood group
11.	Rh	Rhesis blood group
12.	Xg	Xg blood group

Enzyme Markers

13.	ACP1	Acid phosphatase-1
14.	ADA	Adenosine deaminase
15.	AK1	Adenylate kinase
16.	AMY2	Amylase-2
17.	CHE2	Cholinesterase-2
18.	ESD	Esterase D
19.	GALT	Galactose-1-phosphate uridyltransferase
20.	GLO1	Glyoxylase-1
21.	GPT	Glutamic pyruvic transaminase
22.	PGD	Phosphogluconate dehydrogenase
23.	PGM1	Phosphoglucomutase-1
24.	PGP	Phosphoglycolate phosphatase

Protein Markers

25.	C3	Complement 3
26.	F13A	Factor XIIIA
27.	F13B	Factor XIIIB
28.	GC	Group-specific component
29.	HP	Haptoglobin
30.	PI	Protease inhibitor system
31.	TF	Transferrin

Chromosomal Markers

32.	FRAXQ27	Fragile site at (X)(q27)
33.	FRA2Q11	Fragile site at (2)(q11)
34.	FRA2Q13	Fragile site at (2)(q13)
35.	FRA6P23	Fragile site at (6)(p23)
36.	FRA7P11	Fragile site at (7)(p11)
37.	FRA8Q23	Fragile site at (8)(q23)
38.	FRA9Q32	Fragile site at (9)(q32)
39.	FRA10Q23	Fragile site at (10)(q23)
40.	FRA10Q25	Fragile site at (10)(q25)
41.	FRA11Q13	Fragile site at (11)(q13)
42.	FRA11Q23	Fragile site at (11)(q23)
43.	FRA12Q13	Fragile site at (12)(q13)
44.	FRA16P12	Fragile site at (16)(p12)
45.	FRA16Q22	Fragile site at (16)(q22)
46.	INV(3)(P25;Q23)	Inversion at (3)(p25;q23)
47.	9QH	Centromere heteromorphism on chromosome 9
48.	T(1;3)(Q43;P21)	Translocation at (1;3)(q43;p21)
49.	T(10;18)(Q26;Q21)	Translocation at (10;18)(q26;q21)
50.	T(11;22)(Q23;Q11)	Translocation at (11;12)(q23;q11)
51.	T(13;14)(P11;P11)	Translocation at (13;14)(p11;p11)

(b)   RESEARCH OUTLINE

Genetic markers are valuable tools for the study of human genetics. The aims of the investigations to follow can be briefly summarised as follows :

1) Deletion mapping using individuals with monosomic segments defined by G-banding.

2) Mapping by linkage analysis within families having heritable chromosomal markers or disease loci. The possibility of linkage allowing gene assignment or gene localisation to specific chromosomal regions will be examined between:

   i) Unassigned genetic markers and chromosomal markers, particularly autosomal fragile sites,

   ii) Unassigned genetic markers and other marker loci and

   iii) Unassigned disease loci and other marker loci.

3) Investigation of associations.

These investigations will be based on the genetic markers listed in Table 1.1.

(c)   HISTORICAL BACKGROUND

i)   Early History

The foundations upon which gene mapping and association studies are based will now be identified. These encompass many of the key developments that have occurred in human genetics. These developments can be broadly divided into three periods:

(1)   Early history culminating in the rediscovery of Mendelism,

(2)   Proliferation of specialisations based on Mendelian genetics and

(3)   The introduction of recombinant DNA technology.

Adams in 1814 summarised the early pre-Mendelian knowledge of human genetics. He published "A Treatise on the Supposed Hereditary Properties of Diseases" as a guide to counselling in heredity (Motulsky, 1959). Adams

(1)   differentiated between congenital conditions with properties now recognised as recessive and dominant,

(2)   recognised frequent consanguinity in what are now recognised as recessive conditions,

(3)   recognised variable age of onset of hereditary diseases not present at birth,

(4)   recognised the existence of environmental stimuli as a trigger to disease in individuals with a genetic predispostion,

(5)   recognised the existence of disease heterogeneity (different genetic basis for similar clinical conditions),

conditions),

(6)   recognised effects of inbreeding in expressing recessive alleles in small populations, and

(7)   recognised reduction in fitness of the carriers of some of the hereditary diseases, and that the frequency of these diseases would therefore diminish without new input.

The general concept of heredity had therefore been established by the early nineteenth century. Although the concept of natural selection was not to be firmly established for animal populations until much later (Darwin, 1859), there was already the recognition of a reduction in fitness of sufferers of some hereditary diseases in human populations.

Adams was unable to develop explanations for his observations. The medical literature of the nineteenth century documents numerous attempts to attribute the influence of heredity to disease causation (Vogel and Motulsky, 1979). The existence of heredity was clearly recognised, but there was no mechanism expressed in the clear and simple terms of single genes.

The science of human genetics began in about 1865 with the advent of biometrical methods (Vogel and Motulsky, 1979). Unfortunately, Mendel's laws of inheritance, which were published simultaneously (Mendel, 1866), remained in obscurity for another 35 years. The unification of biometrical genetics and Mendelian genetics came when Fisher (1918) proposed that biometrical phenomena were

quantitative characteristic. Biometrical methods do not require detailed knowledge of the genetic architecture of a character.

Credit for the particulate theory of inheritance is due to Mendel. His findings were rediscovered by Correns, Tschermak and de Vries in 1900 (Barthelmess, 1952). The gene concept arising from Mendel's experiments became the central concept of all genetics, including human genetics. The evolutionary concept so well documented by Darwin (1859) had now been generally accepted by the scientific community. The realisation that the laws of heredity which apply to animal and plant species were also valid for humans arose from the knowledge that humans are part of the animal kingdom, originating from more primitive primates. Hence, Mendel's laws eventually were applied to human pedigrees.

Mendel discovered three laws. The law of uniformity states that the crossing of homozygotes of different alleles produces F1 progeny all identical and heterozygous at that locus. The law of random segregation describes observations of 1:2:1 segregation in intercrosses of heterozygotes and 1:1 segregation in backcrosses of heterozygotes with recessive homozygotes. The law of independent assortment states that traits controlled by different loci are transmitted independently. Departure from independence is evidence for linkage between loci, a property later to be applied to gene mapping by linkage analysis.

The proliferation of human genetics was now possible. Numerous specialisations evolved in close association with medical aspects of genetics. The major areas of specialisation recognised today are:

(1)   cytogenetics,

(2)   biochemical genetics,

(3)   immunogenetics,

(4)   population genetics,

(5)   clinical genetics, and

(6)   molecular genetics.

Gene mapping and gene association studies encompass all of these specialisations.

ii)   Cytogenetics

Cytogenetics is the study of chromosomes in relation to inheritance and the origin of pathological conditions. Cytogenetics includes somatic cell genetics. There was little progress in gene mapping until the development of human cytogenetics for chromosomal identification and regional definition of chromosomes.

Hamerton (1971) and Hsu (1979) have reviewed the milestones in human cytogenetics. The most significant of all early developments responsible for the development of human cytogenetics to a level where gene mapping could begin were:

(1)   the introduction of the squash technique (Ford and Hamerton, 1956a),

(2)   colchicine pretreatment of cells (Ford and Hamerton, 1956b,c),

(3)   use of hypotonic solution (Hsu, 1952; Makino and Nishimura, 1952),

(4)   application of lymphocyte culture methods (Hungerford et al., 1959; Moorhead et al., 1960) and

(5)   use of phytohaemagglutinin (Nowell, 1960).

The squash technique preserved each cell intact and spread the chromosome complement of the nucleus into one plane of focus. This technique had already been used for decades in plant and animal cytogenetics (Darlington and La Cour, 1942). Microscopy of a two dimensional karyotype eliminated the interpretative difficulties associated with the slicing of long chromosomes during serial sectioning of a three dimensional nucleus.

Colchicine pretreatment had two effects : it caused further condensation of metaphase chromosomes to reduce the degree of overlap, but more importantly, it arrested cells at metaphase to increase the number of cells suitable for cytogenetic examination.

The use of hypotonic solution for pretreatment of cells was the turning point in mammalian cytogenetics because it dispersed chromosomes and their attached nucleolar material. This permitted the observation of separate sharply defined chromosomes.

The introduction of lymphocyte culture methods allowed biopsy material to be replaced by blood, a tissue much more accessible for routine study.

Phytohaemagglutinin (PHA) aided microscopic analysis by increasing the number of cells available for examination. The observation that PHA stimulated cell division in culture was responsible for the success of lymphocyte culture methods.

The modern era in human cytogenetics began with the identification of all chromosomes. Casperson et al., (1970) did this by staining chromosomes with quinacrine in conjunction with examination under the fluorescence microscope. Each chromosome had a characteristic Q banding pattern arising from preferential binding of quinacrine to DNA. Sumnner et al. (1971) developed the simpler G-banding technique which allowed indefinite storage of banded slides and is now widely used in routine diagnostic laboratories. These techniques allowed the precise identification of relatively minor chromosomal aberrations. The Paris Conference (1971) established nomenclature for metaphase chromosomes and described 320 G bands on the human karyotype. Chromosome identification is the basis for gene mapping, which became highly efficient when combined with somatic cell hybridisation and preferential loss of chromosomes in hybrid lines.

Yunis (1976) devised a method for examination of chromosomes in the late prophase stage of cell division.

This has led to the description of more than 2,000 bands in the human karyotype (Yunis, 1981). Although prophase banding can give up to 2,000 bands, there are associated technical difficulties. The latest nomenclature provides schematic representations of chromosomes at three levels of resolution : 400, 500 and 850 bands (ISCN, 1981).

iii) Population Genetics

Population genetics is concerned with the behaviour of genes in populations. Of central importance is the way in which relative frequencies of genes and genotypes change (or fail to change) from one generation to the next. It is an extension of Mendel's laws from the concept of individual inheritance to the dynamics of genes and genotypes in populations. The useful genes in population studies are the polymorphic ones. These are the genetic marker loci that are applicable to linkage and association studies.

The first locus amenable to widespread population genetic analysis was the ABO blood group. Early population genetics flourished using the monogenic blood group traits as they were discovered (Race and Sanger, 1975). These included disease association studies where gene frequencies were compared between the normal population and segments of the population afflicted with diseases of unknown aetiology (Mourant et al., 1978).

The combination of starch gel electrophoresis (Smithies, 1955) and histochemical staining for specific enzymes (Hunter and Markert, 1957) expanded the number of loci

available for study and provided a more efficient tool for quantifying genetic variation in populations. This was achieved simultaneously in man (Harris, 1966) and Drosophila (Lewontin and Hubby, 1966). About one-third of the sample of loci in man demonstrated common allelic variation. The "average individual" was heterozygous at six per cent of the genome. In fact, such extensive levels of variation were found at the electrophoretic level in most species examined (Manwell and Baker, 1970; Lewontin, 1974; Ayala, 1976).

iv)   Biochemical Genetics

Biochemical genetics encompasses the molecular basis of heredity at the gene (DNA) level and the molecular basis of heritable variation at the gene product level. It encompasses both normal and pathological variation. Until recently, emphasis has been directed at the gene product level using the electrophoretic separation of enzymes and proteins and quantitative determinations of enzyme activity and protein levels. These methods have defined numerous normal variants at structural loci which have been subsequently applied as genetic markers (Harris, 1980). They have also identified many pathologic disorders by detectable deficiency of specific enzyme activities or protein levels associated with gene dosage of defective alleles (Brock, 1972). The extent of genetic heterogeneity for many of these disorders remains poorly understood, but new techniques directed at the gene level rather than the gene product level are presently being applied (Maniatis et al., 1982; Walker

and Gaastra, 1983). Recombinant DNA technology is now at the forefront of advancement in human genetics.

v)   Immunogenetics

Immunogenetics is the study of genetically determined characters called antigens. An antigen is a foreign body which when introduced into an animal elicits an immune response involving antibody formation for destruction of that specific antigen. Antibodies are serum proteins and are used in bloodgrouping to phenotype antigens present in blood of test individuals. Immunogenetics encompasses the blood groups, immunoglobulins of the blood, cellular mechanisms of organ rejection and immune deficiency diseases.

Immunogenetics began with the discovery of the ABO blood group (Landsteiner, 1900). When the different ABO types were shown to be heritable (Dungern and Hirschfeld, 1911) the ABO system became an outstanding example of early Mendelian inheritance applied to a human character. Numerous blood group antigens were subseqently defined (Boettcher, 1972) and applied to disease association studies (Mourant et al., 1978) and to linkage analyses (Race and Sanger, 1975). Their widespread use as genetic markers has been surpassed only by the many electrophoretic markers discovered during the decades since 1960 (Beckman, 1972; Cooper, 1972; Harris and Hopkinson, 1976).

vi)   <u>Clinical Genetics</u>

Clinical genetics is concerned with the description and diagnosis of heritable diseases, genetic counselling associated with these diseases and treatment where possible. Diagnosis is often confirmed by tests in cytogenetic or biochemical laboratories.

The family history, medical history and physical examination are the basic procedures used by the medical geneticist to determine what laboratory test will be appropriate. This information may provide a hint as to whether non-genetic, chromosomal, polygenic or single gene aetiology is involved. If a single gene pattern of inheritance is indicated, then autosomal dominant, autosomal recessive or an X-linked mode of inheritance may be surmised. A term often associated with clinical genetics is "genetic nosology". This is defined as the delineation of genetic diseases (McKusick, 1978).

Identification of the locus involved may influence the genetic counselling, prognosis and management. If the biochemical basis of a disorder is known, simple enzyme assays may confirm the exact nature of the disorder. This can be impossible where the aetiology of the defect is unknown. Diagnosis is then based solely on clinical criteria determined from family history, medical history and physical examination.

Gene mapping of loci responsible for such disorders of unknown aetiology may be useful for an initial

classification of a heterogeneous disease. This classification may then be applied to diagnosis by linkage methods until the discovery of gene products and establishment of techniques for their measurement. The various forms of epidermolysis bullosa (McKusick, 1983) await further delineation by linkage analysis.

vii)   Molecular Genetics

Molecular biology was established by the late 1940's and early 1950's. Pauling et al. (1949) discovered that sickle cell anaemia was a molecular disease attributable to an abnormal haemoglobin molecule. They demonstrated two electrophoretically distinct haemoglobins. During the 1950's the amino acid sequence of both haemoglobins was defined. Many more haemoglobin variants are now known. Most are rare but a few are polymorphic in some populations.

The length of a polypeptide and corresponding DNA coding sequence has many potentially mutable points. A typical protein of about 300 amino acids corresponds to 900 bases in the DNA coding sequence (Harris, 1980). Each base within the triplet (codon) can be substituted by any of three others. A large number of different mutations is theoretically possible even after allowing for degeneracy in the genetic code.

This has been confirmed by observation for haemoglobin, which has been studied in greater detail than any other protein. More than 100 variants have been documented from the β-chain in the minute sample of the human

species examined. This represents about 40% of the estimated allelic diversity that can theoretically occur (Harris, 1980).

Electrophoretic screening underestimates the total amount of genetic variation present within populations because of

(1)   degeneracy of the genetic code,

(2)   failure of all amino acid substitutions to alter the net electrostatic charge of the polypeptide molecule,

(3)   different amino acid substitutions can produce the same degree of altered mobility,

(4)   discriminative power of electrophoretic methods for some enzymes or proteins is suboptimal and

(5)   variants with little or no activity are not detectable.

These problems are becoming redundent. The emphasis is presently shifting from gene products to direct gene analysis using DNA restriction techniques and Southern transfer (Southern, 1975).

The number of structural genes in man is thought to be of the order of 50,000 (McKusick and Ruddle, 1977). Structural genes occur in single copies so that mutations are inherited in a Mendelian fashion. The structural genes code for all the enzymes involved in transcription, protein processing and intermediary metabolism, and all proteins that are structural or have specialised functions. The amount of human DNA is sufficient   for   50

to 100 times as many genes of average length, but a large amount of this DNA is in repetitive form or in other non-coding regions of the genome. The role of repetitive DNA may be regulatory, structural (centromeric DNA) or functional (McKusick and Ruddle, 1977).

The key historical developments and diversification identified in this section were the prelude for both gene mapping (section (d) this chapter) and associations (section (e) this chapter).

(d) GENE MAPPING

The aim of gene mapping is to determine for each gene its locus, or position in the genome. This involves

(1) assignment to respective chromosomes,

(2) subsequent regional localisation, and

(3) the establishment of linear order.

Gene mapping is relevant to both disease loci and loci with no known disease association.

What is the motivation for gene mapping? Several reasons can be recognised and these have either an academic or applied basis. These reasons are:

1) Man wishes to investigate himself as far as existing techniques allow, if only for academic reasons,

2) Comparative mapping with other mammals, especially primates, to study the conservation of linkage groups during evolution,

3) To determine if genes with related functions cluster on the same chromosome,

4) To compare the genetic map with the chiasma map and the mitotic map,

5) To provide a genetical classification of heterogeneous disorders,

6) To apply gene dosage technology for identification of submicroscopic chromosomal deletions and duplications and

7) For application in a predictive fashion to prenatal diagnosis and genetic counselling by linkage for deleterious loci not amenable to direct investigation because

i) gene product unknown

ii) gene product known but tissue not amenable to safe biopsy
for testing.

iii) existing tests unreliable or equivocal.

iv) delayed onset of symptoms.

There are several approaches to gene mapping. These are:

1) linkage analysis,

2) deletion mapping,

3) somatic cell genetics and

4) in situ hybridisation.

Linkage analysis was the first successful mapping technique   for
human autosomal loci. This approach is used in Chapters 4, 5 and 6.
Linkage analysis is defined and discussed in section (f) of this
chapter.

Deletion mapping is defined and applied in Chapter 3.   This
technique has contributed relatively little information to the gene
map by comparison with linkage analysis and somatic cell genetics.

Somatic cell genetics has dominated gene mapping until the
recent re-emergence of linkage analysis associated with the discovery
of DNA restriction polymorphisms. Somatic cell hybrids are produced
by fusing somatic cells from different species, such as man and the
mouse, or man and the chinese hamster. Fusion is promoted by viral
or chemical agents. The cell hybrid possesses a single nucleous with
functional chromosomes of both parental cells. The human
chromosomes are preferentially lost in an irregular fashion from a
hybrid   cell   during   cell   division   in

culture. The chromosomal complement of cloned cells can be precisely defined using G-banding. Gene products are visualised as isozymes after electrophoresis and histochemical staining. Homologous human and animal isozymes are distinguished by their respective electrophoretic mobilities; non-expression of the human component demonstrates loss of the human gene. If a number of independently derived clones are found to have only mouse gene products for a specific locus under test, then the corresponding human locus may be assigned to whatever human chromosome is absent from all such clones. Conversely, clones expressing both mouse and human allelic products would always possess the human chromosome with the corresponding structural gene. The power of the technique is that loci need not be polymorphic : interspecific enzyme products need only be electrophoretically distinguishable.

In situ hybridisation is another technique associated with molecular biology. This involves the hybridisation of cloned single copy DNA sequences labelled with tritium. Sequences are hybridised directly to denatured complimentary sequences on metaphase chromosomes.

Advancement in gene mapping has been rapid as evidenced from reports of seven consecutive International Workshops on Human Gene Mapping (HGM) since 1973 (HGM1, 1974; HGM2, 1975; HGM3, 1976; HGM4, 1978; HGM5, 1979; HGM6, 1982; HGM7, 1984). The accelerated progress in recent years resulted from the development and application of techniques for chromosome banding, electrophoresis and somatic cell hybridisation. The first autosomal assignment was made by linkage analysis (Donahue et al., 1968). At least one structural locus was assigned to every

human chromosome by 1976 (McKusick, 1978). By 1977 about 110 loci were assigned to specific autosomes (McKusick and Ruddle, 1977). Linkage studies between polymorphic loci using pedigree analysis accounted for the establishment of about 40 linkages in the ten years since 1968 (McKusick, 1978). Molecular methods are now providing renewed impetus to gene mapping, either directly via the mode of in situ hybridisation or indirectly by linkage analysis using restriction fragment length polymorphisms (Human Gene Mapping 7, 1984).

Given rapid progress in gene mapping, the map status of some markers changed dramatically during the study period. Of the marker loci examined, their status on the human gene map at the commencement and completion of this study is given in Table 1.2.

TABLE 1.2

THE NON-CHROMOSOMAL MARKERS EXAMINED : STATUS ON THE GENE MAP AT COMMENCEMENT AND COMPLETION OF STUDY

Marker	Region and Status* during study period			
	At commencement**		At completion***	
ABO	(9)(q34)	C	Same	
ACP1	(2)(p25) or (2)(p23)	C	Same	
ADA	(20)(q13.2→qter)	C	Same	
AK1	(9)(q34)	C	Same	
AMY2	(1)(p)	C	(1)(p21)	
C3	6	P	(19)(pter→q13.2)	C
CHE2	(16)(cent→q22)	P	Unassigned	
ESD	(13)(q)	C	(13)(q14.1)	
F13A	Unassigned		6	
F13B	Unassigned		Unassigned	
Fy	1	C	(1)(q12→q21)	
GALT	(9)(p)	C	(9)(p21→p13)	
GC	(4)(q11→q13)	C	Same	
GLO1	(6)(p23→p21.2)	C	(6)(p21.3→p21.2)	
GPT	10	T	(8)(q13→qter) or (16)(pter→p11)	I
HLA	(6)(p23→p21.2)	C	(6)(p21.3)	
HP	(16)(cent→q22)	C	(16)(q21→q22)	
IGH	Unassigned		(14)(q32.3)	C
IGK	7	P	(2)(p12)	C
Jk	(7)(q)	P	2	C
K	Unassigned		Unassigned	
Lu	Unassigned		19	C
MNS	(4)(q28→q31)	C	(4)(q28→q31)	C
P	6	T	Same	
PGD	1	C	(1)(pter→p36.13)	
PGM1	(1)(p34→p33) or (1)(p32) or (1)(p31.1→p22.1)	C	(1)(p22.1)	
PGP	16	C	(16)(p12→p13)	
PI	6 or 8	I	(14)(q24.3→32.1)	C
Rh	(1)(pter→p32)	C	(1)(p36→p34)	
TF	Unassigned		Same	
Xg	X	C	(X)(pter→p22.3)	C

*   C - confirmed
  I - inconsistent
  P - provisional
  T - tentative

**   According to Human Gene Mapping 5 (1979), latest Human Gene Mapping Proceedings available at commencement of study.

***   According to Human Gene Mapping 7 (1984), latest Human Gene Mapping Proceedings available at completion of study.

(e)   ASSOCIATIONS

The associations usually investigated in human genetics   are disease associations. A specific definition for disease association is: the correlation of an allele with the presence or absence of disease. Chromosomal abnormalities are regarded as forms of genetic disease in disease association studies. Not all associations involve diseases. Associations with mutation to fragile sites and segregation distortion will be investigated in Chapters 7 and 8. A general definition for association applicable to all of the above is: the correlation of an allele with the presence or absence of the character being studied.

The cause for an association in the population can be:

1)   The marker locus is the major gene affecting the trait,

2)   the marker locus is in linkage disequilibrium with the major gene affecting the trait,

3)   The marker locus exerts a pleiotropic effect on the trait, or

4)   the marker locus is in linkage disequilibrium with the gene affecting the trait pleiotropically.

The relationship is causal or direct only in cases 1) and 3). Clearly, if a marker locus and a disease locus are tightly linked, then associations will also be detectable within families. This is the basis for linkage analysis. Such an association in no way implies a causal relationship. This association will not exist at the population level unless alleles at these loci are in linkage disequilibrium in the population studied.

Disease associations with polymorphic marker loci have been extensively investigated since about 1945 (Mourant et al., 1978). Differences between individuals are manifested in physical appearance, physiological function and susceptibility to disease. This "genetic background", acting in concert with environmental factors, accounts for variation in the expressivity, penetrance and age of onset of diseases genetically determined by genes of large effect. Similarly, genetic background is relevant to diseases of polygenic, multifactorial or primarily of non-genetic origin.

That functional differences between the polypeptide products of different alleles are likely to exist is suspected from common observations of asymmetrical electrophoretic patterns in heterozygotes. Asymmetrical patterns arise from allelic variation in the expression of enzyme activity or protein concentration. Such observations are characteristic of PGD, ACP1, GPT, GALT and PI (Harris and Hopkinson, 1976; Fagerhol and Cox, 1981). Many of these functional differences perhaps represent only slight or even immeasurable effects on the fitness of the individual when each is considered separately.

The small sample of known polymorphisms is likely to reveal only a fraction of the yet undiscovered associations, except in some instances where relationships of a pathological or physiological nature are known. Such is the case with the PI (protease inhibitor) system which is related to diseases involving tissue destruction (Laurell and Eriksson, 1963; Sharp et al., 1969). Totally fortuitous associations without any known basis have been detected; such as the PC 1 polymorphism with depressive psychosis and multiple sclerosis (Comings, 1979).

Extensive documentation supports many specific disease relationships with blood groups (particularly the much studied ABO) and histocompatibility antigens (Giblett, 1977; Mourant et al., 1978; Harris, 1980). Little is known regarding enzymes and plasma proteins except for instances where gross variations in enzyme activity or protein concentration are responsible for inborn errors of metabolism. Although numerous associations have been postulated, strong associations have been established and verified at least between blood group A and carcinoma of the stomach (Aird et al., 1953), Duffy blood group and resistance to malaria (Miller et al., 1975,1976) and HLA-B27 antigen and anklosing spondylitis (Brewerton et al., 1973, Schlosstein et al., 1973).

(f) LINKAGE ANALYSIS

The basics of linkage analysis are discussed in Publication 11 (Mulley, 1985) of Chapter 4. Linkage analysis is the mapping technique predominantly used in this thesis.

The difference in recombination rate between men and women was first recognised in 1965 (Cook, 1965; Renwick and Schulze, 1965). Crossing over in certain well studied chromosomal segments is almost twice as frequent in females compared with males. If this is general, the female genetic map would therefore be nearly twice as long as the male map. Genetic distances are standardised by the presentation of map distances in units of male recombination frequency.

The standard lod table may be subdivided into male and female components when linkage is suspected from limited data or when linkage is known from a large body of data. Linkage is easier to

demonstrate in data from an informative male parent because crossing over is reduced in males. An accurate estimate of the relationship between male and female recombination rates is possible only from extensive data.

The basis of sex differences in recombination rate and differential recombination rate within chromosome arms is not understood. It could be speculated that sex differences in recombination rate could result from differential chromosomal condensation at meiosis. Differential crossing-over in proximal compared with distal chromosomal segments is presumably determined somehow by the physical presence of the centromere.

Another generalization about the human genome is less clear, less well documented, and of a magnitude that for practical purposes may be ignored in exploratory linkage analysis. There is possibly a reduction in recombination with age (Reid and Parsons, 1963; Henderson and Edwards, 1968). If this correlation exists, it has not been clearly demonstrated.

The maximum likelihood estimate of the recombination fraction (θ) is determined from families as follows:

The frequency of recombinant gametes is θ, by definition. Therefore the frequency of non-recombinant gametes is $1 - \theta$. Two strand crossing-over produces two recombinant gametes in equal frequency, the frequency of each being $\theta/2$, and two non-recombinant gametes in equal frequency, the frequency of each being $(1-\theta)/2$. If loci are on different chromosomes, or syntenic but unlinked, then all four gametes would be produced in equal frequency as stated in Mendel's law of independent assortment.

The probability of obtaining an observed segregation pattern within a family is determined from simple probability theory as the product of the frequency of gametes from the informative parent. Offspring are assessed as recombinants or non-recombinants, firstly on the assumption of coupling phase in the informative parent. Thus, in a four child family, for example, the probability of getting three non-recombinants and one recombinant is:

$$\frac{(1-\theta)^3}{2} \frac{(\theta)}{2} = \frac{\theta(1-\theta)^3}{16}$$

However, it is equally likely that the phase of the informative parent was not in coupling as assumed, but in repulsion. The corresponding probability is:

$$\frac{(1-\theta)}{2} \frac{(\theta)^3}{2} = \frac{\theta^3(1-\theta)}{16}$$

If linkage phase is known, as is often the case in kindreds of three or more generations, one or other of the above expressions determines the probability of obtaining the family. If linkage phase is unknown, as in the example given, there is an equal likelihood of coupling or repulsion, and the probability of obtaining the family is the average of the above probabilities and is:

$$\frac{1}{2} \left[\frac{\theta(1-\theta)^3}{16} + \frac{\theta^3(1-\theta)}{16} \right]$$

The probability of obtaining this family for various values of θ from 0 to 0.5 can then be calculated. Similar examples are given by Emery (1976).

The maximum likelihood estimate of θ is obtained from the probability ratio (Pr) of having obtained a given value of θ, where

$$Pr = \frac{P(\text{family given } \theta=0,0.05,0.1,....0.5)}{P(\text{family given } \theta = 0.5)}$$

Log $_{10}$Pr is the lod score (see Publication 11).

The calculation of lod scores from these expressions are extremely tedious, even for simple families. When parental data is complete there are only a finite number of possible lod scores for any θ in a family of given size. Such tables are available from sources such as Morton (1955), Smith (1968) and Emery (1976).

Computer facilites provide a means of more rapid analysis and a means of analysing families where manual computations are virtually impossible. Algebraic procedures (Elston and Stewart, 1971; Lange and Elston, 1975) have been applied to the computer analysis of extended families. The ubiquitous program is LIPED (Ott, 1974, 1976) written in FORTRAN IV and exportable to most computer installations. Other programs have been described (Woien, 1970; Friedhoff and Chase, 1975; Sturt, 1978). The program LINKAGE (Lathrop et al., 1984) written in PASCAL now allows multilocus linkage analysis.

(g)   SUMMARY

This chapter defined the general objectives of the project using genetic markers. The basis for gene mapping and association studies was developed by documenting the key historical developments relevant to these studies. These developments were considered on a background of the evolution of specialisations in human genetics which proliferated after the rediscovery of Mendelism. Finally, gene mapping, associations and linkage were discussed as a prelude to the specific investigations to be presented in this thesis.

CHAPTER 2

DISCUSSION OF MATERIALS, METHODS AND THE STUDY POPULATION

(a)   INTRODUCTION

(b)   SAMPLES

(c)   MARKERS DETERMINED

(d)   ELECTROPHORESIS

(e)   ISOELECTRIC FOCUSING

(f)   INTERPRETATION

(g)   BLOOD GROUPING

(h)   FAMILY DATA

(i)   ALLELE FREQUENCIES

(j)   SUMMARY

Publication Numbers

1   Mulley (1980)

2   Mulley (1982a)

3   Nicholls and Mulley (1982a)

4   Nicholls and Mulley (1982b)

5   Mulley (1982b)

(a)   INTRODUCTION

This chapter has the following aims:

1)   To document the materials used by the investigator

2)   To discuss the principles behind the methods

3)   To discuss characteristics of the genetic markers used for the investigations

4)   To present the phenotyping results derived from the families to be analysed in subsequent chapters

5)   To genetically characterise the base population for validation of subsequent linkage analyses.

Care was taken to ensure

1)   Proper choice of materials and methods to maximise the efficiency of gathering data,

2)   full understanding of methodologies in order to extract as much information as possible from the samples, and

3)   development of technical expertise with each of the markers under usual laboratory conditions in order to correctly interpret observations for provision of valid data for subsequent analysis.

(b)   SAMPLES

Blood is the most common source for the determination of human genetic markers. It is readily obtainable from living individuals. Markers restricted to other tissues may normally be available only at autopsy. These are impractical for family study because of the generation length in humans. Their heritable nature may be difficult to demonstrate because of this.

The major advantage of blood as the source material is the large number of markers that can be characterised from a small sample. These are generally determined by bloodgrouping (Race and Sanger, 1975; or electrophoretic separation (Beckman, 1972; Cooper, 1972; Harris and Hopkinson, 1976). The range of blood markers is now potentially limitless with the discovery of extensive restriction fragment length polymorphism (RFLP) as described by Botstein et al., (1980). These are detectable by the electrophoretic separation of restriction endonuclease digests of DNA, transfer to nitrocellulose and hybridisation with radioactive probes.

Wherever possible a blood sample of 10 ml was obtained for cytogenetic and genetic marker studies. The 6 ml portion for genetic marker studies was subdivided as follows:

2 ml   allowed to clot for serum collection.

2 ml   placed in a lithium heparin tube for subsequent separation of red cells and plasma by centrifugation.

2 ml   added to 1 ml ACD [disodium hydrogen citrate (3%) dextrose (3%) anticoagulent solution, pH 5.2].

ACD provided an energy source for red cell metabolism until the sample was processed.

Serum, plasma, red cells from the heparinised tube (washed twice in physiological saline) and red cells from the ACD tube (ACD and plasma discarded, equal volume of buffered glycerol added) were frozen at $-20^{\circ}C$ until required for electrophoresis or blood grouping. Buffered glycerol comprised:

9.75g potassium citrate

1.8 g potassium hydrogen phosphate

1.41g potassium dihydrogen phosphate

300 ml water

200 ml glycerol

pH 7.0

Bloodgrouping was usually performed on a fresh aliquot prior to freezing. When frozen cells were used for blood-grouping the cell suspension in glycerol was dialised at 5OC overnight in physiological saline to obtain a high yield of intact red cells.

Thawed serum, plasma and red cell haemolysates were used for electrophoresis. Haemolysates prepared from the frozen intact red cells in glycerol (one wash in 9 per cent saline, refrozen to haemolyse) were preferable for all red cell electrophoretic markers, except GALT and GLO1, for which haemolysate from heparinised tubes provided clearer electrophoretograms. Cells stored in glycerol were washed in 9% saline rather than physiological saline in order to reduce the amount of haemolysis during washing. Haemolysates were used without further treatment for the electrophoresis of ACP1 and PGP. Haemolysates were routinely treated with the reducing agent β-mercaptoethanol (1%) for half an hour at room temperature prior to the electrophoresis of the remaining red cell enzymes. The ratio of β-mercaptoethanol to sample was 1:3.

The use of reducing agents reverses oxidation of thiol (sulphydryl) groups in cysteine residues which occurs during storage (Harris and Hopkinson, 1976). Oxidation by glutathione (which accumulates in stored red cells) can create discrete stepwise changes seen as secondary isozymes. β-Mercaptoethanol was incorporated into the gels rather than the sample for ACP1 and PGP, but not for gels stained using electron transfer dyes. This would create heavy background interference and prevent visualisation of bands.

(c)   MARKERS DETERMINED

The genetic markers routinely examined during this study are listed in Table 2.1. They are described further in Appendix 1. Detailed composition of the electrophoretic systems used are given in Table 2.2. All markers were determined using established electrophoretic or serological techniques. Staining details for most loci are summarised in Appendix 1. Details for the remaining loci are presented in Publications 1-5 (Mulley,1980; 1982a; 1982b; Nicholls and Mulley, 1982a, 1982b).

The allele frequencies given for each locus in Appendix 1 fall within established limits for populations of Anglo-Saxon origin. These allele frequencies were supplied as data to the computer program LIPED, which was used for linkage analysis. Upon completion of the study, these allele frequencies were compared with those obtained from the study population. See section (h) this chapter. Allele frequencies for PI, GC, TF and GALT were also determined directly from the Adelaide population in separate investigations (Publications 1-5).

All procedures, except those for cytogenetic, HLA, IGH and IGK determinations were carried out within my own laboratory. Cytogenetic markers and aberrations were determined within the same department (Department of Histopathology, Adelaide Children's Hospital), IGH and IGK in the Department of Applied Biology, Royal Institute of Medical Technology, Melbourne, and HLA in the Department of Serology, Adelaide Blood Centre of the Red Cross Society.

The genotypic and phenotypic system of human gene nomenclature for all markers is based on that adopted by the 5th

International Gene Mapping Workshop in Edinburgh, July 1979, and subsequently published (Shows et al., 1979) with modifications to blood group nomenclature according to Shows and McAlpine (1982).

TABLE 2.1

GENETIC MARKERS EXAMINED : SUMMARY OF COMMON VARIANTS, ELECTROPHORETIC SYSTEMS AND VISUALISATION METHODS

Marker	*Common Variants	**Electro-phoretic System	Visualisation Method
(a)	**Red cell electrophoretic markers:**		
1. ACP1	A, B and C	SGE 1***	Fluorescence
2. ADA	1 and 2	SGE 2	Enzyme linked
3. AK1	1 and 2	SGE 2	Enzyme linked
4. ESD	2 and 1	SGE 3	Fluorescence
5. GALT	D, LA and N	SGE 4	Enzyme linked
6. GPT	2 and 1	SGE 5	Enzyme linked
7. GLO1	2 and 1	SGE 6	Chemical
8. PGM1	2 and 1	SGE 3	Enzyme linked
9. PGM2****	Monomorphic	SGE 3	Enzyme linked
10. PGD	A and C	SGE 7	Electron transfer dye
11. PGP	1, 3 and 2	SGE 8***	Chemical
12. SOD****	Monomorphic	SGE 7	Electron transfer dye
(b)	**Electrophoretic markers of serum and plasma:**		
13. AMY2	A and B	PAGE 9	Chemical
14 CHE2	C_5+ and C_5-	SGE 10	Chromogenic
15. C3	1 and 2	AGE 11	Non-specific protein stain
16. F13A	1 and 2	AGE 11	Fluorescence
17. F13B	1 and 3	AGE 11	Specific protein stain
18. GC	1F, 1S and 2	PAGIEF 12	Protein precipitation
19. HP	1 and 2	SGE 13	Chemical
20. PI	M1, M3, M2, S and Z	PAGIEF 14	Non-specific protein staining
21. TF	C1, C3 and C2	PAGIEF 15	Non-specific protein staining

(c)   Blood groups

22.	ABO	A, B and O	-	Agglutination
23.	Fy	A and B	-	Agglutination
24.	K	K and k	-	Agglutination
25.	Jk	A and B	-	Agglutination
26.	Lu	A and B	-	Agglutination
27.	MNS	MS, Ms, NS and Ns	-	Agglutination
28.	P	P1 and P2	-	Agglutination
29.	Rh	R_1, r, R_2 and R_0	-	Agglutination
30.	Xg	Xga and Xg-	-	Agglutination

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*   In order of electrophoretic mobility from anode to cathode [blood groups, CHE2, GALT (D and LA) excepted].

**   SGE   - starch gel electrophoresis
PAGE   - polyacrylamide gel electrophoreis
AGE   - agarose gel electrophoresis
PAGIEF - polyacrylamide gel isoelectric focusing

***   1% β-mercaptoethanol added to gel

****   Non-polymorphic markers detected simultaneously with polymorphic markers.

TABLE 2.2

DETAILS OF ELECTROPHORETIC SYSTEMS USED

Buffer System	Electrode (Ingredients/litre)	Gel*	Volts	Time
SGE 1 (Swallow and Harris, 1972)	Sodium dihydrogen phosphate (38.2g). Trisodium citrate (44.1g). EDTA** (1.86g). pH 6.0	1:100 dilution	150	Overnight
SGE 2 (Anonymous)	Potassium dihydrogen phosphate (6.37g). Disodium hydrogen phosphate (2.7g). pH 6.5	1:10 dilution	150	Overnight
SGE 3 (Spencer et al., 1964)	Tris (12.2g). Maleic acid (11.6g). EDTA (3.7g). Magnesium chloride (2.5g). Adjust pH to 7.4 with 10M sodium hydroxide.	1:15 dilution	175	Overnight
SGE 4 (Sparkes et al., 1977)	Tris (18.2g). EDTA (acid) (1.2g), DL-Histidine (19.15g). pH 7.8	1:10 dilution	200	Overnight
SGE 5 (Chen and Giblett, 1971)	Tris (12.1g). Citric acid (5.9g). pH 7.4	1:10 dilution	250	Overnight
SGE 6 (Parr et al., 1977)	Disodium hydrogen phosphate (14.2g). Sodium disodium phosphate (15.6g). pH 6.7	3.75:100 dilution	125	Overnight
SGE 7 (Anonymous)	Disodium hydrogen phosphate (28g). Citric acid (4.6g). pH 7.0	1:20 dilution	150	Overnight
SGE 8 (Barker and Hopkinson, 1978)	Tris (12.1g). EDTA (3.7g) Magnesium chloride (2.46g) Maleic anhydride (9.8g) pH to 7.2 with 10 M sodium hydroxide	1:10 dilution	150	Overnight
PAGE 9 (Ward et al., 1971)	Tris (24.2g) Glycine (112.6g) pH 8.3	Tris (4.5g) pH 8.6	150	4 hours

Contd.

			*	
SGE 10 (Harris and Hopkinson, 1976)	Citric acid (86.16g) pH to 5.0 with 10 M Sodium hydroxide	Tris (2.23g) Succinic acid (1.89g) pH 5.0	150	Overnight
AGE 11*** (Ashton and Braden, 1961)	Solution A	1:9 of A:B	400	Determined by haemoglobin marker
PAGIEF 12 (Kuhnl et al., 1978)	See Publication 3		1,000	4 hours
SGE 13 (Poulik, 1957)	Boric acid (18.55g) Sodium hydroxide (2.4g) pH to 8.0	Tris (9.2g) Citric acid (1.05g)	300	4 hours
PAGIEF 14 (Frants and Eriksson, 1979)	See Publications 1, 2		1,000	4 hours
PAGIEF 15 (Constans et al., 1980)	See Publication 4		1,200	4 hours

* Dilution of electrode buffer, or ingredients/litre.

** Ethylenediamine tetra-acetic acid

*** Solution A : Lithium hydroxide (1.2g), boric acid (11.9g), pH 8.0

   Solution B : Tris (6.2g), citric acid (1.6g), pH 8.4

(d) ELECTROPHORESIS

Standard electrophoretic procedures use an electrical field to separate charged protein variants in a constant pH gel. Good resolution is due to molecular sieving because the pore size of the gel is approximately of the same distribution as the molecular size of proteins. The electrophoretic separation of variants for a given protein depends on the differences in net electrostatic charge on the surface of the molecule. The magnitude of these charge differences vary with the pH of the supporting medium. When allelic variation determines an aminoacid substitution in the corresponding polypeptide which alters the net electrostatic charge, allelic variation is demonstrated simply by differential electrophoretic mobility of the corresponding protein. The rate of migration is determined by the magnitude of applied voltage, magnitude of the electrostatic charge and molecular size of the polypeptide.

Starch gel electrophoresis was carried out using Connaught hydrolysed starch. Starch (46g) was suspended in 120 ml of gel buffer while the remaining 280 ml of gel buffer was brought to the boil. The boiling portion was mixed with the suspension, the cooked starch solution evacuated, then poured into horizontal moulds. The ingredients were increased proportionately when a number of gels were cast simultaneously, the dimensions of each gel being 260x 170x 5mm. After standing for 1-2 hours the top of the gel was sliced off flush, and 12 samples applied approximately 3cm from the cathodal end by pushing into the starch along a marked line. In most cases samples were absorbed into Whatman 3MM chromatography paper. Samples for the detection of GPT, GALT, and PGP were loaded onto thicker inserts, Beckman No. 319329. All starch gels were run

overnight, except for HP which was run for four hours. Gels were stacked one on top of the other, but separated by frozen freezer bricks to maintain low temperature during electrophoresis.

Agarose gel electrophoresis was performed using 1% agarose (Sigma A-6877). Agarose was suspended in the gel buffer and heated to boiling point, then maintained at 60oC until used. All glassware coming into contact with the agarose, such as stirring rods, measuring cylinders and glass plates, were maintained at 60oC until used. For each gel, 60 ml of 1% agarose was made. Portion of this was initially painted onto the glass plates, particularly near the edges, and the plates dried at 60oC. The plates were then placed on a flat surface and 35 ml of agarose poured and spread to edges with a glass rod to produce gels 26 x 12.5 x 0.1cm. After standing 20 minutes in airtight conditions, gels were mounted on an LKB Multiphor and cooled by circulating chilled water. Slots were formed 3cm from the cathodal edge and samples loaded and run for five minutes before wells were sealed with molten agarose, covered with plastic wrap, and electrophoresis completed. Twenty-four samples were run on each gel.

Although many electrophoretic procedures can be done using either starch, agarose or polyacrylamide as the supporting matrix for protein separation, wherever possible starch was used. Apart from isoelectric focusing, electrophoresis was done in polyacrylamide only for AMY2. The non-gradient 5.6% gels were made from cyanogum (3.7g) in 64 ml gel buffer, the solution degassed, and polymerisation achieved with ammonium persulphate (2 ml of 150mg/10 ml solution) and TEMED (50 µl). The gel was moulded with slots on plates to fit the LKB Multiphor. Glycerol (10-20%) was added to the samples to prevent drying out of slots.

The apparatus used for starch gel electrophoresis is shown in Fig 2.1. The apparatus used for agarose gel and polyacrylamide gel electrophoresis is shown in Fig 2.2.

FIG. 2.1 Apparatus used for starch gel electrophoresis.
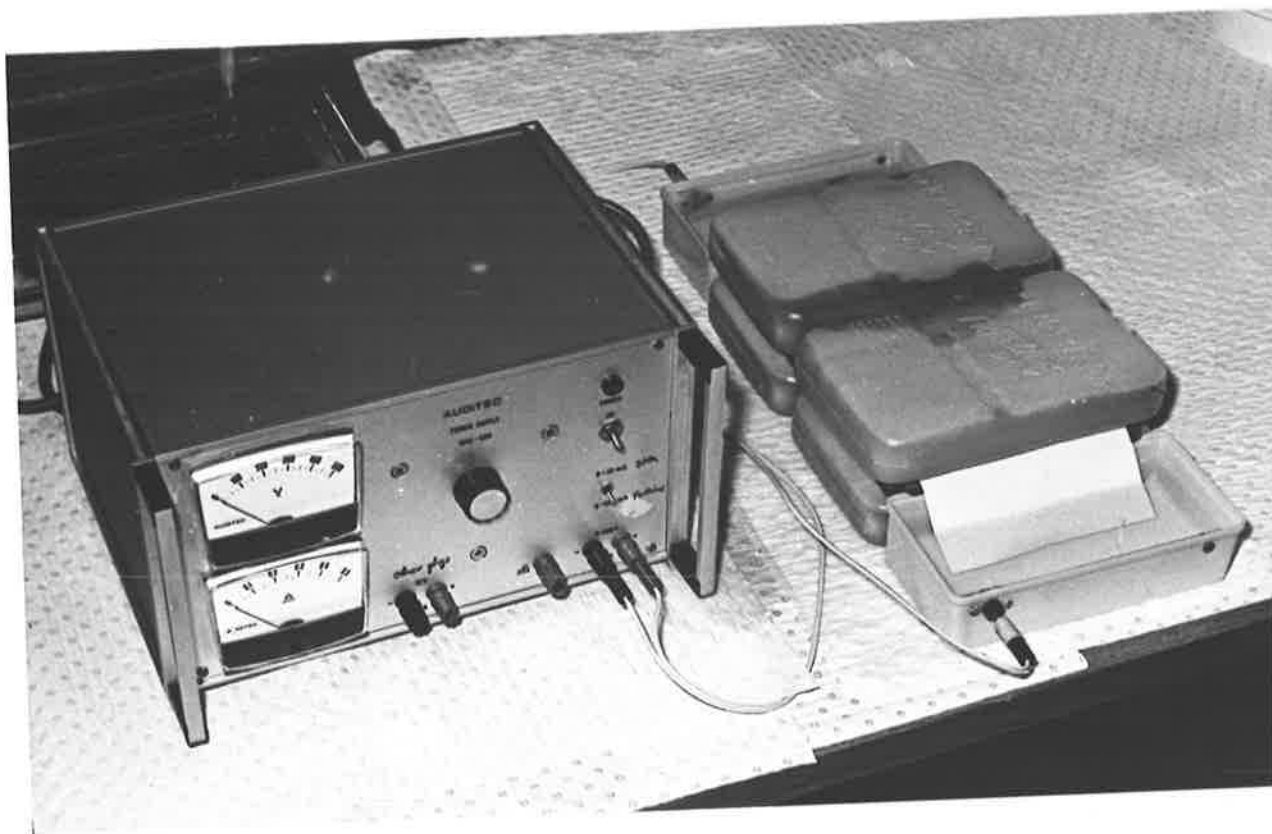
FIG. 2.2   Apparatus used for agarose and polyacrylamide gel electrophoresis and for isoelectric focussing.
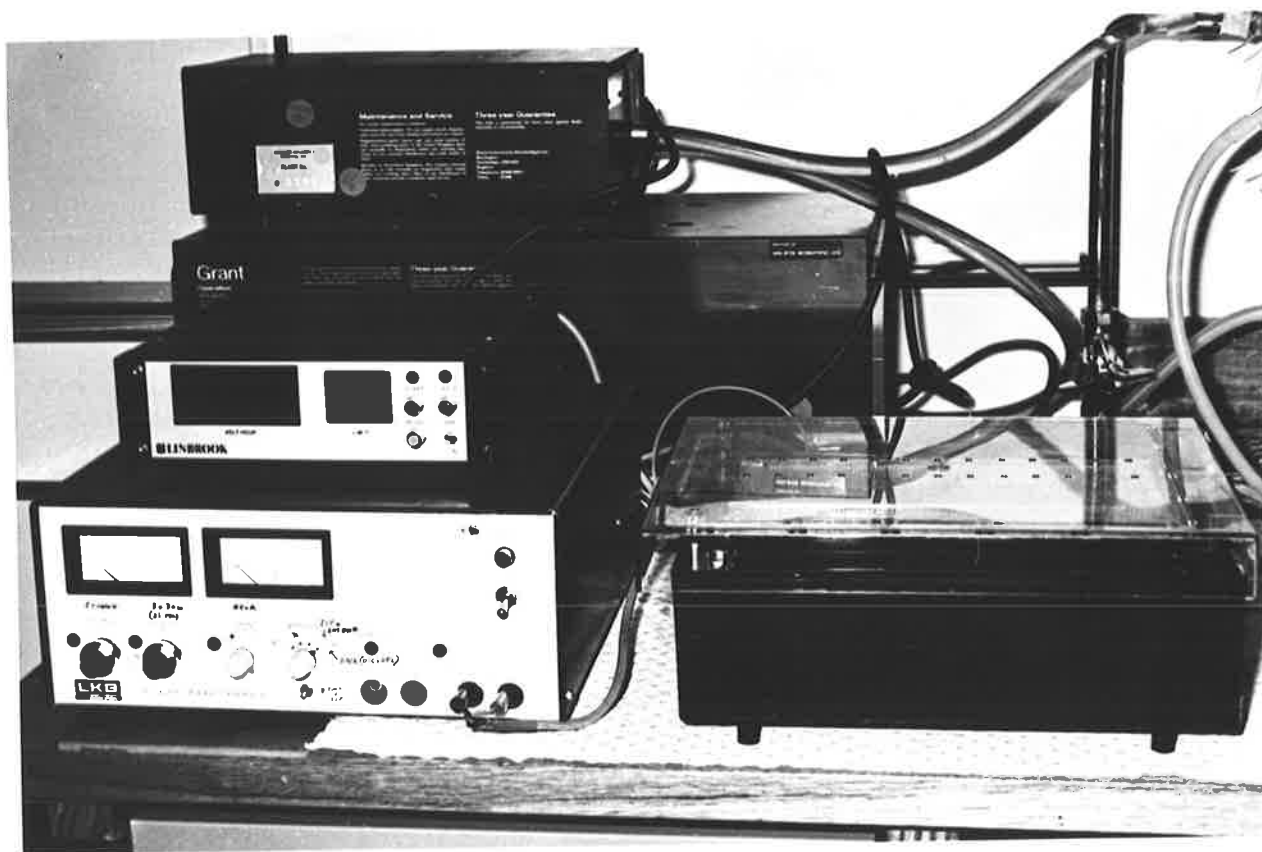
(e) ISOELECTRIC FOCUSING

Isoelectric focusing (IEF) is electrophoresis in a pH gradient rather than at constant pH. The technique was developed in the early 1960's and has been in widespread use in recent years. It is based on electrochemical reactions at the electrodes which affect a mixture of amphoteric substances added during gel preparation. The anode becomes acidic and the cathode alkaline under the influence of an electrical current forming steep micro pH gradients in the vicinity of the electrodes. Under these conditions the amphoteric substances migrate to their isoelectric points. They have buffering capacity at their isoelectric points forming a small pH plateau around the zone of maximum concentration. A large number of such amphoteric substances with a range of isoelectric points will generate a continuous pH gradient. These synthetic substances are designed for various pH intervals and commercially available as Ampholine, Pharmalyte and Servalyt from different suppliers.

Proteins added to the pH gradient will migrate under high voltage to their isoelectric points where they are concentrated. Departure from the isoelectric point by diffusion confers a net electrostatic charge which immediately forces the protein back to its isoelectric point. The diffusion associated with electrophoresis is therefore prevented in IEF and this results in greater resolution. Resolution can be of the order of 0.001 of a pH unit. Additional common variants were detected from PGM1,PI, TF and GC when IEF superseded electrophoresis in the study of these markers.

Details of IEF procedures used are given in Publications 1-4. The apparatus used for IEF was similar to that used for agarose gel and polyacrylamide gel electrophoresis. This is shown in Fig 2.2. It

differed by the substitution of an electrofocusing lid for the simple electrodes used for electrophoresis in gels of uniform pH.

(f)   INTERPRETATION OF ELECTROPHORETOGRAMS

Specific staining methods were first developed for esterases (Hunter and Markert, 1957) and then for dehydrogenases (Markert and Moller, 1959). These were subsequently extended to other enzyme systems. The simplicity of sample requirements, electrophoresis and staining was readily adaptable to mass population screening, in the same way as blood-grouping was in previous decades. Methods for the visualisation of isozymes fall into the following classes:

1)   Chromogenic staining

This involves the conversion of a colourless substrate into a coloured product with an azo dye at the site of enzyme activity (Fig 2.3).

2)   Fluorescence

Both positive and negative fluorescent methods are used. Usually a non-fluorescent substrate is converted to a fluorescent product on a non-fluorescent background (Fig. 2.4). Sometimes the reverse, a non-fluorescent product on a fluorescent background, is used for detection.

FIG. 2.3   Detection of CHE2: cleavage of substrate by CHE2 yields a product which couples to an azo dye to produce an insoluble precipitate.

α - NA

Che

ACETATE   α - N   Fast red TR

COLOURED DYE (Red)
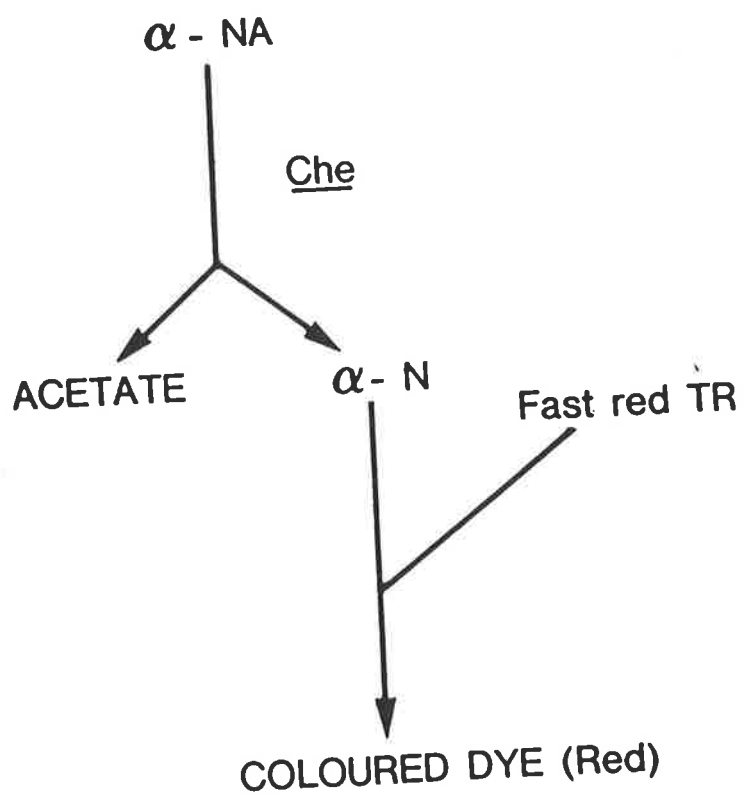
FIG. 2.4   Detection of ESD: cleavage of substrate by ESD yields a fluorescent cleavage product.

4 - M - ACETATE

EsD

ACETATE   4 - M - UMBELLIFERONE
                        (Fluorescent)
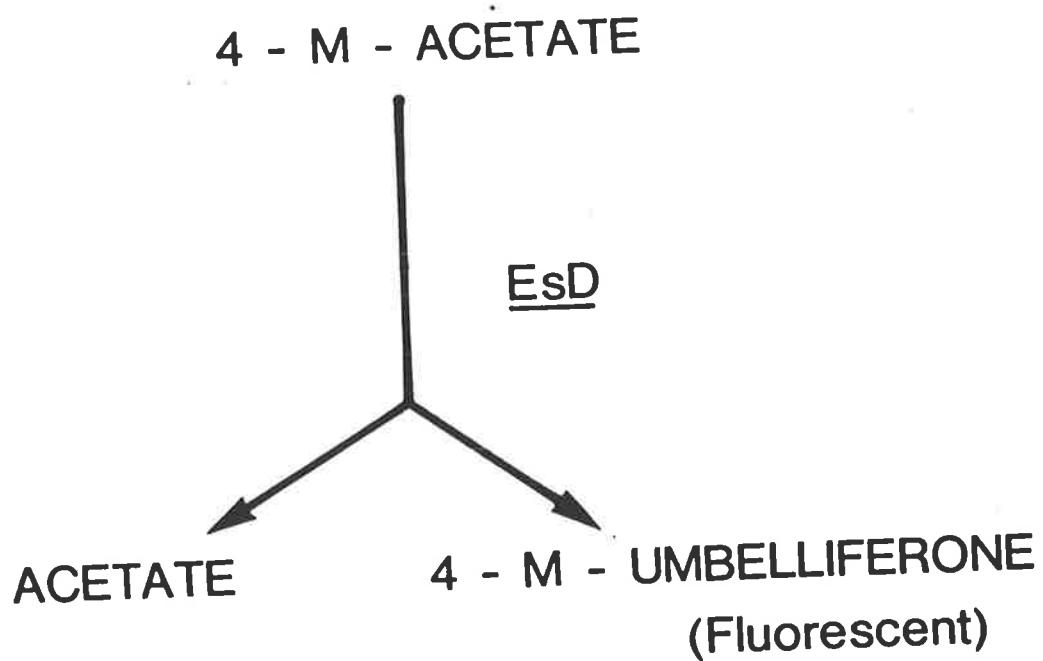
3) Autoradiography (Cavalli-Sforza et al. (1977)

A radioactive product is detected on an x-ray film after the electrophoresis of ligating proteins that have been radiolabelled.

4) Bioautography (Naylor and Klebe, 1977)

Isozymes are located by use of a microbial reagent such as a mutant bacterium. The microorganism cannot utilise the substrate on the plate, but grows at the site of enzyme activity if the enzyme converts substrate to a product necessary for growth.

5) Electron transfer dyes

These substances, colourless when oxidised, form precipitates at the site of enzyme activity when reduced. MTT (Sigma M2128) is the most widely used electron transfer dye forming a formazan precipitate (Fig 2.5).

6) Enzyme linked staining methods

Methods may not be available for the detection of the product of the enzyme reaction under test, but use of an added enzyme converts that product to a substance which can be detected, often by electron transfer dyes (Fig 2.6).

FIG. 2.5   Detection of PGD: reduction of an electron transfer dye forms
an insoluble precipitate.

6 - PG   NADP   PMS   MTT$^+$

Pgd

R - 5 - P   NADPH   PMS$^+$ MTT↓ (Blue)
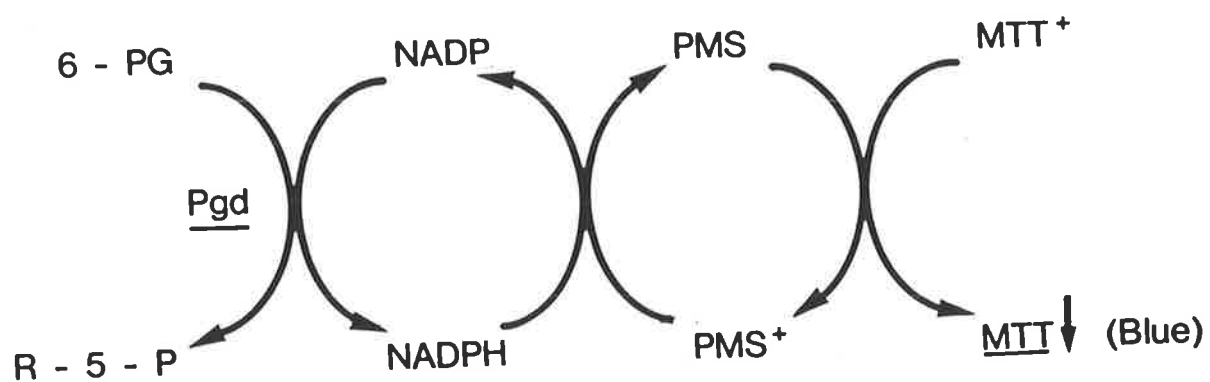
FIG. 2.6 Detection of GALT: the enzyme of interest is detected by a series of conversions linking it to an electron transfer dye.
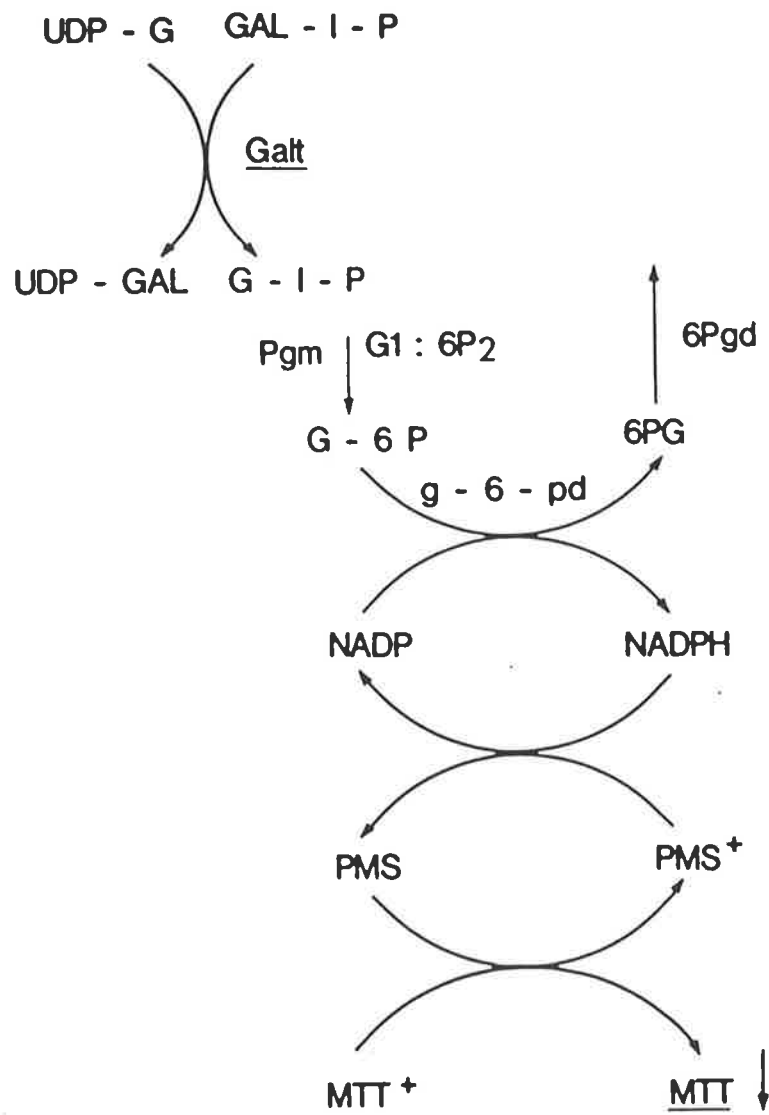
7) Chemical detection

These methods employ chemical reagents other than the electron
transfer dyes or the azo dyes used in the chromogenic staining
procedures. The method may be used to detect the product of
an enzyme reaction, or else lack of activity may be seen on a
background produced by enzymatic activity (Fig 2.7).

8) Non-specific protein staining

Dyes such as Coomassie Blue R bind to protein and are useful
for demonstrating the positions of proteins present in sufficient
concentration (Publications 1 and 4).

9) Specific protein staining (e.g. F13B)

Proteins present in low concentration are precipitated by specific
antisera. Non-specific protein is then preferentially removed
from the gel by washing. The precipitated protein is then
detected as in (8) above.

10) Protein precipitation

Immersion of the gel in fixing solution precipitates the protein.
Opaque bands may be seen on a dark background in transparent
gels, such as polyacrylamide, using side illumination (Publication
3).

FIG. 2.7   Detection of GLO1: the enzyme is detected by a colourless
conversion product.
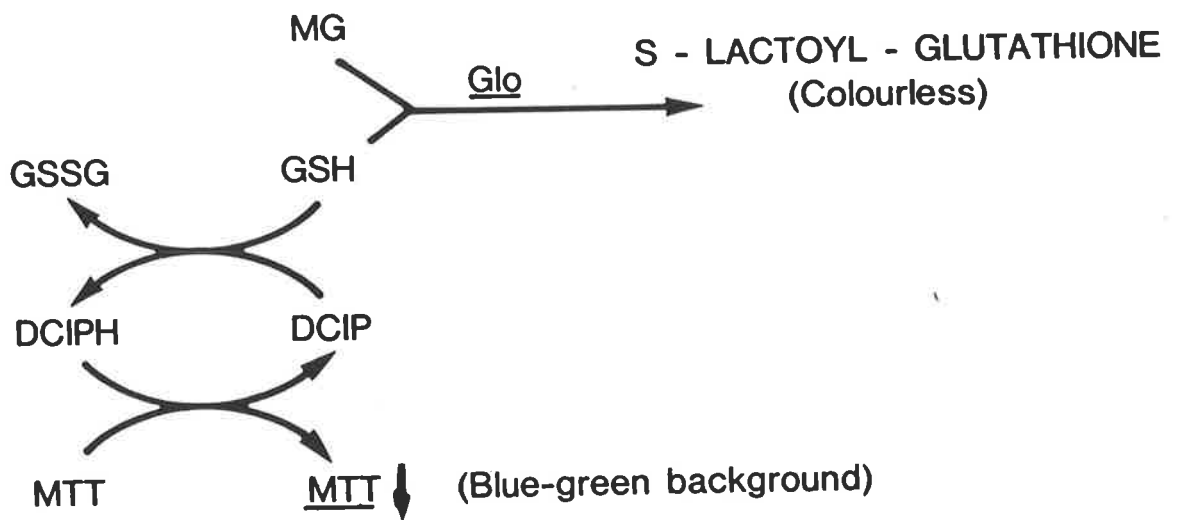
Details of all staining procedures used in this study are given in Appendix 1.

Visualisation of enzymes and proteins after electrophoresis can give complex patterns. These are caused by isozymes which are multiple molecular forms of a given enzyme or protein. Several isozymes can be present in an individual and the isozyme pattern may differ between individuals. The term "isozyme" was introduced by Markert and Moller (1959) as a functional definition at the level of the stained electrophoretic pattern, the zymogram. It has no specific genetic implication. The more narrow term, allozyme, does have genetic connotations : allozymes are allelic isozymes which usually differ by a single amino acid (the basis for electrophoretic separation).

Disregard any additional secondary isozymes and consider the simplest case of an enzyme with monomeric subunit structure. The total number of primary allozyme bands within a population sample can be demonstrated as electrophoretically distinct bands. Within any diploid individual, only one or two such allozymes can occur, corresponding to homozygosity and heterozygosity respectively (Fig.2.8). Alleles controlling electrophoretic phenotypes are usually codominant because both allelic products are displayed irrespective of genotype. The electrophoretic display is phenotypic, not genotypic, because inactive enzymes are not detected and allozymes with different aminoacid sequences but identical charge states are not distinguished. Exact genotyping is only available from DNA sequencing and this is impractical for population screening.

FIG. 2.8   Simple electrophoretic pattern associated with monomeric enzyme
or protein structure.   Phenotypes from left to right are C3 2, C3
2, C3 2, C3 1-2.
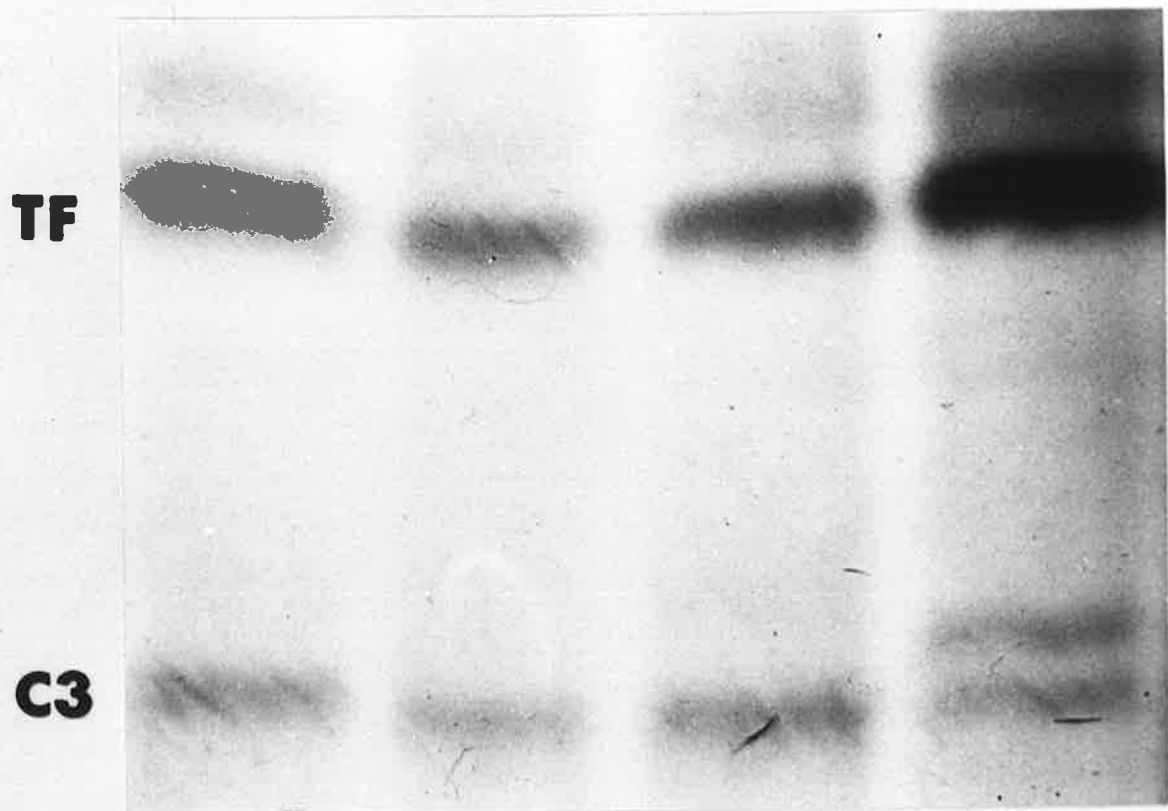
The expected number of electrophoretic phenotypes for any locus coding for a monomeric gene product is given by $n(n+1)/2$, where n is the number of electrophoretically detectable "alleles", or electromorphs, at a locus.

Most genetically determined enzyme and protein variation has two basic electrophoretic patterns: monomeric or multimeric. In both cases homozygotes are represented by a single band of enzyme activity or protein concentration. Monomeric enzymes and proteins appear in heterozygotes simply as the summation of individual bands seen in the homozygotes. This was the case discussed in the previous paragraph.

The number of primary bands seen in the heterozygote when the functional enzyme is multimeric is normally not simply the summation of bands present in the corresponding homozygotes. Any heterozygote displays $(n+1)$ primary isozymes consisting of two homodimeric bands and $(n-1)$ heterodimeric bands, where n is the number of subunits in the active form of the enzyme. Usually $n=2$ and the enzyme or protein is dimeric.

Dimeric molecules usually display one additional hybrid band on zymograms, the heterodimer, comprising subunits with different electrostatic charges or isoelectric points (Fig.2.9). Homodimers consist of two identical subunits. While the presence of hybrid bands is evidence for dimeric structure, their absence is not necessarily evidence for monomeric structure. Phenotypic absence of a hybrid band can be caused by

FIG. 2.9 Simple electrophoretic pattern associated with dimeric structure.

An additional heterodimer is seen for

(a)   GLO1: phenotypes from left to right are 1, 2 and 1-2.

(b)   PGD: phenotypes from left to right are A-FAST and A.

(c)   SOD: phenotypes from left to right are 1-2 and 1.

The variants shown for PGD and SOD are rare.
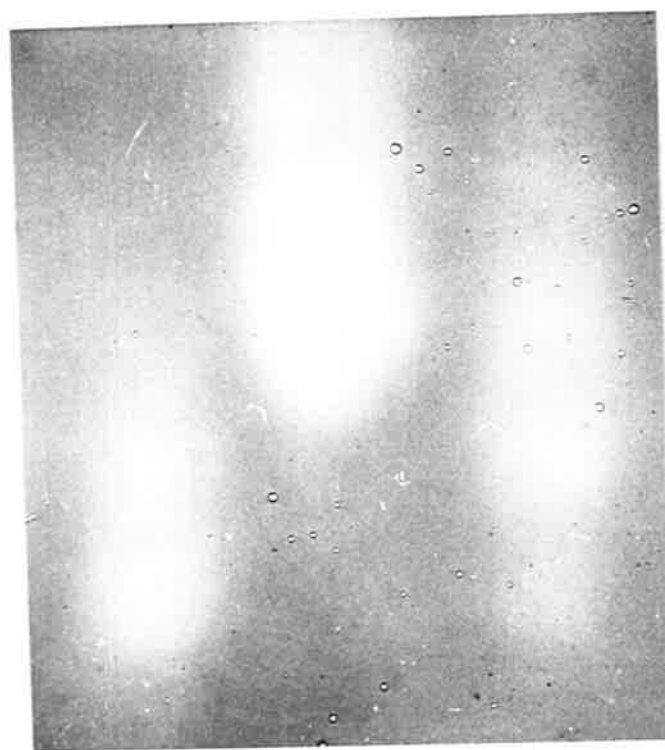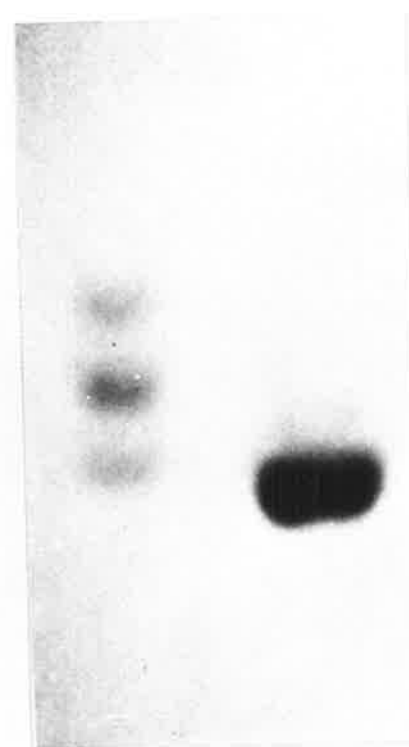
GLO1   PGD   SOD

1)   dissociation during electrophoresis, e.g. haemoglobin,

2)   structural limitation on formation of heterodimers from alternative polypeptide chains, but not for homodimers, or

3)   polypeptide components are formed in different cells, e.g. human glucose-6-phosphate dehydrogenase.

Symmetrical isozyme patterns arise from multimeric isozymes if all polypeptides contribute equally to enzyme activity. The activity of each functional component with random combination of subunits is proportional to the binomial coefficients of the expansion $(A+B)^n$ where A and B represent different polypeptides and n the number of subunits in the functionally active molecule. This pattern of activity determines the band intensity of each isozyme. If all polypeptides do not contribute equally to the phenotype, then activity patterns are asymmetrical.

Electrophoretic patterns are usually more complex than the simple patterns described above. Different activity levels, different rates of synthesis and differential stability can cause asymmetrical patterns in heterozygotes. Superimposed on these basic patterns can be extensive modifications caused by secondary isozyme formation.

Secondary isozymes have a variety of causes involving the modification of polypeptides to affect electrophoretic mobility. (Harris, 1980). These include:

1)   Deamination of glutamine and asparagine residues to glutamic acid and aspartic acid,

2)   acetylation,

3) addition of phosphate groups,

4) oxidation of sulphydryls on cysteine residues (e.g. ADA)

5) addition or removal of carbohydrate groups with various numbers of sialic acid residues,

6) partial cleavage of the amino acid sequence by proteolytic enzymes,

7) conformational isomerism if more than one stable configuration exists (e.g. ACP1),

8) differential saturation of dehydrogenases with coenzyme (e.g. alcohol dehydrogenase) or inorganic ions (e.g. TF),

9) aggregation and polymerisation (e.g. HP).

Some of these changes are reversible. Usually only a fraction of the molecules are subject to secondary modification. Modification is more likely to residues on the outer surface of the molecule. Extensive modification of poorly preserved specimens can result in the misinterpretation of isozyme patterns, especially if secondary bands are in the approximate position of known allelic variants. The degree of secondary isozyme formation can vary markedly between cells of different tissues even in fresh or well preserved specimens (e.g. GALT). Enzymes of red cells (with their long lifespan in which protein synthesis stops at an early stage) commonly exhibit secondary isozymes.

(g) BLOOD GROUPING

The investigator carried out the electrophoretic procedures but had only limited experience of bloodgrouping. Hence, the technical responsibility for this aspect of the work was given to trained haematologists in order to ensure quality of results. The early bloodgrouping was carried out in the Department of Haematology, Adelaide Children's Hospital until it could be carried out within my own laboratory under my direct supervision. Technical responsibility for HLA, IGH and IGK was also given to experts for the same reason. Since these determinations were not directly carried out myself, specific details of the methodology employed are not given.

Blood grouping was performed by observing agglutination using the spin technique according to manufacturers' instructions accompanying the antisera. Centrifugation for bloodgrouping procedures was carried out using a Dade immufuge. Antisera was obtained from Gamma, Biotest and Ortho depending on the current advice of haematologists in this field. Reverse grouping determined ABO groups from serum if cells were not available for study. Reverse grouping was not applied to infants less than six months of age.

(h) FAMILY DATA

Kindreds were routinely typed for the marker loci described above (Table 2.1). Kindreds in this study were broadly defined as a group of related individuals from simple two generation families to complex kindreds of more than 100 individuals. Pedigree information in tabular form with results only from informative markers are fully documented for each kindred in Appendix 2.

The families in Appendix 2 were analysed by LIPED (Ott, 1974; 1976) on a Hewlett-Packard HP3000 computer. The procedure of Ott (1978) was adopted wherever more than four alleles were segregating within a family. Allele frequencies given in this chapter (Appendix 1) were incorporated as data for computations involving missing individuals and loci with dominance. LIPED does not correct for ascertainment bias, which is trivial for families of more than two generations (Fraser, 1968) and unnecessary for two generation families in the context of linkage exploration. For the same reason, prior probabilities for linkage (Renwick, 1969, 1971) were ignored. Prior odds would be affected by differential recombination rates in males and females, known chromosomal location of one of the markers and the relative lengths of the chromosomes.

Identification codes were assigned to individuals merely for the purpose of computer analysis of that pedigree (Appendix 2). For some loci, data were sparse in portions of the pedigree. These pedigrees were analysed twice, once for these loci in the informative portion of the pedigree, and once for all other loci in the total pedigree. This was done merely to avoid excessive use of computer time, and applied only to pedigrees 6047CR, 10351HO and 10556DO. One branch of kindred 5656EH was included in the FRA10Q25 group and another branch in the group with more than one fragile site. These branches were separated for analysis because branches to relatives went upwards rather than downwards, and LIPED is unable to analyse pedigrees in that form.

(i)   ALLELE FREQUENCIES

Gene frequencies for most of the marker loci studied were not initially known. The gene frequencies presented in Table 2.3[a] were assumed at commencement of study to allow LIPED analysis of family data to proceed. These assumptions were based on published data from other caucasian populations. At the completion of all family studies, these values were compared with values derived directly from the study population (Table 2.3[b]). The differences observed are not of sufficient magnitude to affect conclusions from the linkage investigations presented. Virtually all estimates of allele frequency (shown with 95% confidence limits) were similar to values assumed at commencement of study. The reliability of the phenotyping procedures used can be assessed from the goodness of fit between observed phenotypic distributions and Hardy-Weinberg expectations (Table 2.4). Phenotypic classes with low numbers were grouped as described in Publication 2 (Materials and Methods). There was reasonable agreement between observed and expected phenotypic distributions in all cases.

The allele frequencies from the study population (Table 2.3[b]) were derived from the data presented in Appendix 2. To avoid bias (most individuals within families are related and carry genes identical by descent) only persons marrying into families and only parents at the head of kindreds were counted. Only observed phenotypes from these persons, never inferred phenotypes if these persons were missing, were counted. These estimates should be representative for the local population, and are at least representative for the group of

families studied. They are, however, associated with considerable standard errors (Table 2.3). The standard errors themselves are approximations since they were calculated from the sample allele frequencies, not from the population frequencies (which are unknown). The extent to which the individuals represent a random sample of the population depends on the extent to which certain alleles might be associated with chromosomal abnormalities or variations, or if certain racial groups are more prone to chromosomal abnormalities or variations.

Allele frequencies and expected phenotypic distributions for codominant markers were calculated by MULTAL. This is an unpublished program in FORTRAN IV originally written by the author. This program has previously been extensively used for the analysis of animal population data (Mulley, 1975; Barker and Mulley, 1976; Mulley and Latter, 1981a, 1981b). Statistics for ABO and P were calculated manually. Frequencies for recessive alleles were simply determined as the square root of the recessive phenotype. MNS haplotype frequencies were also calculated manually. Haplotypes for the MNSs phenotype were determined by family analysis where possible; otherwise they were divided into the same proportion as those which could be classified.

TABLE 2.3

ALLELE FREQUENCIES

[a]   Values assumed at commencement of study (used for LIPED analysis)

[b]   Values determined at completion of study (from the study population)

Marker	Allele Frequencies			(a) References (b) Sample Sizes (Study population)

(a) Red Cell Electrophoretic Markers

ACP1	A	B	C	
(a)	.31	.64	.05	Harris & Hopkinson, 1976
(b)	.34+.05	.64+.05	.02+.01	2N* = 372

ADA	1	2		
(a)	.95	.05		Harris & Hopkinson, 1976
(b)	.96+.02	.04		2N = 332

AK1	1	2		
(a)	.96	.04		Harris & Hopkinson, 1976
(b)	.96+.02	.04		2N = 366

ESD	1	2		
(a)	.90	.10		Harris & Hopkinson, 1976
(b)	.88+.03	.12		2N = 366

GALT	N	D	LA	
(a)	.93	.05	.03	Mulley, 1982b
(b)	.95+.02	.04	.003	2N = 350

GPT	1	2		
(a)	.52	.48		Harris & Hopkinson, 1976
(b)	.58+.05	.42		2N = 362

GLO1	1	2		
(a)	.44	.56		Parr et al., 1978
(b)	.44	.56+.05		2N = 360

PGM1	1	2		
(a)	.78	.22		Harris & Hopkinson, 1976
(b)	.73+.05	.27		2N = 366

Contd.

PGD	1	2		
(a)	.97	.03		Harris & Hopkinson, 1976
(b)	$.99\pm.01$	.01		2N = 238

PGP	1	2	3	
(a)	.83	.13	.04	Barker & Hopkinson, 1978
(b)	$.88\pm.03$	$.12\pm.03$	$.01\pm0.0$	2N = 352

(b) Electrophoretic Markers of Serum and Plasma

AMY2	A	B	
(a)	.95	.05	Komph et al., 1980

CHE2	C_{5+}	C_{5-}	
(a)	.05	.95	Steegmuller, 1975

C3	1	2	
(a)	.25	.75	Alper and Propp, 1968
(b)	.17	$.83\pm.04$	2N = 298

F13A	1	2	
(a)	.79	.21	Board, 1979
(b)	$.85\pm.04$	.15	2N = 308

F13B	1**	3	
(a)	.83	.17	Board, 1980
(b)	$.89\pm.03$	.11	2N = 368

GC	Other	1F	1S	2	
(a)	-	.14	.56	.30	Nicholls & Mulley, 1982a
(b)	.003	$.15\pm.04$	$.55\pm.05$	$.30\pm.05$	2N = 390

HP	1	2	
(a)	.39	.61	Queen & Peacock, 1966
(b)	.41	$.59\pm.05$	2N = 382

PI	G	I	M1	M2	M3	S	Z	
(a)	-	-	.70	.14	.10	.03	.01	Mulley, 1980, 1982a
(b)	.003	.005	$.67\pm.05$	$.15\pm.04$	$.08\pm.03$	$.07\pm03$	.02	2N = 388

TF	B	C1	C2	C3	
(a)	-	.82	.15	.03	Nicholls & Mulley, 1982b
(b)	.01	$.81\pm.04$	$.17\pm.04$	$.01\pm.01$	2N = 386

Contd.

(c) **Blood Groups*****

ABO	A1	A2	B	O	
(a)	.18	.06	.08	.68	
(b)	.14+.04	.06+.03	.09+.03	.70+.05	2N = 280

Fy	A	B		
(a)	.44	.56		
(b)	.48+.06	.52		2N = 260

K	K	k		
(a)	.06	.94		
(b)	.05	.95+.03		2N = 260

Jk	A	B		
(a)	.51	.49		
(b)	.53+.06	.47		2N = 258

Lu	A	B		
(a)	.04	.96		
(b)	.06	.94+.03		2N = 242

MNS	MS	Ms	NS	Ns	
(a)	.26	.29	.10	.35	
(b)	.21+.05	.32+.06	.07+.03	.41+.06	2N = 266

P	P1	P2		
(a)	.50	.50		
(b)	.54+.08	.46+.08		2N = 160

Rh	R1	r	R2	Ro	r'	r"	
(a)	.42	.41	.15	.01	.002	.004	
(b)	.37+.06	.46+.06	.15+.02	.001	.004	.004	2N = 254

Xg	Xga	Xg-	
(a)	.64	.36	

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*   2N = number of alleles (or haplotypes for MNS) sampled

** $\underline{F13B*1}$ pooled with $\underline{F13B*2}$

***   Gene frequencies at commencement of study calculated from phenotype frequencies given in the CSL Blood Group Reference Guide (1977)

TABLE 2.4

CHI-SQUARE GOODNESS OF FIT TEST (WITH SIGNIFICANCE LEVELS) BETWEEN OBSERVED (O) AND EXPECTED (E) PHENOTYPIC DISTRIBUTIONS

ACP1   (0.5 < P < 0.75)

	AA	AB	BB	AC	BC	CC
O	23	79	77	1	6	0
E	21.3	81.0	76.8	2.4	4.5	0.1

ADA   (0.5 < P < 0.75)

	1-1	1-2	2-2
O	154	12	0
E	154.2	11.6	0.2

AK1   (0.5 < P < 0.75)

	1-1	1-2	2-2
O	170	13	0
E	170.2	12.5	0.2

ESD   (0.25 < P < 0.5)

	1-1	1-2	2-2
O	140	42	1
E	141.6	38.7	2.6

GALT   (0.5 < P < .75)

	NN	ND	DD	NLA	DLA	LALA
O	159	15	0	1	0	0
E	159.4	14.3	.30	0.0	1.0	0.0

GPT   (0.25 < P < 0.5)

	1-1	1-2	2-2
O	64	83	34
E	61.5	88.0	31.5

GLO1   (0.05 < P < 0.1)

	1-1	1-2	2-2
O	29	101	50
E	35.1	88.8	56.1

PGM1 (0.25 < P < 0.5)

	1-1	1-2	2-2
O	96	76	11
E	98.1	71.8	13.1

PGD (P > 0.9)

	AA	AC	CC
O	116	3	0
E	116.0	3.0	0.0

PGP (0.5 < P < 0.75)

	1-1	1-2	2-2	1-3	2-3	3-3
O	136	34	2	4	0	0
E	134.8	36.8	1.8	2.5	0.2	0.0

C3 (0.75 < P < 0.9)

	1-1	1-2	2-2
O	5	42	102
E	4.5	42.9	101.5

F13A (0.1 < P < 0.25)

	1-1	1-2	2-2
O	110	43	1
E	112.3	38.4	3.3

F13B (0.1 < P < 0.25)

	1-1	1-3	3-3
O	147	33	4
E	145.3	36.4	2.3

GC (0.25 < P < 0.5)

	1S1S	1S1F	2F1F	1S2	1F2	2-2	Other
O	63	29	7	59	16	20	1
E	59.0	30.6	4.4	64.4	17.6	17.6	0.0

HP (0.5 < P < 0.75)

	1-1	1-2	2-2
O	34	90	67
E	32.7	92.7	65.7

PI (0.10 < P < 0.25)

	M1M1	M1M2	M2-M2	M1M3	M2M3	M3M3	MS	MZ	Other
O	93	33	5	18	8	1	25	7	4
E	87.1	39.0	4.4	20.8	4.7	1.2	24.4	7.0	5.4

Fy   (0.25 < P < 0.5)

	AA	AB	BB
O	33	60	37
E	30.5	64.9	34.5

K   (0.5 < P < 0.75)

	KK	Kk	kk
O	0	14	116
E	0.4	33.3	116.4

Jk   (P > 0.9)

	AA	AB	BB
O	36	64	29
E	35.8	64.3	28.8

Lu   (0.5 < P < 0.75)

	AA	AB	BB
O	2	10	109
E	0.4	13.2	107.4

Footnote: O and E for MNS, P and Rh are not given.   MNS and Rh are each a system of linked loci in linkage disequilibrium (Race and Sanger, 1975).   There are only two phenotypes for P.

Insufficient individuals were typed for AMY2, CHE2, HLA, IGH, IGK or Xg to estimate realistic allele frequencies. Genetic variation at AMY2 and CHE2 was so low that typing was impractical and was stopped soon after commencement of study. HLA, IGH and IGK were only typed in certain kindreds by co-investigators specialising in those markers. Insufficient persons were typed for Xg. This bloodgroup was only determined in the early fragile X families, prior to knowledge of a precise localisation for Xg.

The estimated allele frequencies should be useful as future data for the calculations of probability of paternity, probability of zygosity, probability of linkage and probability for association in the study population. Blood groups and electrophoretic markers will remain the techniques of choice for paternity and zygosity testing with existing technology. Their determination is far cheaper and faster than it is for typing restriction fragment length polymorphisms.

(j)   SUMMARY

This chapter described details of sample collection and preparation, electrophoretic separation and histochemical staining. These techniques provided the data for this thesis. All family results were documented in this chapter and form the basis for subsequent chapters. Allele frequencies and genotypic distributions were determined from the individuals examined in the study population. These confirmed the validity of the loci examined as reliable genetic markers, and validated the choice of allele frequencies initially selected as data input to LIPED.

CHAPTER 3

DELETION MAPPING USING PERSONS WITH MONOSOMIC SEGMENTS

(a)   INTRODUCTION

(b)   MATERIALS AND METHODS

(c)   RESULTS

(d)   DISCUSSION

(e)   SUMMARY

Publication Numbers

6   Mulley, Bryant and Sutherland (1980)

7   Mulley and Sutherland (1982)

(a)   INTRODUCTION

The first gene mapping investigations in this thesis will now be described using the technique of deletion mapping. Deletion mapping is a specific form of exclusion mapping where exclusion limits are defined by chromosomal breakpoints. The precision of the limits depends on the level of resolution achieved by chromosome banding. In contrast, limits derived from linkage analysis are defined only in probability terms. The main advantage of deletion mapping is its simplicity. Problems include the presence of rare null alleles making the technique susceptible to occasional error and the relative rarity of liveborn individuals with different chromosmal deletions.

(b)   MATERIALS AND METHODS

The methods used for phenotyping are described in Chapter 2. The early bloodgrouping data presented in Publication 6 was carried out in the Department of Haematology, Adelaide Children's Hospital. Soon after, all bloodgrouping was performed within my own laboratory under my control in order to minimise the possibility of error. The deletions examined are described in Publication 6 (Mulley et al., 1980) and Publication 7 (Mulley and Sutherland, 1982). Blood samples for cytogenetic and genetic marker investigation were obtained before death, at necropsy, or if compatible with life, whenever associated mental impairment became evident.

(c)   RESULTS

Tables 3.1 and 3.2 show the data upon which the exclusions were made.   All exclusions are presented in Publications 6 and 7.   The GM locus referred to in Publication 7 has since been renamed IGH (Human Gene Mapping 7, 1984).

(d)   DISCUSSION

Subsequent to the time when this project was initiated, C3, IGH, Jk and PI were assigned and the MNS assignment proven (see Chapter 9).

Historically, deletion mapping has been inefficient for gene assignment compared with genetic linkage analysis and somatic cell hybridisation.   However, deletion mapping has been useful for narrowing regional localisations (Aitken and Ferguson-Smith, 1978; Aitken et al., 1975;   Ferguson-Smith and Aitken, 1978, 1982;   Turleau et al., 1978;   Emanuel et al., 1979).   It has provided initial gene assignments for ACP1 and GC and confirmed the assignment AK1 (Ferguson-Smith et al., 1973;   Mikkelsen et al., 1977; Ferguson-Smith, 1978).   The application of deletion mapping is usually restricted to liveborn infants and consequently most of the deletions are relatively minor, minimising the efficiency of the technique for gene assignment.

TABLE 3.1

BASIS FOR EXCLUSIONS OF INFORMATIVE MARKERS OF INTEREST

(PUBLICATION 6)

Patient No.	Phenotypes		
	Mother	Father	Affected Proband
1	-	NNSs, Jk A, GPT 1, PI M2M3	NNSs, Jk AB, GPT 13, PI M1M3
2	F13B 1	F13B 12	F13B 12
3	-	-	Jk AB, PI M1M2, C3 12, F13A 12
4	NNss, Lu AB	-	MNSs, Lu AB
5	GPT 12	GPT 12	GPT 12, F13A 12
6	MNss	MNSS	MNSs
7	C3 2	C3 12	GPT 12, C3 12
8	-	-	MMSs, GPT 12, PI M1F
10	MNSs, Jk B F13A 12	MMSs, Jk AB F13A 12	MNSs, Jk AB, F13A 12

TABLE 3.2

BASIS FOR EXCLUSIONS OF INFORMATIVE MARKERS OF INTEREST

(PUBLICATION 7)

Patient No.	Phenotypes		
	Mother	Father	Affected Proband
2	F13A 12, F13B 13 PI M1, TF C1C3	F13A 12, F13B 13, PI M2M3, TF C1	F13A 12, F13B 13 PI M1M2, TF C1C3
3	–	–	GPT 12
4	–	TF C1C2	TF C1C3
5	PI M1M3	PI M1M2	PI M1M2
7	C3 2, PI M1M2 GALT N	C3 12, PI M1M3 GALT ND	C3 12, F13B 13 PI M1M2, GALT ND
8	–	–	PI M1M2
9	IGH35, TF C1C2	IGH1235, TF C1	IGH1235, TF C1C2
10	–	–	TF C1C2
11	C3 12	C3 12	C3 12
12	IGH 1235	IGH 1235	IGH 1235
13	F13A 12, Jk B, kk, Lu B, MNSs	F13A 1, Jk A, Kk, Lu AB, MMSs	F13A 12, Jk AB, Kk, Lu AB, MMSs

Cook et al., (1980) recognised another source of information not usually utilised. Some markers, such as $\underline{P}$ and $\underline{CHE2}$, cannot show heterozygosity by phenotyping due to their dominant mode of inheritance. If the monosomic individual in these cases can be shown to have inherited the dominant allele from the parent from whom the deletion clearly orginated (because of familial translocation or centromeric polymorphism) then these loci can also be excluded.

Magenis et al., (1979) recognised two additional sources of information. Duplications are informative for exclusion if both parents were heterozygous and the affected offspring homozygous. Duplications are also informative if there was heterozygosity in the parent of origin and homozygosity in the affected offspring.

Determination of gene dosage by the enzymatic activity of gene products is another form of deletion mapping, but using quantitative rather than qualitative criteria. Retarded children with bilateral retinoblastoma usually have a deletion involving band 13q14 (Yunis and Ramsay, 1978). Mental deficiency is the outcome of deletion of a block of genes, which include the $\underline{ESD}$ locus. The absence of another unidentified gene within 13q14 is equivalent to its mutation to a dominant allele : deletion or mutation predisposes to retinoblastoma. This assignment of the retinoblastoma gene has recently been confirmed by linkage with $\underline{ESD}$ (Sparkes et al., 1983). Similarly, the gene for Wilm's tumour - aniridia syndrome is associated with a deletion at 11p13 near $\underline{CAT}$ (catalase) (de Grouchy, 1981). Half the expected levels of ESD and CAT can be used to diagnose these respective conditions. Consequently, the assignments of $\underline{ESD}$ and $\underline{CAT}$ are confirmed by deletion mapping, but by quantitative rather than qualitative criteria.

The use of quantitation for large trisomies such as Down syndrome has been discredited because of disruption to regulation of enzymes irrespective of their chromosomal localisations (Ferguson-Smith and Aitken, 1982). Gene dosage is now an established technique for the regional localisation of assigned genes using individuals with small monosomic or trisomic segments for chromosomal regions of interest.

Gene dosage studies are most appropriately applied to red cell enzymes. Red cells are non-nucleated end cells giving high reproducibility from different samples in the same individual (Ferguson-Smith and Aitken, 1982). Samples with high reticulocyte counts should be excluded to guard against over-representation of immature cells. Although fibroblasts and white cells have been used to demonstrate dosage effects, variation in activity is generally greater than is found in red cells. The first gene assignment by deletion mapping (ACP1 to chromosome 2) was confirmed by gene dosage (Ferguson-Smith et al., 1973) and this was the first confirmation of an assignment and localisation by gene dosage. The method has since become firmly recognised as a valuable technique for chromosomal localisation of assigned genes (Ferguson-Smith et al., 1976; Aitken and Ferguson-Smith, 1979; Ferguson-Smith and Aitken, 1982).

Partial trisomy (duplication) can give comparable results to deletion mapping by either dosage or qualitative presence of three alleles for multiallelic loci. This can be useful for gene localisation and has been achieved with trisomic codominant expression of HLA antigens (Pearson et al., 1979) and similarly for PGM1 isozymes (Robson, 1982). The method is theoretically possible for ACP1, GC,

IGH, PI, TF and any other codominant system with more than two common alleles segregating.

Exclusion in the present study was not possible using duplications. Similarly, demonstration of a dominant allele in a proband inherited from the parent responsible for the deletion was not possible. There are now insufficient unassigned markers of the conventional type to make deletion mapping by qualitative criteria a worthwhile proposition, except where the chromosomal aberrations involve chromosomal regions suspected of containing the locus of interest.

Daiger and Chakravanti (1983) described a new application, the assignment of DNA polymorphisms. They presented a general method for calculating the probability that a deletion at a specific locus would provide an assignment. This application of deletion mapping is arguable, given the viable alternatives of in situ hybridisation and analysis of somatic cell panels of partial hybrids, which are also applicable to non-polymorphic sequences.

(e)   SUMMARY

New additions to the exclusion map of man (at the time of study) were determined by deletion mapping. Alternative strategies of exclusion were discussed but not applied. The chromosomal localisation for GALT was determined by deletion mapping to be (9)(p13).

CHAPTER 4

LINKAGE ANALYSIS USING FRAGILE SITES AS MARKERS

(a) INTRODUCTION

(b) MATERIALS AND METHODS

(c) RESULTS

(d) DISCUSSION

(e) SUMMARY

Publication Numbers

8   Sutherland, Baker and Mulley (1982)

9   Mulley, Hay, Sheffield and Sutherland (1983a)

10   Mulley, Nicholls and Sutherland (1983b)

11   Mulley (1985)

12   Mulley, Cox and Sutherland (1983c)

13   Mulley, Hill and McPhee (1983d)

(a)   INTRODUCTION

The technique of linkage analysis has been described in Chapter 1. This chapter describes the use of fragile sites in linkage analysis.

Fragile sites were first reported by Dekaban (1965) and subsequently found to be heritable (Lejeune et al., 1968). The fragile site situated on the X chromosome was soon shown to be associated with a form of X-linked mental retardation (Lubs, 1969). The significance of this finding was not immediately recognised. It is the second most common cause of mental retardation (McKusick, 1983).

Fragile sites may correspond to cancer breakpoints on chromosomes (Hecht and Sutherland, 1984). This is based on similar positioning of cancer breakpoints and some fragile sites, though these points have not been located precisely enough to prove correspondence. Whether fragile sites correspond with oncogenes is yet to be determined.

True fragile sites have been defined by Sutherland (1979) as

1)   gaps in chromosomes usually involving both chromatids,

2)   always at the same locus in cells from any one individual or kindred,

3)   inherited as simple codominant variants, and

4)   fragility must be evident under the appropriate cell culture conditions by the production of acentric fragments, deleted chromosomes, triradial configurations, and other such abnormalities. The autosomal fragile sites have been

presumed to be fully, or nearly fully, penetrant. However, the one at (X)(q27) is not always expressed cytogenetically in females (Lubs, 1969) and there is a suggestion that it is also not penetrant in some males (Sherman et al., 1983, 1985).

Many different fragile sites are now recognised (Figs. 4.1 and 4.2). The one at 10q25 requires BrdU for expression (Sutherland et al., 1980) and is present in the study population at a carrier frequency of one in 40 (Sutherland, 1982). All other fragile sites are rare. The fragile sites at 16q22 and 17p12 are often expressed spontaneously, but in some individuals expression is enhanced by the addition of BrdU or Distamycin A (Schmid et al., 1980; Croci, 1983; Sutherland et al., 1984). The remaining fragile sites are folate sensitive. Requirements for their expression include

1)   absence of folic acid and thymidine in the culture media, or

2)   inhibition of folate metabolism with methotrexate, or

3)   inhibition of thymidylate synthetase with fluorodeoxyuridine. These conditions presumably lead to a deficiency of DNA precursor substances (Glover, 1985).

FIG. 4.1   Ideogram of known fragile sites.
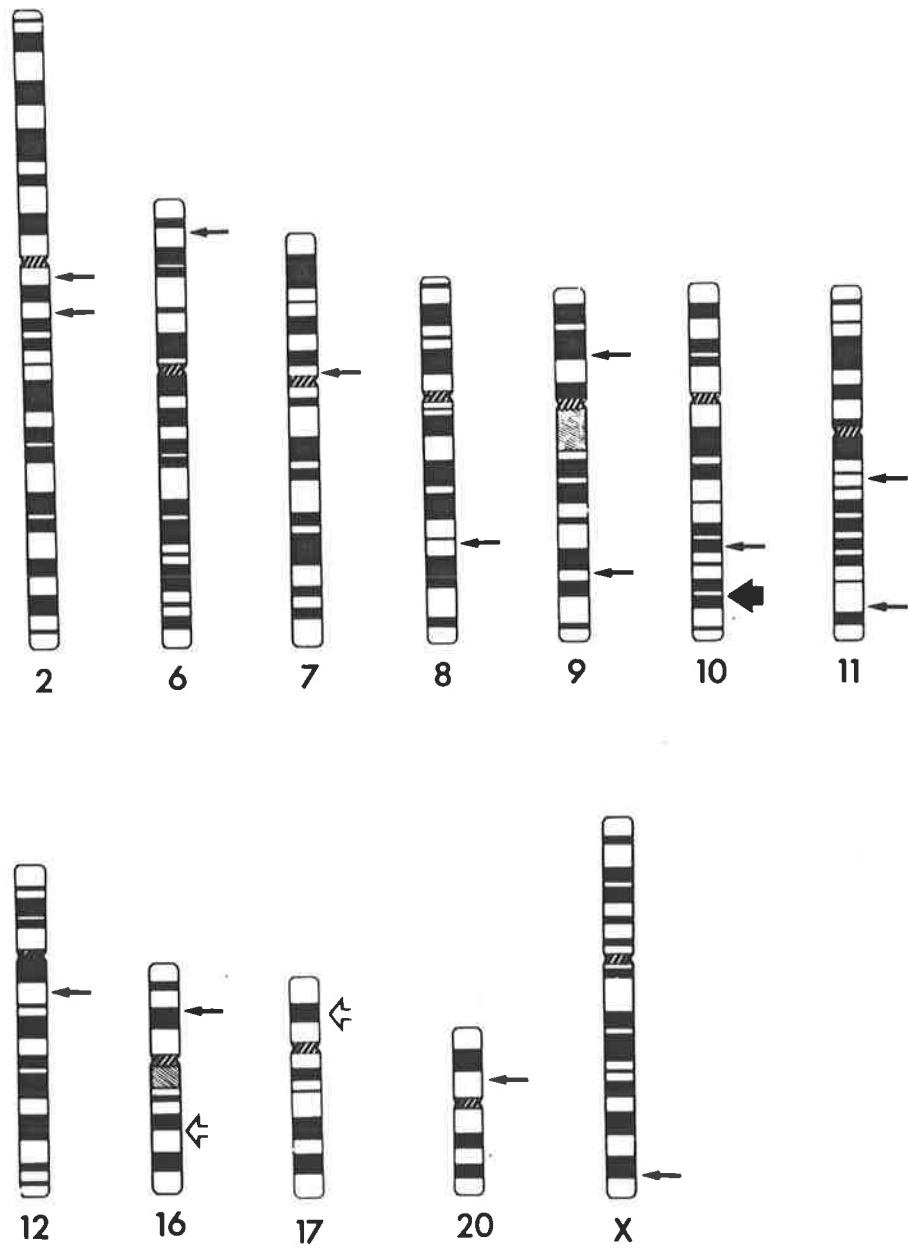
FIG. 4.2 Partial karyotypes showing all known fragile sites (supplied by Dr. G.R. Sutherland).

2q11 2q13 6p23 7p11
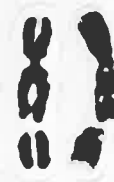
8q22 9p21 9q32 10q23
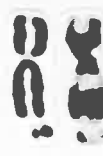
10q25 11q13 11q23 12q13

16p12 16q22 17p12 20p11 Xq27

Linkage analysis was not feasible using the BrdU dependent fragile site and folate sensitive fragile sites until the appropriate lymphocyte culture conditions necessary for their demonstration were determined (Sutherland, 1977; Sutherland et al., 1980). Heritable fragile sites occur at a defined map location, presumably the region carrying the DNA responsible for fragile site expression. These chromosomal locations may be used as genetic markers, in addition to the standard blood group, enzyme and protein polymorphisms. Common non-random gaps and breaks are seen at 1qter, 2q23, 3p14, 6q26, 9q13 and 13qter. These are now accepted as a fourth class of fragile site (Glover et al., 1984), known as common fragile sites. These are unsuitable as markers for linkage analysis because man is probably homozygous for all of them. Knowledge of fragile sites to date has been collated by Sutherland and Hecht (1985) to which the reader is referred for more detailed information.

(b)   MATERIALS AND METHODS

The methods used for phenotyping are described in Chapter 2. The kindreds studied are described in Publications 8-11 (Sutherland et al., 1982; Mulley et al., 1983a,b; Mulley, 1985) and Appendix 2.

(c)   RESULTS

Results are presented in Publications 8-11. Linkage comparisons involving test loci assigned by other investigators during the course of these studies were no longer of interest. These unpublished results are tabulated separately (Tables 4.1 and 4.2). They do not challenge

TABLE 4.1

LOD SCORES BETWEEN TEST LOCI AND FRAGILE SITES.
THESE TEST LOCI WERE ASSIGNED BY OTHER INVESTIGATORS DURING
THE COURSE OF THIS STUDY

Linkage Comparison		θ				
		.05	.1	.2	.3	.4
C3 :	FRA2Q3	-0.5	-0.2	-0.1	0.0	0.0
	FRA6P23	-1.3	-0.7	-0.3	-0.1	0.0
	FRA8Q23	-0.4	-0.2	0.0	0.1	0.1
	FRA10Q23	-4.8	-2.6	-0.8	-0.1	0.1
	FRA10Q25	-7.2	-3.7	-0.9	0.0	0.1
	FRA11Q13	-0.8	-0.3	0.0	0.0	0.0
	FRA12Q13	-1.4	-0.7	-0.1	0.1	0.1
GPT:	FRA6P23	-0.9	-0.2	0.2	0.2	0.1
	FRA11Q13	-4.2	-2.4	-0.9	-0.3	0.0
	FRA11Q23	-0.5	-0.2	-0.1	0.0	0.0
	FRA12Q13	-2.3	-1.2	-0.3	0.0	0.1
JK :	FRA6P23	-4.2	-2.8	-1.4	-0.7	-0.3
	FRA10Q23	-0.8	-0.4	-0.1	0.0	0.0
	FRA10Q25	-0.6	-0.1	0.1	0.1	0.0
	FRA11Q13	-4.4	-2.7	-1.1	-0.4	-0.1
	FRA16Q22	-0.8	-0.5	-0.2	-0.1	0.0
MNS:	FRA2Q13	-0.5	-0.2	-0.1	0.0	0.0
	FRA6P23	-3.6	-2.2	-0.9	-0.4	-0.1
	FRA8Q23	-1.7	-1.1	-0.5	-0.2	0.0
	FRA9Q32	0.7	0.4	0.2	0.1	0.0
	FRA10Q23	0.4	0.5	0.5	0.3	0.2

Contd.

	FRA10Q25	-5.3	-2.5	-0.6	0.1	0.0
	FRA11Q13	-2.0	-1.4	-0.7	-0.3	-0.1
	FRA12Q13	-5.4	-3.6	-1.9	-1.0	-0.4
	FRA16P12	-2.8	-1.7	-0.6	-0.2	0.0
	FRA16Q22	0.4	0.4	0.4	0.3	0.1
PI :	FRA2Q11	-0.7	-0.4	-0.2	-0.1	0.0
	FRA6P23	-7.7	-5.2	-2.7	-1.3	-0.5
	FRA8Q23	-0.6	-0.1	0.2	0.3	0.2
	FRA10Q23	-2.9	-1.8	-0.8	-0.4	-0.1
	FRA1OQ25	-11.9	-7.8	-3.0	-0.1	-0.3
	FRA11Q13	-10.1	-6.6	-3.0	-1.4	-0.5
	FRA11Q23	-0.6	-0.2	0.1	0.1	0.1
	FRA12Q13	-3.1	-2.2	-0.8	-0.2	0.0
	FRA16P12	0.6	0.7	0.7	0.6	0.3
	FRA16Q22	0.5	0.6	0.6	0.4	0.2

TABLE 4.2

LOD SCORES BETWEEN TEST LOCI AND MARKER LOCI FROM KINDREDS WITH FRAGILE SITES. THESE TEST LOCI WERE ASSIGNED BY OTHER INVESTIGATORS DURING THE COURSE OF THIS STUDY.

Linkage Comparison	θ				
	.05	.1	.2	.3	.4
C3 : ABO	-8.9	-5.1	-1.9	-0.5	0.0
ACP1	-10.6	-5.7	-2.1	-0.7	-0.2
ADA	-2.1	-1.1	-0.3	-0.1	0.0
AK1	-3.0	-1.9	-0.9	-0.3	-0.1
ESD	-8.1	-3.5	-0.7	0.3	0.4
Fy	-2.7	-1.7	-0.5	-0.1	0.0
GALT	-2.7	-1.3	-0.2	0.0	0.1
GC	-19.6	-10.9	-4.5	-1.9	-0.6
GLO1	-2.9	-0.9	-0.3	0.4	0.3
GPT	-11.8	-6.8	-4.0	-0.8	-0.1
HP	-6.5	-2.9	-0.3	0.3	0.2
IGH	-0.9	-0.4	0.0	0.1	0.1
IGK	0.5	0.4	0.3	0.1	0.0
Jk	-7.1	-4.3	-0.9	-0.8	-0.3
MNS	-10.6	-5.9	-2.2	-0.8	-0.4
PGM1	-7.7	-3.8	-0.7	0.2	0.3
PGP	-3.7	-1.5	0.2	0.6	0.3
PI	-13.2	-7.0	-2.1	-0.1	0.1
Rh	-2.6	-4.7	-1.6	-0.5	0.0
GPT*: ABO	-3.8	-1.2	0.4	0.5	0.2
ACP1	-19.0	-11.0	-4.3	-1.6	-0.6
ADA	-0.7	0.0	0.4	0.3	0.1
AK1	-1.2	-0.8	-0.4	-0.2	0.0
ESD	-5.7	-2.7	-0.4	0.2	0.2
Fy	-10.1	-6.2	-2.7	-1.2	-0.4

Contd.

	GALT	-9.8	-5.7	-2.4	-1.0	-0.3
	GC	-30.7	-16.5	-5.4	-1.3	0.0
	GLO1	-15.8	-8.0	-2.3	-0.6	-0.2
	IGH	-2.4	-1.5	-0.7	-0.3	-0.1
	Jk	-9.2	-4.6	-1.3	-0.3	-0.1
	MNS	-17.8	-10.1	-1.8	-1.5	-0.4
	PGM1	-12.7	-9.2	-3.5	-0.9	0.1
	PI	-15.8	-8.7	-2.8	-0.8	-0.1
	Rh	-11.7	-6.5	-2.4	-0.8	-0.2
Jk :	ABO	-7.3	-3.9	-1.2	-0.2	0.1
	ADA	-0.5	-0.2	0.0	0.0	0.0
	AK1	2.0	1.8	1.3	0.6	0.2
	ESD	-1.3	-0.3	0.2	0.1	0.0
	Fy	-9.0	-4.8	-1.3	-0.4	-0.1
	GALT	-0.9	-0.4	-0.1	0.0	0.0
	GC	-19.0	-11.1	-4.6	-1.6	-0.3
	GLO1	-7.4	-3.1	-0.4	0.0	-0.1
	HP	-11.6	-6.4	-2.2	-0.9	-0.3
	IGH	-0.9	-0.4	-0.2	-0.1	0.0
	MNS	-9.4	-4.1	-0.2	0.5	0.5
	PGP	-0.8	-0.5	-0.2	-0.1	0.0
	PGM1	-9.7	-5.3	-1.5	-0.7	-0.3
	PGP	-3.3	-1.9	-0.9	-0.4	-0.2
	PI	-9.4	-4.4	-0.9	0.1	0.2
	Rh	-9.2	-4.3	-0.8	0.1	0.1
Lu :	ABO	-1.1	-0.5	-0.5	0.1	0.0
	ACP1	-4.6	-2.4	-0.8	-0.2	0.0
	AK1	0.6	0.5	0.3	0.2	0.0
	ESD	0.7	0.6	0.4	0.2	0.1
	Fy	-2.4	-1.6	-0.8	-0.4	-0.2
	GC	-4.1	-2.4	-0.9	-0.3	-0.1
	GLO1	-1.9	-1.1	-0.5	-0.2	0.0
	GPT	-5.6	-3.4	-1.4	-0.5	-0.2
	HP	0.3	1.0	1.0	0.7	0.2
	Jk	-3.8	-2.3	-1.0	-0.3	-0.1
	MNS	-3.0	-1.8	-0.8	-0.3	-0.1

Contd.

	PGM1	-0.3	0.3	0.7	0.6	0.3
	PGP	-1.6	-0.9	-0.3	-0.1	0.0
	PI	-5.7	-3.3	-1.6	-0.7	-0.2
	Rh	-0.9	-0.3	-0.4	0.0	0.0
MNS:	ABO	-13.6	-8.0	-3.2	-1.2	-0.3
	ACP1	-12.3	-6.4	-2.7	-0.1	0.1
	ADA	-2.3	-1.4	-0.7	-0.4	-0.2
	AK1	-6.1	-3.6	-1.4	-0.5	-0.1
	ESD	-3.8	-2.1	-0.7	-0.4	-0.1
	Fy	-18.6	-10.9	-4.5	-1.8	-0.4
	GALT	-1.1	-0.6	-0.2	0.0	0.0
	GLO1	-13.9	-6.6	-1.4	0.0	0.2
	HP	-17.5	-9.9	-4.0	-1.7	-0.6
	IGH	-4.4	-2.4	-0.9	-0.3	0.0
	IGK	-1.1	-0.3	0.2	0.3	0.2
	PGM1	-9.7	-4.6	-1.0	-0.1	0.0
	PGP	-4.6	-2.5	-1.0	-0.5	-0.5
	PI	-20.5	-11.6	-3.8	-1.6	-0.4
	Rh	-24.5	-12.4	-4.3	-1.1	-0.2
PI :	ABO	-15.8	-8.8	-3.2	-1.0	-0.2
	ACP1	-21.5	-11.5	-3.6	-0.9	-0.6
	ADA	-1.3	-0.6	0.0	0.1	0.1
	AK1	-3.1	-1.3	0.0	0.3	0.2
	ESD	-8.8	-5.1	-1.8	-0.6	-0.1
	Fy	-7.9	-3.9	-0.9	0.0	0.1
	GALT	-1.0	-0.5	-0.2	-0.1	0.0
	GC	-37.2	-20.2	-6.5	-1.6	-0.1
	GLO1	-12.9	-5.0	-0.6	0.4	0.1
	HP	-18.1	-9.5	-2.8	-0.5	0.1
	IGK	-1.6	-0.9	-0.4	0.1	0.0
	PGD	-3.1	-2.0	-1.0	-0.5	-0.2
	PGM1	-21.9	-13.0	-5.4	-2.1	-0.6
	PGP	-7.2	-3.5	-1.0	-0.1	0.1
	Rh	-19.5	-11.3	-4.6	-1.7	-0.4
	SOD	0.3	0.2	0.1	0.1	0.0

* GPT is on either chromosome 8 or 16 (Human Gene Mapping 7, 1984).

any confirmed assignments.   MNS was included in these tabulations because it was first assigned indirectly by exclusion (Cook et al., 1980) rather than by direct methods.

(d)   DISCUSSION

The studies described in Publications 8, 9 and 11 provide a basis for the interpretation of linkage studies with fragile sites.   The fortuitous double ascertainment of both fragile sites on the long arm of chromosome 10 (Publication 8), the discovery of a new fragile site linked to HLA (Publication 9) and the results from fragile sites at 16q22 and Xq27 (Publication 11) have provided the basis for generalisations probably applicable to all fragile sites.   Subsequent to Publication 11, HP has been localised to 16q22 by in situ hybridisation (McGill et al., 1984) and found to be distal to the fragile site at 16q22.1 by in situ hybridisation to metaphase chromosomes expressing the fragile site (Simmers, 1985 - personal communication).   The presence of fragile site DNA has no gross effect on recombination frequency, and probably has very little or no effect even in the immediate vicinity of the lesion.   The fragile site DNA maps to the chromosomal locus of its phenotypic expression. Linkage studies involving DNA fragments near fragile sites should therefore be interpreted in the same way as a linkage comparison between any two markers.   However, tightly linked flanking markers are needed in order to evaluate the amount of recombination that occurs at the point of fragile site expression as distinct from the amount of recombination which occurs on the chromosomal segment between the fragile site and closely linked marker.   A battery of tightly linked flanking markers are also required before accurate

prenatal diagnosis of the site at (X)(q27) is routinely undertaken. These flanking markers are now being found (Camerino et al., 1983; Drayna, 1984).

That the DNA responsible for fragile site expression mapped to the locus of phenotypic expression was not known for certain at the commencement of study. Linkage analysis could have included comparisons between fragile site loci and all assigned markers, not just syntenic loci, in a search for possible fragile site determining genes remote from the lesion. This was not carried out. The results that emerged during this study confirmed that this approach would have proved fruitless: the genes responsible for fragile site expression are indeed at or near the locus of their phenotypic expression.

An application of families segregating with fragile sites is in gene assignment. Despite extensive investigations, no unassigned genes were detected near fragile site markers. This is evident from both the preliminary report (Publication 10) and the final assessment (Publication 11) from all families ascertained with fragile sites in Appendix 2. Extensive exclusion data was accumulated (Publication 11) which may be of value if loci are subsequently assigned to chromosomes where partial exclusions have been made. Recombination between HLA and FRA6P23 (Publication 9) contributed to the precise localisation of HLA to sub-band 6p21.3 (Human Gene Mapping 7, 1984).

Several linkage comparisons did in fact generate lod scores in excess of +1. These are compiled in Table 4.3 as an extract from Publication 11 and Table 4.2. Surprisingly the ABO:AK1 comparison is not included as it only reached 0.8 at $\theta = 0.2$.

This merely reflects the lack of sufficient informative families. As expected, lod scores involving FRA6P23 and HLA, and FRA6P23 and GLO1, were suggestive of linkage. Perhaps the assignment of CHE2 to chromosome 16 needs to be reconsidered on the basis of the CHE2:HP comparison. Provisional assignment of CHE2 to chromosome 16 was withdrawn at Human Gene Mapping 6 (1982). The F13A locus showed indications of linkage to markers on three different chromosomes (1, 4 and 14) but not to chromosome 6 to which it was provisionally assigned at Human Gene Mapping 7 (1984) by Olaisen et al. (1984). Provisional assignment was based on linkage to HLA. HLA and GLO1 are linked, but the F13A:GLO1 linkage comparison in this study did not contribute worthwhile information with a lod score of 0.3 at $\theta = 0.4$. The positive GPT:PGP comparison is interesting given the inconsistent assignment of GPT to chromosomes 8 and 16. The result for Jk:AK1 is surprising, since Jk has now been confirmed to chromosome 2. Occasional chance findings such as this are explicable on the basis of the large number of comparisons being made. This lod score may be dismissed, given existing large negative lod scores for this same comparison (Keats, 1981). Similarly, the positive Lu:HP and P:MNS lod scores may be dismissed (see Keats et al., 1979).

TABLE 4.3

SUMMARY OF HINTS OF LINKAGE

DETECTED IN FRAGILE SITE KINDREDS EXAMINED

Linkage Comparison	Maximum Lod Score
FRA6P23 : HLA	1.4 at $\theta = 0.2$
CHE2 : HP	1.5 at $\theta = 0.05$
F13A : FY	1.0 at $\theta = 0.4$
F13A : GC	1.1 at $\theta = 0.4$
F13A : IGH	1.6 at $\theta = 0.05$
GLO : HLA	1.3 at $\theta = 0.1$
GPT : PGP	1.5 at $\theta = 0.2$
Jk : AK1	2.0 at $\theta = 0.5$
Lu : HP	1.0 at $\theta = 0.1$ and 0.2
P : MNS	1.3 at $\theta = 0.1$

There has been doubt cast upon the use of the fragile X as a reliable X-linked marker (Sherman et al., 1983, 1985). This does not invalidate the use of fragile sites for gene assignment in the linkage studies reported here. If fragile sites, including the autosomal ones, are not fully penetrant, recombination fractions will have been underestimated and suggestions of linkage would be stronger than the results indicate. Demonstration of non-expression of fragile sites in family members would be possible using series of lightly linked flanking markers.

Simple segregation analysis has been reported from kindreds with autosomal fragile sites. This simply means counting carriers and non-carriers in each kindred, but omitting the index case and each carrier through whom the next generation of carriers was ascertained (in the line of descent between the earliest ancestor and the index case). Segregation was not significantly different from a 1:1 ratio in a large kindred segregating for fra(16)(q22) (Magenis et al., 1970), many kindreds segregating for fra(10)(q25) (Sutherland, 1982) and several kindreds segregating for autosomal folate sensitive fragile sites (Sutherland, 1985). Though the ratio of carriers to non-carriers did not differ significantly, there was however a deficiency (86:100) in kindreds with autosomal folate sensitive fragile sites. Complex segregation analysis by computer is required from a large number of such families to investigate in detail the phenotypic segregation of autosomal fragile sites.

This analysis has now been carried out (Sherman, 1985 - personal communication). Segregation of fra(16)(q22) from a

total of five kindreds was random overall, but with a tendency for an excess of normal sons and an excess of carrier daughters among offspring of carrier mothers. There is no deviation from simple codominance among all fra(10)(q25) families studied. Segregation of autosomal folate sensitive fragile sites is apparently codominant when transmitted by females but there is evidence for reduced penetrance (or selection of male gametes) when fragile sites are transmitted through males. These results for fra(16)(q22) and the autosomal folate sensitive fragile sites are only preliminary at present.

Associated with these linkage studies was the discovery of a new PI variant in one of the families. This variant is fully described in Publication 12 (Mulley et al., 1983c). Forty-three variants were already described at this locus (Cox, 1981). Among subsequent variants to be described was Nnagato, with an isoelectric point between Nadelaide and Nhampton (Yuasa et al., 1984). These variants are heritable, in contrast to the one described in Publication 13 (Mulley et al., 1983d).

(e)   SUMMARY

There is now little doubt from the work presented that fragile site DNA has no gross effect on crossing over and is situated at the site of the chromosomal lesion. The use of fragile site markers did not contribute any new assignments to the human genome despite an extensive exploration. Numerous additions to the exclusion map of man were made. A new PI variant was identified from one family with a fragile site. This variant was subsequently described and then accepted by the International PI Committee as a new variant, Nadelaide.

CHAPTER 5

LINKAGE ANALYSIS USING CHROMOSOMAL VARIANTS AS MARKERS

(a)   INTRODUCTION

(b)   MATERIALS AND METHODS

(c)   RESULTS

(d)   DISCUSSION

(e)   SUMMARY

Publication Number

14   Sutherland, Mulley and Goldblatt (1981).

(a)   INTRODUCTION

Chapter 4 described the use of fragile sites in linkage analysis. This chapter describes the use of other chromosomal markers for this purpose.

The application of chromosomal heteromorphisms to linkage analysis has been discussed in Publication 11. Centromeric markers have not been very useful for the purpose of gene assignment. The application of segregating structural rearrangements (balanced translocations and inversions) has been more successful. These aberrations have been responsible for a number of assignments, including HP to chromosome 16 (Robson et al., 1969), HLA to chromosome 6 (Lamm et al., 1974) and MNS to chromosome 4 (Cook et al., 1981). Such rearrangements have breakpoints scattered throughout the genome. Like fragile sites, they provide markers which are not restricted to centromeric or telomeric regions. The familial centromere markers found on chromosomes 1, 3, 4, 9, 13, 14, 15, 16, 21 and 22 are the more common, but the rare familial structural rearrangements have proven to be more informative (Cook et al., 1980).

(b)   MATERIALS AND METHODS

The methods used for phenotyping were described in Chapter 2.

Linkage analysis was carried out in nine kindreds with a variety of structural markers. Two of these kindreds (6047CR, 557FI) were segregating with unusual 9QH variants as previously described (Sutherland and Eyre, 1981). Linkage results between GALT and 9QH from a portion of one of these kindreds was previously presented in

Publication 7. The whole kindred was reanalysed by LIPED. Another kindred (5001KR) had a large inversion inv(3)(p25;q23) and linkage analysis from part of this kindred has been described in Publication 14 (Sutherland et al., 1981). The whole kindred was reanalysed using LIPED. The other kindreds had a variety of balanced translocations : t(10;18)(q26;q21) in kindred 10199HU, t(11;22)(q23;q11) in kindred 10048MU, t(1;3)(q43;p21) in kindred 109285SC, t(5;10)(p14;q15) in kindred 10556DO, and t(13;14)(p11;p11) in kindred 3017CH. These kindreds have not previously been analysed for linkage. All kindreds are documented for pedigree structure and markers examined in Appendix 2.

(c)   RESULTS

Although most of the families analysed were large, little information of value was forthcoming from linkage comparisons involving the chromosomal markers (Table 5.1). F13B was excluded from 5cM either side of 1q43 or 3p21. Results for C3, GPT, IGH, IGK, Jk, MNS and PI became redundant during the study period (see Chapter 9, Tables 9.1 and 9.2).

Addition of the GALT:9QH comparison to existing data is presented in Table 5.2. There is clearly close linkage between GALT and 9QH. GALT must be very close to the centromere on chromosome 9, or else there is little crossing over in the proximal part of 9p.

Lod scores for loci assigned during the progress of this study given in Table 5.3. Status of assignment is that determined by Human Gene Mapping 7 (1984). In no instance was a confirmed assignment seriously challenged. The C3:GLO1 comparison reached 1.2 at $\theta = 0.05$, but this has previously been negated (Chapter 4, Table 4.2). GPT has been included here because it is on either chromosome 8 or 16.

Linkage analysis between unassigned test loci (determined at Human Gene Mapping 4, 1984) and assigned marker loci in families with segregating chromosomal abnormalities are given in Table 5.4. Linkage comparisons involving marker loci that are assigned to rearranged chromosomes are excluded because of possible bias. The positive lod scores that were obtained (the largest being TF:GC with a lod score of 2.1 at $\theta = .05$ and 0.1) were negated by more extensive data presented in Publication 11, with the exception of the K:C3 comparison. Combined data from fragile site families and these families with chromosome rearrangements and variations gives a maximum lod score of 1.4 at $\theta = 0.05$. This possible linkage requires further study.

TABLE 5.1

LOD SCORES BETWEEN TEST LOCI AND CHROMOSOMAL MARKERS
(INVERSION OR TRANSLOCATION BREAKPOINTS AND
HETEROMORPHISMS)

Linkage Comparison		θ				
		.05	.1	.2	.3	.4
C3 :	10q26 or 18q21	-0.4	-0.2	0.0	0.0	0.0
	11q23 or 22q11	-0.4	-0.2	0.0	0.1	0.1
	9qh	0.3	0.2	0.1	0.1	0.0
	1q43 or 3p21	-1.6	-0.9	-0.3	-0.1	0.0
F13A :	3p25 or 3q23	-0.8	-0.7	-0.4	-0.2	-0.1
F13B :	3p25 or 3q23	0.3	0.2	0.2	0.1	0.1
	9qh	-1.5	-0.9	-0.4	-0.1	0.0
	1q43 or 3p21	-2.0	-1.0	-0.3	0.0	0.0
	5p14 or 10p15	0.3	0.2	0.1	0.1	0.0
GPT :	3p25 or 3q23	-0.6	-0.3	-0.1	0.1	0.0
	9qh	-2.0	-1.3	-0.7	-0.4	-0.1
	11q23 or 22q11	-1.0	-0.6	-0.2	-0.1	0.0
	1q43 or 3p21	-2.3	-1.5	-0.8	-0.4	-0.2
IGH :	3p25 or 3q23	-6.6	-4.5	-2.3	-1.2	-0.5
Jk :	11q23 or 22q11	-5.1	-3.4	-1.8	-0.9	-0.3
MNS :	3p25 or 3q23	-1.7	-1.0	-0.4	-0.1	0.0
	11q23 or 22q11	-2.0	-1.2	-0.5	-0.2	0.0
	1q43 or 3p21	-0.5	-0.3	-0.1	0.0	0.0
P :	3p25 or 3q23	0.4	0.4	0.3	0.2	0.0
	11q23 or 22q11	-1.5	-0.9	-0.4	-0.2	0.0
PI :	3p25 or 3q23	-1.4	-0.7	-0.1	0.1	0.1
	9qh	-1.4	-0.9	-0.4	-0.2	0.0
	13p11 or 14p11	0.5	0.5	0.3	0.2	0.0
	1q43 or 3p21	0.4	0.3	0.1	0.0	0.0
TF :	3p25 or 3q23	-1.6	-1.0	-0.5	-0.2	0.0
	10q26 or 18q21	-1.5	-0.9	-0.5	-0.2	0.0
	13p11 or 14p11	-0.7	-0.4	-0.2	-0.1	0.0
	1q43 or 3p21	-0.2	-0.4	-0.5	-0.4	-0.2
ABO :	9qh	-0.7	-0.4	-0.2	-0.1	0.0
GALT :	9qh	0.2	0.3	0.3	0.2	0.1

TABLE 5.2

LINKAGE BETWEEN 9QH AND GALT

Reference	θ				
	.05	.1	.2	.3	.4
Sparkes et al. (1980)	3.4	3.1	2.4	1.7	0.9
Ferguson-Smith and Aitken (1982)	2.3	2.4	2.1	1.5	0.9
This study	0.2	0.3	0.3	0.2	0.1
Total	5.9	5.8	4.8	3.4	1.9

TABLE 5.3

LOD SCORES BETWEEN TEST LOCI AND MARKER LOCI FROM KINDREDS WITH CHROMOSOMAL MARKERS.
THESE LOCI WERE ASSIGNED BY OTHER INVESTIGATORS DURING THE COURSE OF THIS STUDY.

Linkage Comparison		θ				
		.05	.1	.2	.3	.4
C3 :	ABO	-1.6	-1.0	-0.5	-0.3	-0.1
	ADA	-1.0	-0.6	-0.3	-0.1	0.0
	GPT	0.2	0.3	0.2	0.0	0.0
	GALT	-0.7	-0.4	-0.2	-0.1	0.0
	GC	0.7	0.7	0.6	0.5	0.2
	GLO1	1.2	1.0	0.6	0.3	0.1
	Jk	-1.5	-0.9	-0.5	-0.2	-0.1
	MNS	-1.0	-0.4	0.0	0.1	0.0
	PGM1	-0.6	-0.4	-0.2	-0.1	0.0
	PI	-1.2	-0.8	-0.4	-0.2	0.0
	RH	0.8	0.8	0.7	0.5	0.3
GPT* :	ADA	-0.6	-0.1	0.1	0.1	0.0
	ESD	-2.7	-1.7	-0.8	-0.3	-0.1
	Fy	-0.4	-0.3	-0.2	-0.1	0.0
	GC	-3.2	-1.8	-0.7	0.0	0.0
	GLO1	-2.7	-1.7	-0.7	-0.3	-0.1
	HP	-0.8	-0.4	-0.1	-0.1	0.0
	IGH	-1.3	-0.9	-0.5	-0.2	-0.1
	Jk	0.3	0.3	0.2	0.1	0.0
	MNS	-2.0	-1.1	-0.4	-0.1	0.0
	PGM1	-0.7	-0.2	0.2	0.1	0.0
	PGP	-1.2	-0.8	-0.4	-0.1	0.0
	PI	0.6	0.5	0.4	0.2	0.1
	Rh	-0.5	-0.3	-0.2	0.1	0.0
IGH :	ABO	-1.5	-0.9	-0.3	-0.1	0.0
	ACP1	-0.9	-0.6	-0.2	-0.1	0.0
	Fy	-0.6	-0.4	-0.2	-0.1	0.0
	GALT	0.3	0.3	0.3	0.2	0.1
	GC	-1.6	-0.6	0.2	0.4	0.2
	GLO1	-0.9	-0.6	-0.3	-0.1	0.0
	HP	-1.0	-0.8	-0.3	-0.1	0.0
	MNS	-2.9	-1.8	-0.8	-0.3	-0.1
	PGM	-1.2	-0.8	-0.5	-0.2	-0.1

Contd.

IGK :	ACP1	-0.8	-0.5	-0.2	-0.1	0.0
	AK1	0.5	0.5	0.3	0.2	0.0
	GC	-2.0	-1.3	-0.6	-0.2	-0.1
	HP	-0.5	-0.3	-0.1	0.0	0.0
	MNS	-1.1	-0.7	-0.3	-0.1	0.0
	PGM1	-0.7	-0.4	-0.2	-0.1	0.0
Jk :	ABO	-3.4	-2.1	-1.4	-0.4	-0.1
	ADA	-2.7	-1.6	-0.7	-0.3	-0.1
	Fy	-1.4	-0.9	-0.4	-0.2	0.0
	GC	-2.5	-1.7	-0.8	-0.4	-0.1
	GLO1	-1.9	-1.1	-0.4	-0.2	0.0
	HP	-1.7	-1.1	-0.5	-0.2	0.0
	MNS	-1.2	-0.7	-0.3	-0.1	0.0
	PGP	-1.5	-0.9	-0.4	-0.2	0.0
	Rh	-2.3	-1.5	-0.7	-0.3	-0.1
MNS :	ABO	-2.4	-1.4	-0.6	-0.2	0.0
	ACP1	-1.8	-1.1	-0.5	-0.2	0.0
	ADA	-1.4	-0.7	-0.2	0.0	0.0
	AK1	-0.7	-0.5	-0.2	-0.1	0.0
	ESD	-0.4	-0.2	0.0	0.0	0.0
	GALT	-1.0	-0.6	-0.2	-0.1	0.0
	GC	-3.5	-2.0	-0.7	-0.2	-0.1
	GLO1	-3.6	-2.1	-0.8	-0.3	-0.1
	HP	-1.1	-0.4	-0.1	0.0	0.0
	PGM1	-2.1	-1.1	-0.6	-0.1	0.0
	PGP	-2.5	-1.5	-0.6	-0.2	-0.1
	Rh	-1.1	-0.6	-0.2	0.0	0.0
PI :	ABO	-1.8	-0.9	-0.2	0.1	0.1
	ACP1	-1.6	-1.0	-0.4	-0.2	0.0
	ESD	-0.6	-0.4	-0.2	-0.1	0.0
	GALT	0.5	0.5	0.4	0.3	0.1
	GC	-2.5	-1.7	-0.6	-0.1	0.0
	GLO1	-1.1	-0.6	-0.2	0.0	0.0
	HP	0.4	0.4	0.2	0.1	0.0
	IGH	-3.1	-2.0	-1.0	-0.5	-0.2
	Jk	-0.4	-0.3	-0.1	0.0	0.0
	PGM1	-3.4	-2.1	-1.0	-0.4	-0.1
	Rh	-0.7	-0.5	-0.3	-0.1	0.0

* GPT is on either chromosome 8 or 16 (Human Gene Mapping 7, 1984)

TABLE 5.4

LOD SCORES BETWEEN TEST LOCI AND MARKER LOCI OTHER THAN CHROMOSOMAL MARKERS.
THESE MARKERS REMAINED UNASSIGNED DURING THE COURSE OF THIS STUDY.

Linkage Comparison		θ .05	.1	.2	.3	.4
F13A :	ABO	-1.5	-1.0	-0.5	-0.2	0.0
	ACP1	-1.3	-0.8	-0.3	-0.1	0.0
	C3	0.8	0.7	0.5	0.3	0.1
	Fy	-0.3	-0.2	-0.1	0.0	0.0
	GC	-0.9	-0.6	-0.2	-0.1	0.0
	GLO1	-1.4	-1.1	-0.6	-0.3	0.0
	HP	-0.4	-0.3	-0.1	-0.1	0.0
	IGH	-1.3	-0.9	-0.4	-0.1	0.0
	MNS	0.6	0.6	0.5	0.3	0.1
	PGM1	-1.1	-0.7	-0.3	-0.1	0.0
	PI	0.8	0.7	0.4	0.2	0.1
F13B :	ABO	-3.3	-2.1	-0.9	-0.3	-0.1
	ACP1	0.0	-0.4	-0.2	-0.1	0.0
	ESD	-0.9	-0.6	-0.3	-0.1	0.0
	C3	-1.4	-0.9	-0.4	-0.1	0.0
	GALT	-1.5	-0.9	-0.4	-0.2	0.0
	GC	-3.1	-1.8	-0.7	-0.1	0.0
	GPT	-0.3	0.1	0.4	0.3	0.2
	HP	-1.4	-0.8	-0.2	0.0	0.0
	IGH	0.3	0.3	0.2	0.1	0.0
	PGM1	0.5	0.4	0.4	0.3	0.1
	PGP	-0.4	-0.2	0.0	0.0	0.0
K :	C3	0.7	0.6	0.4	0.1	0.1
	GC	-0.4	-0.3	-0.1	-0.1	0.0
	GPT	-0.4	-0.2	-0.0	0.0	0.0
	MNS	-1.4	-0.9	-0.4	-0.2	0.0
	PGM1	-1.4	-0.9	-0.4	-0.2	0.0
Lu :	GC	-0.8	-0.6	-0.2	-0.1	0.0
	GLO1	-1.1	-0.7	-0.3	-0.1	0.0
	GPT	-0.9	-0.5	-0.2	-0.1	0.0
	IGH	-0.5	-0.4	-0.2	-0.1	0.0

Contd.

P	:	ABO	-1.7	-1.1	-0.5	-0.2	0.0
		ACP1	0.4	0.4	0.3	0.1	0.0
		AK1	-0.7	-0.5	-0.2	-0.1	0.0
		C3	0.5	0.5	0.3	0.2	0.0
		ESD	0.3	0.2	0.1	0.1	0.0
		Fy	0.5	0.5	0.2	0.1	0.0
		GALT	0.3	0.2	0.1	0.1	0.0
		GC	0.4	0.6	0.5	0.3	0.1
		HP	0.5	0.5	0.3	0.1	0.0
		IGK	-0.8	-0.5	-0.2	-0.1	0.0
		MNS	-0.8	-0.3	0.0	0.0	0.0
		PGM1	-0.9	-0.6	-0.2	-0.1	0.0
		PI	-0.4	-0.3	-0.2	-0.1	0.0
		Rh	-0.4	-0.2	0.0	0.1	0.0
TF	:	ABO	0.6	0.4	0.2	0.1	0.0
		ACP1	-1.3	-0.8	-0.4	-0.2	0.0
		ADA	-0.3	-0.2	-0.1	0.0	0.0
		C3	-1.6	-1.0	-0.5	-0.3	-0.1
		GC	2.1	2.1	1.7	1.0	0.3
		GLO	-1.3	-0.6	-0.1	0.1	0.0
		GPT	-0.9	-0.5	-0.1	0.0	0.0
		HP	-1.8	-1.1	-0.4	-0.1	0.0
		IGH	-1.2	-0.9	-0.5	-0.2	0.0
		IGK	-0.4	-0.3	-0.2	-0.1	0.0
		MNS	-0.9	-0.4	0.0	0.1	0.0
		PGM1	-1.1	-0.7	-0.3	-0.1	0.0
		PI	-0.5	-0.3	-0.1	0.0	0.0

(d)   DISCUSSION

The effect of translocations on recombination in balanced carriers is not exactly known. The map of chromosome 1 constructed from families with chromosomal rearrangements closely resembled the map determined from the segregation of markers in chromosomally normal families (Cook et al., 1980), suggesting that chromosomal rearrangements do not markedly effect recombination in any of the chromosomes of a balanced carrier. The genetic location of the translocation relative to other markers is defined as one or other of the breakpoints of the rearrangement.

Inversions in man tend to reduce recombination within the inversion breakpoints (Publication 13). No effect is assumed outside the inversion breakpoints. The genetic location of the inversion in relation to other markers is the closest breakpoint of the inversion. There is little or no crossing-over within small inversions. Tight linkage between a marker and such an inversion implies that the marker is within the inversion or not very far outside it. Tight linkage between a marker and a large inversion only implies that the marker is near one of the breakpoints, because crossing-over can occur within "large" inversions.

No definite recombinant between GALT and 9QH was observed in the family presented by Sparkes et al. (1980). One recombinant from 13 has been observed by Ferguson-Smith and Aitken (1982), and corresponding lod scores were given in Table 5.2. There was a probable recombinant in kindred 557 presented in this study. The most likely distance between GALT and 9QH is 5-10 cM

corresponding to band 9p12-13. This agrees with deletion mapping (Publication 7) and the placement of GALT at 9p13 rather than other alternatives at pter->p24 or p22 (Human Gene Mapping 6, 1982). The 9p13 location has now been accepted (Human Gene Mapping 7, 1984).

So far, only the validity of linkage relationships between test loci and the chromosomal markers have been discussed. What of the validity of lod scores from comparisons between test loci and marker loci other than chromosomal markers in these families?

Validity of lod scores depends upon the effects that chromosomal rearrangements have on crossing-over. Possible small physical effects of the translocations and of the large inversion on rates of crossing-over in the families studied may be ignored. Lod scores from the families with centromeric variants will be unbiased. However, structural rearrangements destroy some linkage groups and create others. This occurs only within chromosomes involved in the rearrangement. By excluding all linkage comparisons with marker loci that are assigned to rearranged chromosomes, validity of lod scores is maintained. These lod scores were presented in Tables 5.3 and 5.4. They are additive over families, including fragile site families and other families without cytogenetic aberrations or variations.

The only lod scores not yet considered are those involving two test loci: that is unassigned loci as defined at Human Gene Mapping 7 (1984). The extent or existence of bias cannot be determined because the position of neither locus is known relative to the rearrangement. Therefore such data are usually useless for gene exclusion and were not presented.

These data could be of value under certain circumstances. If lod scores suggest linkage between two unassigned loci in a family in which a rearrangement is segregating, then this could be the direct result of creation of a new linkage group by the rearrangement. Such data could provide clues to chromosomal locations as demonstrated in the hypothetical example (Fig. 5.1). Positive lod scores between unassigned loci could not be found from the kindreds studied. If they had been, suspected locations could have been tested using appropriate restriction fragment length polymorphisms.

(d)   SUMMARY

Although structural chromosomal rearrangements have historically proven useful for gene assignment , they provided no new gene assignments in this study. They did provide further additions to the exclusion map of man. The conditions under which linkage comparisons in such families are valid were discussed.

FIG. 5.1   Hypothetic translocation responsible for the creation of a new linkage group involving locus A and locus B.   Linkage is defined here as the proximity of two loci indicated by a sufficiently positive lod score in families segregating for this translocation.

Loci A and B on
separate chromosomes

reciprocal
translocation

Loci A and B
now linked
on one chromosome
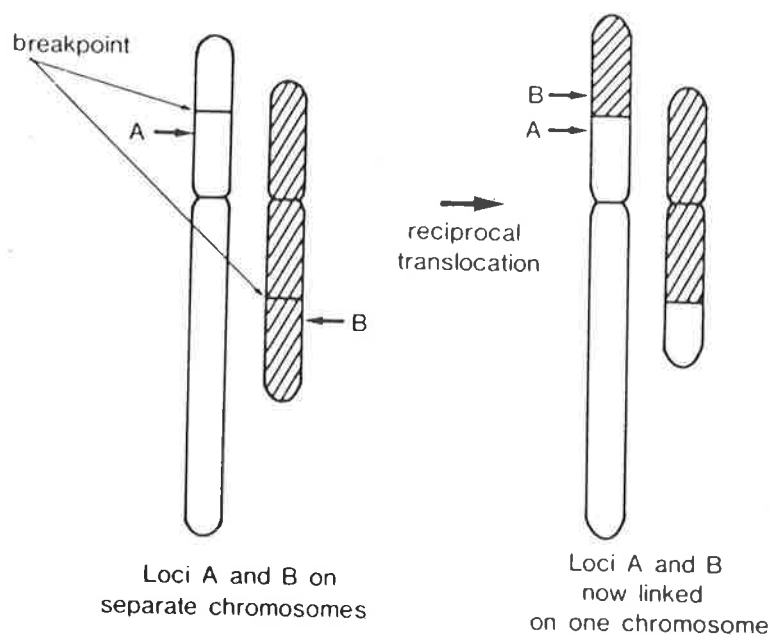
CHAPTER 6

LINKAGE ANALYSIS USING FORMS OF EPIDERMOLYSIS BULLOSA AS DISEASE LOCI

(a) INTRODUCTION

(b) MATERIALS AND METHODS

(c) RESULTS

(d) DISCUSSION

(e) SUMMARY

<u>Publication Numbers</u>

15 Mulley, Nicholls, Propert, Turner and Sutherland (1984).

16 Mulley, Turner, Nicholls, Propert and Sutherland (1985).

(a) INTRODUCTION

Linkage analysis has been applied in the previous two chapters to families segregating for chromosomal markers. Emphasis now shifts to single disease loci.

Some syndromes and diseases once thought of as homogeneous genetic entities are instead a collection of disorders arising from different mutations. Delineation is achieved by refinements in clinical observation, genetic analysis and laboratory testing. The basis for genetic heterogeneity can be :

1) Different mutations at the same locus
2) Mutations at different loci
    (a)   affecting the same enzyme or protein
    (b)   affecting different enzymes or proteins

Mapping the locations of disease loci by linkage analysis is one approach to defining genetic heterogeneity among a group of similar disorders with unknown gene products. This underlying genetic heterogeneity is the basis for the spectrum of clinical observations associated with many disorders. Those diseases with essentially single locus determination are amenable to mapping, and hence identification as discrete entities.

Clinical geneticists classify familial single locus conditions according to their mode of inheritance and spectrum of phenotypes. The mode of inheritance may be autosomal dominant, autosomal recessive, X-linked recessive or X-linked dominant. The major difference between autosomal inheritance and X-linked inheritance is that for X-linked inheritance every mating involving a carrier female is a Mendelian backcross with regard

to male offspring, irrespective of dominance or recessivity. All of these modes of inheritance differ from codominant inheritance in that the various genotypes are not necessarily associated with a unique phenotype, even though the expression is primarily under single locus control.

Autosomal dominant traits are transmitted from one parent to half the offspring. If penetrance is complete, unaffected parents cannot transmit such traits except as new mutations. Transmission is usually through either sex and affected individuals are almost always heterozygotes. Persistence of such deleterious dominant alleles is unlikely if they affect reproductive fitness. If deleterious effects are minor, large kindreds could be available for study, as is the situation with the milder forms of epidermolysis bullosa (EB). Such conditions are amenable to linkage analysis but this is frequently complicated by reduced penetrance (some carriers of the gene remain unaffected while still transmitting it to their offspring) and variable expressivity (a spectrum of phenotypes within a family, though not usually as great as between families).

This chapter describes an attempt to map at least two distinct EB genes. These studies developed from one of the kindreds with a rare folate sensitive fragile site which also segregated for a form of EB.

(b)   MATERIALS AND METHODS

The methods used for phenotyping were described in Chapter 2. The kindreds studied for EB are presented in Appendix 2. Kindred 10531FR had Epidermolysis bullosa simplex - Köbner type (EBS-K) and was investigated in Publication 15 (Mulley et al., 1984). Portion of this kindred was thought previously to have had Epidermolysis bullosa simplex - Weber Cockayne type (EBS-WC), but that diagnosis was undoubtedly due to variable expressivity of a single gene (EBS-K) in the whole kindred derived from one mutational event. Kindred 11366JO also had EBS-K but provided insufficient data for publication. Kindred 368JO had Epidermolysis bullosa dystrophica - Cockayne Touraine type (EBD-CT) and was investigated in Publication 16 (Mulley et al., 1985). The distinctions between the above forms of EB are discussed in Publications 15 and 16. The complete classification and distinguishing features of all forms of EB are given by McKusick (1983).

(c)   RESULTS

For kindreds 10531FR and 368JO the lod scores involving EB loci are given in Publications 15 and 16. Lod scores involving EBS-K for kindred 11366JO are given in Table 6.1. Unfortunately, none of the lod scores were of interest. There was no information linkage between Fy and EBS-K, the comparison of interest identified from kindred 10531FR (Publication 15), but there was an extension to the exclusion limits for EBS-K as indicated in Table 6.1.

Linkage comparisons among loci other than EB are given in Tables 6.2 and 6.3. Table 6.2 includes test loci assigned during the study period (1984). Table 6.3 includes test loci that remained

unassigned during the same period. These tables include data from Families 2 and 3 only. Data from Family 1 was incorporated into lod scores for fragile site families included in Publication 11 in Chapter 4. It was segregating for both EBS-K and FRA12Q13.

TABLE 6.1

LOD SCORES BETWEEN <u>EBS-K</u> AND FOUR MARKER LOCI FROM KINDRED 11366JO

Linkage Comparison	θ				
	.05	.1	.2	.3	.4
EBSK: GPT	-0.7	-0.4	-0.2	-0.1	-0.0
C3	-1.0	-0.7	-0.4	-0.2	-0.1
ACP1	-1.7	-1.1	-0.6	-0.3	-0.1
ESD	-1.0	-0.7	-0.4	-0.2	0.1

TABLE 6.2

LOD SCORES BETWEEN TEST LOCI
AND MARKER LOCI FROM KINDREDS WITH EPIDERMOLYSIS BULLOSA.
THESE TEST LOCI WERE ASSIGNED BY OTHER INVESTIGATORS DURING
THE COURSE OF THIS STUDY.

Linkage Comparison		θ				
		.05	.1	.2	.3	.4
C3	: ABO	-3.1	-1.7	-0.7	-0.2	0.0
	AK1	-0.5	-0.8	-0.2	0.0	0.0
	GPT	-0.4	0.0	0.2	0.2	0.1
	ESD	2.0	1.8	1.2	0.6	0.2
	Fy	-2.4	-1.4	-0.5	-0.2	0.0
	GALT	0.7	0.7	0.5	0.3	0.1
	GC	-3.8	-2.0	-0.7	-0.3	-0.1
	GLO1	-1.8	-1.2	-0.6	-0.3	0.0
	HP	-2.8	-1.8	-0.8	-0.3	0.0
	IGH	-3.0	-1.6	-0.6	-0.2	0.0
	Jk	-3.1	-1.7	-0.6	-0.2	-0.1
	Lu	-0.7	-0.4	-0.1	0.0	0.0
	MNS	-6.6	-4.2	-2.0	-0.8	-0.2
	PGM1	-1.7	-0.9	-0.2	0.0	0.0
	PGP	-1.9	-1.1	-0.4	-0.1	0.0
	PI	-1.6	-0.6	0.0	0.1	0.1
	Rh	-3.3	-1.9	-0.8	-0.3	-0.1
GPT*	: ABO	-0.6	-0.1	0.2	0.2	0.0
	ACP1	-2.3	-1.3	-0.5	-0.2	0.0
	ESD	-1.1	-0.6	-0.2	-0.1	0.0
	Fy	-1.0	-0.6	-0.2	-0.1	0.0
	GC	-2.9	-1.6	-0.5	-0.2	0.0
	GLO1	-1.9	-1.1	-0.3	-0.1	0.0
	HP	-1.2	-0.4	0.1	0.2	0.1
	IGH	-0.8	-0.2	0.1	0.2	0.1
	IGK	-0.8	-0.7	-0.4	-0.2	0.0
	Jk	-1.5	-0.8	-0.2	0.0	0.0
	Lu	0.5	0.5	0.3	0.2	0.0
	MNS	-4.5	-2.7	-1.1	-0.3	0.0
	PGM1	-1.2	-0.7	-0.2	-0.1	0.0
	PGP	-1.6	-1.0	-0.4	-0.2	0.0
	PI	-0.7	-0.3	0.0	0.1	0.0
	Rh	-1.2	-0.6	-0.2	0.0	0.0

Contd.

IGH	:	ABO	1.6	2.0	1.8	1.1	0.3
		ACP1	-1.0	-0.5	-0.1	0.0	0.0
		AK1	1.0	0.9	0.7	0.4	0.1
		ESD	-0.7	-0.4	-0.2	-0.1	0.0
		Fy	-2.2	-1.2	-0.3	0.0	0.0
		GC	-3.7	-2.0	-0.6	-0.2	0.0
		GLO1	-1.2	-0.7	-0.2	-0.1	0.0
		HP	0.8	0.8	0.6	0.4	0.1
		IGK	-1.3	-0.7	-0.2	0.0	0.0
		Lu	-0.5	-0.3	-0.2	-0.1	0.0
		MNS	-2.0	-1.1	-0.4	-0.1	0.0
		PGM1	-2.8	-1.8	-0.7	-0.2	0.0
		PGP	-0.7	-0.4	-0.1	0.0	0.0
		PI	-2.3	-1.4	-0.5	-0.2	0.0
		Rh	-0.3	0.0	0.1	0.1	0.0
IGK	:	ABO	-1.5	-1.2	-0.7	-0.3	-0.1
		GC	-0.6	-0.3	-0.1	0.0	0.1
		GLO1	-0.8	-0.5	-0.2	0.0	0.0
		Lu	0.5	0.4	0.3	0.2	0.1
		PGD	-1.4	-0.9	-0.4	-0.2	0.0
		PI	-0.8	-0.4	-0.1	0.0	0.0
		MNS	0.5	0.4	0.2	0.1	0.0
		Rh	-1.1	-0.7	-0.3	-0.1	0.0
Jk	:	ABO	-2.6	0.0	0.4	0.3	0.1
		ACP1	-1.0	-0.7	-0.4	-0.2	-0.1
		AK1	-1.3	-0.6	-0.1	0.0	0.0
		ESD	-5.7	-3.3	-1.3	-0.4	-0.1
		Fy	-2.1	-1.3	-0.6	-0.3	-0.2
		GC	-4.5	-2.6	-0.9	-0.2	0.1
		GLO1	-3.7	-2.2	-1.0	-0.4	-0.1
		HP	-1.6	-1.0	-0.4	-0.2	-0.1
		Lu	-1.3	-0.8	-0.3	-0.1	0.0
		MNS	-3.7	-2.0	-0.8	-0.3	0.0
		PGD	-2.4	-1.6	-0.7	-0.3	-0.1
		PGM1	-2.9	-1.8	-0.8	-0.3	-0.1
		PGP	-1.5	-0.9	-0.4	-0.1	0.0
		PI	-1.1	-0.3	0.1	0.2	0.0
		Rh	-1.4	-0.4	0.2	0.2	0.1
Lu	:	GC	-1.2	-0.8	-0.4	-0.2	0.0
		PI	-0.9	-0.4	-0.1	0.0	0.0
MNS	:	ABO	-6.4	-4.0	-1.8	-0.8	-0.2
		ACP1	-0.8	0.1	0.3	0.3	0.2
		AK1	-1.2	-0.7	-0.3	-0.1	0.0
		ESD	-4.6	-2.6	-1.0	-0.4	-0.1
		Fy	-5.7	-3.4	-1.4	-0.6	-0.1
		GC	-8.0	-4.5	-1.6	-0.4	-0.2
		GLO1	-0.7	-0.4	-0.2	-0.1	0.0
		HP	-4.2	-3.3	-0.8	-0.1	0.0
		PGD	0.7	0.9	0.8	0.5	0.2
		PGM1	-4.0	-2.2	-0.8	-0.2	0.0
		PGP	-1.0	-0.4	0.0	0.0	0.0

		PI	-4.4	-2.3	-0.8	-0.3	-0.1
		Rh	-7.4	-4.1	-1.3	-0.4	-0.1
PI	:	ABO	-2.4	-1.4	-0.6	-0.1	-0.1
		ACP1	-5.7	-3.9	-2.0	-1.0	-0.4
		AK1	-1.0	-0.6	-0.3	-0.1	0.0
		ESD	-0.5	0.0	0.3	0.3	0.1
		Fy	-2.0	-1.2	-0.5	-0.2	0.0
		GC	-2.8	-1.4	0.4	-0.1	0.0
		GLO1	-2.6	-1.4	-0.5	-0.2	0.0
		HP	0.4	0.7	0.8	0.6	0.3
		PGD	-1.0	-0.4	0.0	0.1	0.1
		PGM1	-1.2	-0.7	-0.2	0.0	0.0
		PGP	-1.5	-0.8	-0.3	-0.1	0.0
		Rh	-8.8	-5.6	-2.7	-1.3	-0.5

* GPT is on either chromosome 8 or 16 (Human Gene Mapping 7, 1984)

TABLE 6.3

LOD SCORES BETWEEN TEST LOCI AND MARKER LOCI
FROM KINDREDS WITH EPIDERMOLYSIS BULLOSA.
THESE MARKERS REMAINED UNASSIGNED DURING THE COURSE OF
THIS STUDY.

Linkage Comparison		θ				
		.05	.1	.2	.3	.4
CHE2:	ABO	-1.5	-0.9	-0.4	-0.1	0.0
	AK1	-0.5	-0.2	0.0	0.0	0.0
	C3	0.5	0.6	0.5	0.3	0.1
	ESD	0.4	0.3	0.2	0.1	0.0
	F13A	-0.5	-0.3	-0.1	0.0	0.0
	GALT	0.8	0.7	0.5	0.3	0.1
	GPT	-0.4	-0.3	-0.1	-0.1	0.0
	HP	-0.5	-0.3	-0.1	0.0	0.0
	IGH	-0.6	-0.3	-0.1	0.0	0.0
	PGM1	0.7	0.6	0.5	0.3	0.1
	TF	-0.7	-0.4	-0.1	0.0	0.0
F13A:	ABO	-1.2	-0.7	-0.2	-0.1	0.0
	F13B	-0.7	-0.4	-0.2	-0.1	0.0
	ACP1	-0.6	-0.3	-0.1	0.0	0.0
	ADA	-0.7	-0.4	-0.2	-0.1	0.0
	AK1	0.5	0.5	0.4	0.3	0.1
	C3	-2.4	-1.3	-0.5	-0.1	0.0
	Fy	-1.9	-1.2	-0.6	-0.2	-0.1
	GALT	-1.2	-0.8	-0.4	-0.1	0.0
	GC	-0.6	0.0	0.2	0.1	0.0
	GLO1	-0.5	-0.4	-0.2	-0.1	0.0
	GPT	0.2	0.2	0.1	0.0	0.0
	HP	-0.9	-0.5	-0.1	0.0	0.0
	IGH	0.2	0.4	0.5	0.3	0.1
	Jk	-0.5	-0.3	-0.1	0.0	0.0
	K	-0.7	-0.4	-0.2	-0.1	0.0
	MNS	-1.0	-0.5	-0.1	0.0	0.0
	PGM1	-1.2	-0.7	-0.2	-0.1	0.0
	PI	-2.2	-1.3	-0.5	-0.2	0.0
	Rh	-1.3	-0.6	-0.1	0.0	0.0
	TF	0.3	0.3	0.3	0.2	0.1

Contd.

F13B :	ABO	-1.3	-0.8	-0.3	-0.1	0.0
	C3	-1.1	-0.7	-0.3	-0.1	0.0
	ESD	-0.6	-0.3	0.0	0.1	0.0
	Fy	0.3	0.3	0.2	0.1	0.0
	GC	-2.4	-1.6	-0.7	-0.3	-0.1
	GLO1	-1.8	-1.1	-0.4	-0.1	0.0
	GPT	-0.4	-0.3	-0.2	-0.1	0.0
	IGH	-0.9	-0.6	-0.3	-0.1	0.0
	Jk	-1.7	-1.1	-0.5	-0.2	0.0
	MNS	-1.6	-0.9	-0.3	-0.1	0.0
	P	-1.1	-0.6	-0.2	-0.1	0.0
	PGM1	-0.7	-0.5	-0.3	-0.1	0.0
	PI	-0.9	-0.5	-0.2	-0.1	0.0
	Rh	-2.6	-1.6	-0.7	-0.2	0.0
	TF	-0.5	-0.2	0.0	0.0	0.0
K :	AK1	-0.7	-0.4	-0.2	-0.1	0.0
	Fy	1.1	0.9	0.6	0.3	0.1
	GC	-1.0	-0.5	-0.2	0.0	0.0
	GLO1	-0.7	-0.4	-0.2	-0.1	0.0
	GPT	-1.7	-1.1	-0.6	-0.3	-0.1
	HP	-0.8	-0.4	-0.8	0.0	0.0
	IGH	-0.6	-0.4	-0.2	-0.1	0.0
	IGK	-0.6	-0.5	-0.3	-0.2	-0.1
	Lu	-0.8	-0.6	-0.4	-0.2	-0.1
	MNS	-1.0	-0.4	0.0	0.0	0.0
	P	-0.5	-0.3	-0.1	0.0	0.0
	PI	0.7	0.6	0.4	0.2	0.0
	PGM1	-0.9	-0.4	-0.1	0.0	0.0
	Rh	-2.1	-1.3	-0.5	-0.2	0.0
P :	ABO	-1.1	-0.6	-0.2	-0.1	-0.1
	ACP1	-1.9	-1.1	-0.4	-0.1	0.0
	C3	-1.3	-0.9	-0.4	-0.1	0.0
	ESD	-2.0	-1.3	-0.5	-0.2	0.0
	Fy	-0.9	-0.5	-0.2	-0.1	0.0
	GC	-1.5	-0.6	0.0	0.2	0.1
	GLO1	-0.7	-0.4	-0.1	0.0	0.0
	GPT	-1.3	-0.7	-0.2	0.0	0.0
	HP	-2.3	-1.5	-0.7	-0.3	-0.1
	IGH	-1.1	-0.7	-0.3	-0.1	0.0
	Jk	-1.8	-0.9	-0.3	-0.1	0.0
	MNS	-2.5	-1.3	-0.3	-0.1	0.0
	PGM1	-3.1	-1.9	-0.8	-0.3	-0.1
	PGP	-2.0	-1.2	-0.5	-0.2	0.0
	PI	-2.6	-1.6	-0.7	-0.2	-0.1
	Rh	-1.2	-0.7	-0.2	-0.1	0.0
	TF	-1.4	-1.0	-0.5	-0.2	0.0

<div align="right">Contd.</div>

TF	:	ABO	-2.3	-1.3	-0.3	0.0	0.1
		ACP1	-0.7	-0.4	-0.2	-0.1	0.0
		AK1	-0.6	-0.1	0.2	0.2	0.1
		C3	-1.3	-0.7	-0.2	0.0	0.0
		ESD	-1.2	-0.6	-0.1	0.0	0.0
		Fy	-2.3	-1.4	-0.7	-0.2	-0.1
		GC	-2.3	-1.4	-0.6	-0.3	-0.1
		GLO1	-1.0	-0.4	0.0	0.1	0.0
		GPT	0.2	0.3	0.3	0.2	0.1
		HP	0.0	0.4	0.4	0.3	0.1
		IGH	1.9	1.6	1.2	0.7	0.3
		Jk	-3.1	-1.7	-0.7	-0.2	0.0
		MNS	-2.1	-1.1	-0.3	0.0	0.0
		PGP	-1.0	-0.7	-0.4	-0.2	-0.1
		PGM1	-1.5	-0.7	-0.2	0.0	0.0
		PI	-2.1	-1.3	-0.5	-0.2	0.0
		Rh	-0.5	-0.1	0.1	0.1	0.0

(d)   DISCUSSION

The hints of possible linkage in Tables 6.2 and 6.3 are summarised in Table 6.4. The C3:ESD comparison is negated by the same comparison in Table 4.2 (Chapter 4). Both the IGH:ABO and TF:IGH comparisons are negated by the same comparisons in Table 5.3 (Chapter 5). The IGH:AK1 and K:Fy comparisons were negated by published works (Keats et al., 1979). Hence, none of the results summarised in Table 6.4 require follow-up because all are chance observations.

Chapters 4 and 5 identified a hint of linkage (lod score of 1.4 at θ = 0.05) between K and C3. This chapter provided no further data regarding this observation. None of the families were informative.

There were several other hints of linkage identified in Chapter 4 (Publication 11). Three of these involved F13A. Additional data from Chapters 5 and 6 now provides the data summaried in Table 6.5. The hint of linkage between F13A and Fy disappears. Similarly for GPT:PGP, but the data is not inconsistent with loose linkage. The hints of linkage between F13A and GC, and F13A and IGH, remain. At least one of these is misleading because GC and IGH are firmly assigned to different chromosomes and both are clearly chance observations now that F13A has been assigned to chromosome 6 (Chapter 9). The positive lod score between CHE2 and HP remains interesting.

TABLE 6.4

SUMMARY OF HINTS OF LINKAGE DETECTED

IN KINDREDS 11366JO AND 368JO

Linkage Comparison	Maximum Lod Score
C3 : ESD	2.0 at $\theta = 0.05$
IGH : ABO	2.0 at $\theta = 0.1$
IGH : AK1	1.0 at $\theta = 0.05$
K : Fy	1.1 at $\theta = 0.05$
TF : IGH	1.9 at $\theta = 0.05$

TABLE 6.5

AFFECT OF ADDITIONAL DATA FROM CHAPTERS 5 AND 6
ON HINTS OF LINKAGE DETECTED IN CHAPTER 4 (TABLE 4.3)

Linkage	θ				
Comparison	.05	.1	.2	.3	.4
K : C3	Unchanged from Chapter 5				
F13A: Fy	-2.2	-0.6	0.3	0.3	0.0
F13A : GC	-5.5	-1.4	1.1	1.0	0.4
F13A: IGH	0.5	0.9	1.2	1.0	0.4
GPT : PGP	-7.0	-1.7	0.7	0.8	0.5
CHE2: HP	1.0	1.1	0.9	0.5	-0.1

These attempts at assignment for at least two epidermolysis bullosa genes discussed in this chapter were unsuccessful. According to Solomon and Goodfellow (1983): "For a dominant disorder a linkage study of 15 families, with six children (each) and their parents using classical markers .... will cover only eight per cent of the genome. This number increases to 44 per cent with the currently available RFLP's". The reported linkage studies (Publications 15 and 16) did provide clues for gene assignments, and these can now be pursued using RFLP's. Confirmation of the proposed EBS2 locus (see Publication 15) is being sought using polymorphic restriction sites associated with AT3 (antithrombin III) (Bock and Levitan, 1983). Polymorphic sites are detected using the restriction enzymes PstI and BamHI (Bock and Leviton, 1983; Bock - personal communication). ATIII is assigned to (1q23) (Shows et al., 1984) within measurable linkage distance to Fy (Kao et al., 1984).

The choice of marker loci for further exploration of linkage to EBD-CT depends upon the location of previous exclusions (Fig.6.1). Exclusions (lod scores less than -2 between EBD-CT and marker loci) are made from the midpoint of the marker locus localisation (if this is an interval). The regional localisations are those determined at Human Gene Mapping 7 (1984). Exclusions based on provisional assignments of marker loci are dependent upon subsequent confirmation of the assignment. The genetic map used is the one of Cook et al. (1980). Exclusions near unassigned and inconsistently assigned marker loci (CHE2, F13B, GPT, TF) cannot be given at this time. The search for the EBD-CT gene can now proceed using RFLP's located at regions not yet incorporated in the exclusion map.

The dominant gene, as envisaged by Mendel, is one in which the phenotype of the heterozygote closely resembles one of the homozygotes. Most so-called dominant conditions in man are, strictly speaking, partially dominant. This is the case for EB. The heterozygote is intermediate between the two homozygotes. In human genetics the term dominance has been more loosely applied. Dominant conditions are regarded as those in which the heterozygote differs from the normal homozygote. Near complete dominance is theoretically possible in a case where the protein product is multimeric, because much more than half of the protein formed in the heterozygote might be structurally defective. Pauli (1983) has suggested that, on available evidence, complete dominance does not exist in human genetic diseases.

Several mechanisms of gene action could explain these so-called dominant genes of human genetics (Vogel and Motulsky, 1979). Such mutations may affect regulation at loci coding for enzymes or proteins or affect the function of structural proteins. The dominance condition is brought about either by abnormal amounts of a given gene product, or by incorporation of defective gene product which weakens a structural protein. Diseases which affect the function of enzymes are usually recessive.

The number of Mendelian conditions with single modes of inheritance have been documented by McKusick (1983). There are 1,637 confirmed examples and a further 1,731 examples not yet fully identified or confirmed. Of the confirmed examples, 934 are autosomal dominant, 584 are autosomal recessive and 115 are X-linked. These conditions are amenable to linkage analysis. This is usually more efficient for dominant disorders, except for

those of late onset or low penetrance, and for X-linked disorders.

(e)   <u>SUMMARY</u>

Evidence (albeit inconclusive) was presented for the existence of allelic variation at a proposed <u>EBS2</u> locus responsible for both EBS-K and EBS-WC. This provided the basis for further work to confirm or refute the localisation of <u>EBS2</u> to a segment on chromosome 1. There was no clue to the gene assignment for EBD-CT. The exclusion map derived from the large EBD-CT kindred is presented as the basis for further linkage exploration.

FIG. 6.1 The current exclusion map for EBD-CT determined from kindred 368JO. The exclusions (shown in red) were determined assuming penetrance = 0.7.
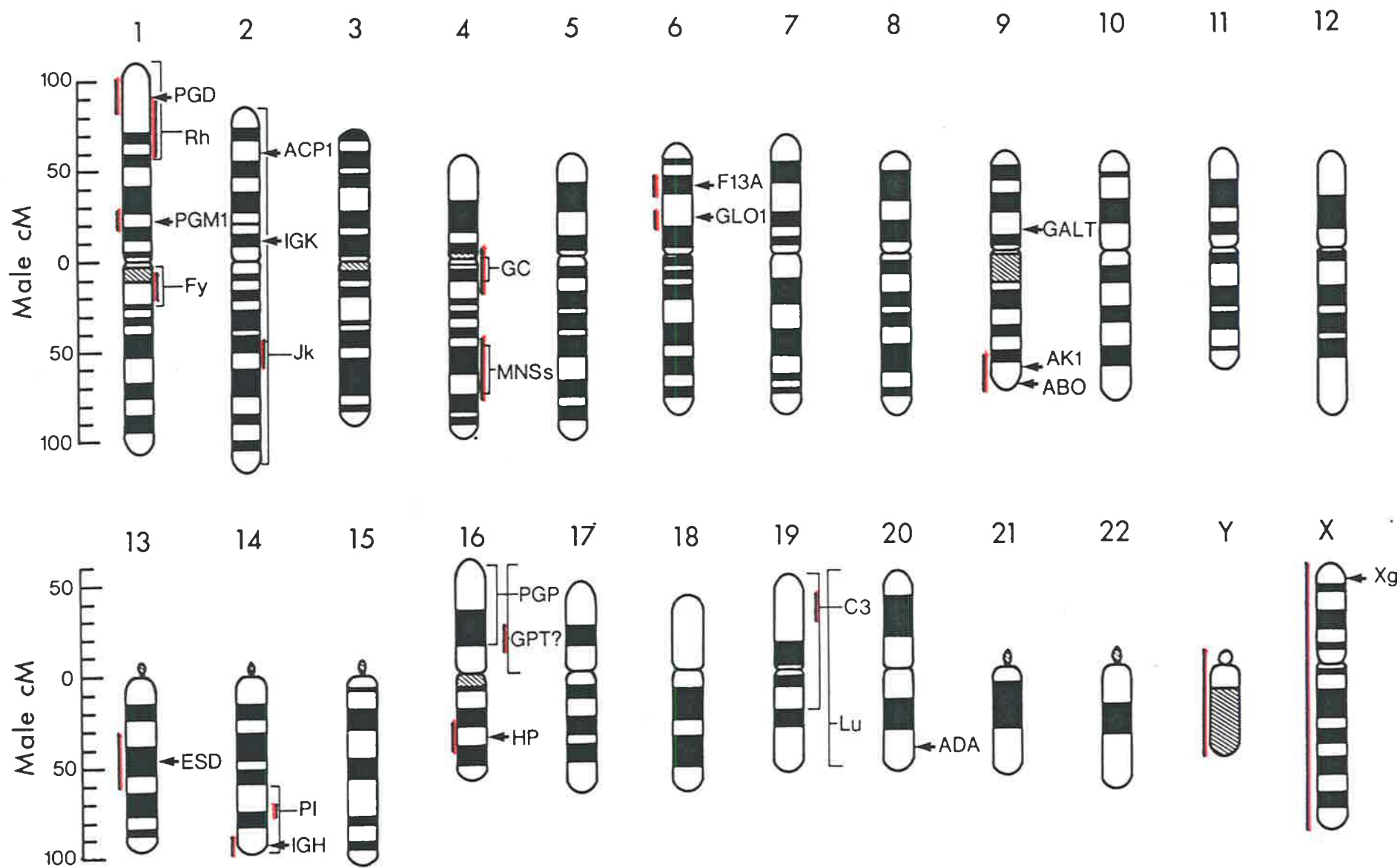
CHAPTER 7

ASSOCIATION BETWEEN PI PHENOTYPE AND MUTATION TO FRAGILE

SITES

(a)   INTRODUCTION

(b)   MATERIALS AND METHODS

(c)   RESULTS

(d)   DISCUSSION

(e)   SUMMARY

Publication Number

17   Mulley and Sutherland (1983).

18   Mulley and Sutherland (1981).

(a)   INTRODUCTION

The nature and origin of fragile sites are not understood. α_1-antitrypsin is associated with diseases involving tissue destruction (Laurell and Eriksson, 1963; Sharp et al., 1969), and when this study commenced, it was under suspicion as a possible factor in the genesis of certain chromosomal aberrations (Aarskog and Fagerhol, 1970; Kueppers et al., 1975; Fineman et al., 1976). Fragile sites are another form of chromosomal aberration. They are heritable chromosomal lesions. On this tenuous pathophysiological basis, data were tested for possible association between α_1-antitrypsin and mutation to fragile sites. Data were already on hand from the families studied in Chapter 4.

(b)   MATERIALS AND METHODS

Two series of data (Series 1 and 2) were collected and analysed. PI phenotyping and allele frequencies from the study population were described in Publications 1 and 2. The families studied were those in Appendix 2 that were segregating with fragile sites. Data from isolated ascertainments were also included, as described in Publication 17 (Mulley and Sutherland, 1983). Publication 17 reported results from Series 1. Results for Series 2 were obtained the same way: from families segregating with fragile sites and from isolated ascertainments where family study could not be undertaken. Results for Series 2 are reported in the text of this chapter. All data refers to the earliest carrier of the fragile site in each kindred as described in Publication 17. Contingency χ^2 tests were applied with a correction for continuity (Steel and Torrie, 1960).

(c)   RESULTS

Series 1

The results from Series 1 are presented in Publication 17. These led to the postulation of a major gene (PI) implicated in the mutagenesis to fragile sites. No mechanism was immediately obvious. In the absence of other series, these results are unconfirmed, and chance must therefore be regarded as a reasonable alternative explanation for the results obtained.

There was also an unexplained high rate of double ascertainment of fragile sites in these families. The double ascertainments involving both folate sensitive and BrdU dependent fragile sites were made from 18 families with folate sensitive fragile sites that were tested for the presence of the BrdU dependent fragile site. Twenty-two of the 40 families with folate sensitive fragile sites were not tested for the presence of the fragile site at 10q25. Given a carrier frequency in the normal population of 1 in 40 for the BrdU dependent fragile site (Sutherland, 1982) the presence of three 10q25 fragile sites among 18 earliest carriers with folate sensitive fragile sites is six times greater than expected. However, this did not reach significance (χ^2_1, = 1.99, 0.10 < P < 0.25). All observations of the fragile site at 16q22 were part of double ascertainments. Three of these were with the fragile site at 10q25. Culture conditions (Sutherland et al., 1984) may have been sub-optimal for the detection of the fragile site at 16q22 in these samples, and many single ascertainments could have been missed. However, the fragile site at 16q22 is sufficiently rare that by chance it should almost always be ascertained alone. It is probably present with a frequency of approximately 1 in 90 individuals (Sutherland, 1985).

Publication 17 raises two separate questions. Firstly, is there a causal relationship between PI phenotype and mutagenesis to fragile sites? Secondly, does the apparently high rate of double ascertainment of fragile sites suggest a common genetic or environmental component involving the PI locus or something else which predisposes to the mutational events for the expression of fragile sites?

The new PI variant NADELAIDE (Publication 12) segregated in one of the families, but was independent of the findings reported. The NADELAIDE and fra(10)(q23) variants had separate ancestry.

Series 2

Another series of fragile site ascertainments was clearly required in order to confirm or refute these findings. Thirty-seven new ascertainments from 36 kindreds have so far been accumulated. This number is, as yet, inadequate for clear evaluation of the possible role of the PI locus in mutagenesis to fragile sites. One of these families did, however, provide another double ascertainment. The double ascertainment involved two rare fragile sites : fra(X)(q27) and fra(16)(q22).

Results from these additional kindreds are summarised in Table 7.1. Data from kindreds with the fragile site at (17)(p12) are now included. These data may be pooled with that from families with the fragile site at (16)(q22). These are both dystamycin A inducible fragile sites (Sutherland et al., 1984). No new data is presented for the polymorphic fragile site at (10)(q25) because no association was originally found with this fragile site. Reasons for this were discussed in Publication 17.

No significant association was found with the 12 additional kindreds with fra(X)(q27) ($\chi_1^2 = 0.07$, $0.75 < P < 0.9$). No association was found with 11 additional ascertainments of the autosomal folate sensitive fragile sites ($\chi_1^2 = 1.80$, $0.10 < P < 0.25$). No association was detected from a total of 19 ascertainments with either fra(16)(q22) or fra(17)(p12) ($\chi_1^2 = 1.32$, $0.25 < P < 0.5$). This second series of fragile site ascertainments failed to confirm the results from the first series. The sample for Series 2 remains, however, of inadequate size.

Since all data was derived from the same population, results from both series were pooled. Significance ($P < .01$) remains for both the fra(X)(q27) ascertainments and the autosomal folate sensitive fragile site ascertainments. However, this is meaningless if the distribution of PI phenotypes obtained from the original series was merely a chance observation.

TABLE 7.1

DISTRIBUTION OF PI PHENOTYPES

AMONG A SECOND SERIES OF FRAGILE SITE GROUPS

Fragile Site Classification	PI Phenotype		
	PI M	Non-PI M	Total
Xq27	10	2	12
Autosomal folate sensitive	8	3	11
16q22 + 17p12*	15	4	19
Control	575	65	640

* Includes five ascertainments of (16)(q22) from first series. These had not previously been analysed for association due to insufficient numbers.

(d) <u>DISCUSSION</u>

Fragile sites have been postulated to arise as specific viral lesions (Lubs, 1969). Morphological changes to chromosomes can be produced by adenovirus-12 (Zur Hausen, 1967; McDougall, 1971; McDougall et al., 1973) and these gaps are in specific chromosomal regions on chromosomes 1 and 17 (Shows et al., 1984). Viral infection could explain the high rate of double ascertainment by inducing more than one fragile site simultaneously.

Reduced regulation of proteases in individuals mildly deficient in protease inhibitor might in some way predispose DNA to viral attack. Protease attack on the histone cores of chromosomes may undermine their structural integrity. This hypothesis is highly speculative, and furthermore, non-specific in regard to the mechanism of action in the absence of full knowledge of metaphase chromosome structure. Development of such knowledge is now proceeding (Kornberg and Klug, 1981; Happala and Nokkala, 1982).

It has now been shown that the <u>PI</u> locus is unlikely to be associated with numerical chromosome abnormalities. Proposals by Aarskog and Fagerhol (1970); Kueppers et al. (1975) and Fineman et al. (1976), have been refuted by Publication 18 (Mulley and Sutherland, 1981) and Bufton et al. (1982). Further study may well show that there is no association between certain PI types and mutation to fragile sites, just as further study refuted the proposed association between certain PI types and nondisjunction resulting in numerical chromosomal abnormalities.

The hypothesis proposed in Publication 17 was that mildly deficient PI phenotypes predispose to mutation to fragile sites. This

hypothesis may be placed in perspective by a passage from Darwin (1859) as cited by Bishop (1983): "False facts are highly injurious to the progress of science, for they often endure long; but false views, if supported by some evidence, do little harm, for everyone takes a salutory pleasure in proving their falseness".

(e)   SUMMARY

An association was detected which suggested that mild α_1-antitrypsin deficiency may predispose to mutation to fragile sites. This association has not yet been verified in the study population and is yet to be tested from other populations. If this association represents a real relationship, its basis is unclear.

CHAPTER 8

THE PI*Z ALLELE AND SEGREGATION DISTORTION

(a)   INTRODUCTION

(b)   MATERIALS AND METHODS

(c)   RESULTS

(d)   DISCUSSION

(e)   SUMMARY

(a)   INTRODUCTION

This chapter explores another potential relationship involving the PI system. The possibility of segregation distortion in the transmission of the PI*Z allele will now be examined.

That there may be preferential transmission of PI*Z by PI MZ males, but not by PI MZ females, was first prposed by Chapis-Cellier and Arnand (1979) and Iammarino et al. (1979). However, the data re-analysed to remove ascertainment bias do not support acceptance of the hypothesis (Cox, 1980), nor do further data (Mittman and Madison, 1980; Constans et al., 1982; Chakraborty et al., 1982), but controversy regarding ascertainment bias remained (Iammarino et al., 1980). Data from the families presented in Appendix 2 are not derived from referred cases of severe α_1-antitrypsin deficiency, and therefore should eliminate the problems of ascertainment bias (unless the relationship between PI phenotype and fragile site mutation is real - see Chapter 7). Preferential transmission has not been observed for PI*S (Suarez et al., 1982).

This chapter reports the outcome of simple segregation analysis for non-PI*M alleles (including PI*Z) in the kindreds in Appendix 2. Mating combinations included the non-PI M phenotypes PI MZ, PI MS, PI MI, PI MNADE and PI MG. All data had been assembled during the course of investigations presented in Chapters 4, 5 and 6.

(b)   MATERIALS AND METHODS

Simple segregation analysis was applied to kindreds in Appendix 2 segregating for non-PI*M alleles. Kindreds were ascertained mainly from fragile sites and never from PI phenotype, hence there should not be ascertainment bias due to severe α^1-antitrypsin deficiency in propositi. Most data was derived from simple sibships.Portion of kindreds where the informative parent was of the phenotype PI SZ were omitted because they obviously had to transmit one or other of their non-PI*M alleles. Heterozygote offspring from families with both parents heterozygous for the same non-PI*M allele were also ignored, because the path of transmission of the alleles could not be determined.

PI was phenotyped as described in Publications 1 and 2.

(c)   RESULTS

Results from 92 offspring are presented in Table 8.1. Simple inspection shows an excess of MZ female offspring (6) from MZ fathers, an excess of MS female offspring (13) from MS fathers, an excess of non-M daughters (21) from non-M fathers (subtotal [1] - Table 8.1) and an overall excess of non-M daughters (31) from all matings (grand total - Table 8.1). The sample is too small for meaningful testing of the first two observations. The excess of female non-M offspring from all matings (grand total - Table 8.1) is not statistically significant ($\chi^2_3 = 3.8$, $0.25 < P < 0.5$). The excess of female non-M offspring from all matings involving non-PI M fathers (subtotal [1] - Table 8.1) is not statistically significant ($\chi^2_3 = 4.8$, $0.10 < P < 0.25$). Similarly, consideration of only female offspring from matings involving non-PI M males (M:non-M 14:21, part of

subtotal [1] - Table 8.1) is not significantly different from expectations ($\chi^2_1 = 1.4$, $0.1 < P < 0.25$).

(d)   <u>DISCUSSION</u>

The controversy described above (Introduction) has apparently been resolved. There was no statistical evidence for the preferential transmission of <u>PI*Z</u> from kindreds examined in this study. Furthermore, there are no additional reports from the literature in support of segregation distortion, apart from those mentioned in the Introduction section. The question of ascertainment bias if there is a real association between PI phenotypes and mutation to fragile sites (Chapter 7) does not arise in the data analysed because no association of <u>PI</u> alleles with segregation distortion could be demonstrated.

TABLE 8.1

RESULTS FROM SIMPLE SEGREGATION ANALYSIS FOR NON-<u>PI*M</u> ALLELES

Parental Combination (Male X Female)	No.	Male		Female	
		M	MZ	M	MZ
PI MZ X PI M	11	2	2	1	6
PI M X PIMZ	7	1	0	2	4
		M	MS	M	MS
PI MS X PI M	35	8	6	8	13
PI M X PI MS	18	5	6	2	5
		M	MI	M	MI
PI MI X PI M	11	2	2	5	2
PI M X PI MI	5	1	1	3	0
		M	MNADE	M	NADE
PI MNADE X PI M	0	0	0	0	0
PI M X PI MNADE	3	0	2	0	1
		M	MG	M	MG
PI MG X PI M	0	0	0	0	0
PI M X PI MG	2	2	0	0	0
Subtotals		M	Non-M	M	Non-M
(1) Non-PI M* x PI M	57	12	10	14	21
(2) PI M x Non-PI M*	35	9	9	7	10
Grand Total	92	21	19	21	31

* Non-PI M phenotypes are either PI MZ, PI MS, PI MI, PI MNADE or PI MG.

(e) SUMMARY

PI alleles segregate randomly in the sample of the population studied. The sample consisted of informative portions of 78 kindreds used in linkage studies (Appendix 2). This result, based on 92 offspring, agrees with the more recent reports from the literature.

CHAPTER 9

CONCLUSIONS

    (a)   GENE MAPPING

    (b)   ASSOCIATIONS

    (c)   IMPACT OF MOLECULAR BIOLOGY

(a)   GENE MAPPING

Two significant factors were evident during the progress of this work. Firstly, the promise of significant advancement by the molecular approach in human genetics became a reality, and is now the dominant method of gene mapping. Secondly, the rate of progress in gene mapping continued to accelerate. Accelerated progress resulted from the continuation of conventional methodology as well as from the introduction of the techniques of molecular biology. This meant that a number of loci not assigned at the beginning of this study are now assigned, making some of the investigations during the study period obsolete.

The status on the gene map of the marker loci investigated at the commencement of study was given in Chapter 1. Advances to the present time in the mapping of relevant loci are summarised in Tables 9.1 and 9.2.   The loci CHE2, F13A, F13B, GPT, K, P and TF remained unassigned, provisionally assigned or inconsistently assigned at Human Gene Mapping 7 (1984). More recent data has now confirmed the assignment of F13A to (6)(p) (Board et al., 1984; Eiberg et al., 1984). The status of F13A will be upgraded to confirmed at the next Human Gene Mapping Workshop. In addition, the loci C3, IGH, IGK, Jk, Lu and PI have been assigned during the study period. When the study commenced, CHE2 was provisionally assigned to chromosome 16, C3 provisionally assigned to chromosome 6, IGK provisionally assigned to chromosome 7, Jk provisionally assigned to chromosome 7, PI inconsistently assigned to chromosomes 6 or 8 and P tentatively assigned to chromosome 6. None of these were subsequently confirmed. Two of the provisional assignments (C3 and Jk) proved to be wrong, the other (CHE2) was downgraded to

TABLE 9.1

CHANGE IN STATUS OF MARKERS AT HUMAN GENE MAPPING 6 (1982)*

Marker	Chromosome	Status**	Region
AMY2	As for HGM5		1p22.1->q11
C3	19	P	
CHE2	Unassigned		
ESD	As for HGM5		13q14
Fy	As for HGM5		1pter->q21 or q25 or q32->qter
GALT	As for HGM5		pter->p13
GLO1	As for HGM5		6p23->p21
GPT	8 or 16	I	8 or 16pter->p11
IGH	14	C	
IGK	2	P	
Jk	2	C	
P	6	P	
PGD	As for HGM5		pter->p34
PGM1	As for HGM5		p22.1
PGP	As for HGM5		p13->p12
PI	14	C	
Rh	As for HGM5		p36->p32
Xg	As for HGM5		pter->p22.3

*   Compared with status at Human Gene Mapping 5 (1979).

**   C - confirmed,   P - provisional,   I - inconsistent.

TABLE 9.2

CHANGE IN STATUS OF MARKERS AT HUMAN GENE MAPPING 7 (1984)

Marker	Chromosome Status*		Region
AMY2	As for HGM5		p21
C3	19	C	pter->q13.2
ESD	As for HGM5		q14.1
F13A	6	P	
Fy	As for HGM5		q12->q21
GALT	As for HGM5		p21->p13
GLO1	As for HGM5		p21.3->p21.2
GPT	As for HGM6		8q13->qter or 16pter->p11
HLA	As for HGM5		p21.3
HP	As for HGM5		q21->q22
IGH	As for HGM6		q32.3
IGK	2	C	p12
Lu	19	C	
P	6	T	
PGD	As for HGM5		pter->p36.13
PI	As for HGM6		q24.3->q32.1
Rh	As for HGM5		p26->p34

*C - confirmed,   P - provisional,   T - inconsistent.

unassigned, the inconsistent assignment (P_I) was wrong and the tentative assignment (P) was downgraded to unassigned. The status of a proposed EBS2 locus on chromosome 1 (Publication 15) may be equally suspect. However, the map locations determined for DNA responsible for fragile sites (Publications 8, 9 and 11) will remain firm.

The status of loci without confirmed assignments are as follows : CHE2, F13B, K, P and TF are unassigned. The status of TF is uncertain. There is no direct evidence for an assignment, although circumstantial evidence from comparative gene mapping supports a provisional assignment to chromosome 3 (Human Gene Mapping 7, 1984). GPT is still inconsistently assigned to chromosomes 8 or 16.

Consider now the effects that recent molecular approaches are having on gene mapping. At Human Gene Mapping 6 (1982) gene mapping had reached "the crossroads" (Lindley, 1979). The gene map was poised for rapid expansion using molecular technology. This is now occurring as evidenced by the content of presentations at Human Gene Mapping 7 (1984).

Solomon and Goodfellow (1983) pointed out that the revolution of recombinant DNA technology has "completed the circle". The imminent discovery of potentially unlimited numbers of new linkage markers by restriction enzymes has reinstated Mendelian genetics as once again the main force in mapping the human genome, but relying heavily on somatic cell genetics and in situ hybridisation. The immediate goal is to cover the genome with markers at regularly spaced intervals, as a means for systematic mapping of all single gene inherited diseases by linkage

analysis. This strategy will facilitate the mapping of the EBS-K and EBD-CT genes discussed in Publications 15 and 16.

(b)   ASSOCIATIONS

The causal connection in associations between alleles at some loci and diseases is often unclear. The marker locus may either be the gene directly involved in the association or in linkage disequilibrium with it if it is closely linked. The type of association examined in Chapter 7 (that mutation to fragile sites is associated with mildly deficient PI phenotypes) would be direct, if it exists, if the mutation to fragile sites is the outcome of protease attack on the chromosome. It could be indirect if a locus causing fragile site mutations had mutator alleles in linkage disequilibrium with non-PI*M alleles. The type of association investigated in Chapter 8 (that PI*Z can be preferentially transmitted) could have been either direct or indirect, depending on the mechanism of distortion, if it existed.

A clear distinction needs to be drawn between association by linkage and direct association. A direct association involves the marker locus as the causal agent in the disease, even if it interacts epistatically with another linked or unlinked locus. Epistatic interaction between a marker locus and an unlinked disease locus can mimic linkage between them if a single disease locus is assumed (Hodge and Spence, 1981). The association of HLA-B27 with ankylosing spondylitis (Brewerton et al., 1973; Schlosstein et al., 1973) is a direct pleiotropic effect (Ebringer et al., 1978) rather than an effect of tightly linked immune response genes in linkage disequilibrium   with   the   HLA

marker. If the association was the outcome of linked immune response genes, then it would have been indirect.

Usually in random mating populations there is some crossing over even between linked loci (such as a marker locus and disease susceptibility locus) and all haplotypes will be randomly distributed. Such a population is in linkage equilibrium and only direct associations will be detected in population samples. Hence a linkage described in a kindred is an association only within the kindred, not an association at the population level. Only associations within kindreds are applicable to linkage analysis.

There are three causes of linkage disequilibrium which could cause association by linkage:

1) Admixture of two populations with different allele frequencies.

2) Population bottleneck.

3) Selection.

Linkage disequilibrium induced by population admixture or a population bottleneck is eroded over successive generations and equilibrium is eventually re-established given sufficient time. Linkage disequilibrium induced by selection is maintained at existing levels unless environmental conditions alter the relative fitnesses of haplotypes.

Interpretative difficulties are characteristic of data analysed for associations. Assuming valid control populations have been chosen, the generally accepted significance level of 5% for comparing control and test populations provides one false

positive association by chance for every 20 tests performed. The statistical methodology described by Mourant et al. (1978) provides not only a relative incidence and a test of significance, but also a homogeneity check with data presented by other investigators. In this way, spurious associations are evident by the lack of regularity with other investigations. In the absence of comparable data sets for checking homogeneity, further independent investigations are needed to verify suspected associations. This applies to the proposed association between PI phenotype and mutation to fragile sites (Chapter 7) and was the reason for the further examination of the possibility of non-random segregation of PI*Z (Chapter 8).

(c)   IMPACT OF MOLECULAR BIOLOGY

The development in molecular biology which has changed the course and intensity of gene mapping, and which has opened up new horizons in medicine, is recombinant DNA technology. The term "recombinant" literally means the joining of two pieces of DNA. It has no connotations whatsoever with crossing over or rates of recombination as analysed by linkage analysis. In general usage, the term has a broader meaning than the joining of two pieces of DNA and encompasses:

(1)   Generation by restriction endonucleases of DNA fragments having part or all of the gene of interest

(2)   Synthesis of DNA by chemical or enzymatic processes

(3)   Incorporation of DNA fragments into vectors as recombinants

(4)   Cloning the fragment of interest to provide usable amounts of DNA for laboratory manipulation

(5)   Sequencing DNA.

Southern blotting (Southern, 1975) is the key technique used to locate specific DNA fragments after electrophoresis. DNA fragments from restriction endonuclease digests are electrophoretically separated by size on agarose gels. The DNA is denatured as a prelude to hybridisation, and transferred by Southern blotting to a nitrocellulose filter. The DNA fragment of interest is hybridised to a denatured radioactive ^{32}P-labelled probe consisting of complementary DNA. The position of the fragment on the filter is visualised by autoradiography.

The main applications of recombinant DNA technology are in the following areas:

(1)   Investigtion of gene structure and function

(2)   Gene mapping

(3)   Prenatal diagnosis, preclinical diagnosis and carrier detection

     i)   using direct DNA probes

     ii)   using tightly linked DNA polymorphisms

(4)   Biosynthesis of biological compounds

(5)   Improvement of production traits of plants and animals

Ultimately, there is the possibility of treatment for genetic disease, but the problems of insertion and control of replacement genes are enormous. For the present, disease detection and determination of disease mechanisms, rather than disease treatment, is the goal of recombinant DNA technology. These goals have been alluded to in Chapters 4 and 6 in relation to both fragile sites and heterogeneous diseases for which aetiology is unknown.

APPENDIX 1

DESCRIPTION OF MARKERS STUDIED
AND HISTOCHEMICAL METHODS FOR THEIR DETECTION

Marker	Page

Marker	Page

The following background on the electrophoretic markers in human blood was compiled from review sources of Beckman (1972), Cooper (1972), Harris and Hopkinson (1976) and Harris (1980) as well as many original references.   Original references are cited for key points.

ACP1   (E.C. 3.1.3.2)

Staining method:

10 ml   Citric acid (0.1M) to pH 5.9 with 1M NaOH

5 mg   4-Methylumbelliferyl phosphate (M-8883, Sigma)

Apply with filter paper overlay (Whatman 3MM).

Incubate for 30 minutes at 37oC.

Remove overlay and view under UV light.

(Modified from Swallow et al., 1973).

The polymorphism was discovered by Hopkinson et al. (1963). ACP1 and PGD were the first red cell electrophoretic enzyme polymorphisms known in man.

Phenotypes :   A, AB, B, AC, BC, C.

ACP1 is a monomeric hydrolytic enzyme with a wide tissue distribution. The isozymes associated with each allele consist of a pair of interconvertible zones : they apparently represent conformational isomers (Harris, 1980). The ACP1 B and ACP1 C isozymes have identical electrophoretic mobility. They differ in the relative intensity of component bands. Activities attributable to ACP1 A, ACP1 B and ACP1 C are in the approximate ratio of 2:3:4 (Beckman, 1972).

The polymorphism is widespread (Beckman, 1972). A fourth allele ACP1*R is common in negroe populations. Numerous rare alleles and a null allele have been reported (Harris and Hopkinson, 1976).

<u>ADA</u>   (E.C. 3.5.4.4)

Staining method:

10 ml	Phosphate buffer (0.02M) pH 7.5
10 mg	Adenosine (A-9251, Sigma)
20 ul	Nucleoside phosphorylase (N3003, Sigma)
20 ul	Xanthine oxidase (X1875, Sigma)
1 ml	MTT (M-2128, Sigma) (5mg/ml)
1 ml	PMS (Phenazine methosulfate) (P-9625, Sigma) (5mg/ml)
12 ml	Agar (2%) at 60ºC

Incubate at 37ºC in dark until bands develop.

(Modified from Spencer et al., 1968).

The polymorphism was first described by Spencer et al. (1968).

Phenotypes : 1, 1-2, 2

ADA functions in the catabolism of purines by the deamination of adenosine. ADA is expressed in all tissues but with great variation between tissues. The subunit structure is monomeric and three isozyme bands are characteristically expressed for each allele. Mean enzyme activity of ADA 1 is greater than ADA 1-2 (Battistuzzi et al., 1981).

Polymorphism is present in most populations (Beckman, 1972). Several rare alleles are known including a null allele associated with severe combined immunodeficiency disease when homozygous (Harris and Hopkinson, 1976).

AK1 (E.C. 2.7.4.3)

Staining method:

10 ml	Tris HCl (0.2M) pH 8.0
40 mg	Glucose
20 mg	MgCl2
25 mg	ADP (Adenine diphosphate) (A-8146, Sigma)
5 mg	NADP (Nicotinamide adenine dinucleotide phosphate) (N-0505, Sigma)
20 ul	Glucose-6-phosphate dehydrogenase (G6PD) (140 u/ml) (G7750, Sigma)
10 mg	Hexokinase (H-5000, Sigma)
1 ml	MTT
1 ml	PMS
13 ml	Agar (2%) at 60oC

Incubate at 37oC in dark until bands develop.

(Modified from Fildes and Harris, 1966).

Fildes and Harris (1966) first described this polymorphism.

Phenotypes : 1, 1-2, 2

AK converts ADP to ATP. It occurs in most tissues except lymphocytoid cells and is one of three human AK loci (Harris and Hopkinson, 1976). AK-1 is monomeric. Enzymatic activity is apparently higher for AK1 1 than for AK1 12 (Beckman, 1972).

This marker is not highly polymorphic. Additional alleles are known. A silent allele is associated with chronic haemolytic anaemia when homozygous (Harris and Hopkinson, 1976).

ESD   (E.C. 3.1.1.1)

Staining method:

10 ml   Phosphate buffer (0.1M) pH 6.5

2.5 mg   4-Methylumbelliferyl acetate (M-0883, Sigma) dissolved in a
           drop of aqueous acetone (1:1).

Apply with filter paper overlay (Whatman 3MM).

Incubate for 10 minutes at 37oC.

Remove overlay.

View under UV.

(Modified from Coates et al., 1975).

This polymorphism is among the more recent of the polymorphic red
cell enzymes to be discovered (Hopkinson et al., 1973).

Phenotypes : 1, 1-2, 2

ESD is one of a number of hydrolytic esterases found in human
tissues.   Tissue distribution is widespread (Harris and Hopkinson, 1976).
The enzyme is a dimer. The electrophoretic phenotype of heterozygotes is
asymmetrical with activity associated with ESD*1 60% greater than ESD*2
(Horai and Matsunaga, 1984).

Polymorphism is present in most populations (Harris and Hopkinson,
1976).   Several rare alleles, including a subtype of ESD*2 and a silent
allele, have been reported (Olaisen et al., 1981).

GALT   (E.C. 2.7.7.12)

Staining method:

10 ml   Tris (0.3M) pH 7.8 with glacial acetic acid 40 mg.

Mixture of glucose-1,6-diphosphate (10mg),

Galactose-1-phosphate (250mg) (G-0380, Sigma), and

UDP-glucose (200mg) (U-4625, Sigma).

10 mg   MgC12

10 mg   NADP

25 ul   PGM (P7502 or 3397, Sigma)

100 ul   G6PD

 50 ul   PGD (P0507, Sigma)

  1 ml   MTT

0.2 ml   PMS

 14 ml   Agar (2%) at 60oC

Incubate at 37oC in dark until bands develop.   (Sparkes et al., 1977).

This marker is described in Publication 5.

GPT   (E.C. 2.6.1.2)

Staining method:

10 ml	Tris (0.1M)
400 mg	L-Alanine (A-7627, Sigma)
150 mg	α-Ketoglutarate (Na) (K-1875, Sigma)

pH to 7.5 with HCl

10 mg	NAD (Nicotinamide adenine dinucleotide) (N-7004, Sigma)
100 ul	Glutamic dehydrogenase (G-2626, Sigma)
1 ml	MTT
0.2 ml	PMS
12 ml	Agar (2%) at 60°C

Incubate at 37°C in dark until bands develop.

The author has used this modified method since 1978 after application to the mouse by Eicher and Womack (1977). Chen and Giblett (1971) discovered GPT in man.

Phenotypes : 1, 1-2, 2

The enzyme GPT catalyses transamination and has a wide tissue distribution. It is typed from red cells. The enzyme is dimeric but the electrophoretic pattern of the heterozygote is asymmetrical. GPT 1 has about three times the catalytic activity of GPT 2 (Harris, 1980) with GPT 2 difficult to detect in aged samples.

The locus is polymorphic in most populations (Harris and Hopkinson, 1976). Rare and silent alleles are known (Harris and Hopkinson, 1976). The silent allele is relatively common (Kompf and Ritter, 1978).

<u>GLO1</u>   (E.C. 4.4.1.5)

Staining method:

20 ml   Phosphate buffer (0.2M) pH 6.8 for four slices

0.9 ml   Pyruvic aldehyde (M0252, Sigma)

30 mg   Glutathione (G4251, Sigma)

Apply with filter paper overlay.

Incubate for 30 minutes at 37oC.

Remove overlay.

4 ml   MTT

1 ml   Dichlorophenolindophenol (1 mg/ml)(D-1878, Sigma)

10 ml   Tris HCl (0.2M) pH 8.5

15 ml   Agar (2%) at 60oC

Expose to light until bands develop.

(Modified from Kompf et al., 1975).

This recently discovered red cell polymorphism was first described as a new marker by Kompf et al. (1975).

Phenotypes :   1, 1-2, 2

GLO1 is a component of the glyoxylase system of two enzymes in red cells which convert methylglyoxal to lactic acid in the presence of reduced glutathione. The enzyme is a dimer and the electrophoretic pattern of the heterozygote is symmetrical.

Polymorphism is widespread (Harris and Hopkinson, 1976). A silent allele has been described (Rittner and Weber, 1978).

PGM1, PGM2, PGM3   (E.C. 2.7.5.1)

Staining method:

10 ml	Tris HCl (0.2M) pH 8.0
30 mg	Glucose-1-phosphate with G-1,6-diphosphate (G-1259, Sigma) (use 100 mg for PGM3).
5 mg	MgCl2
5 mg	NADP
20 ul	G6PD
1 ml	MTT
0.5 ml	PMS
13 ml	Agar (2%) at 60oC.

Incubate at 37oC in dark until bands develop.

(Modified from Spencer et al., 1964).

This system was first applied as a marker by Spencer et al. (1964).

PGM is a component of glycolysis. Three PGM loci are known : PGM1, PGM2 and PGM3 (Harris and Hopkinson, 1976). The major PGM activity (85-95%) is attributable to PGM1 in all tissues except red cells and fibroblasts. PGM1 and PGM2 contribute equally to red cell PGM activity. In fibroblasts PGM3 is responsible for about 7% of PGM activity, but PGM3 contributes nothing in red cells and muscle. PGM1 is more effective in catalysing the phosphoglucomutase reaction. PGM2 is capable of both phosphoglucomutase and phosphoribomutase activity. The primary role of PGM3 is yet to be determined.

PGM1

Phenotypes : 1, 1-2, 2

PGM1 is a monomer. The isozyme associated with each allele is double-banded. Each allele can be further subdivied by IEF. The polymorphism is widespread (Harris and Hopkinson, 1976) at both the two allele (Spencer et al., 1964) and four allele (Bark et al., 1976) degree of classification. Numerous rare variants occur and some have been documented since the introduction of IEF. Some rare variants are associated with reduced enzyme activity and deficiency is attributable to homozygosity for a null allele.

PGM2

PGM2 migrates anodally to PGM1. The usual phenotype is characterised by three isozymes and occurs in all tissues. Numerous rare electrophoretic variants have been described and common variants can occur in negroid populations and certain isolated communities. PGM2 is monomorphic in caucasians. PGM2 is a monomer.

PGM3

Phenotypes : 1, 1-2, 2

PGM3 migrates anodally to both PGM1 and PGM2. PGM3 is a monomer and each phenotype is characterised by a prominent anodal secondary band. Polymorphism is widespread. A rare variant has been reported (van Wierst et al., 1973).

PGD   (E.C. 1.1.1.44)

Staining method:

10 ml	Tris HCl (0.2M) pH 8.0
10 mg	6-Phosphogluconate (Na3) (P-7877, Sigma)
5 mg	NADP
1 ml	MTT
1 ml	PMS
13 ml	Agar (2%) at 60oC

Incubate at 37oC in dark until bands develop.

(Modified from Fildes and Parr, 1963).

Inherited variation at this locus was first described by Fildes and Parr (1963).

Phenotypes :   A, AC, C

PGD is a component of the hexose-monophosphate shunt (pentose cycle) and has a wide tissue distribution. The enzyme is a dimer. The electrophoretic pattern of the heterozygote is symmetrical except for red cell extracts where asymmetry is due to in vivo instability of the PGD C isozyme (Harris and Hopkinson, 1976).

The locus is polymorphic in most populations (Harris and Hopkinson, 1976). Numerous rare alleles are known including two associated with a marked reduction in enzyme activity (Parr and Fitch, 1967).

PGP   (E.C. 3.1.3.18)

Staining method:

25 ml   Tris HCl (0.1M) pH 7.5 for four slices

150 mg   Phosphoglycolic acid (P-4261, Sigma)

10 mg   MgSO4

Apply with filter paper overlay (Whatman 3MM).

Incubate for two hours at 37ºC.

Remove overlay and apply 1.25 g Ascorbic acid, 25 ml 2.5%   Ammonium

molybdate in 4N sulphuric acid with filter paper overlay at room

temperature.   Remove overlay when bands begin to develop.

(Modified from Barker and Hopkinson, 1978).

This marker represents another recently discovered marker in red cells.
The polymorphism was first described by (Barker and Hopkinson, 1978).

Phenotypes : 1, 1-2, 2, 1-3, 2-3, 3

PGP may have some regulatory role in oxygen transport via the
activating effect of its substrate on 2, 3-diphosphoglycerate phosphatase.
(2, 3-Diphosphoglycerate binds with haemoglobin and alters its oxygen
affinity).   PGP is widely distributed in human tissues (Barker and
Hopkinson, 1978).   It is a dimer. The electrophoretic pattern appears to
be symmetrical.

The polymorphism is widespread but not present in all ethnic groups
(Blake and Hayes, 1980).

<u>SOD</u>   (E.C. 1.15.1.1)

Staining method:

Biproduct of staining methods using MTT.

Reaction enhanced by exposure to light and use of alkaline pH, e.g. Tris HCl pH 8.5 - 9. (First reported by Brewer, 1967).

The physiological function of SOD is the neutralisation of potentially harmful superoxide free radicals. The soluble form of SOD is expressed in most tissues. The enzyme is a dimer.

Genetic variation is rare except in certain isolated populations (Harris and Hopkinson, 1976).

AMY2   (E.C. 3.2.1.1)

Staining method:

20 ml   Phosphate buffer (0.02M) pH 6.9

10 mg   Sodium chloride

200 mg   Soluble starch (previously boiled)

Incubate 1 hour at 37oC

Rinse in water

Stain briefly with iodine solution (30g KI, 13g I2 in 1 litre).

(Modified from Merritt et al., 1973).

A clear genetic interpretation of the AMY isozymes was presented by Vacikova and Blockova (1969). Genetic heterogeneity had been described earlier (Kamaryt and Laxova, 1965, 1966).

Phenotypes : A, B

Pancreatic amylase (AMY2) and salivary amylase (AMY1) are coded by two tightly linked loci (Merritt et al., 1972). AMY2 is a monomer and is expressed in serum, plasma and urine as multiple bands with two phenotypes. The phenotype AMY2 A arises from homozygosity of the common allele. AMY2 B is the heterozygote or less common homozygote. Agarose electrophoresis has been used to distinguish all three genotypes determined by AMY2*A and AMY2*B (Kompf et al., 1980). A third phenotype, AMY C, is common in negroe populations.

<u>CHE2</u>   (E.C. 3.1.1.8)

Staining method:

25 ml   Phosphate buffer (0.2M) pH 7.1

 5 mg   a-Napthyl acetate (N-6750, Sigma) in 0.5 ml aqueous acetone

          (1:1)

10 mg   Fast red TR (F-1500, Sigma).

Incubate in dish at 37ºC until bands develop.

(Harris and Hopkinson, 1976).

The polymorphism was detected by Harris et al. (1962) and the dominant mode of inheritance suggested by Harris et al. (1963).

Phenotypes : +, -

<u>CHE2</u> is one of two cholinesterase loci widely expressed in human tissues with other esterases.   CHE is usually studied in serum or plasma where the cholinesterases are the principal esterase isozymes present.   The two phenotypes of CHE2 presence or absence of the C5 isozyme, are determined by two common alleles, one active and one silent.   The active allele is expressed in about 10% of the population (Harris and Hopkinson, 1976).

C3

Staining method:

Terminate electrophoresis when the haemoglobin marker has run 8cm.

Fix in 5:1 saturated picric acid : acetic acid for 15 minutes, rinse briefly in running tapwater, then cover with wet filter paper (Whatman MMm), 1cm thickness of paper towel and compress for 5 minutes with 2 kg weight. Dry with hairdryer until agarose has shiny surface. Stain for 10 minutes in 0.2% Coomassie blue R (B-0630, Sigma) in methanol : H_2O : acetic acid at 9:9:2. Destain until background clear (destained in methanol : H_2O : acetic acid at 9:9:2).
(Modified from Alper and Propp, 1968).

Phenotypes :   1, 1-2, 2

C3 carries out many of the physiological functions necessary for defence against pathogenic microorganisms. C3 deficiency results in recurrent bacterial infection. C3 concentration in serum is by far the highest of all the components of complement. The molecule has an α and β chain (Harris, 1980).

Polymorphism is widespread among caucasian populations but reduced in negroid and mongoloid populations. Many variants have been described. All appear to be allelic, and are all products of either the α or β chain, but it is not known which (Harris, 1980). This protein is unstable when stored.

F13A

Staining method:

Terminate electrophoresis when haemoglobin marker has run 10cm

2 ml	Tris HCl (1M) pH 8.0
2 ml	Calcium chloride (0.05M)
60 mg	Casein in 6 ml Tris HCl (0.2M) pH 8.0
20 mg	Monodansyl cadaverine (D-4008, Sigma) (initially dissolved in drop of 0.1M hydrochloric acid) and made up to 2 ml with Tris HCl (0.1M) pH 8.0
40 ul	2-Mercaptoethanol (1%)

2 units Thrombin

Apply with filter paper overlay (Whatman 3MM).

Incubate at 37oC overnight in humid chamber.

Replace with filter paper overlay of 7.5 % trichloroacetic acid for 5 minutes.

Replace with filter paper overlay of 0.3M disodium hydrogen phosphate for 5 minutes.

Wash in dish of running tapwater for 2 hours (avoiding direct contact of water stream with gel).

View under UV.

(Board, 1979)

F13B

Staining method:

Terminate electrophoresis when haemoglobin marker has run 6.5cm 15% v/v FXIII S antisera (OTOKO5, Calbiochem-Behring) in physiological saline placed on gel 2-4cm behind the haemoglobin marker.

Incubate for 1 hour at room temperature in a humid chamber.

Rinse briefly in dish of running tapwater for 5 minutes (avoiding direct contact of water stream with gel).

Cover with wet filter paper (Whatman 3MM), 1cm thickness of paper towel and compress for 5 minutes with 2 kg weight.

Soak overnight in litre of physiological saline.

Dry with hairdryer until agarose has shiny surface.

Stain for 10 minutes in 0.2% Coomassie blue R in methanol:H20:acetic acid at 9:9:2.

Destain until background clear (destained in methanol:H20:acetic acid at 9:9:2).

(Board, 1980). An improved method was subsequently described using an enzyme-linked immunoblotting technique (Board, 1984).

FACTOR XIII

Coagulation factor XIII (F13) is the precursor for fibrinoligase. Fibrinoligase forms crosslinks between fibrin molecules for the stabilisation of clot structure. Plasma F13 comprises two A subunits and two B subunits : the B units probably acting as carrier molecules for the A subunit in the plasma (Board, 1979; 1980).

F13A

Phenotypes : 1, 1-2, 2

The A subunits of F13 are detectable from plasma and platelets. F13A is a dimer and the electrophoretic pattern of the heterozygote is symmetrical.

The populations so far studied suggest that the A subunit polymorphism is widespread (Board, 1979; Board and Coggan, 1981; Kera and Nishimukai, 1982). Rare alleles have been described (Board and Coggan, 1981). A silent allele is responsible for herited deficiency (Rodeghiero and Barbui, 1980).

F13B

Phenotypes : 1, 1-2, 2, 1-3, 2-3, 3

The B subunits are detectable from either serum or plasma. This polymorphism may also be widespread (Board, 1980; Nakamura and Abe, 1982).

The F13A and F13B polymorphisms were only recently discovered (Board, 1979; 1980). Subsequently, Kera et al. (1981) challenged theclassification of Board (1980) on the basis of IEF studies in Japanese. Board (1984) resolved the problem. There are only the two common alleles in Japanese populations (1 and 3) and IEF fails to separate the 1 and the 2 variants. Distinction between the one and two variant was often ambiguous in the hands of the present investigator using the technique described. Results are reported in Appendix 2 only as 1 or 3, where 1 is the sum of the 1 and 2 variants reported by Board (1980).

GC

See Publication 3. The polymorphism was discovered by Hirschfeld (1959).

HP

Staining method:

(Saturate samples with haemoglobin prior to electrophoresis).

Lower pH of gel in 1M acetic acid for 15 minutes.

10% Guaicol mixture in fume cupboard comprising

20 ml   Guaicol (G-5502, Sigma),

90 ml   Acetic acid

7 g   Sodium hydroxide in 90 ml H20, for four gels.

Immediately before use add 3 ml hydrogen peroxide. (Queen and Peacock, 1966).

This was the first electrophoretically detectable protein polymorphism described in man (Smithies and Walker, 1955).

Phenotypes : 1, 1-2, 2

HP is an α2-glycoprotein of serum and plasma. The physiological role of HP is in binding haemoglobin with subsequent removal from circulation by the liver. Low or undetectable levels of HP occur in neonates (Cooper, 1972).

Dissociation of HP gives two different subunits : α and β chains. The gene determining the a chain is polymorphic, and the common alleles are designated HP*1 and HP*2 (Smithies and Walker, 1955). Rare β chain variants have been found. The HP polymorphism is

worldwide. Rare HP alleles and a null allele have been described (Cooper, 1982).

Subunit structure associated with each genotype are :

HP 1-1   : $\alpha 1_2 \beta 2$

HP 1-2   : $\alpha 1 \alpha 2 \beta n$ $n = 3 \dots 7\text{-}10$

HP 2-2   : $\alpha 22 \beta n$

where subscripts refer to the number of such chains. HP 1 is a single electrophoretic band. HP 2 consists of multiple bands of varying intensity. The heterozygote is asymmetric with bands unique to the heterozygote formed by random association of the a dimer. HP 2 has a tendency for polymerisation.

HP 1 can be subtyped into HP 1S and HP 1F controlled by two alleles HP*1S and HP*1F (Smithies et al., 1962). There are thus three common HP alleles when subtyping is done. Subtyping involves the electrophoresis of purified HP subsequent to reductive cleavage by β-mercaptoethanol in the presence of 8M urea. HP*2 arose as a duplication by unequal crossing over between HP*1S and HP*1F. HP 2 has α chains almost twice as large as HP 1, as a result of this duplication.

PI

See Publications 1 and 2. Polymorphism has been known since Fagerhol and Braend (1965).

TF

See Publication 4. The polymorphism was first discovered by Smithies (1957). It was the second electrophoretically detectable protein polymorphism described in man.

The following background on blood groups was compiled from Boettcher (1972) and Race and Sanger (1975) : Original references are cited only for key points. These blood groups were applied to investigations described in this thesis merely as genetic markers for linkage studies.

ABO

This was the first blood group discovered in man (Landsteiner, 1900).

Genotypes		Phenotypes
OO		O
A2O) A2A2)		A2
A1O) A1A2) A1A1)		A1
BO) BB)		B
A2B A1B		A2B A1B

Alleles $\underline{A}$ and $\underline{B}$ are codominant to each other, but dominant to $\underline{O}$. Conversely, $\underline{O}$ is recessive to $\underline{A}$ and $\underline{B}$; being an amorph, producing no detectable antigen. Further subdivision of ABO groups resulted from the splitting of A into A1 and A2. A1 reacts with anti A1 while A2 does not, hence A1A2, A1O and A1A1 are indistinguishable. Numerous additional variants of group A are known, but are rare in Europeans. B subgroups are rare. The ABO polymorphism is worldwide.

The ABO system is important in transfusion. If a patient lacks A or B antigens, corresponding antibodies develop in sera which rapidly destroy incompatible cells following transfusion. The ABO antigens of cells and ABO antibodies of sera are not fully developed at birth. Neonates typing as A2 at birth have been shown to be A1 at six months of age. Subgroups of A can be difficult to determine without family study because of the weakening of antigen A in the presence of antigen B.

Fy

This blood group was not discovered until 1950 (Cutbush et al., 1950).

Phenotypes : A, AB, B

The Duffy (Fy) locus was the first autosomal gene assignment in man (Donahue et al., 1968) and is now part of a linkage group on chromosome 1. Polymorphism is widespread, except in asian populations. A third allele is commonly present in negroes for which no antisera has been found. Antigens are well developed at birth.

K

The existence of this system was first shown by Coombs et al. (1946).

Phenotypes : k, Kk, K Kpa, Kpab, Kpb

Polymorphism is widely distributed but not worldwide. Heterozygosity is relatively low. Gene K and k are allelic. Kpa and Kpb are allelic at a second closely linked locus. JsA and JsB are antigens at a third tightly linked locus. Numerous other antigens are known. K negative individuals exposed to K positive cells have a risk of forming anti-K antibody resulting in haemolytic transfusion reactions. Both K and k antigens are well developed in neonates. Similarly, Kpa and Kpb antigens are well developed in neonates.

Jk

This group was recognised soon after the discovery of Fy. It was reported by Allen et al. (1951).

Phenotypes : A, AB, B

Polymorphism of Kidd (Jk) is widespread. A rare allele with no antigenic activity has been found in Europeans. Perhaps this silent allele has a frequency as high as 0.01. A common difficulty with the Jk system is the instability of Jk antisera. Delayed haemolytic transfusion reactions may occur with Jk antibodies. Antigens are well developed at birth.

Lu

This locus was first recognised by Callendar et al. (1945).

Phenotypes : A, AB, B

The Lutheran (Lu) system has two common codominant alleles. The polymorphism is widespread but the level of heterozygosity is low. Numerous other antigens are known but their relationship to A and B is unclear. Antigens are not fully developed on the red cells of neonates. The Lu locus provided the first known autosomal linkage in man (Mohr, 1951) to the locus for salivary secretion of the ABO antigens (secretor status).

MNS

The MN locus was the second blood group discovered (Landsteiner and Levine, 1927a). Anti S was not discovered for another 20 years (Walsh and Montgomery, 1947).

Phenotypes : MMSS, MMSs, MMss, MNSS, MNSs, MNss, NNSS, NNSs, NNss.

Phenotypes correspond to genotypes except for MNSs which can be either MS/Ns or Ms/NS. The MN and Ss loci are tightly linked (Sanger and Race, 1947; Sanger et al., 1948). Inheritance is codominant. Linkage disequilibrium is present : S is more usually in coupling with M than with N. A rare variant is known at the Ss locus and a number of rare variants are known at MN. M may be subtyped but this is not generally done because of the rarity of the necessary antisera. Polymorphism is worldwide.

A technical error sometimes arises in typing the MN system because M bloods give a slight agglutination with anti-N sera. In the absence

of MN and N controls some M bloods can be typed as MN, producing an apparent excess of MN offspring from MN x MN matings.

P

This was another of the early blood groups and was discovered during the same work that disclosed the existence of the MN group (Landsteiner and Levine, 1927b).

Genotypes	Phenotypes
P1P1	P1
P1P2	
P2P2	P2

P1 is dominant to P2. A third rare allele exists. The polymorphism is worldwide. The P1 antigen is labile and in poor samples P1P1 and P1P2 genotypes can be mistakenly phenotyped as P2. The antigen strength is considerably weaker in children.

Rh

The Rhesus (Rh) system (Levine and Stetson, 1939; Landsteiner and Wiener, 1940) consists of three closely linked loci : C, D and E (serial order probably DCE) (Fisher and Race, 1946; Fisher, 1947). The notational representation of genotypes has two common forms : a CDE formula originating from Fisher and a shorthand R form. A range of antisera (anti-D, anti-C, anti-c, anti-E, anti-e) allows classification of samples into most probable genotypes. A single phenotype can sometimes represent any one of a number of different genotypes and this pitfall must be recognised when testing paternity. Each of the three loci have two common alleles and many rare alleles. The polymorphism is widespread.

Many series of observations are based on the D antigen alone because of the greater availability of anti-D during the early days and because of the medical significance of the D antigen. Individuals lacking the D antigen are regarded as Rh negative. The Rh group is the most significant system involving haemolytic disease of the newborn arising from maternal immunisation by transfusion (Levine et al., 1941). Rh antigens are well developed before birth.

Xg

Phenotypes : A, -

The X-linked Xg system (Mann et al., 1962) was the first blood group
gene assigned to a specific chromosome in man. X-linked loci are readily
recognised by the characteristic inheritance pattern of their alleles.
Unlike most X-linked loci, Xg is not subject to random inactiviation in
females when carried on a structurally normal X chromosome (Fialkow,
1970). It is preferentially inactivated on structurally abnormal
chromosomes (Race, 1971). The polymorphism is widespread. A is
dominant. The Xg antigen is well developed at birth, but not well
developed until late in foetal life. Full development does not occur until
well after birth.

HUMAN LEUCOCYTE ANTIGENS

Allele frequencies (>0.05) (Tait, 1977).

A locus:			B locus:			C locus:		
HLA-A1	=	0.17	HLA-B5	=	0.05	HLA-CW3	=	0.19
-A2	=	0.32	-B7	=	0.14	-CW4	=	0.09
-A3	=	0.15	-B8	=	0.13	-T7	=	0.18
-A9	=	0.09	-B12	=	0.14	Blank	=	0.46
-A11	=	0.05	-BW15	=	0.09			
-A28	=	0.05	-BW35	=	0.07			
-AW19	=	0.10	-BW40	=	0.10			

Numerous other antigens exist at these loci, and at the D locus. The HLA system of leucocyte antigens is the most polymorphic set of loci yet known in man. At least four loci, HLA-A, HLA-B, HLA-C and HLA-D (order : centromere - D-B-C-A) are well established (Tait, 1977). HLA is the major histocompatibility system in man and as such is of major significance in renal transplantation.

IMMUNOGLOBULIN SERUM GROUPS

Human immunoglobulin (IG) is built from heavy and light chains:

Chain Composition		
IG Classes	Heavy Chains	Light Chains
IgG	γ	κ or λ
IgA	α	κ or λ
IgM	μ	κ or λ
IgD	δ	κ or λ
IgE	ϵ	κ or λ

The function of the immunoglobulins is to form soluble complexes with foreign materials such as proteins and viruses. IgG is quantitatively the predominant class. the IgG molecule is built from two identical heavy chains (γ) and two identical light chains. The gammaglobulin (IGH) system is polymorphic and is characteristic of the IgG heavy chains. The kappa (IGK) system is also polymorphic and is characteristic of light chains of the κ type only, found in all Ig classes. Polymorphism is detected using the haemagglutination inhibition test (Grubb, 1970). Infants less than six months of age are not typed because of transplacental movement of maternal immunoglobulin.

IGH

Common haplotypes : 1, 17, 21 = 0.22

1, 2, 17, 21 = 0.11

3, 5, 13, 14 = 0.67

(Propert, 1977).

These are recognised using antigens GM(1), GM(2), GM(3) and GM(5).

Genotypes	Phenotypes
1-1	1
1, 2-1, 2	1,2
1, 2-1	1,2
1-3, 5	1, 3, 5
1, 2-3, 5	1, 2, 3, 5
3, 5-3, 5	3, 5

Numerous antigens have been recognised. The haplotypes are determined by four tightly linked loci on the γ chain.

IGK

Common alleles : 1 = 0.09

3 = 0.91

(Propert, 1977)

Phenotypes   : 1+, 1- using only KM(1) antisera.

APPENDIX 2

FAMILY DATA PRESENTED IN THIS THESIS

INDEX TO FAMILIES

		Number of Families
(a)	AUTOSOMAL FOLATE SENSITIVE FRAGILE SITES	17
(b)	X-LINKED FOLATE SENSITIVE FRAGILE SITE *	22
(c)	BRDU DEPENDENT FRAGILE SITE **	23
(d)	DISTAMYCIN A INDUCIBLE FRAGILE SITE	1
(e)	MORE THAN ONE FRAGILE SITE	4
(f)	FRAGILE SITES AND CHROMOSOMAL HETEROMORPHISMS	2
(g)	FRAGILE SITES AND EPIDERMOLYSIS BULLOSA	1
(h)	CHROMOSOMAL HETEROMORPHISMS	6
(i)	EPIDERMOLYSIS BULLOSA	2
	TOTAL ***	78

*   Two families informative for Xg only.

**   Kindred 5656EH listed in this group not included in total. Another branch of this kindred is included in the total for section (e).

***   Of these, 70 are fragile site families.

The 78 kindreds documented in this appendix formed the basis for the linkage investigations reported in Chapter 4, Chapter 5 and Chapter 6. All unrelated individuals within these kindreds provided the gene frequency data presented in Chapter 2.

The full range of markers were determined for most kindreds, but only those informative for linkage are presented. Both informative and uninformative markers were considered for the derivation of gene frequency data presented in Chapter 2. The pedigree format is based on that used for linkage analysis by LIPED. The pedigree information presented in this appendix were extracted directly from the stored data file for each kindred, which had been established on the computer for linkage analysis. All pedigree information was built into a single file to which kindred identification, locus abbreviations and nature of chromosomal markers or disease loci were added. Slight improvements to the setting out were performed using a word processor.

The data format is as follows :

Column 1   Identification code for each individual.

Column 2   Identification code for one parent. The numerical prefix identifies the generation to which that individual belongs.

Column 3   Identification code for other parent.

Column 4   Sex of individual (M = male, F = female).

This fully specifies pedigree structure. For example, in the first kindred (878BU) the third individual is in generation 2 is male and has for its parents 1A (a male in generation 1) and 1B (a female in generation 1). The unrelated individuals used for the

calculation of allele frequency in Chapter 2 are readily identified as those without parents (blanks in columns 2 and 3).

The next column shows the phenotype of segregating chromosomal markers or disease loci. If the chromosome marker was sporadic, then this column shows, instead, the first marker locus. All marker loci are readily identified by locus abbreviations at the top of the columns. Chromosomal marker phenotypes and disease loci phenotypes are identified as follows :

NN   normal

NF   carrier of a familial fragile site

FF   male hemizygous for familial FRAXQ27

NE   carrier of familial epidermolysis bullosa

NT   carrier of a familial translocation

NI   carrier of a familial inversion

NV   carrier of a familial chromosomal variant

Where more than one fragile site segregates, each is identified at the top of the respective column. The designation X for any locus implies either the locus was not typed, could not be typed with confidence or could not be typed due to insufficient sample. The designation X at all loci for any individual implies either the individual was deceased, could not be located or would not cooperate for family study. If phenotypes are not given for chromosomal variants in a kindred with such a variant, then this variant was considered to be sporadic and omitted. Linkage between FRAXQ27 and Xg was tested assuming no male transmission and omitting families with possible new mutants. Subsequently, Sherman et al., (1983, 1985) demonstrated probable male transmission in a proportion of families and suggested that all new mutations occurred in sperm.

Hence, isolated males could not be new mutants. Data was not reanalysed using these assumptions because $\underline{Xg}$ and $\underline{FRAXQ27}$ were by then firmly mapped to the extremities of separate chromosome arms (Human Gene Mapping 6, 1982). Two families uninformative for autosomal linkage comparisons were informative for the $\underline{Xg}$ linkage comparison. Including these, the number of fragile site kindreds is 70, not 68 as stated in Publication 11.

The locus abbreviations ACP, AK, GLO and PGM refer to $\underline{ACP1}$, $\underline{AK1}$, $\underline{GLO1}$ and $\underline{PGM1}$ respectively. Genotype designations KZ and ZZ refer to Kk and kk of the Kell system, MNSZ and MNZZ refer to MNSs and MNss of the MNS system, 1, 2 and 3 refer to M1, M2 and M3 in the $\underline{PI}$ system or C1, C2 and C3 in the $\underline{TF}$ system. Otherwise, phenotypes and genotypes are designated as described in Appendix 1. Generally, inferred genotypes are given, rather than phenotypes. For example, for the GC system, a genotype 1S1S is given rather than the phenotype 1S. Information for each individual extends to a second line in many kindreds, otherwise all information is contained within a single line.

(a) AUTOSOMAL FOLATE SENSITIVE FRAGILE SITES

KINDRED 878BU FRA2Q11

					PI	GPT	ESD	PGM	SOD	HP	ACP	ABO
1A			M	NN	11	12	12	12	11	12	AB	O
1B			F	NF	1S	22	11	12	12	22	BB	AB
2A	1A	1B	M	NF	11	22	11	12	12	12	AB	B
2B	1A	1B	F	NF	1S	22	12	22	11	12	BB	A
2C	1A	1B	F	NN	1S	X	X	X	X	X	X	X

KINDRED 12778NI FRA2Q13

					GPT	C3	F13B	Jk	MNS	ACP	AK	ADA	PGM	GALT	GLO	GC	Rh	Fy
2C	1A	1B	M	NN	12	11	13	BB	NNSZ	AB	11	12	11	NL	12	1F2	R1LR	AB
2D	1A	1B	M	NF	22	22	13	BB	MNZZ	AB	11	12	12	NL	12	1F2	R1R1	AB
2A	1A	1B	M	NN	12	22	13	BB	MNZZ	AB	12	12	12	NL	12	1F2	R1R1	AB
1A			M	X	12	12	11	AB	MNSZ	BB	11	11	12	NN	12	1S2	R1LR	BB
1B			F	X	22	12	33	BB	MNSZ	AA	12	12	11	NL	22	1F1F	R1R1	AA
2B	1A	1B	F	NF	12	22	13	BB	MMSZ	AB	11	X	12	NL	12	1S1F	R1LR	AB

KINDRED 10647WA FRA6P23

					GPT	C3	F13B	PI	F13A	Jk	MNS	PGM	GLO	HP	GC	Rh	Fy	PGP	HLA*
3C	2F	2G	M	NF	11	12	13	12	11	AB	MMSZ	12	12	12	1S2	X	AB	11	12
2G			M	NN	12	X	11	11	11	AB	MNZZ	12	12	12	1S2	LRR2	AB	11	23
2F	1A	1B	F	NF	12	22	13	2I	12	AB	MMSZ	12	12	12	1S1F	R1LR	AB	11	13
1A			F	NN	11	12	13	X	11	AA	MNZZ	22	22	12	1F2	R1LR	AA	11	34
2D	1A	1B	F	X	11	12	11	11	12	AB	NNZZ	12	12	12	1S2	R1R1	AB	11	24
2C	1A	1B	F	NN	11	12	11	X	12	AB	MNSZ	12	12	11	1S2	R1LR	AB	11	14
3A	2E	2F	F	NF	22	12	11	12	12	AA	MMZZ	11	11	12	1S1F	R1LR	AA	12	12
3B	2E	2F	F	NN	12	12	13	12	X	BB	MNZZ	12	22	22	1S1F	R1LR	AA	11	23
2B	1A	1B	M	NN	11	12	11	1I	12	AB	NNZZ	12	12	11	1S2	R1R1	AB	11	14
1B			M	NF	12	12	X	1I	12	BB	MNSZ	11	11	11	1S2	R1R1	BB	11	12
2A	1A	1B	M	NN	11	22	13	1I	11	AB	NNZZ	12	12	12	22	R1LR	AB	11	13
2H	1B	1C	M	NN	11	12	11	12	22	AB	MNSS	11	12	12	1S2	R1LR	BB	11	13
2I	1B	1C	F	NF	12	22	11	13	12	AB	MNSS	11	11	12	1S1S	R1LR	BB	11	24
2K	1B	1C	F	NN	22	22	13	2I	12	AB	MNSS	11	12	12	1S1S	R1LR	BB	11	23
2J			M	NN	11	22	11	11	11	AA	MNZZ	12	12	12	1F1F	R1LR	AB	11	13
3D	2I	2J	M	NN	12	22	11	11	12	AA	MNSZ	11	11	22	1S1F	LRLR	BB	11	12
3F	2L	2M	F	NF	X	22	11	1I	12	AB	MNSZ	11	12	12	1S2	LRR2	AB	13	12
4A	3F	3G	M	NF	12	22	11	11	12	AB	MNSS	11	11	12	1S2	LRR2	BB	13	23
3E	2I	2J	F	NN	11	X	X	11	11	AB	MNSZ	11	11	11	1S1F	R1LR	AB	11	14
3H	2L	2M	M	NN	12	X	X	12	11	X	X	11	11	22	1S2	X	X	11	23
4B	3F	3G	F	NN	12	22	X	X	11	BB	MNSS	11	12	11	1S2	R1R2	AB	13	14
4C	3F	3G	F	NF	12	22	X	X	X	BB	MMSZ	12	12	22	22	R1R2	AB	13	24
3G			M	NN	11	22	X	12	11	BB	MNSZ	12	11	12	1S2	R1LR	AB	11	34
3I	2L	2M	F	NN	12	22	X	1I	12	AB	MMSS	11	12	X	1S2	LRLR	AB	11	14
3J	2L	2M	F	NN	11	22	X	1I	12	X	X	11	12	11	1S2	X	BB	11	14
AC			F	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	34
2E			M	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	24
2L	1B	1C	M	NF	X	X	X	X	X	X	X	X	X	X	X	X	X	X	24
2M			F	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	13

* HLA coded by the method of Ott (1978).

KINDRED 2231AR FRA7P11

					GPT	F13B	TF	F13A	Jk	MNS	P	GLO	HP	GC	ABO	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2A	1A	1B	F	NF	22	33	12	12	BB	MNZZ	−	12	12	1S2	O	R1R2	AA
2B	1A	1B	F	NN	12	11	22	12	AB	MMSZ	−	12	12	1S1F	O	R1R2	AB
2F	1A	1B	F	NF	12	33	11	12	BB	MMSZ	−	12	12	1F2	A1	R1R2	AA
2D	1A	1B	F	NN	12	13	11	12	AB	MNZZ	−	12	22	1S2	A1	R1R2	AB
2C	1A	1B	F	NN	12	33	12	12	BB	MNZZ	+	12	12	22	A1	R1LR	AA
2E	1A	1B	F	NF	X	13	12	12	BB	MNZZ	+	11	22	22	O	R1R2	AB
2G	1A	1B	M	NN	X	13	11	12	X	X	X	12	22	1S2	X	X	X

KINDRED 12912ZA FRA8Q23

					C3	TF	PI	Jk	MNS	PGP	ACP	ESD	GLO	HP	GC	ABO
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X
3B	2E	2F	M	NF	22	12	11	AB	NNSZ	12	AA	11	22	22	1S2	A1
3A	2E	2F	F	NF	12	12	11	AA	MNSS	11	AB	11	22	12	1S1S	A1
2E	1B	1C	F	NF	12	11	13	AB	MNSZ	12	AB	11	12	12	1S1F	A1
2F			M	NN	22	12	11	AA	NNSZ	11	AB	11	22	22	1S2	A2
2C	1B	1C	F	NF	12	11	23	AB	MMZZ	11	AB	12	11	12	1F1F	A1
2B	1A	1B	F	NF	22	11	23	AB	MNSZ	11	AB	11	12	12	1S1S	A1B
2D	1B	1C	M	NF	12	11	33	AB	MNZZ	11	BB	12	11	12	1F1F	A1
1B			F	NF	22	11	13	AB	MMSZ	12	AB	12	12	22	1S1F	A1
3C	2E	2F	M	NF	22	11	11	X	X	12	AB	11	22	X1	S2	X
1C			M	NN	12	11	23	BB	MNZZ	11	AB	11	12	11	1F1F	A1B
2A	1A	1B	M	NN	22	11	33	X	X	12	AA	11	22	12	1S1F	X

KINDRED 460MC FRA9P21

				GPT	TF	PI	ACP	AK	PGM	GLO	HP	GC
1A			M	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X
2A			F	X	X	X	X	X	X	X	X	X
2B			M	X	X	X	X	X	X	X	X	X
2C			M	X	X	X	X	X	X	X	X	X
3C	2A	2B	F	X	11	1F	AA	11	12	11	12	1S1S
4D	3D	3E	M	12	12	2I	AB	11	12	12	22	1S1S
4C	3D	3E	F	12	12	12	AB	11	11	12	11	1S 2
3E	2C	2D	F	12	11	2I	BB	11	12	12	12	1S1F
3A	2A	2B	M	22	11	11	BB	12	12	12	11	1S1F
4B	3A	3B	M	22	11	11	BB	11	11	12	11	1S1F
4A	3A	3B	M	22	11	11	BB	12	12	11	11	1S1S
2D	1A	1B	F	22	11	12	BB	11	12	22	12	1S1F
3B			F	X	X	X	X	X	X	X	X	X
3D			M	X	X	X	X	X	X	X	X	X

KINDRED 11277WI FRA9Q32

				TF	PI	F13A	MNS	P	ACP	PGM	GLO	GC	ABO	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X
3A	2A	2B	M	NF	11	12	11	MMSS	+	AB	12	12	1S1F	A1	R1R2	AA
2B			M	NN	11	12	12	MMSZ	−	AB	12	12	1S1F	O	R1LR	AB
3B	2A	2B	M	NN	11	11	22	MMSS	+	BB	12	22	1S1S	A1	R1LR	AB
2A	1A	1B	F	NF	12	11	12	MNSS	+	AB	11	22	1S1S	A1	LRR2	AA
1B			F	NN	12	11	11	X	X	AA	12	22	1S1S	X	X	X

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KINDRED 10920BL FRA9O32

				C3	Jk	MNS	ACP	PGM	GLO	HP	ABO	Rh	Fy
2F	1A	1B	M	X	AA	MMZZ	AA	12	22	X	A1	LRLR	AB
1B			F	12	AA	MNZZ	AB	11	12	12	A2B	R1LR	AB
1A			M	22	AB	MMSZ	AB	12	12	12	A1B	LRLR	AB
2D	1A	1B	F	12	AA	MMSZ	AB	11	11	11	A1	R1LR	AB
2B	1A	1B	F	22	AA	MMSZ	AA	11	12	12	B	R1LR	BB
2C	1A	1B	F	12	AB	MMSZ	BB	11	11	22	B	R1LR	BB
2A	1A	1B	F	12	AB	MNZZ	BB	12	12	11	A1B	LRLR	AB
2E	1A	1B	M	22	AA	MNSZ	AB	12	22	12	A2B	LRLR	AB

KINDRED 653AY FRA10O23

					F13B	PI	GC
1A			M	X	X	X	X
1B			F	NF	13	23	1S2
2A	1A	1B	M	NF	13	2N	1S1S
2B	1A	1B	M	NF	13	3N	22
2C	1A	1B	F	NF	13	2N	1S2
2D			M	NN	11	12	1S2
3A	2C	2D	F	NF	11	2N	X

KINDRED 751MO FRA10O23

					C3	F13B	TF	PI	HP	GC
1A			M	NN	22	11	12	12	12	1S1F
1B			F	NF	12	13	11	11	22	1S2
2A	1A	1B	F	NN	12	11	12	11	12	1S1S
2B	1A	1B	M	NF	12	13	12	12	22	1S1F

KINDRED 10555WH FRA10O23

			Sex	GPT	C3	F13B	F13A	K	Jk	Lu		MNS	PGP	ACP	ESD	PGM	GLO	HP	GC	ABO	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2D			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2F			M	NN	11	22	11	12	ZZ	AA	AB	NNSZ	12	AB	X	11	12	12	1S1F	O	LRR2	AB
2E	1A	1B	F	NF	12	X	11	11	KZ	AB	BB	MMSZ	11	BB	11	12	12	12	1S1S	O	R1LR	AB
1B			F	NF	22	22	11	X	ZZ	AB	BB	MNSZ	11	AB	11	12	22	12	1S1S	O	R1LR	AB
3G	2F	2E	M	NN	11	22	11	11	ZZ	AA	BB	MNZZ	11	BB	11	11	12	12	1S1F	O	R1R2	AA
3F	2F	2E	M	NN	12	22	11	11	ZZ	AA	BB	MNSZ	11	BB	11	11	11	11	1S1F	O	R1LR	AA
3E	2F	2E	F	NF	11	22	11	11	KZ	AB	AB	MNSZ	11	BB	11	12	22	11	1S1S	O	R1R2	AA
2B	1A	1B	M	NF	22	22	13	11	ZZ	BB	AB	MNSZ	11	BB	11	12	12	22	1S1S	O	R1LR	BB
2A			F	NN	12	22	11	11	ZZ	AA	BB	MMZZ	11	AB	11	12	12	22	1S2	A2	LRR2	BB
3A	2B	2A	M	NN	12	22	11	11	ZZ	AB	AB	MNZZ	11	BB	11	22	12	X	1S1S	O	LRR2	BB
3B	2B	2A	M	NN	12	22	11	11	ZZ	AB	AB	MMSZ	11	AB	11	11	12	22	1S1S	O	R1LR	BB
2C	1A	1B	F	NF	12	22	11	12	ZZ	AA	AB	MMSZ	13	AB	11	12	22	22	1S1S	O	R1LR	BB
3C	2D	2C	M	NF	11	12	11	12	ZZ	AB	BB	MMSZ	13	AB	12	12	22	X	1S2	O	R1LR	AB
3D	2D	2C	F	NN	12	22	11	X	ZZ	AB	BB	MNZZ	11	BB	12	12	12	X	1S2	O	R1LR	AB
3H	2F	2E	F	NF	11	22	11	12	X	X	X	X	11	AB	11	12	11	X	X	X	X	X

KINDRED 10049LA FRA10O23

			Sex		TF	PI	F13A	PGP	ACP	AK	ESD	PGM	GALT	GLO	HP	GC	ABO
2F	1A	1B	M	NN	12	12	12	12	AB	11	12	12	ND	22	22	1S2	O
1B			F	NN	11	11	12	12	BB	11	12	12	ND	12	12	1S1F	O
1A			M	NF	12	12	12	11	AB	12	12	11	NN	12	12	1S2	A
2B	1A	1B	F	NF	11	11	12	11	BB	11	22	12	NN	22	12	1S1F	O
3A	2A	2B	M	NN	11	11	11	11	BB	11	22	12	NN	22	22	X	X
2A			M	NN	12	12	11	11	BB	12	12	11	ND	22	22	1S2	X
2C	1A	1B	F	NN	11	11	X	12	BB	12	11	11	ND	22	22	1F2	A
2E	1A	1B	F	NF	11	11	11	11	BB	12	12	12	NN	12	11	1F2	A
2D	1A	1B	F	NN	11	11	12	12	BB	12	11	12	NN	22	22	1F2	A
2G	1A	1B	F	NF	11	11	12	12	BB	12	12	11	NN	22	12	1S1S	A

KINDRED 10635GI FRA11Q13

					GPT	F13B	TF	PI	PGP	ACP	ADA	PGM	GLO	GC	ABO
2D	1A	1B	M	NF	12	13	12	13	X	AB	11	22	12	1S2	O
1A			M	X	22	33	12	11	11	AB	12	12	11	1S1S	O
1B			F	X	11	11	11	13	12	AB	11	12	12	1S2	B
2E	1A	1B	F	NF	12	13	12	11	11	BB	11	12	12	1S2	B
3A	2E	2F	M	NN	X	11	12	13	11	AB	11	22	12	1F2	X
2A	1A	1B	M	NN	12	13	11	11	12	AB	11	22	12	1S2	O
2I	1A	1B	F	NN	12	13	11	13	11	AA	12	22	11	1S1S	B
2H	1A	1B	F	NF	12	13	11	11	11	AB	12	22	12	1S2	B
2G	1A	1B	F	NF	12	13	12	13	11	BB	11	22	12	1S1S	B
2B	1A	1B	F	NN	12	13	12	13	12	BB	12	22	11	1S2	B
2C	1A	1B	F	NN	12	13	11	11	12	AB	11	11	11	1S2	B
2F			M	X	X	X	X	X	X	X	X	X	X	X	X

KINDRED 10614MC FRA11Q13

					GPT	C3	F13B	TF	PI	F13A	Jk	Lu	MNS	PGP	ACP	ESD	PGM	GLO	HP	GC	ABO	Rh	Fy
AA			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2B			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2N	1A	1B	M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2W			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2Z			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3Y	2V	2T	F	NF	12	22	11	12	12	11	AB	AB	NNZZ	11	AB	12	11	11	22	1S1S	O	R1R1	AB
2U			M	NN	12	12	11	11	13	12	AB	BB	NNZZ	11	BB	12	12	12	22	1S2	A1	LRZ	AA
3Z	2T	2Z	F	NF	12	22	11	11	13	11	AA	BB	NNZZ	11	BB	11	11	12	22	1S1S	A1	R1R1	AB
3X	2T	2U	F	NF	12	22	11	12	12	12	BB	BB	NNZZ	11	BB	11	12	12	12	1S1F	A1	R1LR	AB
4B	3W	3X	M	NN	X	22	11	X	12	X	BB	BB	X	X	AB	12	12	11	22	X	X	X	X
4A	3W	3X	F	NF	11	22	11	12	11	11	BB	BB	MNSZ	11	BB	12	11	12	12	1S1F	O	LRR2	BB
3W			M	NN	12	22	11	11	11	12	BB	BB	MMSS	11	AB	12	12	12	22	1S1S	A1	LRR2	BB
2V			M	NN	12	12	11	11	11	11	AA	BB	NNZZ	11	BB	12	11	12	22	1S1F	O	R1LR	AB
2T	1A	1B	F	NF	11	X	11	12	23	11	AB	AB	NNZZ	X	AB	11	11	11	12	1S1F	A1	R1R1	AB
4F	AA	3Z	F	NN	11	12	11	11	33	X	X	X	X	11	BB	11	12	12	X1	S2	X	X	X
1B			F	X	11	22	11	11	13	11	AB	BB	MNZZ	11	AB	11	11	12	12	1F1F	O	R1R2	AB
1A			M	X	12	12	11	22	22	11	AB	AB	NNZZ	11	AB	11	12	12	12	1S1S	A1	R1R1	BB
AB	2T	2U	F	NF	12	12	11	11	13	12	AA	AB	NNZZ	11	BB	11	12	12	22	1F2	A1	R1LR	AA
2K	1A	1B	F	NF	11	12	11	12	23	11	AB	AB	NNZZ	11	AB	11	12	12	12	1S1F	A1	R1R1	BB
2C	1A	1B	M	NF	12	12	11	12	23	11	AB	BB	MNZZ	11	AA	11	11	11	11	1S1F	A1	R1R1	BB
2R	1A	1B	M	NF	11	22	11	12	23	11	AB	BB	NNZZ	11	AB	11	12	12	11	1S1F	A1	R1R1	AB
2L	1A	1B	M	NN	11	22	11	12	12	11	AA	BB	NNZZ	11	AB	11	12	12	12	1S1F	A1	R1R2	BB
2P	1A	1B	M	NN	11	12	11	12	23	11	AB	AB	NNZZ	11	AB	11	12	22		1S1F	A1	R1R2	BB
2E	1A	1B	F	NF	12	12	11	12	23	11	AA	AB	MNZZ	11	AB	11	12	12		1S1F	A1	R1R2	BB
2A	1A	1B	F	NF	11	12	11	12	12	11	AA	AB	NNZZ	11	BB	11	12	12		1S1F	A1	R1R1	BB
2G	1A	1B	F	NN	12	22	11	12	23	11	AB	BB	NNZZ	11	BB	11	12	12		1S1F	A1	R1R2	BB
3F	2C	2D	M	NN	22	12	11	11	23	12	AA	BB	MNZZ	11	AB	11	12	12		1S1F	A1	R1R1	AB
3G	2C	2D	M	NN	12	12	11	12	13	12	AA	BB	MNZZ	11	AB	11	12	11		1S1F	O	R1R1	AB
2D			F	NN	22	12	11	11	12	12	AA	BB	MMZZ	12	BB	12	12	22	12	1F2	O	R1R1	AB
3P	2J	2K	M	NN	11	12	11	11	13	11	AB	BB	NNZZ	11	AA	11	12	12		1S1S	A1	R1R1	AB
3Q	2J	2K	F	NN	11	12	11	12	12	11	BB	AB	NNZZ	11	BB	12	11	22	22	1S1S	A1	R1LR	BB
2J			M	NN	11	12	11	11	11	12	AB	BB	NNZZ	11	AB	12	11	12	22	1S1S	A1	R1LR	AB
3R	2J	2K	M	NN	11	22	11	11	13	11	AB	AB	NNZZ	11	AA	12	11	12	22	1S1F	O	R1R1	AB
2S			F	NN	22	22	11	12	11	11	AB	BB	NNSZ	11	AB	12	12	12	11	1S1F	A1	R1LR	AB
3U	2R	2S	M	NN	12	22	11	22	12	11	AB	BB	NNSZ	11	AA	12	12	11		1S1S	A1	R1R1	AB

2I	1A	1B	F	NN	12	12	11	12	12	11	X	X	X	11	BB	11	12	12	11	1S1F	X	X	X
3H	2E	2W	F	NN	11	12	11	22	22	X	X	X	X	11	AB	11	11	12	12	1S1F	X	X	X
3K	2E	2F	M	NN	11	12	11	12	22	11	X	X	X	11	AB	11	11	12	12	1S1F	A1	X	X
3L	2E	2F	F	NN	11	12	11	12	13	11	X	X	X	11	AB	11	11	12	X1	S1F	A1	X	X
3N	2E	2F	F	NN	11	22	13	12	13	12	X	X	X	11	BB	11	11	11	22	1S1S	X	X	X
3V	2R	2S	F	NN	X	22	X	22	X	11	X	X	X	X	BB	12	22	X	11	X	X	X	X
3O	2J	2K	M	NF	11	X	11	12	12	12	BB	BB	NNZZ	11	BB	12	1	12	12	1S1S	O	R1R1	BB
3T	2R	2S	F	NF	X	X	X	12	X	11	X	X	X	X	BB	X	X	X	11	X	X	X	X
2O			F	NN	22	12	11	11	X	11	AB	AB	MMSZ	11	AB	11	11	12	12	1S1S	O	LRLR	BB
3M	2E	2F	F	NF	11	22	11	12	13	12	AA	AA	MNZZ	11	BB	11	11	22	22	1S1S	A1	LRR2	BB
3A	2A	2B	F	NN	11	11	11	12	11	11	AA	AB	MNZZ	11	BB	12	12	12	11	1S2	A1	R1LR	BB
3J	2E	2W	M	NN	12	X	13	11	22	12	X	X	X	11	AB	X	X	X	11	1S1S	O	X	X
3S	2N	2O	M	X	X	11	11	12	11	11	X	X	X	11	BB	X	X	X	22	1S1S	O	X	X
2H	1A	1B	F	NN	12	12	11	12	23	11	AB	BB	MNZZ	11	AA	11	12	12	11	1S1F	A1	R1R2	BB
2F			M	NN	12	22	13	11	12	12	AA	AB	MMZZ	11	BB	11	11	12	22	1S1S	A1	R1LR	BB

KINDRED 12301DO FRA11013

					GPT	PI	Jk	P	PGP	ACP	GALT	GLO	HP	GC	Rh
1A			M	X	X	X	X	X	X	X	X	X	X	X	X
2C	1A	1B	M	NF	12	12	BB	–	11	BB	NN	11	22	1S1S	R2R2
1B			F	NF	11	12	AB	–	11	AB	NN	11	12	1S1S	R2R2
2A	1A	1B	M	NN	11	11	BB	+	11	BB	NN	12	12	1S1S	R1R2
2B	1A	1B	M	NN	11	12	AB	+	11	AB	ND	11	12	1S2	R2R2
2D	1A	1B	M	NF	11	11	AB	+	12	AA	NN	12	12	1S2	R1R2

KINDRED 10531DA FRA12013

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                GPT C3 TF PI Jk Lu MNS  P ACP ESD PGM GLO HP  GC  ABO  Rh  Fy

2C         M     X  X  X  X  X  X   X   X  X   X   X   X   X   X    X   X   X
4A 3B 3A M NF   22 12 12 1S  X  X   X   X  AB  12  22  22  22  1S2  X   X   X
3A       M NN   22 22 12 2S AB BB MNZZ  - AA  11  12  22  12  1S2  A2 R1LR AB
3B 2B 2C F NF   22  X 12 1Z BB BB MMZZ  + AB  12  12  22  22  1S2  A1 R1LR BB
2B 1A 1B F NF   12 12 11 SZ BB BB MNZZ  + AB  12  12  12  12  1S2  A1 LRLR BB
3C 2B 2C F NN   22  X 12 1Z AB BB NNZZ  + AA  11  11  22  12  1S2  A1 LRR2 AB
3D 2B 2C F NN   22 11 12 1S AB BB MNZZ  + BB  11  12  12  11 1S1S  A1 R1LR AB
1A       M NF   22  X 11 2S BB AB MNSZ  + BB  12  12  11  22  1S2  A1 LRR2 BB
2A 1A 1B F NN   22 22 11 SZ AB AB MMSS  + BB  11  11  12  12 1S1S  A1 LRR2 BB
1B       F NN   12 22 11 1Z  X  X   X   X  AB  11  12  12  11  1S2  X   X   X
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KINDRED 10463GR FRA16P12

				PI	GLO
1A			M	11	12
1B			F	12	22
2A			M	1Z	12
2B	1A	1B	F	11	22
3A	2A	2B	M	1Z	22
3B	2A	2B	M	11	22

(b) <u>X-LINKED FOLATE SENSITIVE FRAGILE SITE</u>

<u>KINDRED 6053MA FRAXO27</u>

				TF	PI	HP	GC
1A			M	X	X	X	X
1B			F	X	X	X	X
2A	1A	1B	F	X	X	X	X
2B			M	X	X	X	X
2C	1A	1B	F	11	3S	12	1S2
2D			M	X	X	X	X
3A			M	X	X	X	X
3B	2A	2B	F	12	12	22	1S1S
3C	2A	2B	F	12	11	22	22
3D			M	X	X	X	X
3E			M	X	X	X	X
3F	2C	2D	F	11	1S	12	1S2
3G			M	X	X	X	X
4A	3A	3B	F	22	12	22	1S1S
4B	3A	3B	F	11	11	22	1S2
4C	3A	3B	F	12	12	22	1S1S
4D	3A	3B	F	12	12	22	1S1S
4E	3C	3D	F	11	11	22	1S2
4F	3E	3F	F	11	1S	12	1S1S
4G	3E	3F	F	11	1S	11	1S2
4H	3E	3F	M	11	11	11	1S2
4I	3F	3G	M	11	11	12	1S1F

KINDRED 10923EL FRAXO27

				TF	PI	HP	GC
1A			M	X	X	X	X
1B			F	12	1S	11	1F2
2A	1A	1B	F	12	1S	11	1S1F
2B			M	X	X	X	X
2C	1A	1B	M	12	X	11	1S2
2D	1A	1B	F	12	1S	12	1S2
2E			M	12	11	22	1S2
3A	2A	2B	M	11	1S	12	1S1S
3B	2A	2B	F	11	1S	11	1F2
3D	2D	2E	M	12	X	22	22
3E	2D	2E	M	12	X	22	22
3F	2D	2E	M	X	1S	12	22

KINDRED 1075SP FRAXO27

				TF	PI	HP	GC
1A			M	X	X	X	X
1B			F	X	X	X	X
2C			M	X	X	X	X
2B	1A	1B	F	12	12	12	1S1F
3A	2B	2C	M	22	12	11	1F1F
3B	2B	2C	F	22	11	12	1F1F
2A	1A	1B	F	11	12	12	1S1F
3C	2B	2C	M	11	11	X	1S1S

KINDRED 11525FE FRAXO27

				TF	PI	HP	GC
1A			M	12	12	11	1S2
1B			F	11	3S	22	1S1S
2A	1A	1B	M	11	13	12	1S1S
2B	1A	1B	F	11	1S	12	1S2
2C	1A	1B	F	11	23	12	1S1S

KINDRED 840PA FRAXO27

				TF	PI	HP	GC
1A			M	X	X	X	X
1B			F	X	X	X	X
2C			M	X	11	11	X
2B	1A	1B	F	11	3S	22	1S1F
3A	2B	2C	M	11	1S	12	1F2
3B	2B	2C	M	11	13	12	1F1F
2A	1A	1B	F	11	11	22	1S1F

KINDRED 13010MC FRAXO27

				TF	PI	HP	GC
1A			M	X	X	X	X
1B			F	11	11	22	1S2
2A	1A	1B	F	12	11	12	1S2
2C	1A	1B	M	12	11	12	1S2
2B	1A	1B	F	11	11	12	1S2

KINDRED 14840T FRAXO27

				TF	PI	HP	GC	ABO
1A			M	X	X	X	X	X
1B			F	11	1S	22	1S1S	A
2A	1A	1B	M	11	1S	22	1S1S	A
2B	1A	1B	F	12	1S	12	1S1S	A
2C	1A	1B	F	11	11	12	1S1S	A

KINDRED 994PE FRAXO27

				TF	PI	PGP	ACP	AK	ESD	PGM	GLO	HP	GC	ABO
1A			M	X	X	X	X	X	X	X	X	X	X	X
1B			F	11	13	11	BC	11	12	11	12	11	1S1S	A
2A	1A	1B	F	11	13	11	AB	11	22	12	11	12	1S2	A
2B	1A	1B	F	11	3S	11	AB	11	12	11	12	12	1S1S	A
2C	1A	1B	F	12	1S	12	AB	11	22	12	22	12	1S1S	A
2D	1A	1B	M	11	3S	12	AC	12	22	12	12	12	1S1S	A

KINDRED 11049EV FRAXO27

				F13B	PI	F13A	Jk	MNS	ACP	ESD	PGM	GLO	ABO	Fy
1A			M	11	12	12	X	X	BB	11	22	22	X	X
1B			F	X	X	X	X	X	X	X	X	X	X	X
3A	2A	2B	M	11	12	11	AB	NNZZ	AB	11	12	12	A1	AB
3B	2A	2B	M	11	11	11	AA	MNSZ	AB	11	12	22	A1	AB
3C	2A	2B	M	11	11	11	AA	NNZZ	X	X	X	X	A1	BB
3D	2A	2B	F	13	12	12	AA	NNZZ	BB	12	12	22	A1	AB
2A	1A	1B	F	11	12	11	AA	NNZZ	BB	11	12	22	A1	AB
3E	2A	2B	F	13	12	12	AA	NNZZ	BB	11	22	22	A1	AB
2B			M	13	11	12	AB	MNSZ	AB	12	22	12	A1	BB

KINDRED 1398TR FRAXQ27

				GPT	TF	PI	ACP	HP	GC
1A			M	22	12	1I	AB	22	22
1B			F	12	11	1Z	BB	11	1S1F
2C	1A	1B	F	22	11	1I	BB	12	1S2
2D	1A	1B	F	12	12	IZ	BB	12	1F2
2E	1A	1B	F	X	11	1Z	X	12	1S2
2A	1A	1B	F	12	12	11	AB	12	1S2
3B	2A	2B	M	22	12	11	BB	12	1S2
3A	2A	2B	F	X	11	11	X	12	1S1S
2B			M	X	X	X	X	X	X

KINDRED 1047LO FRAXQ27

				TF	PI	F13A	PGM	GLO	HP
1A			M	11	13	12	11	12	12
2A	1A	1B	M	11	13	11	12	22	12
1B			F	12	11	11	12	22	11
2B	1A	1B	M	11	13	11	12	22	11

KINDRED 594KO FRAXQ27

				GPT	F13B	TF	PI	PGP	ACP	PGM	GLO	HP	GC
1A			M	X	X	X	X	X	X	X	X	X	X
2A	1A	1B	F	12	11	11	13	12	BB	11	12	12	1S2
1B			F	22	13	13	11	11	AB	11	12	12	1S2
2B	1A	1B	M	22	11	13	11	11	AB	11	12	12	1S2

KINDRED 12721WA FRAXO27

				PI	Jk	MNS	ACP	ESD	PGM	GALT	GC		Rh	Fy
2A	1A	1B	M	1S	AA	NNSZ	AB	12	22	NN	1S	2	R1LR	AB
1A			F	11	AA	NNSZ	AB	12	12	ND	1S	2	R1LR	AB
2B	1A	1B	F	1S	AA	NNZZ	BB	11	12	ND	1S1S		R1LR	BB
1B			M	1S	AB	NNSZ	BB	11	22	NN	1S1S		R1R1	BB

KINDRED 12714ST FRAXO27

				TF	Jk	MNS	ACP	AK	ADA	GC		ABO	Rh	Fy
2A	1A	1B	M	12	AA	MNZZ	AB	11	11	1S	2	A1	LRR2	AB
1A			F	12	AA	NNSZ	AA	12	11	1S	2	A1	R2R2	AB
1B			M	11	AB	MMZZ	AB	11	12	1F	2	O	R1LR	AA
2B	1A	1B	F	12	AB	MNSZ	AB	11	11	1S	2	A2	R1R2	AB

KINDRED 11922DA FRAXO27

				C3	TF	PI	K	Jk	MNS	P	PGP	GLO	HP	GC	ABO	Rh
1B			M	X	X	X	X	X	X	X	X	X	X	X	X	X
3A	2A	2B	M	11	12	11	KZ	BB	MNSZ	+	13	11	12	1S1S	B	R1R1
2A	1A	1B	F	12	12	13	KZ	AB	MNSZ	+	11	11	11	1S1S	A1	R1R1
2B			M	11	11	1S	ZZ	AB	MNSZ	+	13	12	X	1S2	A1B	R1LR
3B	2A	2B	F	11	11	3S	KZ	AA	MMSS	+	13	12	12	1S1S	A1B	R1LR
1A			F	X	11	23	ZZ	AA	MNZZ	−	11	11	11	1S1S	A1	R1R2

KINDRED 12765BU FRAXQ27

				C3	TF	PI	Jk	MNS	P	PGP	GLO	HP	GC	Rh	Fy
2F			M	X	X	X	X	X	X	X	X	X	X	X	X
2C			M	X	X	X	X	X	X	X	X	X	X	X	X
2E			M	X	X	X	X	X	X	X	X	X	X	X	X
3D	2C	2D	M	X	11	23	AB	MNZZ	+	11	22	12	1S2	R1R1	AA
2D	1A	1B	F	22	11	22	AB	MNZZ	+	11	22	11	1S2	R1LR	AA
3E	2D	2E	F	22	11	12	AB	MMZZ	+	11	12	11	1S2	LRLR	AA
2B	1A	1B	F	22	12	22	AB	MNZZ	+	11	22	11	1S2	R1R1	AA
3C	2B	2F	F	12	12	12	AB	NNZZ	−	12	22	12	1S2	R1LR	AB
3B	2B	2F	M	12	22	12	AB	MNZZ	+	11	12	12	1S1S	R1LR	AB
3A	2B	2F	M	22	11	12	BB	MNZZ	+	12	12	12	22	R1LR	AB
2A	1A	1B	M	22	11	12	AB	MMZZ	+	11	22	22	1S2	R1R1	AA
1B			F	12	X	22	BB	MMSZ	+	11	22	12	1S1S	R1LR	AA
1A			M	22	X	12	AA	MNZZ	+	11	22	12	22	R1R2	AA

KINDRED 14320LE FRAXQ27

				GPT	F13A	Jk	Lu	MNS	P	ACP	AK	ESD	PGM	GLO	GC	ABO	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2C			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3A	2B	2C	F	12	12	AB	BB	MMSZ	−	AB	11	12	11	22	1S2	A1	R1R2	AB
2B	1A	1B	F	12	11	AA	BB	MMSZ	+	AA	12	11	12	12	1S2	A1	R1LR	AB
3B	2B	2C	M	12	11	AB	AB	MMZZ	−	AA	11	11	12	12	1S1F	A1	R1R2	AB
2A	1A	1B	F	12	11	AA	BB	MMSZ	+	BB	12	11	22	22	1F2	O	R1R1	BB

KINDRED 2127GI FRAXO27

Ind	Fa	Mo	Sex	GPT	TF	PI	Jk	MNS	ACP	AK	ESD	PGM	GLO	HP	ABO	Rh	Fy
			M	22	12	11	BB	NNZZ	AA	12	12	22	22	11	O	R1LR	AA
2A	1A	1B	M	12	11	13	AB	MNZZ	AB	11	11	12	12	12	A2	LRLR	AB
1A			M	12	11	13	AB	MNZZ	AB	11	11	12	12	12	A1	R1R2	AB
1B			F	22	12	13	AB	NNZZ	AB	12	12	12	12	12	O	R1LR	AA
2B	1A	1B	F	X	11	33	AB	MNZZ	AB	12	11	22	12	12	A2	R1LR	AB
2C	1A	1B	F	12	11	13	AB	MNZZ	AA	11	11	11	12	12			

KINDRED 12757WI FRAXO27

Ind	Fa	Mo	Sex	GPT	C3	F13B	PI	F13A	K	Jk	MNS	P	ACP	AK	PGM	GALT	GLO	HP	GC	ABO	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3B	2C	2D	F	12	X	33	12	11	ZZ	AB	MMSS	+	AB	12	11	ND	11	12	1F 2	B	LRLR	AA
2C	1A	1B	F	22	12	13	12	11	ZZ	AB	MNSZ	−	AA	11	11	NN	12	22	1F 2	O	R1LR	AB
2D			M	12	12	13	11	12	KZ	AB	MMSS	+	BB	12	11	ND	12	11	1S 2	B	LRLR	AB
3A	2C	2D	M	22	12	11	11	12	ZZ	AA	MMSS	+	AB	12	11	ND	12	12	1S1F	B	LRLR	AB
3C	2C	2D	F	12	12	11	11	11	ZZ	AA	MNSZ	−	AB	12	11	NN	12	12	2 2	B	LRLR	AB
2A	1A	1B	M	22	12	11	12	11	ZZ	AB	MNSZ	−	AA	11	22	NN	11	12	1F 2	O	LRLR	AB
2B	1A	1B	M	22	22	11	2Z	11	ZZ	AB	MNSZ	+	AA	11	11	NN	12	22	1F1F	O	R1LR	BE

KINDRED 12901BI FRAXO27

Ind	Fa	Mo	Sex	GPT	C3	F13B	TF	PI	F13A	Jk	MNS	P	PGP	ACP	PGM	GLO	HP	GC	ABO	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2D			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	R1LR	AA
3A	2E	2F	F	11	X	11	1B	1S	11	BB	NNZZ	+	11	AB	11	12	11	1S2	O	R1LR	AB
2E	1A	1B	F	12	22	11	1B	2S	11	BB	NNZZ	+	11	AB	12	22	12	1S2	O	R1R2	AB
1B			F	22	12	11	1B	2S	X	AB	NNSZ	−	11	AB	22	12	22	1S1S	O	LRLR	AB
2F			M	12	22	11	11	11	12	BB	NNSZ	+	12	BB	12	12	11	1S1S	O	R1LR	AA
3B			M	11	22	11	11	12	12	AB	NNZZ	+	11	AB	22	11	12	1S2	A1	R1R1	BB
2C	1A	1B	F	12	12	11	11	SS	11	AA	NNSZ	+	11	AB	22	11	12	1S1S	A1	R1R1	AB
3C	2C	2D	M	X	22	11	12	1S	11	AA	MNSS	−	11	AB	22	12	12	1S1S	A1	R1LR	X
3D	2C	2D	F	X	22	13	11	SS	11	AA	MNZZ	+	11	AA	22	11	12	1S2	O	R1R2	BB
2B	1A	1B	M	X	X	11	1B	1S	11	AB	NNZZ	+	11	AA	22	12	22	1S1F	O	R1R1	BE
2A	1A	1B	F	22	22	11	12	X	11	AB	NNZZ	+	11	AA	22	12	22	1S1F	O	R1R1	BE

FAMILIES INFORMATIVE FOR XG

KINDRED 12243BO

1A			M	NN	X
1B			F	NF	+
2A	1A	1B	M	FF	+
2B	1A	1B	M	FF	+

KINDRED 13009FI

1A			M	NN	X
1B			F	X	+
2A	1A	1B	F	NN	+
2B	1A	1B	M	FF	−

KINDRED 13010MC

1A			M	NN	X
1B			F	NF	+
2A	1A	1B	F	NF	−
2B	1A	1B	M	FF	+

KINDRED 1075SP

1A			M	NN	X
1B			F	X	X
2A	1A	1B	F	NN	+
2B	1A	1B	F	NF	+
3A	2C	2B	M	FF	+
3B	2C	2B	F	NF	+
2C			M	NN	X

KINDRED 1484OT

1A			M	NN	X
1B			F	X	+
2A	1A	1B	M	FF	+
2B	1A	1B	F	NN	+
2C	1A	1B	F	NN	+

KINDRED 1398TR

1A			M	NN	+
1B			F	NF	+
2A	1A	1B	F	NF	+
2B			M	NN	X
2C	1A	1B	F	NF	+
2D	1A	1B	F	NF	+
2E	1A	1B	F	NF	X
3A	2A	2B	F	NN	X
3B	2A	2B	M	FF	−

KINDRED 1047LO

1A			M	NN	X
1B			F	X	X
2A	1A	1B	F	X	+
2B	1A	1B	F	X	+
2C	1A	1B	F	X	+
2D			M	NN	+
3A	2C	2D	M	NN	+
3B	2C	2D	M	FF	+

KINDRED 11525FE

1A			M	NN	+
1B			F	NF	+
2A	1A	1B	M	FF	+
2B	1A	1B	F	NN	+
2C	1A	1B	F	NF	+

KINDRED 11049EV

1A			M	NN	+
1B			F	X	X
2A	1A	1B	F	NF	+
2B			M	NN	X
3A	2A	2B	M	FF	+
3B	2A	2B	M	FF	+
3C	2A	2B	M	FF	+
3D	2A	2B	F	NF	X
3E	2A	2B	F	NN	X

KINDRED 10923EL

1A			M	NN	X
1B			F	X	X
2A	1A	1B	F	X	+
2B			M	NN	X
2C	1A	1B	F	X	+
3A	2A	2B	F	NF	+
3B	2A	2B	M	FF	+
3C	2A	2B	F	NF	+

KINDRED 6053MA

1A			M	NN	+
1B			F	X	X
2A	1A	1B	F	NF	+
2B			M	NN	X
2C	1A	1B	F	NF	X
2D			M	NN	X
3A	2A	2B	M	FF	−
3B	2C	2D	M	FF	+
3C	2C	2D	M	FF	X
3D	2C	2D	M	FF	X

KINDRED 840PA

1A			M	NN	X
1B			F	X	X
2A	1A	1B	F	X	+
2B	1A	1B	F	X	+
2C			M	NN	+
3A	2B	2C	M	NN	+
3B	2B	2C	M	FF	+

KINDRED 12714ST

2A	1A	1B	M	FF	−
1A			F	NF	+
1B			M	NN	−
2B	1A	1B	F	NN	+

KINDRED 11922DA

3A	2A	2B	M	FF	+
2A			F	NF	+
2B			M	NN	−
3B	2A	2B	F	NF	−

KINDRED 12765BU

2F			M	NN	X
2C			M	NN	X
2E			M	NN	X
3D	2C	2D	M	FF	+
2D	1A	1B	F	NF	+
3E	2D	2E	F	NN	+
2B	1A	1B	F	X	+
3C	2B	2F	F	NN	+
3B	2B	2F	M	NN	+
3A	2B	2F	M	NN	−
2A	1A	1B	M	FF	+
1B			F	NF	+
1A			M	NN	+

KINDRED 12901BI

2F			M	NN	+
2E			F	NF	+
3A	2E	2F	F	NF	+
3B	2E	2F	M	FF	−

KINDRED 12273LI

2B			M	NN	X
2A			F	NF	+
3A			M	NN	X
3B	2A	2B	F	NF	+
3C	2A	2B	F	NF	+
3D			M	NN	+
4A	3A	3B	M	NN	+
4B	3C	3D	F	NF	+
4C	3C	3D	M	FF	+

KINDRED 2127GI

1A			M	NN	+
1B			F	NF	+
2A	1A	1B	M	FF	+
2B	1A	1B	F	NF	+
2C	1A	1B	F	NN	+

KINDRED 14320LE

2C			M	NN	X
2B			F	NF	−
3A	2C	2B	F	NF	−
3B	2C	2B	M	FF	−

(c) BRDU DEPENDENT FRAGILE SITE

KINDRED 6026LE FRA10Q25

Ped	Sex	(?)	GPT	F13B	TF	PI	F13A	PGD	ACP	ESD	PGM	GALT	GLO	HP	GC
1B	M	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2C	M	NN	12	11	12	SZ	X	AC	AA	11	11	NN	12	22	1F2
2B 1A 1B	F	NF	12	13	11	11	12	AA	AB	12	22	DD	12	22	1S2
2E 1A 1B	F	NN	12	11	13	11	12	AA	AB	12	11	ND	12	12	1S2
1A	F	NF	12	13	11	11	12	AA	BB	12	12	ND	12	12	1S2
2D 1A 1B	F	NF	12	13	11	11	X	AA	AB	11	12	NN	11	22	1S1S
3B 2B 2C	M	NF	22	13	12	1S	12	AA	AA	12	12	ND	12	22	2 2
2A 1A 1B	F	NF	11	13	11	11	12	AA	AB	11	11	NN	22	12	1S2
3A 2B 2C	M	NF	11	13	11	1Z	11	X	AA	11	12	ND	12	22	1S1F

KINDRED 6027HA FRA10Q25

Ped	Sex	(?)	GPT	F13B	PI	F13A	MNS	PGP	ACP	ADA	PGM	GLO	HP	GC	ABO
3A 2A 2B	M	NF	11	11	11	12	X	X	BB	11	12	22	11	1S2	X
2B 1A 1B	M	NF	11	11	11	12	MNSZ	11	AB	11	12	12	12	1S1F	A
2A	F	NN	11	11	1G	11	NNZZ	22	AB	11	11	12	11	1S2	O
3B 2A 2B	M	NF	11	11	11	11	MNZZ	12	AB	11	11	11	11	1S2	A
1A	M	NF	11	13	13	11	MNSS	11	AA	11	12	12	12	1F2	A
3D 2D 2E	F	NF	12	13	11	11	MNSZ	11	AB	12	12	12	X	1S1F	O
2C 1A 1B	M	NN	12	33	11	12	MMSZ	11	AB	11	12	11	12	1S2	O
2D 1A 1B	F	NF	12	11	13	11	MNS/	11	AB	11	12	22	12	1F2	O
3C 2D 2E	M	NF	12	11	11	11	MNSZ	12	AB	11	12	12	12	1S2	A
2E	M	NN	22	13	13	11	MNZZ	12	BB	12	11	11	22	1S1S	A
2F 1A 1B	F	NN	12	13	13	12	MNSS	11	AB	11	12	12	11	1S1F	O
1B	F	X	X	X	X	X	X	X	X	X	X	X	X	X	X

KINDRED 10864WH FRA10Q25

					GPT	TF	PGP	ACP	ESD	PGM	HP	GC
1A			M	X	X	X	X	X	X	X	X	X
2D			M	X	X	X	X	X	X	X	X	X
2E	1A	1B	F	NF	11	12	11	AB	11	11	11	1F 2
3C	2E	2F	M	NF	X	22	11	BB	11	11	X	1F 2
1B			F	NN	12	12	12	AB	12	12	12	1S 2
2B	1A	1B	F	NF	11	11	11	AA	12	11	12	1S1S
2A	1A	1B	M	NF	12	12	12	AB	12	11	11	1S 2
2C			M	NN	22	11	11	BB	11	11	12	1S1F
3A	2B	2C	M	NF	12	11	11	AB	11	11	22	1S1F
2F			M	NN	11	12	11	AB	11	11	12	2 2
3B	2D	2E	F	NN	12	12	12	AB	11	11	12	1F 2

KINDRED 10582CL FRA10Q25

					GPT	PI	PGP	ACP	ADA	PGM	GLO	HP	GC
1A			M	X	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X	X
3B			M	NN	12	3Z	11	BB	11	12	12	11	2 2
4B	3B	3C	M	NF	12	X	X	X	X	11	12	12	1F 2
3C	1A	1B	F	NF	12	11	12	AB	11	11	11	22	1F1F
3F			F	NN	22	11	X	AB	11	11	12	11	1S1S
4F	3E	3F	M	NF	12	11	X	AB	X	11	12	11	1S1F
4E	3E	3F	M	NF	12	11	X	BB	X	11	12	11	1S1F
4G	3E	3F	M	NN	12	11	X	BB	X	11	11	12	1S1F
3D	1A	1B	F	NN	12	11	12	AA	12	12	11	12	1F1F
3E	1A	1B	M	NF	12	11	12	BB	22	11	11	12	1F1F
3A	1A	1B	F	NN	12	11	11	AA	22	11	11	22	1S 2
4A	3B	3C	M	NF	22	13	11	AB	X	X	X	X	1F 2
4C	3B	3C	F	NF	12	1Z	11	AB	11	11	11	12	1F 2
4H	3E	3F	F	NN	12	11	12	AB	X	11	11	11	1S1F
3G	1A	1B	F	NN	X	11	X	X	X	11	11	22	1S 2
4D	3B	3C	F	NF	11	X	12	AB	X	12	X	X	1F 2

KINDRED 6032FA FRA10O25

ID			Sex	GPT	F13B	PI	F13A	CHE2	Jk	Lu	MNS	ACP	ESD	GLO	HP	GC	ABO	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2C			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4B	3D	3E	F	NF	12	X	13	12	X	X	X	BB	12	12	11	1S1F	X	X	X
3E			M	NN	12	11	11	11	−	AA	MMZZ	BB	11	12	11	1F2	O	R2R2	AB
3D	2B	2C	F	NF	12	11	33	12	+	AB	MNZZ	BB	12	11	11	1S1S	B	R1R2	AA
4A	3D	3E	M	NN	12	11	13	11	−	AB	MMZZ	BB	11	11	11	1S1F	O	R1R2	AB
4C	3D	3E	M	NN	22	11	13	11	−	AB	MMZZ	BB	11	11	11	1S 2	O	R2R2	AB
3C	2B	2C	F	NN	12	11	1Z	12	−	X	X	AB	12	12	22	1S1S	X	X	X
2B	1A	1B	F	NF	11	13	13	12	+	X	X	AB	12	12	12	1S1S	X	X	X
2A	1A	1B	F	NF	11	13	33	12	−	X	X	AA	11	12	22	1S1S	X	X	X
3B	2A	2D	F	NN	12	11	13	11	−	X	X	AA	11	12	12	1S1S	X	X	X
3A	2A	2D	F	NN	12	13	13	11	−	X	X	AA	11	12	12	1S1S	X	X	X
2D			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

KINDRED 6039DE FRA10O25

ID			Sex	GPT	F13B	PI	F13A	Jk	Lu	MNS	ACP	ESD	GALT	GLO	HP	GC	ABO	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4A	3A	3B	F	NF	11	11	11	X	X	X	AB	11	NN	12	X	1S2	O	X	X
3A			M	NN	11	11	11	X	X	X	BB	11	NN	22	22	22	O	X	X
3B	2A	2B	F	NF	11	13	11	AB	BB	NNZZ	AB	11	NN	11	22	1S1F	O	R1R2	AB
2B	1A	1B	M	NF	12	13	11	BB	AB	NNZZ	AB	11	NN	12	22	1F2	A2	R1R1	BB
2A			F	NN	11	11	11	AB	BB	NNZZ	AA	11	NN	12	22	1S1F	A1	R1R2	AA
2C	1A	1B	M	NF	12	13	11	AB	AB	NNSZ	AB	11	NN	12	22	1F2	A2	R1R1	BB
2D			F	NN	12	11	1S	AB	BB	MMZZ	AB	12	NN	22	12	1S1S	A1	R1LR	AB
2E	1A	1B	M	NN	12	11	12	BB	AB	MNSZ	AB	11	NN	22	12	1S2	O	R1LR	AB
3E	2C	2D	M	NF	12	11	1S	AB	AB	MNZZ	AB	11	NN	22	12	1S1F	A2	R1LR	BB
3C			F	NN	22	11	12	X	X	X	AB	12	NN	22	12	X	X	X	X
4B	3C	3D	F	NF	12	11	1S	X	X	X	AA	11	NN	12	22	22	X	X	X
3D	2C	2D	M	NF	11	11	1S	AB	AB	MNZZ	AA	11	NN	12	22	1S2	A2	R1LR	BB

KINDRED 5003SE FRA10O25

					C3	TF	F13A	HP	GC	
2A	1A	1B	M	NF	X	22	X	X	2	2
1B			F	NF	22	12	12	121S	2	
1A			M	NN	12	12	11	121S	2	
2C	1A	1B	F	NF	12	22	12	11	2	2
2B	1A	1B	M	NN	22	12	12	22	2	2

KINDRED 6036RO FRA10O25

					GPT	C3	TF	PI	ACP	ADA	PGM	GLO	HP	GC
1A			M	X	X	X	X	X	X	X	X	X	X	X
2E			M	X	X	X	X	X	X	X	X	X	X	X
3A	2D	2E	M	NF	22	12	12	23	AB	11	11	12	22	1S1F
2D	1A	1B	F	NF	22	22	11	12	AC	12	11	11	12	1S1F
1B			F	NN	12	22	X	12	BC	11	12	12	22	X
2A	1A	1B	M	NN	12	22	12	23	BC	11	11	12	12	1S 2
2B	1A	1B	M	NN	12	12	11	13	BC	12	12	12	12	1S 2
2C	1A	1B	M	NN	12	12	11	12	BC	12	12	12	12	1S1S
2F	1A	1B	F	NN	12	22	11	13	BC	11	22	12	12	1S1S

KINDRED 6041AX FRA10O25

					GPT	C3	F13B	TF	HP	GC
1A			M	X	X	X	X	X	X	X
3A	2A	2B	F	NF	22	22	11	11	22	1S 2
2A			M	NN	12	22	13	12	12	1S1F
2B	1A	1B	F	NF	12	12	11	11	22	1S 2
3B	2A	2B	F	NF	12	22	11	11	X	1S1S
1B			F	NN	11	X	13	11	22	1S1S

KINDRED 5096LA FRA10Q25

					GPT	TF	F13A	ACP	ESD	GLO	HP	GC	ABO
3A	2B	2C	M	NF	12	12	22	BB	11	22	12	1S1S	B
2C			M	NN	12	11	12	AB	11	12	12	1S 2	A
2B	1A	1B	F	NF	11	12	12	BC	11	22	11	1S1S	B
3B	2B	2C	M	NF	12	11	22	X	X	X	11	1S 2	X
1A			M	NN	11	11	12	BC	11	22	11	1S1S	B
1B			F	NF	12	12	12	BB	12	22	12	1S1S	B
2A	1A	1B	M	NN	11	11	22	BB	12	22	11	1S1S	B

KINDRED 10901DU FRA10Q25

					GPT	C3	TF	PI	Jk	MNS	ACP	ADA	ESD	PGM	GALT	GLO	HP	GC	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3A	2B	2C	M	NF	11	22	11	11	BB	MNZZ	BB	12	12	11	NN	12	22	1S1F	R1LR	AA
2B	1A	1B	F	NF	12	22	12	11	AB	MMSZ	BB	11	11	11	NN	11	12	1S1S	LRLR	AA
3B	2B	2C	F	NF	11	12	11	11	AB	MNZZ	AB	11	12	11	NN	12	22	1S1F	LRLR	AB
3D	2B	2C	F	NN	22	12	12	11	AB	MNSZ	BB	12	11	11	NN	12	12	1S1F	R1LR	AB
3E	2B	2C	F	NF	12	12	12	11	BB	MNSZ	BB	12	12	11	NN	12	22	1S1F	R1LR	AA
2C			M	NN	12	12	11	11	AB	NNZZ	AB	12	12	11	NN	22	22	1S1F	R1LR	AB
1B			F	NF	22	22	11	11	X	X	BB	12	11	11	NN	12	12	1S1S	X	X
2A	1A	1B	M	NN	22	22	13	11	X	X	BB	11	11	11	NN	12	11	1S1S	X	X
4B	3B	3C	M	X	12	22	11	11	X	X	BB	11	11	12	NN	12	X	1F2	X	X
4C	3B	3C	M	NF	12	12	11	11	X	X	BB	11	11	12	NN	12	X	1S2	X	X
4A	3B	3C	M	NN	12	12	11	11	X	X	AB	11	12	12	NN	12	X	1S2	X	X
3C			M	NN	22	22	11	12	AA	NNZZ	AB	11	12	12	NN	11	12	2 2	LR Z	AB

KINDRED 6044AR FRA10Q25

					GPT	TF	PI	F13A	MNS	PGP	ACP	ESD	PGM	GLO	GC	ABO
1A			M	X	12	12	13	11	MMZZ	12	BB	12	12	22	1F2	A1
1B			F	X	12	11	11	12	MNZZ	11	AB	11	22	12	1S1S	B
2A	1A	1B	M	NN	11	11	11	11	MMZZ	12	BB	11	22	12	1S1F	O
2B	1A	1B	F	NF	22	12	11	11	MMZZ	11	AB	11	22	12	1S1F	A1B

KINDRED 5274NE FRA10Q25

				GPT	C3	TF	PI	FA	Jk	MNS	P	PGD	PGP	ACP	ESD	PGM	GA	GLO	HP	GC	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4A	3A	3B	M	NF	12	22	12	11	11	X	X	X	AA	11	BB	11	11	ND	22	X	1S1F	X	X
3A			M	NN	12	12	12	11	X	AB	MNSZ	+	AA	11	AB	12	11	NN	12	12	1S1F	LRLR	AB
3B	2A	2B	F	NF	12	22	12	11	12	BB	MNZZ	+	AF	11	BB	11	11	ND	12	11	1S1F	LRR2	AB
2A			M	NN	22	22	11	11	12	BB	MNSZ	+	AA	11	AB	11	12	NN	12	12	1S1F	R1LR	AA
2B	1A	1B	F	NF	11	22	12	13	X	AB	MMZZ	+	AF	11	BB	11	11	ND	22	11	1S 2	R1R2	AB
3C	2A	2B	F	NF	12	22	12	13	11	BB	MMSZ	−	AA	11	BB	11	12	ND	12	11	1S 2	R1R2	AA
1B			F	NF	11	22	13	11	11	X	X	X	AA	11	AB	11	11	ND	12	12	1S 2	X	X
3E			M	NN	12	X	11	11	11	X	X	X	AA	11	BB	11	22	NN	22	22	1S 2	X	X
3D	2A	2B	F	NF	12	22	11	11	22	X	X	X	AA	11	AB	11	12	ND	12	12	1S1F	X	X
4F	3D	3E	M	NN	12	22	11	11	12	X	X	X	AA	11	AB	11	22	NN	12	22	1S1F	X	X
4D	3D	3E	F	NF	11	22	11	11	12	X	X	X	AA	12	AB	11	12	NN	12	22	1S1F	X	X
4E	3D	3E	F	NN	22	22	11	11	12	X	X	X	AA	12	AB	11	22	ND	22	12	1S1S	X	X
4C	3D	3E	M	NN	12	22	11	11	12	X	X	X	AA	12	AB	11	22	ND	12	12	1S1F	X	X
3F	2A	2B	F	NF	12	22	12	11	11	X	X	X	AF	11	AB	11	11	NN	12	12	1S1F	X	X
4B	3A	3B	F	NN	22	12	11	11	11	AB	MMSZ	X	X	11	BB	11	11	X	12	X	1S1F	LRR2	AB

KINDRED 5294TM FRA10Q25

				GPT	C3	TF	PI	F13A	K	Jk	MNS	PGP	ACP	ADA	PGM	GA	GLO	HP	GC	ABO	Rh	Fy
3C	2B	2C	M	NF	12	22	12	12	12	X	X	X	12	AA	11	11	NN	12	X	1S1S	X	X	X
2C	1A	1B	F	NN	12	22	11	12	11	X	X	X	12	AB	11	11	NN	22	11	1S 2	O	X	X
1A			M	NN	11	22	12	11	11	X	X	X	12	AA	12	11	NN	22	12	2 2	O	X	X
1B			F	NN	12	22	11	23	12	X	X	X	12	BB	11	11	NN	12	11	1S1S	O	X	X
2B			M	NF	12	22	22	1S	X	ZZ	AB	MMSS	11	AA	11	11	ND	11	12	1S 2	A1	R1LR	AB
3B	2A	2B	F	NN	12	22	12	1S	12	ZZ	AA	MMSZ	11	AB	11	12	ND	11	12	1S 2	A1B	LRR2	BB
3A	2A	2B	F	NF	22	22	22	1S	12	ZZ	AB	MMSZ	11	AB	11	12	ND	11	12	1S 2	A1B	R1R1	BB
2A			F	NN	12	12	12	1S	11	KZ	AB	MMSZ	11	BB	11	12	ND	12	12	1S 2	B	R1R2	AB
3D	2B	2C	M	NN	12	22	12	12	X	X	X	X	12	AA	X	11	NN	12	X	1S 2	O	X	X

KINDRED 5179F0 FRA10Q25

					GPT	F13B	PI	F13A	PGD	ACP	PGM	GLO	HP	GC	ABO
1A			M	NN	22	13	1S	12	AC	BB	11	22	12	1S 2	X
2D	1A	1B	F	NF	12	11	11	12	AC	AB	12	22	12	1S1S	A
2E	1A	1B	M	NN	12	11	11	12	AC	AB	12	12	11	1S1F	A
1B			F	NF	11	11	11	12	AA	AA	22	12	12	1S1F	A
2A	1A	1B	M	NF	12	11	1S	22	AC	AB	12	12	22	1S 2	AB
2C			M	NN	12	11	11	11	AA	BB	12	12	X	1S 2	X
2B	1A	1B	F	NF	12	11	1S	11	AA	AB	12	22	12	1F 2	A
3A	2B	2C	F	NF	X	11	1S	11	AA	X	X	X	X	2 2	X

KINDRED 10003CO FRA10Q25

					GPT	C3	F13B	TF	PI	F13A	ACP	PGM	GLO	HP	GC	ABO
1A			M		X	X	X	X	X	X	X	X	X	X	X	X
1B			F		X	X	X	X	X	X	X	X	X	X	X	X
3J	2I	2J	F	NF	11	12	11	11	13	X	BB	11	11	11	1S1S	A
2I	1A	1B	F	NF	11	12	11	11	13	12	BC	11	12	11	1S1S	B
3L	2I	2J	M	NN	11	22	13	12	13	22	BB	11	12	11	1S1S	A
2J			M	NN	12	22	13	12	1S	X	BB	11	12	11	1S1S	A
3K	2I	2J	F	NF	12	12	11	11	3S	X	BB	11	22	11	1S1S	O
3M	2I	2J	M	NN	11	12	11	12	13	X	BB	11	11	11	1S1S	O
2G	1A	1B	F	NN	X	22	13	11	11	11	BB	11	12	12	1F 2	X
2C	1A	1B	F	NF	12	22	11	11	11	11	BB	11	12	12	1S1S	X
3E	2C	2D	M	NF	11	22	11	11	11	11	BB	11	12	11	1S1S	X
2H	1A	1B	F	NN	11	22	11	11	33	11	AB	11	12	11	1F 2	X
3F	2C	2D	F	NN	11	22	11	11	11	11	BB	11	11	22	1S 2	X
2E	1A	1B	M	NF	11	X	11	11	13	12	AB	11	12	12	1S 2	O
2F			F	NN	12	22	11	11	12	22	BB	12	12	12	1S1S	A
3G	2E	2F	M	NF	12	X	11	X	13	X	BB	11	11	X	1S1S	X
3H	2E	2F	M	NN	12	22	11	11	11	X	BB	11	12	12	1S1S	X
3I	2E	2F	F	NN	X	X	X	11	12	X	BB	12	11	X	1S1S	X
2B	1A	1B	F	NF	12	12	11	11	13	X	AC	11	12	11	1S 2	B
3D	2C	2D	M	NN	11	22	11	11	11	11	BB	11	12	12	1S1S	O
2D			M	NN	11	22	11	11	11	11	BB	11	12	12	1S 2	O

KINDRED 5656EH FRA10Q25

Ind.	Parents	Sex	—	GPT	F13B	TF	F13A	Jk	MNS	PGP	ACP	ESD	PGM	GALT	HP	GC	ABO	Rh	Fy
1A		M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B		F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2J	1A 1B	F	NF	12	11	11	11	AA	MNSZ	11	BB	11	12	NN	12	1S1S	O	R1LR	AB
2E	1A 1B	M	NN	11	13	11	11	AB	MMSS	12	BB	11	12	NN	12	1S1F	O	R1LR	AB
2H	1A 1B	M	NF	11	13	11	11	AB	MNSZ	11	BB	11	11	NN	12	1S1S	A1	R1R1	AB
2D	1A 1B	M	NN	11	11	11	12	AB	MMSS	11	BB	11	12	NN	12	1S1S	A1	R1LR	BB
2B	1A 1B	F	NF	11	11	11	11	AB	MNSZ	11	BB	11	11	NN	12	1S1S	A1	R1R1	AB
2C	1A 1B	M	NN	11	11	12	11	AB	MNSZ	11	BB	11	12	NN	12	1S1F	A1	R1R1	AB
3A	2G 2H	M	NF	X	X	11	11	X	X	X	AB	X	X	X	12	1S1S	X	X	X
3B	2G 2H	M	NN	X	11	11	11	X	X	X	X	X	X	X	12	X	X	X	X
3C	2G 2H	M	NF	X	11	11	11	X	X	12	AB	11	11	NN	12	1S 2	X	X	X
2G		F	NN	22	11	13	11	BB	MMSS	12	AB	12	11	ND	11	1S 2	A1	R1LR	AA
2F	1A 1B	M	NN	11	11	12		X	X	11	BB	11	11	NN	12	1S1S	X	X	X
2I	1A 1B	F	NN	11	11	12		X	X	X	BB	11	12	NN	12	1S1S	X	X	X
2A	1A 1B	F	NN	12	11	11	11	X	X	X	BB	11	11	NN	X	1S1S	X	X	X

KINDRED 5716CO FRA10Q25

Ind.	Parents	Sex	—	GPT	ACP	ESD	GLO	HP	GC
3A		M	X	X	X	X	X	X	X
3C		M	X	X	X	X	X	X	X
1B		F	X	X	X	X	X	X	X
4B	3C 3D	F	NF	11	AB	X	22	X	1S1S
3D	2A 2B	F	NF	12	AA	12	12	12	1S 2
1A		M	NN	12	AA	12	12	11	1S 2
3B	2A 2B	F	NN	22	AB	12	12	12	1S 2
2B		F	NF	22	AB	11	22	12	2 2
4A	3A 3B	M	NN	12	BB	12	12	X	X
3E	2A 2B	M	NN	12	AB	12	12	11	1S 2
2A	1A 1B	M	NN	12	AA	12	11	11	1S1S

KINDRED 10549KE FRA10O25

				GPT	C3	F13B	TF	PI	K	Jk		MNS	PGP	ACP	ESD	PGM	GLO	HP	GC	ABO	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2D			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4A	3C	3D	F	NF	22	X	13	X	22	X	X	X	X	BB	11	11	22	X	F 2	X	X	X
3D			M	NN	12	12	33	11	12	ZZ	BB	MNZZ	X	AB	11	11	12	12	1F1F	B	R1R2	BB
3C	2A	2B	F	NF	22	12	11	12	12	ZZ	AB	MNZZ	11	BB	11	11	12	12	1S 2	A1	R1R2	AB
2A			M	NN	12	12	13	12	12	ZZ	AB	MNZZ	12	AB	11	12	11	22	1S 2	A1	R1R1	AA
2B	1A	1B	F	NF	22	X	11	11	11	ZZ	AB	MNZZ	11	AB	12	11	12	12	1S1S	A1	LRR2	BB
2C	1A	1B	F	NF	22	22	11	13	12	KZ	AB	MMZZ	11	AB	12	11	22	22	1S 2	A1	LRR2	AB
3F	2C	2D	M	NF	22	22	11	13	11	ZZ	AA	MNZZ	11	AA	11	11	22	12	1S 2	A1	LRR2	BB
3E	2C	2D	M	NN	12	22	11	11	11	ZZ	BB	MNZZ	11	AB	11	11	22	12	1S 2	A1	R2R2	AA
3B	2A	2B	M	NN	12	22	11	11	12	ZZ	AA	NNZZ	11	AA	11	11	12	22	1S 2	A1	R1LR	AB
3A	2A	2B	M	NN	22	X	11	11	12	ZZ	AB	MMZZ	12	AB	12	12	12	22	1S 2	A2	R1LR	AB

KINDRED 10562GO FRA10O25

				GPT	C3	F13B	TF	PI	K	Jk	Lu		MNS	ACP	ADA	ESD	PGM	GLO	HP	GC	ABO	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4B	3A	3B	M	NF	22	22	11	11	11	X	X	X	X	AB	11	11	11	22	X	1S1S	X	X	X
3A			M	NN	22	12	13	12	11	ZZ	AB	BB	MNZZ	AB	12	12	12	22	11	1S1S	A	R1R1	BB
3B	2A	2B	F	NF	12	22	11	11	11	ZZ	AA	BB	MNSZ	AB	11	11	11	12	12	1S1S	A	R1LR	AA
4A	3A	3B	F	NF	12	22	13	X	X	X	X	X	X	X	X	X	X	X	12	X	X	X	X
4C	3C	3D	M	X	11	22	11	13	11	ZZ	AA	AB	MMSZ	AB	11	11	11	12	22	1F 2	O	R1R1	BB
3D	2A	2B	F	NF	12	22	11	11	11	ZZ	AB	BB	MNSZ	AB	11	11	12	11	22	1S1F	O	R1LR	AB
2A			M	NN	22	22	11	11	11	ZZ	AB	BB	NNZZ	AB	11	11	12	12	12	1S 2	B	R1R1	AB
2B	1A	1B	F	NF	11	22	11	12	11	ZZ	AA	BB	MNSZ	AA	11	11	12	12	22	1S1F	A	LR Z	AA
3C			M	NN	11	22	13	13	11	X	X	X	X	AA	11	11	11	22	22	1S 2	X	X	X
4D	3C	3D	F	NF	11	22	11	13	11	X	X	X	X	AA	11	11	12	12	22	1S1F	X	X	X
3E	2A	2B	F	NN	12	22	11	12	11	X	X	X	X	AB	11	11	11	12	12	1F 2	A	X	X
2D	1A	1B	M	NF	11	22	11	12	11	ZZ	AA	BB	MMSS	AA	11	11	11	22		1S1S	O	R1 Z	AB
2C			F	NN	12	12	11	11	12	KZ	AB	BB	MNSZ	BB	12	11	11	12	22	1S1S	O	LRLR	BB
3F	2C	2D	F	NN	12	22	11	12	11	KZ	AB	BB	MMSZ	AB	11	11	11	12	22	1S1S	O	R1LR	BB
3G	2C	2D	F	NN	11	22	11	11	12	KZ	AB	BB	MMSZ	AB	11	11	11	12	22	1S1S	O	LR Z	AB

KINDRED 10094GL FRA10Q25

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                  GPT C3 FB TF PI FA  K  Lu Jk MNS  ACP ESD PGM GA GLO HP GC   ABO Rh   Fy

1A          M NF  22 22 11 11 23 11  KZ AB BB NNSZ AA  12  12  ND 12  11 1S 2  B   R1R2 AB
3B 2A 2B    F NF  X  X  X  X  X  X   X  X  X  X    AB  11  11  NN 22  X  X  X   X        X
2C 1A 1B    M NF  22 12 13 11 23 11  KZ AB BB MNZZ AB  11  11  ND 11  12 1S1F B   R2R2 AB
2E 1A 1B    F NN  12 12 11 11 23 11  KZ AB BB MNZZ AB  12  12  ND 12  11 1F 2  B   R1R2 AB
2B 1A 1B    M NF  12 22 13 11 23 11  X  X  X  X    AB  11  12  NN 12  11 1F 2  B   X    X
2A          F NN  11 22 11 12 11 11  X  X  X  X    AB  11  11  NN 22  12 1S1F O   X    X
1B          F NN  12 12 13 11 33 11  KZ AB BB MNSZ AB  11  12  NN 11  12 1S1F B   LRR2 AA
3A 2A 2B    F X   X  22 X  12 X  X   X  X  X  X    X   X   X   X  X   12 1F 2  X   X    X
2D          F NN  11 12 11 12 1S X   ZZ AB BB MNSZ BB  11  11  NN 22  12 2 2   O   X    BB
3C 2C 2D    M NN  12 12 11 12 X  X   ZZ AB BB MMSZ BB  11  11  ND 12  11 1F 2  O   X    X
3D 2C 2D    F NN  12 12 13 11 X  12  KZ BB BB NNZZ AB  11  11  ND 12  12 1F 2  B   X    X
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KINDRED 10297GO FRA10Q25

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                  GPT F13B TF PI F13A Jk MNS  ACP ADA ESD PGM GLO HP GC    ABO  Rh    Fy

1A          M X   X    X  X  X    X  X    X   X   X   X   X   X  X     X    X     X
1B          F X   X    X  X  X    X  X    X   X   X   X   X   X  X     X    X     X
2F          F X   X    X  X  X    X  X    X   X   X   X   X   X  X     X    X     X
4A 3D 3E    M NF  X    11 12 12   11 X    X   BB  11  11  11  22 X  1S 2   X    X     X
4B 3D 3E    F NF  X    11 11 11   11 X    X   BB  11  11  11  22 X  1S 2   X    X     X
3D 2A 2B    F NF  12   11 11 12   12 AB MNSZ  AB  11  11  12  22 22 2 2    A1   LRR2  AB
3E          M NN  22   11 12 1S   11 AA MNSZ  AB  11  12  11  22 12 1S1S   A1   R1LR  AB
3C 2A 2B    M NN  11   11 11 12   11 AB MMSZ  AB  11  11  22  11 11 2 2    A2B  LR Z  AB
3A 2A 2B    M NF  11   11 11 12   11 AB MMSZ  AB  11  11  11  12 12 2 2    A1B  LRR2  AB
3B 2A 2B    M NN  12   11 11 12   11 AB MMSZ  AB  11  11  12  12 12 1F 2   A1   LR Z  AB
2B 1A 1B    F NF  12   11 11 11   11 AB MNSZ  AA  11  11  12  12 12 1F 2   A1B  LR Z  AB
2A          M NN  12   11 11 23   X  AB MMSZ  BB  11  11  12  12 12 2 2    A1   LRR2  AA
2D 1A 1B    M NF  12   11 11 11   12 AA MNSZ  AA  12  11  12  12 12 1F 2   A1B  LRLR  BB
3H 2D 2E    M NN  11   13 11 11   11 AB NNZZ  AB  11  11  12  12 12 1S 2   B    R1LR  BB
3K 2D 2E    M NN  12   13 11 11   12 AB MNZZ  AB  12  11  11  12 22 1S1F   A1B  LRLR  BB
3G 2D 2E    M NN  12   11 11 11   11 AB MMSZ  AB  11  11  11  12 12 1S1F   A1B  LRLR  BB
2C 1A 1B    M NF  11   11 11 11   12 AA MNSZ  AA  11  11  12  12 12 1F 2   O    LRLR  AB
3I 2D 2E    M NF  11   11 11 11   12 AB NNZZ  AB  11  11  12  22 12 1S1F   B    LRLR  BB
3J          F NN  11   11 11 12   11 AB MNSZ  AB  11  11  12  22 12 1F 2   O    R1LR  AA
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Ind	Fa	Mo	Sex		GPT	C3	PI	F13A	Jk	Lu	MNS	PGP	ACP	AK	ESD	PGM	GLO	HP	GC	ABO	Rh	Fy
2E			F	NN	12	13	11	11	11	BB	MNZZ		BB	12	11	12	12	12	1S1S	B	R1LR	BB
3F	2C	2F	M	NN	12	11	11	X	X	AA	MNSZ		AB	X	11	12	12	11	1S 2	O	LRLR	AB
4D	3I	3J	F	NN	X	11	11	12	X	X	X		AA	X	11	11	X	12	1F1F	X	X	X
4E	3I	3J	F	NF	X	11	X	X	X	X	X		AA	X	X	X	X	12	X	X	X	X
4C	3D	3E	F	NN	X	11	12	1S	X	X	X		BB	X	11	11	22	X	1S 2	X	X	X

KINDRED 1053700 FRA10Q25

Ind	Fa	Mo	Sex		GPT	C3	PI	F13A	Jk	Lu	MNS	PGP	ACP	AK	ESD	PGM	GLO	HP	GC	ABO	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2E			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4C			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4B	3D	3E	F	NF	12	22	12	11	AB	BB	MNSZ	12	AB	11	11	12	12	11	1F 2	O	LRLR	AB
3D	2D	2E	F	NF	22	22	11	11	AB	BB	MMSS	12	BB	11	12	12	12	12	1F1F	O	LRLR	BB
3E			M	NN	12	12	12	12	AB	BB	MNZZ	11	AA	11	12	11	12	12	1S 2	A1	R1LR	AA
5A	4B	4C	M	NF	22	22	11	X	X	X	X	12	AB	X	X	X	X	12	2 2	X	X	X
4A	3D	3E	M	NF	22	22	12	12	AA	BB	MNSZ	11	AB	11	12	11	12	22	1S1F	A1	R1LR	AB
3B	2D	2E	M	NN	12	22	11	11	AB	BB	MMSZ	12	BB	11	12	11	12	12	1S1F	O	LRLR	AB
3C	2D	2E	M	NN	22	22	11	X	BB	BB	MNSZ	12	BB	11	12	11	12	12	1S1F	O	R1LR	AB
3A	2D	2E	M	NF	22	22	11	11	BB	BB	MNSZ	11	BB	11	12	12	12	12	1F1F	O	R1LR	AB
2D	1A	1B	M	NF	22	12	11	11	BB	BB	MMSZ	12	BB	11	22	11	12	12	1F 2	O	LRLR	BB
2C	1A	1B	M	NN	22	12	11	12	AB	AB	MMSS	11	BB	12	11	12	12	22	1F 2	O	R1LR	AB
2B	1A	1B	M	NN	22	22	11	12	AB	BB	MNSS	22	AB	11	12	12	12	22	1S 2	O	LRLR	BB
2A	1A	1B	F	NN	12	12	11	11	X	X	X	X	BC	11	12	12	22	12	1S 2	X	X	X
4D	3D	3E	M	NF	22	12	12	X	AB	BB	MNSZ	11	AB	11	11	12	11	12	X	O	LRLR	AB
5B	4B	4C	M	NN	12	12	23	X	AB	BB	MNZZ	X	AB	12	11	11	12	X	2 2	O	R1LR	AB

KINDRED 10681CL FRA10Q25

					GPT	F13B	PI	PGP	ACP	GLO	HP	GC	ABO
1A			M	X	X	X	X	X	X	X	X	X	X
3D			M	X	X	X	X	X	X	X	X	X	X
3C	2A	2B	F	NF	12	13	1S	11	X	12	12	1S 2	O
4B	3C	3D	M	NF	X	13	1S	X	X	X	X	1S1S	X
2B			M	NF	12	11	2S	12	AB	12	22	1S1S	A
2A			F	NN	22	13	11	11	AB	12	12	2 2	B
3E	2A	2B	M	NN	22	11	1S	12	AB	22	X	1S 2	O
3B	2A	2B	M	NF	X	11	12	X	X	X	22	1S 2	B
3A			F	NN	X	11	1Z	X	X	X	11	1F 2	A
1B			F	NN	12	13	12	12	BB	12	22	1S1F	O
4A	3A	3B	F	NN	12	11	11	X	BB	12	X	1S 2	AB

(d) DYSTAMYCIN A INDUCIBLE FRAGILE SITES

KINDRED 12058EG FRA16Q22

					GPT	C3	FB	TF	PI	FA	K	Jk	MNS	P	PGD	PGP	ACP	ESD	PGM	GLO	HP	GC	ABO	Rh	Fy
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3G	2C	2D	M	NF	12	X	33	11	3S	11	KZ	BB	MMZZ	+	AA	12	AB	11	12	12	X	1S1S	A1	LRR2	AA
2D			F	NN	12	X	13	11	1S	11	KZ	AB	MNZZ	+	AA	12	AB	12	12	12	22	1S1F	A1	R1LR	AA
2C	1A	1B	M	NF	12	12	13	11	33	12	ZZ	AB	MNZZ	+	AA	12	AA	11	11	11	22	1S1F	O	R1R2	AB
2A	1A	1B	M	NN	12	12	13	11	13	12	ZZ	AB	MNZZ	+	AC	12	AB	11	11	11	12	1S1F	O	R1R2	BB
2B	1A	1B	F	NN	12	11	13	11	13	12	ZZ	AB	MNZZ	+	AC	12	AB	11	11	12	12	1S 2	O	R1R2	AB
3B	2C	2D	F	NF	12	12	11	11	13	11	ZZ	AB	MMZZ	+	AA	X	AA	11	12	12	22	1F1F	O	R1LR	AA
3F	2C	2D	F	NN	11	12	11	11	13	12	KZ	AA	NNZZ	+	AA	12	AB	12	11	11	22	1S1S	A1	R1R1	AB
3A	2C	2D	F	NN	22	12	11	11	13	12	ZZ	AB	NNZZ	+	AA	11	AA	11	12	11	X	1S1S	A1	LRR2	AA
3D	2C	2D	F	NF	12	12	X	11	3S	11	KZ	AB	MNZZ	+	AA	11	AA	11	11	12	22	1S1F	O	R1LR	AA
3E	2C	2D	M	NF	11	X	X	11	13	12	KZ	AA	MNZZ	+	AA	22	AB	X	11	11	X	1S1S	A1	R1R1	AB
4B	3B	3C	M	NF	12	22	11	11	13	11	ZZ	AB	MMZZ	+	AA	22	AB	11	12	12	X	1S1F	O	LRLR	AB
3C			M	NN	12	22	11	11	1I	11	ZZ	AB	MMZZ	–	AA	12	BB	11	12	11	22	1S1S	O	R1LR	BB
4A	3B	3C	F	NF	11	22	11	11	11	11	ZZ	AB	MMZZ	–	AA	22	AB	11	22	12	22	1S1F	O	R1LR	AB

(e) MORE THAN ONE FRAGILE SITE

KINDRED 12273LI FRA10Q25, FRA16Q22 AND FRAXQ27

Ped	Sex	Q25	Q22	GPT	FB	TF	PI	FA	Jk	Lu	MNS	P	ACP	AK	ESD	GA	GLO	HP	GC	ABO	Rh	Fy
1A	M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B	F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2B 1A 1B	M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2D	F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2F	F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3A	M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4A 3A 3B	M	NN	NF	12	11	12	2S	11	BB	BB	MNSZ	+	AA	11	11	NN	22	12	1S 2	A1	R1R2	AB
4B 3C 3D	F	NF	NF	12	11	12	1S	11	AB	BB	MMZZ	+	BB	11	11	ND	22	12	1S1S	A2	LRR2	AB
3D	M	NF	NN	12	11	12	11	11	AB	BB	MNZZ	+	BB	11	12	ND	12	12	1S1F	O	LRR2	AB
3B 2A 2B	F	NN	NF	22	11	12	1S	11	BB	BB	MNZZ	−	AB	12	12	NN	22	22	1S1S	A1	R2R2	AB
3C 2A 2B	F	NN	NF	22	11	12	1S	12	AB	AB	MMZZ	+	AB	11	11	NN	22	12	1S1S	A2	LRLR	AB
2A	F	NN	NF	X	11	22	1S	11	BB	BB	MNZZ	+	BB	11	11	NN	12	12	1S 2	A2	LRR2	AA
4C 3C 3D	M	NN	NN	12	11	12	11	12	X	X	X	X	AB	11	12	NN	22	X	1S1S	X	X	X
3E 2C 2D	F	NN	NN	11	13	11	11	11	AB	BB	NNZZ	−	AA	11	11	ND	12	22	1S 2	A1	LRR2	AB
3F 2E 2F	F	NN	NN	22	13	11	11	12	AA	BB	MNZZ	+	AB	11	11	NN	22	22	1S1S	A1	LRR2	BB
2E 1A 1B	M	NN	NN	12	11	11	11	22	X	X	X	X	AB	11	12	NN	12	22	1S1S	A1	R1R2	X
2C 1A 1B	M	NN	NN	X	11	11	11	12	X	X	X	X	AB	11	11	NN	12	12	1S 2	X	X	X

KINDRED 10351HO FRA10Q23 AND FRA10Q25 (PORTION BLOODGROUPED)

Ped	Sex	Q23	Q25	GPT	C3	TF	Jk	Lu	MNS	PGP	ACP	ESD	PGM	GALT	GLO	HP	GC	ABO	Rh	Fy
2E	M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3B 2A 2B	F	NF	NF	22	22	11	AB	AB	MMSZ	11	BB	11	12	NN	12	22	1S1F	B	R1LR	AB
2A 1A 1B	F	NF	NF	12	12	12	AB	AB	MMSZ	12	AB	11	12	NN	12	22	1S1F	O	R1R1	AB
3A 2E 2A	F	NF	NF	12	12	13	AB	BB	MMSZ	12	AB	11	11	NN	12	12	1S1F	A	R1R1	AB
2B	M	NN	NN	12	22	11	AB	BB	MMSZ	11	AB	12	12	NN	22	22	1S1S	B	R1LR	AB
2D	M	NN	NN	12	12	1B	BB	BB	MMZZ	11	BB	11	11	NN	11	22	1S1S	A	R1LR	AB
3C 2C 2D	F	NN	NN	22	12	1B	BB	BB	MNZZ	11	BC	11	11	ND	12	X	1S1F	AB	R1LR	AB
3D 2C 2D	M	NN	NN	22	12	1B	BB	BB	MNZZ	11	BC	11	11	NN	12	22	1S1F	AB	LR Z	AA
2C 1A 1B	F	NF	NF	12	12	11	X	X	X	11	AC	11	11	ND	12	12	1S1F	B	X	X
1A	F	NF	NF	12	12	11	X	X	X	12	AA	12	12	ND	22	12	1S1F	B	X	X
1B	M	NN	NN	11	11	12	X	X	X	11	BC	11	11	NN	12	12	1S1F	O	X	X

ID			Sex	Q23	Q25	GPT	C3	TF	PGP	ACP	ESD	PGM	GALT	GLO	HP	GC	ABO
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2D			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3V			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4C	3C	3D	F	NF	NF	22	22	11	11	BB	11	12	NN	12	22	1S1F	B
3C	2E	2F	F	NF	NF	12	12	12	12	AB	11	12	NN	12	22	1S1F	O
4B	3V	3C	F	NF	NF	12	12	13	12	AB	11	11	NN	12	12	1S1F	A
3D			M	NN	NN	12	22	11	11	AB	12	12	NN	22	22	1S1S	B
3A	2C	2D	M	NN	NN	12	12	11	11	BB	11	11	ND	22	22	1S1S	B
3K			M	NN	NN	12	12	1B	11	BB	11	11	NN	11	22	1S1S	A
4G	3J	3K	F	NN	NN	22	12	1B	11	BC	11	11	ND	12	X	1S1F	AB
4I	3J	3K	M	NN	NN	22	12	1B	11	BC	11	11	NN	12	22	1S1F	AB
3J	2E	2F	F	NF	NF	12	12	11	11	AC	11	11	ND	12	12	1S1F	B
5A	4A	4B	F	NN	NF	X	12	13	X	AB	X	11	NN	12	22	1S1F	X
2E	1A	1B	F	NF	NF	12	12	11	12	AA	12	12	ND	22	12	1S1F	B
2F			M	NN	NN	12	11	12	11	BC	11	11	ND	12	12	1S1F	O
3T	2E	2F	M	NN	NN	12	12	11	12	AB	11	11	NN	22	22	1S1S	O
3U	2E	2F	F	NN	NN	X	12	12	11	AB	11	11	NN	22	22	1S1F	O
3O	2E	2F	M	NF	NF	11	11	12	12	AC	12	12	NN	12	11	1S1S	B
3G	2E	2F	M	NF	NN	11	11	11	12	AC	12	12	NN	12	12	1S1F	O
2A	1A	1B	F	NN	NN	11	11	11	11	AB	11	11	NN	22	12	1S1F	X
2C	1A	1B	M	NN	NN	11	12	11	12	AB	11	12	NN	22	12	1S1F	X
2B	1A	1B	F	NN	NN	11	22	11	11	AA	11	12	NN	22	12	1S1F	X
3R	2E	2F	F	NF	NF	12	11	11	12	AC	11	12	NN	12	12	1S1F	O
3E	2E	2F	F	NN	NN	12	12	12	11	AB	11	11	NN	12	11	1S1F	B
3F	2E	2F	F	NN	NN	12	12	11	11	AC	11	11	NN	22	11	1F1F	O
3M	2E	2F	F	NN	NN	12	12	12	11	AB	11	11	NN	12	22	1S1F	O
3I	2E	2F	F	NN	NN	22	11	11	11	AC	11	12	NN	22	22	1S1F	B
3B	2E	2F	F	NN	NN	22	12	11	11	AB	11	11	NN	22	22	1S1F	B
3S			M	NN	NN	11	22	11	11	BB	11	11	NN	12	22	1S1S	X
4J	3R	3S	M	NN	NF	11	12	11	12	AB	11	11	NN	12	12	1S1S	X
4H	3J	3K	F	NF	NF	22	X	11	11	BC	11	11	NN	12	22	1S1S	X
4K	3R	3S	M	NN	NN	12	12	11	12	AB	11	12	NN	22	22	1S1F	X
3Q	2E	2F	F	NN	NN	12	12	12	11	AB	11	12	ND	12	22	1S1F	B
4F	3J	3K	F	NF	NF	12	22	1B	11	BC	11	11	NN	12	22	1S1S	X

ID	P1	P2	Sex														
3H			F	NN	NN	12	22	12	11	AB	11	11	ND	22	12	1S 2	O
4D	3G	3H	F	NN	NN	11	X	11	11	AA	11	11	ND	12	11	1S1F	O
3L	2E	2F	F	NF	NF	22	12	11	11	AB	11	12	ND	22	11	1F1F	B
3W			M	NN	NN	12	22	11	12	BB	11	12	NN	22	22	1S 2	X
4L	3L	3W	F	NN	NN	12	22	11	11	BB	11	12	NN	22	12	1F 2	X
4M	3L	3W	M	X	X	X	X	11	X	X	X	X	X	X	12	X	X
3N	2E	2F	F	NN	NN	11	12	12	12	AC	11	12	NN	12	22	1S1F	B
4E	3G	3H	F	NN	NN	X	12	11	X	AA	12	12	X	12	22	1S1S	O

KINDRED 10833CH FRA10Q23 AND FRA10Q25

				Q23	Q25	F13B	F13A	Jk	Lu	MNS	AK	GC	ABO	Rh
1A			M	NN	NN	11	12	AB	BB	MNSZ	12	1S1F	A1	R1LR
2A	1A	1B	F	NF	NF	13	11	BB	AB	MMSZ	22	1S1F	A1	LRLR
1B			F	NF	NF	13	11	AB	AA	MMZZ	12	1F1F	A2	LRLR
2B	1A	1B	F	NN	NN	11	11	AA	AB	MMSZ	11	1S1F	O	LRLR
2C	1A	1B	M	NN	NN	13	12	AB	AB	MNZZ	12	1F1F	A1	R1LR

Ind	Fa	Mo	Sex	Q25	Q23	GPT	C3	FB	TF	PI	FA	Jk	MNS	PGP	ACP	AK	ESD	PGM	GLO	HP	GC	ABO	Rh	Fy
2F			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3A	2A	2B	M	FF	NN	X	X	X	12	13	11	X	X	12	BB	11	11	12	12	X	1S 2	X	X	X
2B	1A	1B	M	NF	NF	12	12	11	12	13	11	BB	MNSZ	12	BB	12	12	11	12	11	1S 2	O	R1LR	BB
2A			F	NF	NN	12	22	11	11	11	11	AA	MNSZ	11	BB	11	11	12	12	12	1S1S	O	R1LR	AB
3B	2A	2B	F	FF	NN	12	12	11	12	11	11	X	X	12	BB	11	11	11	22	12	1S 2	X	X	X
3C	2A	2B	F	NF	NN	12	12	11	X	11	11	X	X	12	BB	12	11	X	12	11	1S1S	X	X	X
3D	2A	2B	F	NF	NN	X	12	11	11	13	11	X	X	12	BB	11	12	11	12	11	1S 2	X	X	X
1B			F	NF	NF	12	12	11	12	13	12	BB	NNZZ	22	AB	11	12	11	12	11	1S 2	O	R1LR	BB
2C	1A	1B	M	NF	NN	11	22	11	11	11	12	BB	NNZZ	12	AB	11	11	11	11	12	1F 2	O	R1R1	AB
2E	1A	1B	F	NF	NN	11	X	13	12	11	11	AB	MNSZ	12	BB	11	11	11	12	11	1S1F	O	R1LR	AB
3E	2F	2E	M	NF	NN	12	12	33	11	11	X	X	X	11	BB	11	11	11	22	11	1S1F	X	X	X
2D	1A	1B	M	NF	NF	11	12	13	12	13	12	BB	MNSZ	12	BB	12	11	11	12	12	1S1F	O	R1R1	AB
1A			M	NN	NN	11	12	13	12	11	11	AB	MNSZ	11	BB	12	11	12	11	12	1F 2	O	R1LR	AB

KINDRED 6047CR FRA10O23 AND 9QH (PART WITH FRAGILE SITE)

					GPT	F13B	TF	PI	F13A	K	Jk	MNS	PGP	ACP	PGM	GLO	HP	GC	ABO	Rh	Fy
2C			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X X	X	X	X
4A	3C	3D	M	NF	22	11	12	13	11	X	X	X	11	BB	12	12	22	1S 2	AB	X	X
3D			M	NN	12	11	11	23	11	X	X	X	11	BB	12	12	22	1S 2	B	X	X
3C	2A	2B	F	NF	12	13	12	11	11	X	X	X	11	BB	11	12	22	1S 2	A	X	X
4B	3C	3D	F	NN	12	13	11	12	11	X	X	X	11	BB	12	12	22	1S1S	A	X	X
2B	1A	1B	M	NF	12	11	12	11	11	ZZ	AB	NNZZ	11	BB	11	12	22	1S 2	A	LRLR	BB
2A			F	NN	12	13	11	11	12	ZZ	AB	NNZZ	11	AB	11	12	22	1S 2	O	R1LR	AB
3E			M	NN	22	11	11	13	11	ZZ	AA	NNSZ	12	BB	11	12	12	2 2	O	R1LR	BB
1B			M	NN	12	11	12	11	11	ZZ	AB	MNZZ	11	BB	11	22	12	2 2	A	R1LR	AB
3B	2A	2B	F	NN	22	13	11	11	11	ZZ	AB	NNZZ	11	AB	11	12	22	1S 2	O	LRLR	BB
3A	2A	2B	M	NN	12	11	12	11	12	ZZ	AA	NNZZ	11	BB	11	22	22	1S 2	O	R1LR	BB
1A			F	NF	12	13	11	11	11	ZZ	AA	MNSZ	11	BB	12	11	22	1S1S	O	R1LR	BB
3G	2C	2D	F	NN	22	13	11	13	11	KZ	AB	NNSZ	12	BB	12	22	12	1S 2	O	LRLR	BB
3F	2C	2D	F	NN	12	13	12	11	11	ZZ	AB	NNSZ	12	BB	11	12	22	1S1S	O	R1LR	AB
2D			F	NN	12	13	11	13	11	KZ	AB	NNSZ	12	BB	11	12	22	1S1S	O	LRLR	AB

KINDRED 6047CR FRA10O23 AND 9QH (PART WITH 9QH)

					GPT	C3	PI	F13A	PGM	GLO	ABO
3A	2A	2B	M	NV	22	22	13	11	12	12	AB
2B	1A	1B	M	NV	12	22	23	11	12	12	B
2A			F	NN	12	22	11	11	11	12	A
3B	2A	2B	F	NV	12	22	12	11	12	12	A
1A			M	NV	11	12	12	11	11	11	O
1B			F	NN	22	22	3S	12	12	12	AB
2C	1A	1B	M	NV	12	22	13	11	11	12	A

KINDRED 10556DO FRA16P12 AND T(5;10)(P14;P15)

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                    GPT F13B TF PI CHE2 MNS  PGP ACP ADA ESD PGM GLO HP  GC

1A          M       X   X   X   X  X    X    X   X   X   X   X   X   X   X
1B          F       X   X   X   X  X    X    X   X   X   X   X   X   X   X
2C 1A 1B    F       X   X   X   X  X    X    X   X   X   X   X   X   X   X
2D          M       X   X   X   X  X    X    X   X   X   X   X   X   X   X
3M          F       X   X   X   X  X    X    X   X   X   X   X   X   X   X
3N          M       X   X   X   X  X    X    X   X   X   X   X   X   X   X
3O          M       X   X   X   X  X    X    X   X   X   X   X   X   X   X
2A 1A 1B    M  NF   12  11  11 11  -    MNSS 11  AB  11  11  11  12  12 1F 2
2B 1A 1B    F  NN   12  11  11 11  -    MMZZ 11  AB  22  11  11  12  12 X
3A 2A 2E    F  NN   12  11  11 11  -    MNSZ 11  AB  11  11  11  22  12 1S 2
3C 2A 2E    F  NF   12  13  12 11  -    MNSZ 11  BB  11  11  11  12  11 1S 2
4A 3B 3M    M  NN   12  X   12 11  -    MMZZ 11  AB  11  11  11  22  22 1S1S
4B 3C 3L    F  NN   11  13  11 11  -    NNSZ 11  BB  11  11  11  12  12 2 2
4C 3C 3L    M  NN   12  11  12 1S  -    MNZZ 11  AB  11  11  11  22  12 1S 2
3D 2C 2D    M  NF   11  11  11 12  +    MMZZ 11  AA  11  11  11  22  12 1S 2
3E 2C 2D    F  NF   22  11  12 11  -    X    11  AA  12  11  12  12  11 1S1S
3F          M  NN   12  11  22 1S  -    MMZZ 12  AB  11  11  12  22  12 1S1S
3G 2C 2D    F  NF   22  11  11 11  +    MMSZ 11  BB  12  11  11  12  12 1S 2
4D 3F 3G    F  NF   12  11  12 11  -    MMSZ 11  BB  11  11  11  22  12 1S 2
4E 3F 3G    F  NN   22  11  12 1S  -    MMSZ 12  AB  11  11  11  12  12 1S1S
3H 2C 2D    F  NF   12  11  11 12  -    MMZZ 11  AA  12  11  11  22  11 1F 2
3I          M  NN   12  11  12 13  -    X    12  BB  11  12  22  12  22 1S1F
4F 3H 3I    F  NF   X   11  12 11  -    X    X   X   X   X   X   X   12 1F 2
4G 3H 3I    F  NF   X   11  12 13  -    X    X   X   X   X   X   X   12 1F 2
3J 2C 2D    F  NF   22  13  11 12  +    X    11  AA  12  11  11  22  12 1S 2
4H 3N 3J    F  NN   X   11  X  23  -    X    X   X   X   X   X   X   12 1S1S
4I 3N 3J    M  NN   X   11  12 12  +    X    X   X   X   X   X   X   22 2 2
4J 3N 3J    M  NN   X   11  12 23  +    X    X   X   X   X   X   X   22 1S 2
4K 3N 3J    F  NF   X   11  11 11  +    X    X   X   X   X   X   X   22 1S1S
4M 3O 3K    M  NN   11  11  11 11  -    NNZZ 12  BB  11  11  11  X   12 1F 2
3L          M  NN   11  11  12 1S  X    NNZZ 11  AB  11  11  11  22  22 1S 2
3K 2C 2D    F  NN   11  13  11 12  -    X    11  BB  11  11  12  22  12 X
4L 3N 3J    M  NF   12  11  11 12  -    X    11  AB  11  11  11  22  11 1S 2
2E          F       X   X   X   X  X    X    X   X   X   X   X   X   X   X
3B 2A 2E    M       X   X   X   X  X    X    X   X   X   X   X   X   X   X
```

KINDRED 10556DO FRA16P12 AND T(5;10)(P14;P15)

					GPT	F13B	TF
3L			M	NN	11	11	12
3C			F	NT	12	13	12
4B	3L	3C	F	NN	11	13	11
4C	3L	3C	M	NT	12	11	12

KINDRED 10531FR FRA12Q13 AND EBS-K

```
                 GPT C3 TF PI FA Jk MNS P ACP AK ESD PGM GLO HP GC ABO Rh Fy IGH IGK

1B         M  X X X X X X X X X X X  X  X  X X X  X X  X  X X
1A         F  X X X X X X X X X X X  X  X  X X X  X X  X  X X
2A         F  X X X X X X X X X X X  X  X  X X X  X X  X  X X
2B 1A 1B   M  X X X X X X X X X X X  X  X  X X X  X X  X  X X
2C 1A 1B   F  NE X X X X X X X X X X X  X  X  X X X  X X  X  X X
2D         M  X X X X X X X X X X X  X  X  X X X  X X  X  X X
3B 2A 2B   M  NE X X X X X X X X X X X  X  X  X X X  X X  X  X X
3C         F  X X X X X X X X X X X  X  X  X X X  X X  X  X X
3D 2C 2D   M  NE X X X X X X X X X X X  X  X  X X X  X X  X  X X
4I 3C 3D   M  NE X X X X X X X X X X X  X  X  X X X  X X  X  X X
4K         F  X X X X X X X X X X X  X  X  X X X  X X  X  X X
6A 5F 5G   M  NE NN 12 11 11 12 X AB MNSZ- BC 11 11 12 22 22 1F 2 O LRR2 AB  35  -
6D 5F 5G   M  NN NF 11 12 11 12 X AB MMSZ+ BB 11 11 11 12 22 1S1S O LRR2 AA  35  -
6E 5F 5G   F  NE NF 11 22 11 11 11 AB MMSZ+ BB 11 11 12 12 22 1S 2 O LRR2 AB1235 -
5F 4F 4G   F  NE NF 12 12 11 12 11 AA MNSS+ BC 11 11 12 22 12 1S 2 O R1R2 AB1235 -
5G         M  NN NN 11 12 11 12 12 BB MNZZ- BB 11 11 11 12 22 1S1F O LRLR AA1235 -
6B 5F 5G   M  NN NF 12 X 11 11 11 AB NNSZ- BB 11 11 12 22 12 1S 2 O LRR2 AA1235 -
6C 5F 5G   M  NN NN 11 X 11 12 11 AB NNSZ+ BB 11 11 12 22 22 1S 2 O LRR2 AA  35  -
5J 4I 4J   F  NN NN 12 22 22 1S 11 AA MNZZ+ AA 11 11 22 12 12 2 2 O R1R2 AA1235 +
4J         F  NN NN 22 X 12 11 11 AB MNZZ+ AA 11 12 12 22 11 1S 2 A1 R1LR AB  12  -
4F 3C 3D   F  NE NF 11 22 11 11 X AA NNSZ- BC 11 12 11 22 22 1S 2 A1 R1R2 BB1235 +
5H 4F 4G   F  NN NF 12 22 12 11 X AB MNZZ+ AC 11 12 11 22 12 1S 2 O R1R2 BB1235 -
5K 4E 4K   M  NN NN 12 12 11 11 12 XM NSZ + AB 11 11 12 22 22 1S 2 O R1R1 AB 135 -
4E 3C 3D   M  NN NF 11 11 11 11 11 AA MNSZ- AB 11 12 12 22 12 1S 2 A1 R1R1 AB 135 -
5L         M  NN NN 12 X 12 11 11 AA NNZZ+ AB 11 11 11 12 X 1S1S B R1LR AB1235 -
6F 5H 5L   F  NN NF 11 X 11 11 X X X X X 11 X 11 12 22 1S1S X X  X  X X
6G 5M 5I   F  NN NF 11 22 11 12 11 X X X X 11 11 22 X 12 1F 2 X X  X  X X
5I 4F 4G   F  NN NF 12 22 11 12 11 AA MNSZ+ AC 11 11 12 22 12 1S 2 A1 R1R2 BB1235 -
5M         M  NN NN 12 22 11 11 11 AB MMSZ+ AB 11 11 12 22 22 1S1F O R1R1 AA  35  -
6H 5M 5I   M  NN NF 12 X 11 11 11 X X X AC 11 11 22 22 22 1S1F X X  X1235 -
3E 2C 2D   F  NE NN X X 12 11 12 X X X AA 11 X 12 12 12 1S1S X X  X 12  -
4B 3A 3B   M  NE NN 12 22 12 13 12 AB MNSS- AA 12 11 11 22 12 X  O R1R1 AB1235 +
4A         F  NN NN 12 12 11 13 11 AB MMSZ+ AB 11 11 12 11 12 1S1F A1 R1R2 BB1235 -
```

```
5B 4A 4B F NE NN 12 12 11 33 11 AB MMSZ- AB 12  11  12  12 12  X      O R1R2 AB   35 -
5C 4A 4B F NE NN 12 22 12 11 11 AA MNSS+ AB 11  11  12  12 22 1S 2    O R1R2 AB   35 -
5E 4A 4B M NE NN 12 12 12 13 12 AA MMSS+ AA 11  11  12  12 12  X     A1 R1R1 AB1235 -
5D 4A 4B F NE NN  X  X 12 11 11 AB MMSS+ AA 11  11  11  12 12 1S1F  A1 R1R1 AB   35 -
5A 4A 4B M NN NN 12 22 12 11 11 BB MMSZ- AB 12  11  12  12 12 1S1F  A1 R1R2 AB1235 -
4C 3A 3B M NN NN 11 22 22 11 11 BB MNSS- BB 12  11  12  12 22  X      O LRR2 AA   35 -
3A       F NN NN  X 22 12 13 11 AB MMSS- AB 11  12  11  12 12 1S1S   O R1R2 AB   35 -
4D 3A 3B F NN NN 11  X 22 33 12 AB MMSZ- AB 11  11  12  11 12 1S 2    O LRR2 AB1235 -
4L 3A 3B F NN NN  X 22 12 13 11 AA MMSZ- AB 11  12  11  22 12 1S1S   O R1R1 AA   35 -
4G       M  X  X  X  X  X  X  X  X  X  X  X   X   X   X   X  X         X  X    X    X  X
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(h) CHROMOSOMAL HETEROMORPHISMS

KINDRED 5001KR INV(3)(P25;Q23)

ID	Parents	GPT	FB	TF	PI	FA	Lu	MNS	P	PGP	ACP	AK	ESD	PGM	GA	GLO	HP	GC	ABO	Rh	Fy	IGH	IGK
1A		M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B		F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2A	1A 1B	F	NI	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2B		M	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2C	1A 1B	F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2D		M	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2E	1A 1B	F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2F		M	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2G	1A 1B	M	NI	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2H		F	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2I	1A 1B	F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2J		M	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2L	1A 1B	M	NI	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2M		F	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2N	1A 1B	F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2O		M	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

```
2Q        M NN  X  X  X  X  X  X   X  X  X   X   X   X   X   X  X  X  X   X   X   X
   X   X

3G        F NN  X  X  X  X  X  X   X  X  X   X   X   X   X   X  X  X  X   X   X   X
   X   X

3H 2C 2D  M    X  X  X  X  X  X  X   X  X  X   X   X   X   X   X  X  X  X   X   X   X
   X   X

3I        F NN  X  X  X  X  X  X   X  X  X   X   X   X   X   X  X  X  X   X   X   X
   X   X

3N        F NN  X  X  X  X  X  X   X  X  X   X   X   X   X   X  X  X  X   X   X   X
   X   X

3O        M NN  X  X  X  X  X  X   X  X  X   X   X   X   X   X  X  X  X   X   X   X
   X   X

3P 21 2J  F  X  X  X  X  X  X  X   X  X  X   X   X   X   X   X  X  X  X   X   X   X
   X   X

3T        F NN  X  X  X  X  X  X   X  X  X   X   X   X   X   X  X  X  X   X   X   X
   X   X

3A 2A 2B  F NN 11 11 11 11 12 BB MMSS + 12  BB  11  12  12  NN 22 11 1F1F A  R1LR BB
   35  -

3C 2A 2B  F NI 11 11 11 1S 12 BB MMSS - 11  AA  11  11  22  NN 22 12 1F1F A  R1LR AB
 1235  -

3E 2C 2D  M NN 12 11 12 1S 11 BB MMSZ - 11  BB  11  11  11  NN 11 12 1S1S A  R1LR AB
  135  -

4A 3G 3H  F NN 11 11 11 11 12 BB MMZZ + 11  BB  11  11  11  NN 11 22 1S1S A  LRLR X
    1  -

3M 2G 2H  M NI 11 11 12 11 11 AB MMZZ - 11  AB  11  11  11  NN 12 12 1F 2 O R1R1 AA
   35  -

4E 3M 3N  M NN 12 11 11 11 11 BB MNZZ + 11  AA  11  11  11  NN 22 11 1F 2 O R1R1 AB
  135  +

2R 1A 1B  M NN  X 11 11 11 12  X   X  X  X   X   X   X   X   X  X 22 1S1F X   X    X
 1235  +

4G 3M 3N  M NI 12 11 11 11 11 AB MMSZ - 11  BB  11  11  12  NN 22 22 1S 2 O R1R1 AB
  135  +

3F 2C 2D  F NN 22 11 12 1S 12 BB MMSZ + 11  BB  11  11  11  NN 11 12 1F1F X   X   AA
   12  -

3J 2E 2F  M NN 12 11 12 11 12 BB MMSS + 11  BB  11  11  11  NN 22 12 1F 2 O LRLR X
   12  -

3K 2E 2F  M NN 22 11 12 1Z 11 AB MMZZ X 11  BB  11  11  11  NN 22 12 1F 2 X   X    X
   12  -

3B 2A 2B  F NN 11 11 11 1S 11 BB MNSZ + 12  BB  11  12  12  NN 22 11 1F1F A  R1LR BB
 1235  X
```

```
4H 3O 3P F NN 12 11 11 11 11 ABN NZZ + 12 AB 11 11 22 ND 11 22 1S1S X    X    X
   12 -

3R 2L 2M M NN 12 11 11 12 12 BB  MNZZ + 12 AB 11 11 12 NN 11 22 1S 2 A  R1R1 AB
   12 -

4I 3S 3T F NI 12  X 12 11 12 BB  MNSZ + 11 BB 11 11 11 ND 22 12 1S 2 A  R1LR AB
   135 -

4J 3S 3T M NN 11  X 11 12 11  X  MNZZ - 11 BB 11 11 11 NN 12 22 1S1S X    X    X
   135 -

4K 3S 3T F NI  X 11 12 12 11  X   X    X  X  X  X  X  X  X  X 22 1S 2 X    X    X
   135 -

3U 2N 2O M NN 12 11 12 11 11  X  MMSS X 11 AB 11 11 12 NN 12 12 1F 2 X    X    X
   35 -

3W 2N 2O F NN  X 13 11 1I 11  X   X    X  X  X  X  X  X  X  X 12 1S1F X    X    X
   1235 +

3V 2N 2O F NN 11 13 2D 1I 11 BB  MMSZ - 12 BB 11 11 11 NN 12 12 1F 2 O  R1LR X
   1235 +

4F 3M 3N F NN 12 11 11 11 11 BB   X   - 11 AB 12 11 11 NN 22 12 1S1F O    X    X
   135 -

2P 1A 1B F NI  X 11 12 11 11  X   X    X  X  X  X  X  X  X  X 12 1F 2 A    X    X
   1235 -

4O 3X 3Y F NN  X 11 11 13 12  X   X    X  X  X  X  X  X  X  X 22 1F 2 A    X    X
   1235 -

4N 3X 3Y M NI  X 11 11 11 12  X   X    X  X  X  X  X  X  X  X 22 1F1F B    X    X
   135 -

4P 3X 3Y F NI  X 11 11 11 12  X   X    X  X  X  X  X  X  X  X 22 1S 2 A    X    X
   135 -

3L 2E 2F F NN 12 11 11 1Z  X  X  MMSZ X 11 BB 11 11 11 NN 22 11 1S1F X    X    X
   135 -

3Q 2I 2J F NN 11 11 11 11  X  X  MNSZ X 11 BB 11 11 12 NN 11 12 1S 2 X    X    X
   12 -

4M 3X 3Y M NI 11 11 11 23  X BB  NNZZ + 11 BB 12 11 12 ND 11 22 1S1F AB R1LR AB
   135 -

5A 4L 4M M NI 12 11 11 12  X BB  MNSZ + 12 BC 12 11 11 NN 11 12 1F1F  B R1LR BB
   35 -

3X 2P 2Q F NI 11 11 11 13  X BB  NNZZ + 11 BB 11 11 11 ND 11 22 1S1F  A LRLR AB
   12 -

3Y        M NN 12 13 11 12  X BB  NNZZ + 12 BB 12 12 12 NN 12 221 F 2  B R1LR AB
   35 -

3S 2L 2M M NI 12 13 11 12  X BB  MMZZ - 11 BB 11 11 11 NN 22 22 1S1S  A R1LR AA
   35 -
```

```
4B 3H 3I F NN 11 11 12 11 11  X MMSS X 11 BB  11  11  11  NN 22  22 1F 2  X   X   X
 135 -

4C 3H 3I F NN 11 11 12 11 12  X MMZZ X 11 AB  11  11  11  NN 22  22 1F 2  X   X   X
1235 -

4D 3H 3I M NN 11 11 11 11 12 BB MMZZ X 11 BB  11  11  11  NN 22   X 1S 2  X   X   X
 135 -

3D 2A 2B M NN  X 11 11 1S 12  X  X   X  X  X   X   X   X   X  X  11 1F1F  X   X   X
1235 -

4L       F NN  X  X  X  X  X  X  X   X  X  X  11  11  12   X  X   X   X    X   X   X
   X  X
```

KINDRED 557FI 9OH

			Sex		GPT	F13B	PI	F13A	PGP	ACP	ADA	ESD	PGM	GALT	GLO	HP	GC
1A			M		X	X	X	X	X	X	X	X	X	X	X	X	X
1B			F		X	X	X	X	X	X	X	X	X	X	X	X	X
2F			F		X	X	X	X	X	X	X	X	X	X	X	X	X
3G			M	NN	11	11	12	12	13	AB	11	11	11	NN	12	22	1S 2
3F	2G	2H	F	NV	X	13	11	11	11	AA	12	11	12	NN	11	12	1S1S
4A	3F	3G	F	NV	11	13	12	12	13	AA	12	11	11	NN	12	22	1S1S
2G	1A	1B	M	NV	12	13	11	11	11	AA	12	11	11	ND	11	22	1S 2
2H			F	NN	11	11	13	11	11	AA	11	11	12	NN	12	11	1S1S
2D	1A	1B	M	NN	12	13	11	X	12	AA	11	12	11	ND	12	22	1S 2
2A	1A	1B	F	NN	12	13	11	12	11	BB	11	12	11	ND	11	22	1S 2
2B	1A	1B	F	NN	12	11	11	12	12	AB	11	11	11	ND	12	22	1S 2
2C	1A	1B	F	NV	12	13	11	12	12	AA	11	11	11	ND	12	22	1S 2
2E	1A	1B	M	NV	12	11	11	11	12	AA	11	11	11	ND	12	22	1S1S
3C	2G	2H	F	NN	12	13	13	11	11	AA	11	11	12	ND	12	12	1S1S
3E	2G	2H	F	NV	12	13	11	11	11	AA	11	11	12	NN	12	12	1S1S
3D	2G	2H	F	NN	12	13	11	11	11	AA	11	11	12	ND	11	12	1S 2
3A	2E	2F	F	NN	12	11	11	11	12	AA	11	11	11	ND	12	12	1S1S
3B	2E	2F	F	NV	12	11	11	11	11	AB	11	11	11	ND	12	22	1S1S

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                      GPT C3 FB TF PI FA  K  Jk Lu MNS  PGP ACP ESD PGM GLO HP GC   ABO  Rh   Fy

1A           M        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
1B           F        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
2A 1A 1B     M        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
2B           F        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
2C 1A 1B     M        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
2D           F        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
2E 1A 1B     M        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
2F           F        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
2G 1A 1B     F        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
2H           M        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
3B           M        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
3F           M        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
3I           M        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
4K           M        X  X  X  X  X  X  X  X  X  X    X   X   X   X   X  X  X    X    X    X
4G 3E 3F     M   NT  12 22 13 12 11 11 KZ AB AB MMZZ  11  BB  11  12  22  X 1S1S  A1  LRR2 AB
4H           F   NN  11 22 11 12 2S 11 ZZ AB BB MMSZ  11  BB  11  11  12 12 1S1S  B   R1LR AA
5C 4G 4H     M   NN  11 22 11 22 1S 11 KZ AB AB MMSZ  11  BB  11  11  12 22 1S1S  B   R1R2 AB
4F           F   NN  11 22 33 12 12 11 ZZ AA BB MNZZ  11  AB  11  11  12 22 1S1S  O   R1R1 AA
4E 3E 3F     M   NT  12 12 13 12 11 11 ZZ BB BB MNSZ  11  BB  12  12  12 22 1S1S  O   R1R2 BB
5A 4E 4F     F   NT  11 22 13 11 11 11 ZZ AB BB MNSZ  11  AB  12  12  12 22 1S1S  O   R1R2 AB
5B 4E 4F     F   NT  11 12 13 12 11 11  X  X  X  X    X   BB  11   X  12  X 1S1S  O    X    X
4D 3C 3D     M   NN  22 22 13 11 12 11 KZ AA BB NNZZ  12  BB  11  12  12 11 1S 2  B   R1LR BB
3A 2A 2B     F   NN   X 22 13 11 2S 12 ZZ AB BB MNSZ  11  AB  11  12  22 12 1S1S  A2  LRR2 BB
4A 3A 3B     M   NN   X 22 11 11 22 12 ZZ AB BB MNSZ  12  AB  11  11  12 22 1S1F  O   R1R2 BB
4B 3A 3B     M   NN   X 12 13 11 2S 11 ZZ AB BB MMSS  12  BB  11  11  22 12 1S1F  O   LRR2 BB
4I 3E 3F     F   NT  12  X 11 12 11 11  X  X  X  X    11  BB  11  22  11 22 1S 2   X    X    X
4J 3E 3F     M   NN   X 11 12 11 11  X  X  X  X  X    11  BB  11  11  12 22 1S 2   X    X    X
3E 2E 2F     F   NT  22  X 13 12 11 11  X  X  X  X    11  BB  11  12  12 22 1S1S   X    X    X
4L 3E 3F     F   NT  22 12 13 11 11  X  X  X  X  X    11  BB  11  11  12 22 1S 2   X    X    X
3D           M   NN  12 22 13 11 11 11  X  X  X  X    12  BB  11  22  22 12 1S1S   X    X    X
3C 2C 2D     F   NT  12 22 11 11 12 11  X  X  X  X    11  BB  11  12  11 12 1F 2   X    X    X
4C 3C 3D     M   NN  22 22 11 11 12 11  X  X  X  X    12  BB  11  12  12 11 1S 2   X    X    X
3H 2G 2H     F   NN  22 11 11 12 11 11  X  X  X  X    11  AB  11  12  12 12 1S1S   X    X    X
4M 3H 3I     F   NN  12 11 11 11 11 11  X  X  X  X    11  BB  11  11  22 22 1S1S   X    X    X
4N 3H 3I     M   NN  12 11 13 12 1S  X  X  X  X  X    12  BB  11  12  12 22 1S1S   X    X    X
```

```
5D 4K 4L F NT 22 12 13 11 11  X ZZ BB BB MMSZ 11 BB 12  X  X 12 1S1S B R1R2 BB
5E 4K 4L F NN 12 22 11 11  X 12 ZZ AB BB MMZZ 11 BB 12 11 22 12 1S 2 O R1R1 BB
3G 2G 2H M NN  X 12 11 12 11 11  X  X  X  X 12 AB  X 11 22 12 1S1S X  X   X
```

KINDRED 10199HU T(10;18)(Q26;Q21)

ID	Fa	Mo	Sex		C3	F13B	TF	F13A	ADA	ESD	PGM	HP	GC
2D			M	X	X	X	X	X	X	X	X	X	X
2B			F	X	X	X	X	X	X	X	X	X	X
2A	1A	1B	M	NT	12	11	11	12	11	11	11	12	1S1S
1B			F	NN	X	X	11	11	12	11	12	12	1S1S
1A			M	NT	12	13	12	12	11	11	11	12	1S1S
3A	2A	2B	M	NN	22	11	12	11	11	11	11	12	1S1S
3B	2C	2D	F	NT	12	11	11	12	12	11	11	12	1S1F
3C	2C	2D	M	NT	22	X	12	11	12	11	11	11	1S1S
2C	1A	1B	F	NT	12	X	12	12	12	11	11	11	1S1S

KINDRED 10048MU T(11;22)(Q23;Q11)

ID	Fa	Mo	Sex		GPT	C3	TF	K	Jk	MNS	P	PGP	ACP	ADA	PGM	GALT	GLO	HP	GC	ABO	Rh	Fy
2D	1A	1B	M	NN	11	12	11	ZZ	AB	MMZZ	+	12	BB	11	11	NN	11	22	1S 2	B	R1LR	AA
2C	1A	1B	M	NT	11	12	11	ZZ	AB	MNZZ	+	11	BB	22	11	NN	12	11	1S 2	A1	R1LR	AA
2A	1A	1B	F	NT	12	12	11	ZZ	AA	MNZZ	+	11	BB	11	11	NN	11	11	1S1F	A1	R1LR	AA
2B	1A	1B	M	NN	X	12	11	ZZ	AA	MNZZ	+	X	X	X	X	X	X	12	1S 2	A2B	R1LR	AA
1B			M	NN	X	22	11	ZZ	AA	MNZZ	+	X	X	X	X	X	X	12	1F 2	A2	R1R1	AA
1A			F	NT	X	11	11	ZZ	AB	MNZZ	+	X	X	X	X	X	X	12	1S1S	A1B	LRLR	AB
3E	2E	2F	F	NT	12	11	11	KZ	AB	MNZZ	+	12	AB	11	12	ND	11	12	1S1S	B	LRR2	AB
3D	2E	2F	F	NT	12	11	12	KZ	AB	MMZZ	+	12	AB	12	12	ND	11	12	1S1S	A2	LRR2	AB
3C	2E	2F	M	NN	12	11	12	KZ	BB	MMZZ	+	12	AB	12	11	NN	11	12	1S1S	A2	LRR2	AB
3B	2E	2F	F	NT	11	12	11	KZ	BB	MMSZ	-	11	AB	11	12	ND	12	12	1S 2	B	LRR2	AB
3A	2E	2F	F	NN	11	12	11	KZ	BB	MNSZ	-	12	AB	11	11	ND	11	12	1S1S	A2	R1R2	AB
2F			M	NN	12	12	12	KZ	BB	MMSZ	-	11	AA	11	12	ND	11	22	1S1S	O	R2R2	BB
2E	1A	1B	F	NT	12	12	11	ZZ	AB	MNZZ	+	12	BB	12	11	NN	12	11	1S 2	A2B	R1LR	AA

KINDRED 3017CH T(13;14)(P11;P11)

					F13B	TF	PI	HP	GC	ABO
1A			M	NT	11	12	13	12	2 2	O
1B			F	NN	13	11	11	12	1S 2	A
2A	1A	1B	F	NN	13	11	11	11	2 2	O
2B	1A	1B	F	NN	11	12	11	12	2 2	A
2C	1A	1B	F	NT	13	12	13	22	1S 2	A

(i) EPIDERMOLYSIS BULLOSA

KINDRED 11366JO EBS-K

					GPT	C3	TF	PI	Jk	MNS	P	ACP	ESD	PGM	GLO	HP	GC	ABO	Fy
3A	2A	2B	M	NE	X	12	11	X	AA	MNZZ	+	BB	12	11	X	11	1F1F	B	AB
2A			F	NN	X	22	11	11	AA	MNZZ	+	BB	11	11	22	12	1S1F	A1B	AB
2B	1A	1B	M	NE	11	12	11	12	AB	MNZZ	+	AB	12	12	11	11	1F 2	A2	AB
1B			M	NE	12	22	12	11	AA	MNZZ	+	AB	11	12	11	12	1F1F	O	AB
2C	1A	1B	M	NE	12	12	12	12	AB	MNSZ	+	BB	12	12	12	11	1S1F	A2	BB
2D	1A	1B	F	X	11	12	11	1S	AA	MNSZ	−	BB	12	12	11	12	1F 2	O	AA
1A			F	NN	11	12	12	2S	AB	MMSZ	+	BB	12	12	12	12	1S 2	A2	AB

KINDRED 368JO EBD-CT

ID	Father	Mother	GPT	C3	F13B	TF	PI	F13A	CHE2	K	Jk	Lu	MNS	P	PGD	PGP	ACP	AK	ADA	ESD	PGM	GALT	GLO	HP	GC	ABO	Rh	Fy	IGH	IGK
1A			M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1B			F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2A			F	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2B	1A	1B	M	NF	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2C	1A	1B	M	NF	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2D			F	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2E	1A	1B	M	NF	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2F			F	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3A			M	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3B	2A	2B	F	NF	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3C			M	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3D	2A	2B	F	NF	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3E	2A	2B	F	NF	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3F			M	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3H			M	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3I			F	NN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3J	2C	2D	M	NF	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

```
3K         F NN  X  X   X   X X   X   X X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

3L 2E 2F M NF  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

3M 2E 2F M NF  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

3N         F NN  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

3O 2E 2F M NF  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

3P         F NN  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

3Q 2E 2F M NF  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

3R         F NN  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

4C         F NN  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

4H         M NN  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

M4 3G 3H M NF  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

N4         F NN  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

4K         F NN  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

4L 3I 3J M NF  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

4P PI 3J M NF  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X
  X   X   X  X  X    X    X  X   X

4R 3K 3L M NF  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X  X
  X   X   X  X  X    X    X  X   X

4S         F NN  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X  X
  X   X   X  X  X    X    X  X   X

4T 3K 3L F NF  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X  X
  X   X   X  X  X    X    X  X   X

4U         M NN  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X  X
  X   X   X  X  X    X    X  X   X

4X         F NN  X  X   X   X X   X   X X X  X X  X  X  X X  X  X  X  X
  X   X   X  X  X    X    X  X   X
```

```
4Y 3M 3N M NF  X   X    X    X   X    X     X  X  X  X   X  X   X   X   X  X   X   X   X
   X    X   X    X   X    X      X   X    X

E4       F NN  X   X    X    X   X    X     X  X  X  X   X  X   X   X   X  X   X   X   X
   X    X   X    X   X    X      X   X    X

G4 3O 3P M NF  X   X    X    X   X    X     X  X  X  X   X  X   X   X   X  X   X   X   X
   X    X   X    X   X    X      X   X    X

J4       M NN  X   X    X    X   X    X     X  X  X  X   X  X   X   X   X  X   X   X   X
   X    X   X    X   X    X      X   X    X

5P       F NN  X   X    X    X   X    X     X  X  X  X   X  X   X   X   X  X   X   X   X
   X    X   X    X   X    X      X   X    X

A5       M NN  X   X    X    X   X    X     X  X  X  X   X  X   X   X   X  X   X   X   X
   X    X   X    X   X    X      X   X    X

G5       M NN  X   X    X    X   X    X     X  X  X  X   X  X   X   X   X  X   X   X   X
   X    X   X    X   X    X      X   X    X

B5 4X 4Y F NF  X  12  11   11  12   12     -  KZ AB AB NNZZ + AA  11  BB 11  11   11   11
   X   12  11   2   2   A1  R1LR  BB 1235   +

K4 3Q 3R F NF 12   X  11   11  11   11     -  ZZ AB BB NNSS + AC  11  AB 11  11   12   11
NN     22  12  1S1S A1  R1LR  AA 1235   -

C4 3M 3N M NN 12  22  11   11  11   12     -  ZZ AA BB MNSZ + AC  11  AB 11  11   12   11
NN     11  11   2   2   A1  R1R2  AA 1235   X

A4 3M 3N F NF 12  12  13   11  11   12     -  ZZ AA BB NNSZ + AA  11  BB 11  11   12   11
NN     11  11       X   A1  R1R2  AA 1235   -

6N D5 E5 M NF 12   X   X   11  11    X     -  ZZ AB BB MNSZ + AA  11  AA 11  11   11   11
NN     11  22  1S1S  O   R1LR  AB    X    X

6M D5 E5 M NF 12   X   X   11  11    X     -  ZZ AA BB MNSZ + AA  11  AB 11  11   11   11
NN     12  12  1S1S  O   R1LR  AB    X    X

E5       F NN 22  22  11   12  11   11     -  ZZ AB BB MMSS + AA  11  AB 12  11   11   11
NN     12  22  1S1S  O   R1R1  BB   35    -

D5 A4 B4 M NF 11  12   X   11  1S   12     -  ZZ AA BB NNSZ + AA   X  AB 11  11   11   11
   X   11  12  1S1S  O    X    AA   35    -

5R 4R 4S M NF 12  22   X   11  11    X     -  ZZ BB BB   X  + AA  11  AA 11  11   11   12
NN     12  12  1S  2   O   R1LR  BB   35    -

I4 3Q 3R F NF 11  22  11   11  11   11     -  ZZ AB BB NNSS + AA   X  AB 11  11   12   11
NN     22  22  1S  2   A2  LRLR  AB   35    -

4V 3K 3L M NF  X  12  11   11  11   12     -  ZZ AA BB MNSZ + AA  12  AA 11  11   11   11
NN     11  11  1S  2   A1  LRR2  AA 1235   -

F4 3O 3P M NF 22  22  11   13  12   11     -  ZZ AB BB MNZZ - AA  12  AA 11  11   12   11
NN     22  12  1S  2   A1  LRR2  AA   35    -

M5 G4 H4 F NF 11  22  11   11  1S   11     -  ZZ AB BB MMSZ + AC  11  AB 11  11   12   12
NN     22  12 1S1F  A1  LRR2  AB 1235   -
```

```
5S 4R 4S M NF 22 22  11  11 11   11   - ZZ AB BB  X  + AA 11 AA 11  11  11  12
NN    11  12  2 2  O  R1LR  BB   35   -

H5 E4 F4 F NF 22 22  11  13 11   11   - ZZ AA BB MNSZ + AA 11 AA 11  11  22  12
NN    22  11 1S 2  A1  LRLR  AA   35   -

4Z 3M 3N F NN 12 12  13  11 11   11   - ZZ AA BB MNSZ + AA 11 BB 11  11  12  12
NN    11  11 1S 2  A1  R1R2  AA 1235   -

N5 G4 H4 F NN  X  X  13  12 1S   11   - ZZ AB BB MNSZ + AC 11 AA 11  11  12  12
NN    22  12 1S1S  O  LRR2  BB 1235   -

4W        F NN 11  X  11  11 11   12   - ZZ AB BB MMZZ + AA 12 AB 11  11  11  12
NN    11  11 1S 2  A1  R1R2  BB 1235   -

5T 4R 4S M NN 22 22  11  11 11   11   - ZZ AB BB  X  + AA 11 AA 11  11  11  12
NN    11  11  2 2  O  R1R1  BB   35   -

5X 4V 4W F NF 11 22  11  11 11   22   - ZZ AA BB MNZZ + AA 12 AB 11  11  11  12
NN    11  11  2 2  A1  R2R2  AB 1235   -

6J 5W 5X M NF 11 22  11  11 11   12   - ZZ  X BB MNZZ + AA 11 AB 11  11  11  11
NN    11  11  2 2  A1  LRR2  AB   35   -

B4        M NN 11 22  11  11 1S   12   - KZ AA BB NNZZ + AA 11 AA 11  11  11  11
NN    12  22 1S1F  O  R1LR  AB   35   -

5U 4T 4U M NN 11  X  11  11 12   11   - ZZ AA BB  X  + AA 11 AB 11  11  11  11
NL    12  12 1S 2  A1  R1LR  AB 1235   -

5W        M NN 11 22  11   X 11   12   - ZZ AA BB MNZZ + AA 11 BB 11  11  11  11
ND    12  12 1S 2  O  LRR2  BB   35   -

T5 I4 J4 F NN 11 22  11  11 11   11   - ZZ BB BB MNSS - AA 11 BB 11  11  11  11
NN    22  12 1S1F  O  R1LR  BB   35   -

5V 4T 4U F NN 11 22  11  11 12   12   - ZZ AB BB  X  + AC 11 AA 11  11  11  11
NL    12  12 1S 2  A1  R1LR  AA 1235   -

6K A5 B5 F NF 11  X  13  12 11   11   - KZ AB BB MNSZ + AA 11 AB 11  11  11  11
NN    12  11  2 2  A2  R1R2  BB   35   -

C5 4X 4Y M NN 11  X  13  11 11   12   - KZ AA BB MNZZ + AA 11 BB 11  11  11  11
NN    22  12  2 2  A2  R1R2  AB   35   -

H4        F NN 11 22  11  11 1S   11   - KZ AA BB MNZZ + AA 11 AB 11  11  11  12
NN    22  22 1S1F  O  LRLR  AB 1235   -

F5 A4 B4 F NF 12 22  11  11 11   11   - KZ AA BB NNSZ + AA 11 AB 11  11  12  11
NN    11  12 1F1F  A1  R1LR  AB   35   -

6Q F5 G5 F NN 22 22  11  11 11   11   - ZZ AA BB MNSZ + AA 11 BB 11  11  12  11
NN    12  12 1S1F  A1  R1LR  AB   35   -

6R F5 G5 F NN 11 12  11  11 11   11   - ZZ AA AB MNZZ + AA 11 AB 11  11  12  11
NN    12  22 1S1F  A1  R1LR  AB   35   -

6S F5 G5 F NN  X 12  11   X 12   11   - ZZ AB BB MNSS + AA 11 BB 11  11  11  11
NN    12  12 1S1F  O  LRLR  AB  135   -
```

```
6T F5 G5 F NF 11 12 11  11 12  11   - ZZ AB AB MNSZ +  AA  11  BB 11  11  12  11
NN    12 12 1S1F  A1  R1LR  AB  135   -

P5 I4 J4 M NN 11 22 11  11 11  11   - ZZ AB BB MNSS +  AA  11  AB 11  11  11  11
NN    22 12 1S 2  A2  R1LR  AB  35    -

6W K5 L5 F NN 11  X  X   X  11   X   - ZZ AA BB MMZZ +   X   X   X 11   X  11  12
X     12 12 1S1S  O   R1LR  AB   X    X

K5 G4 H4 M NF 11 22 11  11 1S  11   - ZZ AB BB MMZZ +  AA  11  AB 11  11  12  12
NN    12 12 1S 2  O   LRLR  AB  35    -

L5       F NN 11 12 11  11 12  11   - ZZ AB BB MNZZ +  AA  12  AB 11  12  11  12
NN    12 22 1S 2  O   R1LR  AB  135   -

S5 I4 J4 F NN 11 22 11   X 11  11   - ZZ AB BB MNSZ -  AA  11  BB 11  11  12  11
NN    22 12 1S 2  O   LRR2  AB  35    -

D4 3M 3N M NN 11 22 13  13 11  11   - ZZ AB BB NNSZ +  AA  11  AB 11  11  11  11
NN    11 11 1S1F  O   R1LR  AA  35    -

5Y 4V 4W F NN 11 11 11  11 11  12   - ZZ AB BB MNZZ +  AA  22  AA 11  11  11  12
NN    11 11 1S 2  O   R1R2  AB  35    -

5F 4K 4L F NF 11 22 11  12 12  22   - ZZ AB BB MNSZ -  AA  11  BB 11  11  11  11
NN    22 22 1S1S  O   LR Z  AB  135   -

5G 4K 4L M NF 12 22 11   X 22  12   - ZZ AB BB MMSZ -  AA  11  AB 11  11  12  12
NN    22 11 1S 2  O   R1R1  AA  135   -

5K 4M 4N M NF 11 22 11  11 12  11   - ZZ AA BB MMSS -  AA  12  AB 11  11  12  11
NN    12 22 1S1F  O   R1LR  AA  35    -

5M 4P 4Q F NN 12 12 11  12 11  11   + ZZ AB BB MMSZ -  AA  11  AA 11  11  11  11
NN    12 22 1S1F  A1  X     BB  35    -

4Q       F NN 12 22 11  11 1S  11   - ZZ BB BB MMSS +  AC  11  AB 11  11  11  11
NN    12 22 1S1F  A1  R1R1  BB  135   +

5O 4P 4Q M NN 11 22 11  12  X  11   - ZZ AB BB MMSZ -  AC  11  AB 11  11  11  12
X     12 22 1S1F  B   R1R1  AB  135   -

5N 4P 4Q M NN 11 12 11  12 11  11   + ZZ AB BB MMSZ +  AC  11  AB 11  11  11  11
NN    22 22 1F1F  B   R1R1  BB  135   -

L4       M NN 11  X 11  11 11  11   - ZZ AA AB MNZZ +  AA  11  BB 11  11  11  11
NN    22 12 1F 2  A1  X     AA  1     -

U5 K4 L4 M NN 12 12 11   X 11  11   - ZZ AB ABN NSZ +  AC  11  AB 11  11  11  11
NN    22 12 1S 2  A1  R1LR  AA   X    X

4N 3I 3J F NF 12 12 11  12 11  11   - ZZ AB BB MMSZ +  AA  11  BB 11  11  12  12
NN    22 12 1F 2  B   R1LR  AA  35    -

4M       M NN 12 12 11  12 12  11   - ZZ AB BB MMSZ +  AA  12  AA 11  11  11  12
X     11 22 1S1S  O   R1LR  AB  35    -

5Q 4P 4Q F NN 12 22 11  11 1S  12   - ZZ AB BB MMSZ -  AA  11  AA 12  11  11  12
ND    22 12 1S 2  A1  X     BB  1235  -
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6I 5O 5P F NN 12 22   11   22 12   12    - ZZ AB BB MMSZ - AA 11 AB 11 11 11 22
DD    12 12 1S1F  B   R1R2  AB 1235  -

W5 K4 L4 M NF 11 12   11    X  X   11    - ZZ AB BB MNSZ - AA 11 AB 11 11 11 11
NN    22 11 1S 2  A1   R1LR  AA   12  -

I5       M NN 11 22   11   12 11   11    - ZZ AB BB MMSS - AA 11 BB 11 11 11 11
NN    22 22 2 2   O   LRLR  AB  135  -

6U H5 I5 F NF 12 22   11    X 11   11    - ZZ AB BB MNSZ - AA 11 AB 11 11 12 11
NN    22 12 1S 2   O   LRLR  AB  135  -

6V H5 I5 F NN 12 22   11    X 11   11    - ZZ AB BB MNSZ + AA 11 AB 11 11 12 11
NN    22 12 2 2   A1   LRLR  AB  135  -

Q5 I4 J4 M NF 11  X   11   11 11   11    - ZZ AA BB MNSS + AA 11 BB 11 11 11 11
NN    22 12 1S 2  A2   LRR2  AB   35  -

R5       F NN 12 22   11    X 11   12    - ZZ AB BB NNZZ + AA 11 BB 11 11 11 12
NN    11 22 1S 2  A2   LRR2  AB   35  -

6X Q5 R5 M NF 12 22   11   12 11   11    - ZZ AB BB MNSZ + AA 11 BB 11 11 11 11
NN    12 22 1S1S A2   LRLR  AA   35  -

6H 5I 5J F NN 22 22   11    X 11   11    - KZ BB BB MMSS - AA 11 BB 11 11 12 12
NN    12 22 1S 2   O   R1R1  BB   35  -

6G 5I 5J F NF 12 12   11    X 11   12    - KZ BB BB MMSS + AA 11 AB 12 11 11 22
NN    12 22 1S 2   O   R1LR  AB   35  -

6O D5 E5 F NN 12 12   11   11 11   11    - ZZ AA BB MNSS +  X 11 AB 11 11 11 11
 X    12 12 1S1S   O   R1LR  AB    X  X

6P D5 E5 M NN 12 22   11   11 11   12    - ZZ AB BB MNSS +  X 11 AA 12 11 11 11
 X    12 12 1S1S   O   R1LR  AB    X  X

6L D5 E5 M NN 12 12   11   11 11   12    - ZZ AB BB MNSZ - AA 11 AA 12 11 11 11
NN    12 12 1S1S   O   R1LR  AB   35  -

5I       F NN 12 22   11    X 12   12    - KZ BB BB MMSZ + AA 11 AB 12 12 11 12
NN    12 22 2 2   O   R1LR  BB   35  -

J5 G4 H4 M NF 11 22   11    X  X   11    - ZZ AA BB MMSZ + AC 11 AB 11 11 12 12
NN    12 12 1F 2   O   LRR2  BB   35  -

O5 G4 H4 M NF 11 22   13   11 2S   11    - ZZ AB BB MMZZ + AA 11 AA 11 11 11 11
NN    12 12 1S 2   O   LRLR  BB   35  -

6B 5B 5C M NN 11 12   11   11 11   11    - ZZ BB BB MNSZ + AA 22 AB 12 11 11 11
NN    12 22 1S1S   O   LRLR  BB   35  -

5C       F NN 11 12   11   11 12   11    - ZZ BB BB MNSZ + AA 12 AB 11 11 11 11
NN    12 22 1S1S   O   R1LR  AB   35  -

4G 3E 3F F NF 11 22   11   11 11   11    - ZZ AB BB MNSZ + AA 12 AB 12 11 12 12
NN    22 12 1S1S  A2   R1LR  BB   35  -

5B 4G 4H M NF 11 22   11   12 11   11    - ZZ AB BB MNSZ + AA 22 AB 12 11 12 11
NN    22 22 1S1S   O   LRLR  AB   35  -
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6A 5B 5C F NF 11 22  11  11 11   11   - ZZ AB X MNSZ + AA  22 AB 12  11  11  11
NN   12  22 1S1S  O  R1LR  AA   35   -

3G 2A 2B F NF 12 22  11   X 12   11   - ZZ AB X MMZZ - AA  11 BB 11  11  11  11
NN   12  11 1S1S  A2 LRR2  BB  135   -

4B 3A 3B M NF 11 22  11  11 11    X   - ZZ BB X  X   - AA   X BB 11  12  12  11
NN   22  22 1S 2   O  R1R2  AB   35   -

5D 4G 4H M NF 12 22  11  11 12    X   - ZZ BB X NNZZ - AA  22 BB 12  11  12  12
NN   22  22 1S1S  O  R1LR  AB   35   -

4H       M NN 12 22  11  11 12   11   - ZZ BB X MNSZ + AA   X AB 11  11  11  11
NN   22  22 1S1S  O  R1LR  AA   35   -

5E 4G 4H F NN 12 22  11   X 12   11   - ZZ AB X MNSZ - AA  11 AB 12  11  11  11
NN   22  12 1S1S  O  R1LR  AB   35   -

4J 3G 3H F NN 22 22  11  12 11   11   - ZZ AB X MMSZ + AA  11 BB 11  11  11  12
NN   22  12 1S1S  O  LR Z  AB   35   -

4A 3A 3B M NN 11 22  11  11 11   11   - ZZ AB X  X   + AA  22 BB 11  11  12  11
NN   22  22 1S 2   O  R1R2  BB   35   +

4F 3E 3F M NN 11 22  13  11 11   11   - ZZ AB X MMSZ + AA  11 AB 12  11  12  12
NN   12  12 1S1S  O  R1LR  AA 1235   +

4I 3G 3H M NN 11 22  11  11 22   11   - ZZ AB X MMSZ + AA  11 BB 11  11  11  11
NN   12  11 1S1S  O  LRLR  AB  135   +

5A 4B 4C M NN 11 22  11  11 11   12   - ZZ BB X  X   + AA  11 AB 11   X  11  11
NN   22  22 1S1S  O  R1R1  AB   35   -

5L 4M 4N M NN 11 22  11  12 12   11   - ZZ AA BB MMZZ + AA  11 AB 11  11  12  22
NN   12  22 1S 2   B  LRLR  AB    X   X

5J 4M 4N M NF 12 12  11   X 11   11   - ZZ BB BB MMSS - AA  11 AB 11  11  12  22
NN   12  12 1S 2   O  R1R1  AB   35   -

4D 3C 3D M NN 11 22  11  11 11   11   - ZZ BB BB MNZZ - AA  11 AB 11  11  11  11
NN   12  12 1S1S  O  R1LR  AB 1235   +

4O 3I 3J F NN 11 22  11  12 12   12   - ZZ AB BB MNSS + AA  11 BB 11  11  12  11
NN   22  12 1S1S  O  R1LR  AA    X   X

4E 3C 3D M NN 11 12  11  11 22   12   - ZZ BB BB NNSZ - AA  11 AA 11  11  11  11
NN   12  12 1S1F  O  R1R2  AB   35   -

X5 M4 N4 F NF 22 12  11  11 12   11   - ZZ AB BB  X   + AA  11 AB 11  11  11  12
NN   12  12 1S1S  O  LR Z  AB  135   +
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ADDITIONS TO THE EXCLUSION MAP OF MAN

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ADDITIONS TO THE EXCLUSION MAP OF MAN

J.C. MULLEY [1], G.D. BRYANT [2],
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──── SUMMARY ────

Exclusion mapping was applied to individuals with mono-somic segments defined by chromosomal banding. A range of genetic markers and blood groups was determined resulting in new exclusions for unassigned markers at the following segments : (3)(p25→pter) - JK, GPT, PI ; (4)(q27→31) - MNS, JK, PI, C3, F13A, F13B ; (7)(q22) - LU, F13A ; (12)(p12) - MNS ; (12)(p13) and (15)(q15) - GPT, C3 ; (12) (pter) and (19)(p or qter) - MNS, GPT, PI ; and (18)(q21→23) - MNS, JK, F13A. These exclusions may be useful for narrowing the regional localisations of any genetic markers subsequently assigned to the chromosome involved in the exclusion. No new gene assignments were made from cases where family data was available.

──── RÉSUMÉ ────

Suppléments à la carte génique humaine par exclusions
La technique d'exclusion a été appliquée pour l'obtention de la carte génique chez des individus monosomiques partiels. Une série de marqueurs génétiques et de groupes sanguins ont été déterminés et permettent de nouvelles exclusions pour des marqueurs non encore localisés aux segments suivants : (3)(p25→pter) - JK, GPT, PI ; (4)(q27→31) - MNS, JK, PI, C3, F13A, F13B ; (7)(q22) - LU, F13A ; (12)(p12) - MNS ; (12)(p13) et (15)(q15) - GPT, C3 ; (12) (pter) et (19)(p or qter) - MNS, GPT, PI ; et (18)(q21→23) - MNS, JK, F13A. Ces conclusions peuvent être utiles pour préciser les localisations des marqueurs ultérieurement localisés sur des chromosomes intéressés par l'exclusion. Aucune localisation nouvelle n'a pu être obtenue à partir des cas pour lesquels des données familiales étaient disponibles.

Gene mapping by exclusion is applicable to cases having unbalanced chromosomal rearrangements with defined monosomic segments. The exclusion of codominant loci provides supplementary mapping data to that derived from somatic cell hybridisation and segregation analysis. Test loci are excluded when an individual with a deletion is heterozygous. Gene assignments are indicated when obligate heterozygotes (on the basis of parental phenotypes) are shown to be hemizygous. Possible misclassification, the presence of null or silent alleles and incorrect paternity require careful investigation for the confirmation of assignments by deletion mapping. While deletions can be useful for narrowing regional localisations within chromosomes, the technique is relatively inefficient for gene assignment. Exclusion data is readily interpretable and considerable information has been obtained since the advent of chromosomal banding (Aitken et al., 1975 [1] ; Aitken and Ferguson-Smith, 1978 [2]).

MATERIALS AND METHODS

Genetic markers were examined from patients with deleted autosomal segments (3)(p25→pter), (4)(q27→31), (7)(q22), (12)(p12), (12)(pter), (12) (p13), (15)(q15), (18)(p11), (18) (q21→23) and (19)(p or qter) and a fetus with deleted (5)(p14→pter).

The (3)(p25→pter) deletion resulted from a familial pericentric inversion inv(3)(p25q23). Apart from the ten cases of autosomal deletion, another

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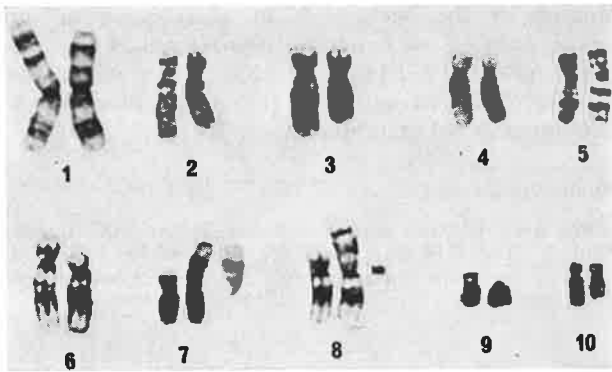
Fig. 1. — Partial karyotypes from 10 cases with autosomal deletions as shown in table I. Full karyotypes are as follows :
1. 46,XY,rec(3)dup(q)del(p)inv(3)(p25q23)pat., 2. and 3. 46,XY,del(4)(q27q31). 4. 46,XY,del(5)(p14), 5. 46,XY,inv(7)(q11q22)del(7)(q22q22), 6. 46,XY,del(12)(p11p13), 7. 45,XY,t(12;15)(p13;q15), 8. 45,XX, ter rea (12;19)(p13;p or q13), 9. 46,XX,del(18)(p11), 10. 46,XX,del(18) (q21q23).

family included a female proposita with del(X)(p11p22). Deletions were delineated by trypsin-Leishman G-banding and partial karyotypes for the autosomal deletions are given in figure 1.

Samples of serum, plasma, red cells or fibroblasts where available were stored and subsequently typed by standard blood grouping and electrophoretic procedures. *PI* was completely subtyped by isoelectric focusing (Mulley, 1980 [11]) and *F13A* and *F13B* typed by agarose gel electrophoresis (Board, 1979, 1980 [3]). Locus designations follow the recommendations of Shows et al. (1979) [13].

RESULTS

The results of autosomal genetic marker studies are presented in table I. No gene assignments were made. New exclusions or extensions to existing exclusions were determined for the unassigned loci *JK, MNS, LU, GPT, PI, FI3A, FI3B* and *C3*. These may prove useful for regional localisation if subsequent gene assignment is made to the chromosome involved in the exclusion. Although new exclusions were determined for the assigned markers *JK, RH, FY, PGM1, PGM3, AK1, ACP1, GLO1, PGP, ESD* and *HPA*, these were irrelevant for regional localisation as all are assigned to other autosomes.

The *XG* result from the family of the propositus with del(X)(p11→p22) indicated anomalous inheritance involving the proposita (fig. 2). Paternity testing (apart from *XG*) indicated that the probability of correct paternity was 97 %. This family either suggests the localisation of *XG* to (X)(p11→p22), or demonstrates inactivation of the paternal *XG* allele present on the deleted chromosome.

TABLE I. — List of exclusions for both assigned and unassigned loci from ten cases with autosomal deletions.

Patient n°	Segment deleted	Assigned Loci	Unassigned Loci
1	(3) (p25→pter)	RH, PGM1, PGP, ACP1, PGM3, HPA	MNS*, JK, GPT, PI
2	(4) (q27→31)	JK, PGM3, HPA	F13B
3	(4) (q27→31)	RH, ESD, PGM1	MNS, JK, PI, C3, F13A
4	(5) (p14→pter)	AK1, PGM3	MNS *, LU,
5	(7) (q22)	ACP1, PGM1	GPT *, F13A
6	(12) (p12)	RH, FY, GLO1, PGP	MNS
7	(12) (p13) and (15) (q15)	PGM1, PGM3, ESD, HPA	GPT, C3
8	(12) (pter) and (19) (p or qter)	ACP1, GLO1, ESD, HPA	MNS, GPT, PI
9	(18) (p11)	HPA*	
10	(18) (q21→23)	HPA*	MNS, JK, F13A

* Previously excluded from the segment investigated (Aitken et al., 1975 ; Aitken and Ferguson-Smith, 1978).

DISCUSSION

The localisation of *ACP1* to the short arm of chromosome 2 (Ferguson-Smith et al., 1973 [7]) remains the only locus to be assigned by deletion mapping. Meanwhile, other unassigned loci have been eliminated from significant regions of the genome : *MNS* being the most notable (Aitken and Ferguson-Smith, 1978 [2]). Additional portions of the genome in the vicinity of assigned markers may be excluded by segregation analysis when the absence of linkage is demonstrated between the unassigned markers and markers previously localised. Recent examples of regional localisations arising from exclusion mapping are *HPA* to (16)(cent→q22) (Ferguson-Smith and Aitken, 1978 [8], *ACP1* to the distal
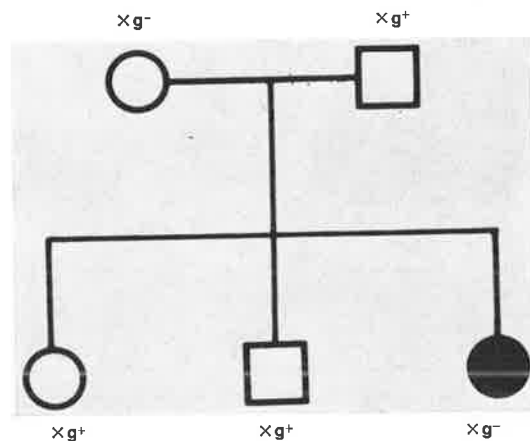
Fig. 2. — Pedigree showing anomalous XG inheritance in the proposita with caryotype 46,X,del(X)(p11p22).

portion of band (2)(p23) (Emanuel et al., 1979 [6]) and *ESD* to (13)(q31) or (13)(q32) pending confirmation of a previous assignment to (13)(q3) (Turleau et al., 1978 [14]).

XG is not normally inactivated (Fialkow, 1970 [9] ; Fialkow et al., 1970 [10] ; Ducos et al., 1971 [5] unlike other studied X-linked loci which are subject to random inactivation. On this basis, *XG* is apparently localised to (X)(p11→p22) given the anomalous inheritance ; but this conclusion is invalidated by the occurrence of inactivation of *XG* alleles carried on structurally abnormal X chromosomes (Race, 1971 [12]). The alternative explanation for the observed result is the inactivation of the abnormal X of paternal origin.

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FURTHER EXCLUSIONS BY DELETION MAPPING

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FURTHER EXCLUSIONS BY DELETION MAPPING

J.C. MULLEY, G.R. SUTHERLAND

SUMMARY : Exclusions for unassigned markers were determined by deletion mapping at the following segments : (4)(p15.1→16.1) - *F13A,, F13B, TF* ; (4)(q27→31) - *TF* ; (9)(p22→pter) - *F13B* ; (12)(p12) - *TF* ; (12)(pter) · *TF* ; (18)(q22) - *F13A, K* and *LU* ; (19)(p or qter) - *TF* ; (22)(pter→q11) - *F13B. GC* was excluded from (4)(p15.1→16.1) and (4)(q27→31). *GALT* was excluded from (9)(p22→pter).

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RÉSUMÉ : Les exclusions suivantes pour les localisations des marqueurs génétiques non encore localisés ont été déterminées par la méthode des délétions : (4)(p15.1→16.1) - *F13A, F13B, TF* ; (4)(q27→31) - *TF* ; (9)(p22→pter) - *F13B* ; (12)(p12) - *TF* ; (12)(pter) - *TF* ; (18)(q22) - *F13A, K* et *LU* ; (19)(p ou qter) - *TF* ; (22)(pter→q11) - *F13B. GC* a été exclu de (4)(p.15.1→16.1) et de (4)(q27→31). *GALT* a été exclu de (9)(p22→pter).

Further exclusions arising from deletion mapping are presented. These were derived from patients with monosomic segments defined by trypsin-Leishman G-banding or BrdU replication banding. The detection of two alleles implies heterozygosity and clearly excludes that locus from the defined monosomic segment in the corresponding individual.

MATERIALS AND METHODS

A large range of genetic markers were examined from individuals with the deleted autosomal segments listed in table I. All deletions examined were spontaneous in origin except the deletion in patient 1 resulting from a familial pericentric inversion inv(3)(p25q23) (Sutherland, Mulley and Goldblatt, 1981) and the deletions in patients 7 and 8 resulting from familial translocations t(9;22)(p22;q11) and t(10;18)(q26;q21) respectively. Partial karyotypes for patients 1, 3, 4, 6, 9, 10 and 12 in table I were given previously (Mulley et al., 1980) and for patients 2, 5, 7, 8, 11 and 13 are shown in figure 1. Isoelectric focusing was used to subtype GC 1 into 1S and 1F, PI M into M1, M2 and M3 and TF C into C1, C2 and C3. No GM results were presented for infants younger than six months of age.

RESULTS AND DISCUSSION

The results of genetic marker studies are given in table I. *F13A, F13B, TF, K* and *LU* are unassigned, although *TF* may be located on chromosome 3 (Oslo Conference, 1981). The exclusions shown for these unassigned markers, and markers known to be syntenic with monosomic segments, are potentially valuable for regional localisation. New exclusions are : *F13A* from (4)(p15.1→16.1), (18)(q22); *F13B* from (4)(p15.1→16.1), (9)(p22→pter), (22)(pter→q11) ; *TF* from (4)(p15.1→16.1), (4)(q27→31), (12)(p12), (12)(pter), (19)(p or qter) ; *K* and *LU* from (18)(q22) (table I). Recent gene assignments have been *JK* to chromosome 2, *MNS* to chromosome 4, *GM* and *PI* to chromosome 14, *GPT* to chromosome 16 and *C3* to chromosome 19 (Oslo Conference, 1981). The numerous exclusions given for these loci (table I) are trivial if assignments for these loci are correct.

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TABLE I. — **Exclusions determined by deletion mapping.**

Patient No.	Segment deleted	Locus excluded
1	(3)(p25→pter)	GC
2	(4)(p15.1→16.1)	F13A, F13B, GC, PI, TF, GALT, PGM1, PGP
3	(4)(q27→31)	GC, GPT
4	(4)(q27→31)	TF
5	(5)(p13)	PI, ADA, ESD, PGM1
6	(7)(q22)	GC
7	(9)(p22→pter) and (22)(pter→q11)	C3, F13B, GC, PI, ACP1, ADA, GALT, GLO, PGM1, PGP
8	(10)(q26)	HPA*, PI, AK1, ESD, GLO1, PGP
9	(12)(p12)	GM, TF
10	(12)(pter) and (19)(p or qter)	TF
11	(18)(p11)	C3, GC*, AK1, GLO1, PGM1*
12	(18)(q21→23)	GM
13	(18)(q22)	F13A, FY*, JK*, K, LU, MNS*, RH, GC*, ACP1*, GALT, PGM1*

* Previously excluded from the segment investigated (Aitken et al., 1975 [1]).
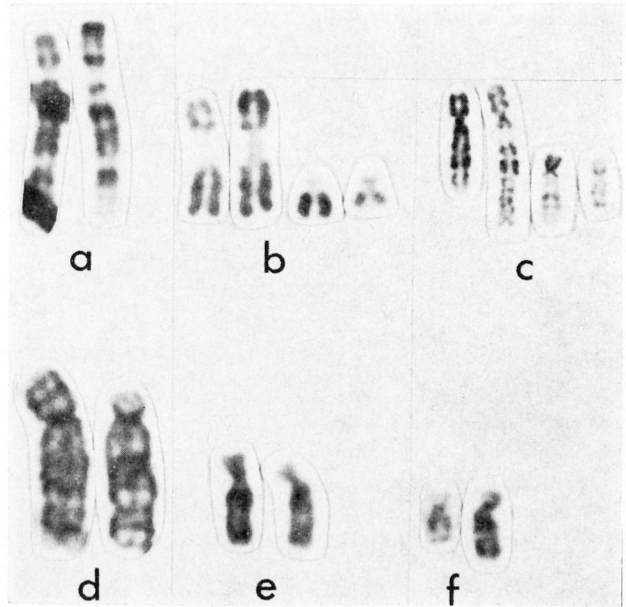
Fig. 1. — **Partial karyotypes from patients 2, 5, 7, 8, 11 and 13. BrdU banded karyotypes : (a) patient 2, del(4)(p15.1→p16.1) ; (b) balanced form of t(9;22)(p22;q11), patient 7 had karyotype 45,XY, −9,−22,+der(9)t(9;22)(p22;q11) ; (c) balanced form of t(10;18)(q26;q21), patient 8 had karyotype 46,XY,der(10)t(10;18)(q26;q21) ; and G-banded karyotypes ; (d) patient 5, del(5)(p13) ; (e) patient 11, del(18)(p11) and (f) patient 13, del(18)(q22).**

GC and *GALT* are each excluded from portions of the chromosome to which they are assigned. *GC* is excluded from (4)(p15.1→16.1) and (4)(q27→31) (table I) which is compatible with the regional assignment of *GC* to (4)(q11→13). *GALT* is excluded from (9)(p22→pter) (table I) which is compatible with the regional localisation of *GALT* to (9) (p13) and tight linkage between *GALT* and 9qh variants with a lod score of 3.67 at θ = 0 (Sparkes et al., 1980). Close linkage of *GALT* to the centromere is further supported in « family 2 » of Sutherland and Eyre (1981) which is segregating for an

unusual G-band variant near be centromere of the short arm of chromosome 9. Portion of this pedigree is informative giving a positive lod score of 0.90 at θ = 0, (z_1 4:0), resulting in a total positive lod score of 4.57 at θ = 0.

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Regional Localization for *HLA* by Recombination with a Fragile Site at 6p23

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SUMMARY

A family with a fragile site on chromosome 6 at band p23 was examined for recombination between the fragile site and *HLA*. Recombination was observed in four of the 20 offspring in whom it could occur. The estimate of the genetic length of chromosome between the fragile site and *HLA* is 20 centimorgans (cM) with a lower 95% probability limit of 8.5 cM, placing *HLA* proximal to the midpoint of 6p22. The most likely regional localization is at 6p21.3, which agrees closely with methods that do not involve recombination with the fragile site. This fragile site does not measurably disrupt recombination frequency, and the allele predisposing to expression of the fragile site is situated at the fragile site.

INTRODUCTION

A family segregating for a fragile site in the distal region of band p23 on chromosome 6 provided an opportunity to localize *HLA* by measuring the recombination rate between *HLA* and the fragile site. The regional localization for *HLA* on chromosome 6 between p21.2 and p23 has been well established [1], and there is strong evidence for its elimination from the distal portion of this interval [2–6]. Given a proximal limit for *HLA* at 6p21.2, the distance between *HLA* and the fragile site must be less than 25 map units according to the male meiotic map presented by Cook et al. [7]. Hence, linkage between *HLA* and this fragile site can be assumed.

MATERIALS AND METHODS

Part of this family and the fragile site at 6p23 have been previously described using standard procedures for detection of folate-sensitive fragile sites [8]. At least 50 metaphases were routinely scored for the presence of fragile sites. *HLA* haplotypes at the A, B, and C loci were determined by the microcytotoxicity test. Chromosome breakpoints defined using different nomenclature systems have been standardized to the ISCN system [9]. It is assumed that the relationship between map distance and recombination frequency is approximately linear up to $\theta = .25$, and that recombination is often greater in females than in males [10].

RESULTS

Four generations were available for study (fig. 1). The fragile site at 6p23 was detected without difficulty in every generation. Eight carriers were detected, and one untested individual (II.11) was an obligate carrier. It is highly improbable that the other untested, unrelated individuals (I.3, II.4, II.12) would carry the fragile site since this is the only family in which this fragile site has been recorded. The frequency of expression was 44% for the propositus (II.13), and ranged from 7% to 30% of cells for the other carriers. For the purpose of linkage analysis, the fragile site in this family may be regarded as a heritable codominant variant.

The inheritance of the fragile site and *HLA* haplotypes are shown in figure 1. *HLA* haplotypes have been coded as indicated in table 1. The HLA phenotypes could be inferred in the untested individuals as 7/8 in I.3, 6/? in II.4, 2/8 in II.11, and 11/12 in II.12. There were three nonrecombinants (NR) between *HLA* and the fragile site in generation IV. There were seven NR and two recombinants (R) in generation III. Given prior knowledge of linkage, it is highly probable that there are six NR and two R in generation II rather than six R and two NR.

The combined male and female recombination frequency (θ) is 20% (4/20), well within the maximum expected male recombination frequency of less than
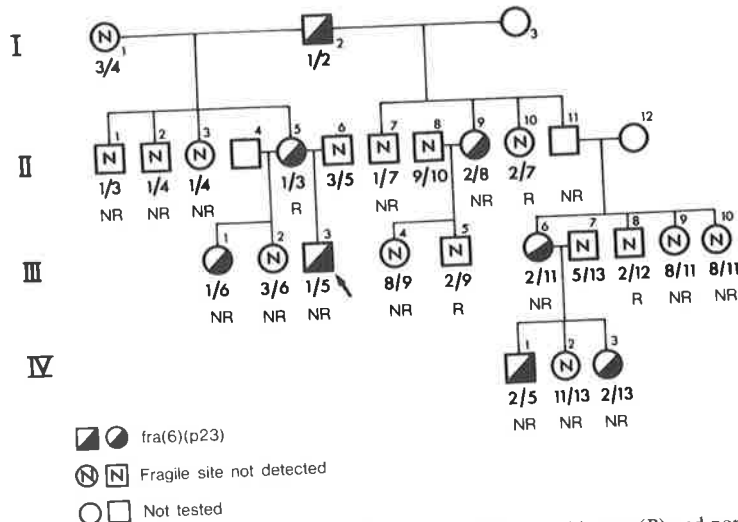
FIG. 1.—Inheritance of *HLA* and the fragile site at 6p23 with recombinants (*R*) and nonrecombinants (*NR*) indicated. See text for probable phenotypes of untested individuals.

TABLE 1

HLA HAPLOTYPE CODE

Code	Haplotype
1	Aw31, Bw57, Bw4, Cw6
2	A1, Bw49, Bw4
3	A1, B8, Bw6
4	Aw32, B27, Bw4, Cw1
5	A2, Bw44, Bw4, Cw5
6	A11, Bw22, Bw6, Cw3
7	A29, B7, Cw6
8	A3, B7, Bw6
9	A26, Bw41, Bw6
10	Aw33, B14
11	Aw30, B18, Bw6, Cw5
12	A2, Bw35, Bw6, Cw4
13	A28, B27

25%. The maximum lod score was 1.7 at $\theta = 0.2$. The recombination frequency is equivalent to map distance expressed as cM because of the approximate linear relationship between map distance and θ up to $\theta = .25$. The lower 95% probability limit for this interval is 8.5 cM and was determined by subtracting 5% of the area under the truncated relative probability curve from one end of the curve. The curve was truncated at 6p21.2. Given that recombination frequency is often greater in females than in males, the estimate of 20 cM for the interval between HLA and the fragile site at 6p23 is possibly an overestimate for comparison with a map expressed in male cM. However, in this family, only one out of eight offspring of informative females were recombinants, compared to three out of 12 from informative males. These results suggest a probable regional localization for HLA between about the midpoint of 6p22 (near to 6p22.2) and the previously known proximal limit for HLA at 6p21.2. HLA is not tightly linked to the fragile site and is unlikely to lie within 6p23 or the distal half of 6p22. The most likely location of HLA is at 6p21.3 (fig. 2).

DISCUSSION

Considerable information already exists for the regional localization of HLA. Family study with a translocation t(6;21)(p22;q11) suggested the probable localization of HLA proximal to 6p22 [2]. A translocation family with t(6;20)(p21;p13) demonstrated close linkage ($\theta = .05$) between the breakpoint (in 6p21 near 6p22) and HLA [3]. While the effect of reciprocal translocations on recombination is not definitely known, there is unlikely to be any major disruption [11]. The absence of a duplicated set of parental HLA antigens in a child partially trisomic for 6p22.2→pter derived from a balanced translocation t(6;10)(p22.2;pter) excluded HLA from 6p22.2→pter [5]. Subsequently, HLA was localized to 6p21 [6]. Berger et al. ([4] and personal communication, 1982) proposed a more precise localization for HLA, suggesting that a chromosomal break at 6p21.2 or at the interface of 6p21.2 and 6p21.3 was within the HLA cluster. These data agree closely with the most likely position for HLA determined by recombination with the fragile site.
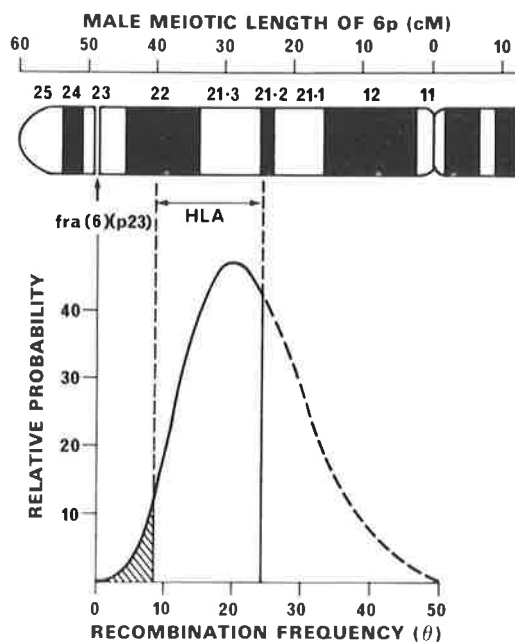
FIG. 2.—Probable localization of *HLA* by recombination with the fragile site at 6p23

The intervals between the fragile sites at 10q23 and 10q25 in families with both fragile sites [12], and now between *HLA* and the fragile site at 6p23, have been estimated by recombination and fall within limits established by other procedures. Consequently, fragile sites do not appear to disrupt recombination in chromosomal segments near the loci of expression and thus are valid markers for linkage analysis giving unbiased recombination values. Hence, the distance between *HP* and the fragile site at 16q22 as estimated by recombination between them [13] is probably a true indication of their distance apart.

The alleles responsible for the expression of fragile sites are probably situated on the chromosomes at or very near to the fragile site. This had been verified for the fragile sites at 10q23, 10q25 [12], and now at 6p23. The interval between genes responsible for the expression of fragile sites at 10p23 and 10q25 was estimated by recombination and is consistent with that expected from their locations on mitotic chromosomes. The positive lod score between *HLA* and the fragile site at 6p23 is consistent with existing knowledge of the location of *HLA* and the position of the fragile site at 6p23. Control of fragile site expression must be within the homolog expressing the fragile site since confirmed heterozygous carriers do not express fragile sites in both homologs.

The rapid rate of discovery of fragile sites [8] indicates that many more of these are likely to exist and be used for linkage studies. Fragile sites have advantages over other chromosomal variants because they are not restricted to paracentromeric regions. They are precisely mapped by banding and are ideal both for use in searching for linkages with unassigned markers in chromosome regions devoid of polymorphic genetic markers and for the regional localization of markers

shown to be near regions of fragile site expression. The fragile site at 6p23 confirms a more exact regional localization for *HLA* and, consequently, the cluster of genes that are known to be linked to *HLA*. Confirmation that the location of DNA responsible for a fragile site is at the locus of expression may now permit the characterization of this DNA by study of the relevant chromosomal segments.

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CASE REPORT

A TRANSIENT CATHODAL PI PHENOTYPE IN AN INFANT WITH MILD PERSISTENT JAUNDICE

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SUMMARY

Polyacrylamide gel isoelectric focusing revealed a transient cathodal alpha-1-antitrypsin phenotype in a two-month-old infant with mild persistent jaundice. After recovery the phenotype reverted to PI M1. The transient phenotype was probably an asialo form of alpha-1-antitrypsin associated with mild liver disease and jaundice.

KEY WORDS   Alpha-1-antitrypsin   Jaundice   Non-genetic variation   Transient variation

INTRODUCTION

Variation between individuals for serum levels of alpha-1-antitrypsin (α_1AT) is controlled by the *PI* locus. The *PI* locus has at least 32 alleles (Cox *et al.*, 1980). Levels fluctuate within individuals as α_1AT is an acute-phase reactant. The genetic variation at this locus was first demonstrated by electrophoresis (Fagerhol and Braend, 1965; Fagerhol and Laurell, 1967), and is now usually characterized by isoelectric focusing in polyacrylamide gels. Severe α_1AT deficiency is genetic in origin and was initially recognized in the sera of adults with emphysema (Laurell and Eriksson, 1963). Subsequently an association was established between severe α_1AT deficiency in infants and children with cirrhosis of the liver (Sharp *et al.*, 1969).

A transient non-genetic variant of α_1AT was observed in an infant with mild persistent jaundice. This variant was extremely cathodal. A similar variant has been reported in an infant with cytomegalovirus and fatty liver (Hug *et al.*, 1982).

METHODS

Alpha-1-antitrypsin was quantitated by radial immunodiffusion (Mancini *et al.*, 1965). PI was phenotyped by polyacrylamide gel isoelectric focusing (PAGIF) as described previously (Mulley, 1980). Print immunofixation was done using human α_1AT antisera (Behring, F.R.G.). Sialic acid was removed from α_1AT with neuraminidase (Sigma N-2876, St. Louis, MO) by three
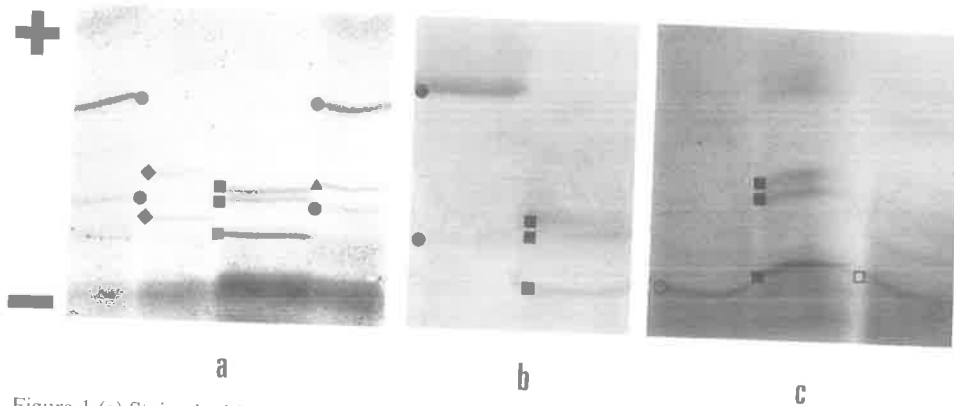
Figure 1.(a) Stained with Coomassie blue R, phenotypes (l to r): MI ●; Z ◆; transient ■; MIS ▲
(b) Print immunofixation, MI ●; transient ■
(c) Desialated normal MI ○; transient ■; desialated transient □

additions of 12·5 IU to 100 µl of serum at 0, 8, and 24 h with incubation at 37°C for a total of 48 h.

CASE REPORT

The index case was a full-term female neonate (birth weight 3300 g) who had mild birth asphyxia (Apgars 5, 7). She was discharged six days after birth fully breast-fed and gaining weight. Forty days after birth she presented with mild persistent jaundice (weight 4950 g). The liver was only 0·5 cm below the costal margin. Fifty-one days after birth the total serum bilirubin was 128 µmol/l, direct 15 µmol/l. Alkaline phosphatase was slightly raised. Albumin and total protein were normal. α_1AT (2 g/l) was at the lower end of the normal range (2–4 g/l). Routine plasma protein electrophoresis on cellulose acetate detected blurring in the cathodal portion of the α_1-globulin region. PAGIF demonstrated the unusual α_1AT pattern shown in Figure 1a. Eighty-eight days after birth the child was well and liver function tests were normal. α_1AT was 2·5 g/l and associated with a normal PI M1 phenotype.

RESULTS

The unusual phenotype determined by PAGIF at day 51 was confirmed as an α_1AT-related protein by print immunofixation (Figure 1b). A normal M1 phenotype determined 88 days after birth was compatible with the maternal M1M3 phenotype. All traces of the transient bands had disappeared.

Neuraminidase treatment of serum suggested an explanation for the transient banding. Desialation of the serum with the transient phenotype, and normal M1 sera, converted both phenotypes into a band corresponding to the most cathodal and most prominent component of the untreated transient variant (Figure 1c). The transient variant is probably a mixture of partially and completely unsialated α_1AT.

DISCUSSION

The variant described is a non-heritable transient type similar to the other cathodal transient variant which has been described (Hug *et al.*, 1982). Transient α_1AT phenotypes are common in association with liver disease, but in an anodal position. Anodal shifts have also been associated with disseminated intravascular coagulation (Inokuma, 1980). Possible reasons proposed for *in vivo* transient cathodal variation are post ribosomal changes in the sialic acid content associated with liver disease, or that another α_1AT locus not normally expressed is switched on by the stress of liver disease (Hug *et al.*, 1982). The transient variant in Figure 1 was probably a mixture of asialo forms of α_1AT associated with mild liver disease and jaundice. However, this pattern is not normally observed in infants with hyperbilirubinemia.

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A LARGE KINDRED WITH AN INV(3)(p25q23) : CLINICAL, CYTOGENETIC AND GENETIC MARKER STUDIES

G.R. SUTHERLAND, J.C. MULLEY, E. GOLDBLATT

A LARGE KINDRED WITH AN INV(3)(p25q23) : CLINICAL, CYTOGENETIC AND GENETIC MARKER STUDIES

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SUMMARY : A large kindred in which an inv(3)(p25q23) is segregating is described. At least two malformed children with the recombinant chromosome rec(3)dup(q23→qter)del(p25→pter) have been produced, both of whom have the characteristic trisomy 3q syndrome. Genetic marker studies showed that **PI** and **GM** are not linked to the inversion break points. The **PI-GM** linkage group has been excluded from much of chromosome 3 by deletion mapping and linkage analysis of this kindred.

RÉSUMÉ : Les auteurs décrivent une grande famille dans laquelle ségrègue une inv(3)(q25q23). Au moins deux enfants malformés avec le chromosome recombiné rec(3) dup(q23→qter)del(p25→ter) sont nés l'un et l'autre avec le phénotype caractéristique de la trisomie 3q. Une étude des marqueurs génétiques a montré que **PI** et **GM** ne sont pas liés au point de cassure de l'inversion. Le **PI-GM** groupe de linkage a été exclu d'une grande partie du chromosome 3 par une analyse de linkage de l'utilisation des délétions dans cette famille.

INTRODUCTION

A number of large pericentric inversions of chromosome 3 have been ascertained via malformed children with recombinant chromosomes (Hirschhorn et al., 1973 ; Fineman et al., 1978 ; Rivera et al., 1979 ; Kawashima and Maruyana, 1979). One large kindred has been described in which such an inversion has resulted in more than 20 recombinants or presumed recombinants (Allderdice et al., 1975). The present report documents another large kindred which was ascertained via a recombinant child in South Australia in 1978. This kindred was independently ascertained in Western Australia and the phenotype of the recombinant described (Mulcahy et al., 1979). The clinical features of the South Australian propositus are recorded, and cytogenetic and genetic marker studies of the family presented.

CASE REPORT

The propositus was the second child born to healthy unrelated parents. Delivery was by caesarean section at 38 weeks gestation and birth weight was 3,01 kg. The appearance was unusual (fig. 1) and the following features were noted on examination : slanting, curved palpebral fissures, an unusual nose, high arched palate, micrognathia, odd ears with pits on the helices, redundant skin folds on the back of the neck, inverted nipples, bilateral simian creases, right talipes calcaneo-valgus, left ectopic and right undescended testes. Investigations at the age of three months showed a hypertonic severely retarded infant, with a clinically diagnosed ventricular septal defect and probably atrial septal defect. Intravenous pyelography showed a dysplastic right kidney with dilated ureter. Radiography revealed retarded skeletal maturity, closed or closing sagittal suture, scapho-

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Fig. 1. — The propositus.

cephaly, mild cervical and sacrococcygeal kyphosis, hypoplastic third right rib and a mildly enlarged heart. The child died in another hospital at 22 months of age; an autopsy was not performed.

Cytogenetics

G-banded chromosome studies (fig. 2) showed that the propositus had a recombinant chromosome rec(3)dup(q23→ qter)del(p25→pter) resulting from a paternal inv(3)(p25q23).

Family studies

Family studies (fig. 3) showed that the inv(3) chromosome was present in many other members of the family. A number of stillbirths and neonatal deaths had occurred, but records were inadequate to indicate whether or not these were due to recombinant chromosomes.

Genetic marker studies

The blood groups LU, MNS, JK, ABO, RH, FY, K (two loci) and P, and enzyme and protein polymorphisms GALT, GPT, PGP, ACP1, AK1, ADA, GLO1, PGD, ESD, PGM1, HPA, E2, AMY2 and GM were determined using standard serological and electrophoretic techniques. Genetic marker results from the propositus and the GM results from infants aged less than six months were not used for linkage analysis. PI was completely subtyped by isoelectric focusing (Mulley, 1980) and F13A and F13B typed by agarose electrophoresis (Board, 1979 and 1980). In no instance were any of the genetic marker results inconsistent with Mendelian inheritance or paternity. The results of the genetic marker studies which were informative are shown in table I and the corresponding lod scores in table II.

TABLE I. — Informative phenotypes for segregation analysis with the marker inv(3)(p25q23).

Subject		Locus		
		PI	GALT	GM
II	10	M1	—	1,2,3,5
III	34	M1-M3	N—D	1,2 (*)
Husband of	34	M1-M2	N	3,5
IV	23	M2-M3	N—D	1,3,5
	24	M1	—	1,3,5
	25	M1-M3	—	1,2,3,5
	26	M1	—	1,3,5
V	13	M1-M2	N	3,5

(*) Phenotypes of offspring indicate genotype to be GM 1/1,2.

Fig. 2. — G-banded chromosomes from three metaphases of the propositus (right) showing the recombinant chromosome, and his father (left) showing the inverted chromosome.
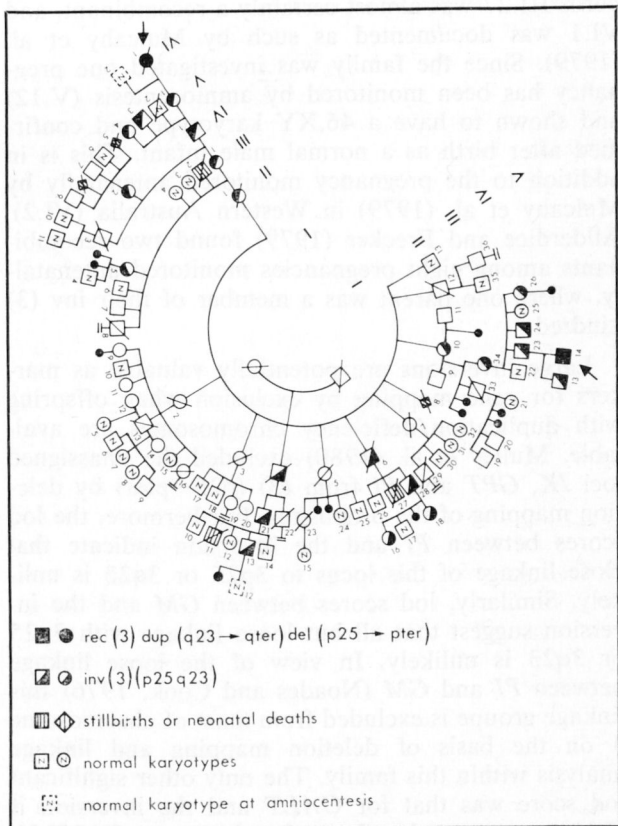
rec (3) dup (q23 → qter) del (p25 → pter)

inv (3)(p25 q23)

stillbirths or neonatal deaths

normal karyotypes

normal karyotype at amniocentesis

Fig. 3. — The family.

TABLE II. — **Lod scores between the inversion and PI, GM and GALT.**

θ

	.01	.05	.10	.15	.20	.25	.30
PI	—2.508	—1.164	—0.632	—0.355	—0.184	—0.074	—0.005
GM	—8.495	—5.000	—3.495	—2.615	—1.990	—1.505	—1.109
GALT	—3.398	—2.000	—1.398	—1.046	—0.796	—0.602	—0.444

DISCUSSION

The phenotype of the propositus is typical of the syndrome produced by partial trisomy 3q, either as a result of recombination within an inversion or malsegregation of a balanced translocation involving this chromosome segment (Mulcahy et al., 1979). The risk of inversion carriers producing recombinant offspring has been previously discussed (Sutherland et al., 1976). In a more recent review Winsor et al. (1978) concluded that the risk of an abnormal child resulting from recombination within a pericentric inversion was greater for « large » inversions than for « small » ones. In the present kindred it is not possible to determine such a risk because of uncertainty about the number of recombinants in the family. On the basis of medical records III.27 was almost certainly a recombinant, and VI.1 was documented as such by Mulcahy et al. (1979). Since the family was investigated one pregnancy has been monitored by amniocentesis (V.12) and shown to have a 46,XY karyotype and confirmed after birth as a normal male infant. This is in addition to the pregnancy monitored antenatally by Mulcahy et al. (1979) in Western Australia (VI.2). Allderdice and Frecker (1979) found two recombinants among eight pregnancies monitored antenatally, where one parent was a member of their inv (3) kindred.

Large inversions are potentially valuable as markers for gene mapping by exclusion when offspring with duplication/deficiency chromosomes are available. Mulley et al. (1980) excluded the unassigned loci *JK*, *GPT* and *PI* from (3) (p25→pter) by deletion mapping of the propositus. Furthermore, the lod scores between *PI* and the inversion indicate that close linkage of this locus to 3p25 or 3q23 is unlikely. Similarly, lod scores between *GM* and the inversion suggest that all but loose linkage with 3p25 or 3q23 is unlikely. In view of the loose linkage between *PI* and *GM* (Noades and Cook, 1976) this linkage groupe is excluded from most of chromosome 3 on the basis of deletion mapping and linkage analysis within this family. The only other significant lod score was that for *GALT* and the inversion if II.10 was assumed to have the phenotype GALT N,

a fair assumption given the absence of other *GALT* alleles in 16 of her nieces and nephews tested. This is, however, of minimal significance now that *GALT* has been assigned to chromosome 9 (Sparkes et al., 1980).

In natural populations where pericentric inversions are present as adaptive polymorphisms there is no recombination within the inverted segments in structural heterozygotes (White and Morley, 1955). However, newly arisen inversions in insects (White and Morley, 1955 ; White, 1961) and in man do not behave in this fashion. Renwick (1971) has considered the theoretical use of inversions for gene mapping. Recombination within the inversion is reduced, especially between inversion breakpoints and markers within small inversions. For large inversions in man recombination can occur within the inverted segment as a result of two-strand double crossovers at meiosis. Van der Linden et al. (1975) observed three out of 40 inversion loops resulting from an inv (4) which they interpreted at diakinesis as having two crossovers within the inverted sequence. However, according to Haldane (1931), « ...if crossing over occurs in any section, the probability of crossing over in adjacent sections is reduced... », hence any inversion would need to be of a minimum size before more than one chiasma would occur within it. If it were possible to determine the minimum size of an inversion necessary for two-strand double crossovers then inversions below this size would be very useful for gene mapping since the observation of a single event of recombination between such an inversion and a gene locus would exclude that locus from within the inversion.

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Genetic linkage analysis of epidermolysis bullosa dystrophica, Cockayne-Touraine type

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Genetic linkage relationships between 27 informative marker loci and the locus for epidermolysis bullosa dystrophica, Cockayne-Touraine type (EBD-CT), were examined in a single large kindred. Linkage could not be demonstrated to any of the marker loci, further adding to the exclusion map for *EBD-CT*. The dominant forms of EBD so far delineated by clinical criteria and electron microscopy remain genetically undefined in terms of loci and allelism. Further investigation will be undertaken using restriction fragment length polymorphisms mapped to regions outside the existing exclusion map.

Epidermolysis bullosa (EB) is a heterogeneous group of skin disorders subdivided into two major classes (McKusick 1983). The EB dystrophica (EBD) class is characterised by scarring bullae located subepidermally and is often associated with nail dystrophies. Apart from several autosomal recessive forms of EBD, three dominant forms have been delineated by clinical criteria and electron microscopy: Cockayne-Touraine (EBD-CT), Bart (EBD-B) and Pasini (EBD-P) (Gedde-Dahl 1978). Their pathogenesis is unknown.

The number of separate major genes associated with EB, and the extent of different allelic mutations, is unclear. In the absence of identifiable gene products, linkage analysis is necessary for the derivation of a genetically based classification. No linkage relationships have been established for genes responsible for any of the EBD types. The genetical basis for clinical heterogeneity has been partially elucidated for the EB simplex (EBS) class (Olaisen & Gedde-Dahl 1973) but resolved only to the extent of dominant versus recessive types within the EBD class.

This investigation considers only the dominant types of EBD. Linkage analysis is reported from a large kindred with a definitive EBD-CT diagnosis.

Material and Methods

Ninety-one available persons from the kindred shown in Fig. 1 were typed for the marker loci in Table 1 using standard electrophoretic and serological procedures. The presence or absence of EB symptoms was determined by report of the family members themselves. All affected individuals in the

kindred studied must possess the same EB mutation identical by descent. This eliminates the possibility of genetic heterogeneity of EB in the data to be presented. The data were analysed for linkage using the computer program LIPED (Ott 1976).

Results

Electron microscopy of intact skin from two affected members of the geneology confirmed the diagnosis of EBD-CT. The anchoring fibrils connecting the underside of the basement membrane to the upper dermis were rudimentary in structure and markedly reduced in number. The defective anchoring fibrils were present only at the site of blister formation. This is diagnostic for EBD-CT (Gedde-Dahl 1981). EBD-P was excluded by the presence of normal anchoring fibrils in non-blistered skin.

The distribution of lesions among affected persons who responded to a questionnaire is summarised in Table 2. Some reported no symptoms until the third or fourth decade of life (Table 3). Hence, some
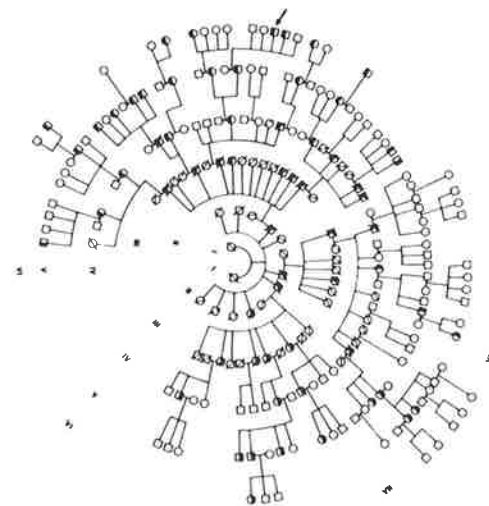
Fig. 1. The pedigree showing segregation of the EBD-CT gene.

Table 1

Lod scores between the locus for EBD-CT and 27 marker loci
(a) assuming complete penetrance
(b) assuming penetrance of 0.7

Linkage Comparison		θ				
		.05	.1	.2	.3	.4
EBD-CT:						
ABO	(a)	−8.6	−5.4	−2.4	−0.9	−0.3
	(b)	−4.8	−3.3	−1.7	−0.8	−0.3
ACP1	(a)	−6.3	−3.9	−1.6	−0.6	−0.1
	(b)	−3.1	−1.9	−0.8	−0.3	0.0
AK1	(a)	−4.4	−2.7	−0.5	−0.5	−0.1
	(b)	−1.8	−1.3	−0.6	−0.3	−0.1
C3	(a)	−5.2	−3.2	−1.4	−0.6	−0.2
	(b)	−2.0	−1.3	−0.6	−0.2	−0.1
CHE2	(a)	−1.4	−0.9	−0.4	−0.2	0.0
	(b)	−0.3	−0.2	−0.1	0.0	0.0
ESD	(a)	−9.1	−5.2	−1.8	−0.5	0.0
	(b)	−5.4	−2.9	−0.8	−0.1	0.1
F13A	(a)	−3.3	−2.0	−0.8	−0.3	−0.1
	(b)	−1.9	−1.1	−0.5	−0.2	−0.1
F13B	(a)	1.0	1.1	1.0	0.7	0.3
	(b)	0.9	1.0	0.8	0.6	0.3
Fy	(a)	−5.3	−3.0	−1.0	−0.3	0.0
	(b)	−3.9	−2.3	−0.8	−0.2	0.0
GALT	(a)	−1.3	−0.8	−0.3	−0.1	0.0
	(b)	−0.1	−0.1	0.0	0.0	0.0
GC	(a)	−8.8	−5.2	−2.2	−0.9	−0.3
	(b)	−6.7	−4.1	−1.7	−0.7	−0.2
GLO1	(a)	−2.5	−1.2	−0.2	0.1	0.1
	(b)	−2.0	−1.0	−0.2	0.0	0.1
GPT	(a)	−5.4	−3.3	−1.5	−0.6	−0.2
	(b)	−3.2	−1.9	−0.9	−0.4	−0.1
HP	(a)	−7.9	−4.7	−2.0	−0.8	−0.3
	(b)	−4.5	−2.6	−1.0	−0.4	−0.1
IGH	(a)	−3.8	−2.4	−1.3	−0.7	−0.2
	(b)	−1.6	−1.1	−0.7	−0.4	−0.1
IGK	(a)	−0.5	0.0	0.3	0.3	0.2
	(b)	0.4	0.5	0.4	0.3	0.2
Jk	(a)	−4.5	−2.3	−0.5	0.1	0.2
	(b)	−4.1	−2.3	−0.7	−0.1	0.1
K	(a)	−3.3	−2.3	−1.3	−0.7	−0.3
	(b)	−1.2	−0.9	−0.6	−0.3	−0.1
Lu	(a)	0.3	0.3	0.2	0.1	0.1
	(b)	0.3	0.3	0.2	0.1	0.1
MNS	(a)	−11.2	−6.9	−3.0	−1.2	−0.4
	(b)	−8.1	−5.1	−2.3	−1.0	−0.3
P	(a)	−1.3	−0.5	0.1	0.2	0.2
	(b)	−0.3	0.1	0.3	0.3	0.2
PGD	(a)	−2.7	−2.2	−1.5	−0.9	−0.3
	(b)	−2.6	−1.9	−1.1	−0.6	−0.2
PGM1	(a)	−4.5	−2.8	−1.2	−0.5	−0.1
	(b)	−1.8	−1.1	−0.4	−0.1	0.0
PGP	(a)	−0.2	0.1	0.3	0.2	0.1
	(b)	0.2	0.3	0.3	0.2	0.1
PI	(a)	−6.8	−4.2	−1.9	−0.7	−0.2
	(b)	−1.9	−1.2	−0.4	−0.1	0.1
Rh	(a)	−7.4	−4.2	−1.6	−0.5	0.0
	(b)	−6.4	−3.8	−1.6	−0.6	−0.1
TF	(a)	−0.2	0.3	0.6	0.6	0.3
	(b)	0.8	0.9	0.8	0.6	0.3

Table 2

Distribution of lesions determined by questionnaire from 33 affected individuals

Affected Regions	Right	Left
Knees	28	26
Inner ankles	28	26
Elbows	13	14
Outer ankles	12	12
Shins	11	10
Knuckles	9	9
Backs of Hands	8	9
Heels	7	7
All toe-nails*	19	19
Some toe-nails*	4	4
Finger nails*		
thumb	9	6
first	6	4
second	4	3
third	4	0
fourth	0	1

* Nails were affected in all individuals. Not all individuals provided complete details of which nails were affected.

members of the kindred classed as unaffected may indeed carry the EBD gene. Simple segregation analysis determined affected and unaffected individuals to be in the ratio of 58:83, just significantly different from 1:1 ($\chi_1^2 = 4.4$, $P < 0.05$). Penetrance could be as low as 0.7.

The data were analysed for linkage assuming (a) complete penetrance of the trait and (b) penetrance of 0.7. There was no detectable linkage between this form of EBD and any of the markers examined (Table 1). The *EBD-CT* locus was excluded from substantial chromosomal segments near *ABO, ACP1, AK1, C3, ESD, F13A, Fy, GC, GPT, HP, IGH, IGK, K, MNS, PGM1, PI,* and *Rh*. Exclusions of a smaller magnitude were detected at the remaining loci.

There was a hint of linkage to *F13B* (Table 1). The lod score reached 1.1 at $\theta = 0.1$. This positive finding could easily represent a chance fluctuation (given the number of loci examined for linkage).

Discussion

The characteristic features of EBD are subepidermal bullae associated with nail dystrophies and usually scarring of the skin. Nails on some of the digits may be unaffected in some individuals. In families where the disease is only mildly expressed, nail dystrophies may be the only manifestation (Gedde-Dahl 1978). Unlike EBS, blistering is on dorsal aspects of extremities

Table 3

Age of onset of skin symptoms from 33 affected individuals

Age of Onset of Skin Condition	No. of Individuals Affected	Involvement of Nails and Teeth*
Birth	11	Nails affected in all sometime before school age, often at birth. Teeth affected in five cases.
Pre-school (<5 yrs)	15	Nails affected simultaneously or sometimes a little later, but one not until 20 yrs. Teeth affected in seven cases.
Early School (5–12 yrs)	3	Nails affected simultaneously or a little later. Teeth affected in one case.
Adult	4	
i) Age 25		Toenails affected since birth. Teeth affected.
ii) Age 35		Nails affected since pre-school. Teeth not affected.
iii) Age 50		Toenails affected at 40. Teeth affected.
iv) No skin symptoms		Nails affected at 50. Teeth not affected.

* Sometimes both fingernails and toenails affected, sometimes toenails more severely affected, sometimes only toenails, not fingernails, affected.

(finger and toe knuckles, elbows and knees) and red, atrophic scarring results from recurrent blisters. The more severely affected individuals of this kindred had extensive involvement of the lower legs. Tooth enamel hypoplasia was evident in many of the affected (Table 3). EBD-CT is less severe than EBD-P in terms of age of onset and extent of blistering (Gedde-Dahl 1978).

Characteristically, EBD-CT is apparent between the first week and as late as the fourth or fifth year of life (Gedde-Dahl 1978). Later age of onset was noted among some family members of this kindred (Table 3). Lod scores based on the assumption of complete penetrance may be biased, given that a number of the younger unaffected members of the kindred may yet manifest symptoms in later life.

The three dominant EBD types involve an unknown number of gene loci. EBD-CT and EBD-P both result from mutations affecting anchoring fibrils (Gedde-Dahl 1981). The two conditions are not necessarily allelic because more than one polypeptide could be necessary for normal structure.

A number of EBD families have been previously examined for linkage. Linkage has been sought from one definite Cockayne-Touraine family (cited as unpublished data — Gedde-Dahl 1978) and two possible Cockayne-Touraine families (Gedde-Dahl 1971, Joensen et al. 1979). There was a low positive lod score between the EBD-CT gene and IGK (Kappa light immunoglobulin chain) in one of these families. This was confirmed in the present study, but very few individuals were informative and only a small positive lod score was detected (Table 1). Two Pasini families have previously been studied (Gedde-Dahl 1971, 1978). One of these provided a hint of linkage between the EBD-P gene and Se (ABH secretor gene). A possible Bart family gave a hint of linkage between EBD-B and PGM1 (phosphoglucomutase1) (Joensen et al. 1979).

There remains no clear evidence for linkage involving any of the EBD genes. The chromosomal location of F13B is unknown (Human Gene Mapping 7, 1983). The possibility of close linkages to many marker loci were excluded in the kindred presented. All EBD types are independent of EBS1 because they are not linked to GPT (glutamic pyruvic transaminase). The GPT:EBS1 linkage was established by Olaisen & Gedde-Dahl (1973). The additions to the exclusion map of EBD-CT from this kindred are reported to enable the concentration of future effort using restriction fragment length polymorphisms to chromosomal locations not yet excluded.

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PROTEASE INHIBITOR (PI) PHENOTYPE OF INDIVIDUALS WITH CHROMOSOMAL FRAGILE SITES

J.C. MULLEY, G.R. SUTHERLAND

SUMMARY : One hundred and four fragile sites were ascertained from 95 unrelated families. In each family the ancestry of the fragile site was traced as far as possible and the PI phenotype of the carrier in the earliest generation determined. Mildly deficient PI phenotypes were more prevalent than expected among earliest carriers of folate sensitive fragile sites, but not for BrdU dependent fragile sites. The significance of this finding is unclear and is based upon relatively small numbers. Another series of fragile site carriers would need to be studied and similar results found before the question of any relationship between PI phenotype and mutagenesis to fragile sites could be raised.

RÉSUMÉ : Cent quatre sites fragiles ont été détectés à partir de 95 familles non apparentées. Dans chaque famille, l'origine ancestrale du site fragile a été retracée aussi loin que possible et le phénotype PI déterminé chez le porteur le plus ancestral. Des phénotypes PI moyennement déficients étaient plus fréquents que prévus parmi les porteurs les plus ancestraux des sites fragiles sensibles au folate, mais non des sites fragiles dépendants du BrdU. La signification de cette observation n'est pas claire et n'est fondée que sur des nombres relativement petits. Une autre série de porteurs de sites fragiles devrait être étudiée et des résultats semblables retrouvés avant que la question d'une relation causale entre phénotype PI et mutation vers des sites fragiles puisse être posée.

INTRODUCTION

Fragile sites are non-staining gaps in chromosomes inherited as codominant markers. The folate sensitive fragile site at Xq27 is associated with mild to severe mental retardation in males, and mild mental retardation in some females (Sutherland, 1983). The significance of the autosomal folate sensitive fragile sites is unclear at present, but in some carriers could possibly be associated with mental retardation (Sutherland, 1982). The common BrdU dependent site at 10q25 is phenotypically harmless even when homozygous (Sutherland, 1983). Similarly, the fragile site at 16q22, which in some individuals has no special tissue culture requirements for expression, appears to be phenotypically harmless in both heterozygous and homozygous carriers (Schmid et al., 1980).

The PI locus (protease inhibitor system) has a major influence upon levels of alpha-1-antitrypsin (α_1AT), the predominant regulator of proteolytic activity in body fluids. The common alleles *PI*M1*, *PI*M2* and *PI*M3* are associated with normal levels of α_1AT, while some of the less frequent alleles, *PI*S, PI*Z, PI*I, PI*F* and others, are associated with reduced levels of α_1AT. Mildly deficient PI phenotypes have been associated with some chromosomal aberrations but the evidence is unconvincing (Fagerhol and Cox, 1981).

The nature of the mutations which give rise to fragile sites are unknown and this investigation examines the possibility of an association between mildly deficient PI phenotypes and mutations to fragile sites.

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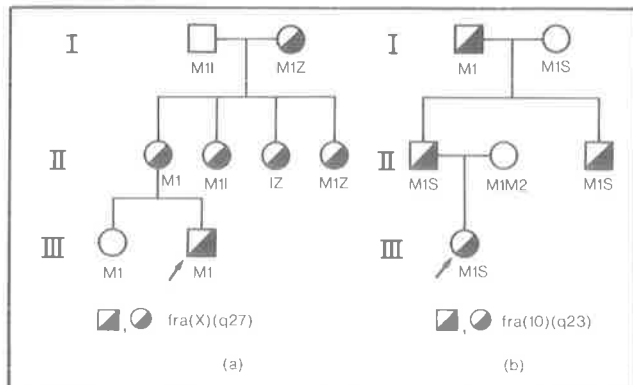
Fig. 1. — Pedigrees showing segregation of fragile sites and *PI* alleles to demonstrate the need to consider only the earliest carrier. a) The true relationship is eroded in generations II and III by independent assortment. b) Association developed in generations II and III is spurious.

PATIENTS AND METHODS

This investigation is unusual in that the chromosomal lesion is a heritable characteristic and any association between mutation to the fragile site and PI phenotype is directly testable in families only at the generation in which the mutation arose. Evidence for such association would be eroded in successive generations by independent assortment (fig. 1a) and non *PI*M* alleles could easily associate with fragile sites by chance (fig. 1b). For this reason only the carrier in the earliest available generation of each family was examined for statistical association to eliminate as far as possible effects of independent assortment and association by chance. This provided a sample of carriers as close as possible to the mutational event required for the expression of fragile sites. The earliest carrier, whether propositus or otherwise (table I), was determined prior to PI typing. The number of generations available for study depended on the availability and co-operation of family members.

All the fragile sites ascertained fit the criteria for classification as true fragile sites (Sutherland, 1979). PI typing was carried out as previously described for the determination of allele frequencies in the normal population (Mulley, 1980 and 1982). Fragile site acertainments were grouped for statistical analysis into three classes : folate sensitive at Xq27, folate sensitive at autosomal loci and BrdU dependent at 10q25. There were insufficient fragile sites at 16q22 for meaningful statistical analysis.

Of the families with folate sensitive fragile sites, 18 of the 40 ascertained had been examined for the presence of the BrdU dependent fragile site. All BrdU dependent fragile site ascertainments had been examined for folate sensitive fragile sites.

RESULTS

A total of 104 fragile sites were ascertained from 95 unrelated families. There were 17 ascertainments for the fragile site at Xq27, 23 for various folate sensitive autosomal sites, 59 for the BrdU dependent site and 5 for the site at 16q22. More than one fragile site was found in 8 families. One family had three fragile sites, two at 10q25 (homozygous in two individuals) and one at 11q23. The remaining seven families were all double ascertainments : an Xq27 with 16q22, two cases of 10q23 with 10q25, three cases of 10q25 with 16q22 and a 20p11 with 16q22. In all 8 families the earliest carrier for one fragile site was also the earliest carrier for another fragile site. The earliest carrier was the propositus for three families, the parent of the propositus in two families and the grandparent of the propositus in three families.

The distribution of PI phenotypes determined from the earliest carrier of all fragile site ascertainments in shown in table II. For example, the pheno-

TABLE I. — **Portion of pedigree from which the earliest carrier was examined.**

Fragile site	Propositus	Carrier parent	Carrier grand-parent	Carrier great-grand-parent	Total
Xq27	3	11	3	0	17
Autosomal-folate sensitive	10	9	3	1	23
10q25	25	20	13	1	59
16q22	3	1	1	0	5
	41	41	20	2	104

TABLE II. — PI phenotype in the earliest carrier of each fragile site ascertainment.

Fragile site	N°	PI M				Non PI M						
		M1	M1M2	M1M3	M2M3	M1S	M2S	M3S	M1Z	M1F	M2F	M1I
Xq27	17	6	4	1	0	2	0	3	1	0	0	—
Autosomal-folate sensitive												
2q13	2	—	1	—	—	1	—	—	—	—	—	—
6p23	1	—	—	—	—	—	—	—	—	—	—	1
9p21	1	—	—	—	—	—	—	—	—	1	—	—
9q32	2	2	—	—	—	—	—	—	—	—	—	—
10q23	9	5	2	1	1	—	—	—	—	—	—	—
11q13	2	—	—	1	1	—	—	—	—	—	—	—
11q23	1	—	—	1	—	—	—	—	—	—	—	—
12q13	1	1	—	—	—	—	—	—	—	—	—	—
16p12	2	—	1	—	—	—	—	—	—	1	—	—
20p11	2	—	—	—	—	1	—	—	—	1	—	—
Total	23	8	4	3	2	2	0	0	2	1	0	1
10q25	59	31	12	6	2	5	1	0	0	1	1	—
16q22	5	3	0	0	0	2	0	0	0	0	0	—

TABLE III. — Distributions of PI phenotypes (PI M subtypes combined for the fragile site groups and a random sample.

Fragile site classification	PI phenotype		Total
	PI M	Non PI M	
Xq27	11	6	17
Autosomal - folate sensitive	17	6	23
10q25	51	8	59
16q22	3	2	5
Control (non-fragile site) (Mulley, 1980, 1982)	575	65	640

types given for the pedigrees in figure 1 are presented in table II as M1Z (fig. 1a) and M1 (fig. 1b). Comparisons of the fragile site groups with those in a random sample from the population (phenotypes classified as either PI M or non PI M) are given in table III. Non PI M phenotypes have at least one allele other than PI^*M1, PI^*M2 or PI^*M3. The distribution of PI phenotypes was similar to that of the general population for the BrdU dependent fragile site sample ($\chi^2_1 = 0.36$, $0.50 < P < 0.75$). The corresponding comparison for the autosomal folate sensitive fragile sites is significant ($\chi^2_1 = 4.34$, $P < .05$). The data derived from the limited sample of 17 ascertainments with the fragile site at Xq27 also detected an association ($\chi^2_1 = 8.41$, $P < .01$).

DISCUSSION

There is strong selection against the folate sensitive fragile site at Xq27, which in terms of reproductive fitness, is virtually lethal in affected males and in some females. A significant proportion of independent Xq27 fragile site ascertainments are expected to result from new mutations and many of the remaining ascertainments are likely to be of relatively recent mutational origin. This group of fragile site families is therefore likely to provide the most sensitive test of any hypothetical relationship between PI phenotype and mutagenesis to fragile sites. An association was indeed found in this study. Unfortunately, the identification of new mutants is uncertain given the difficulty in detecting this fragile site in some female carriers (Sutherland, 1983).

If a relation between PI phenotype and mutation to the fragile site at Xq27 does exist, then a similar relationship may also exist for the other fragile sites. While it is not inconceivable that recent mutations are represented amongst the autosomal folate sensitive fragile site sample, the mutational origin would be expected to be more remote than for the fragile site at Xq27. Most heterozygotes for the autosomal folate sensitive fragile sites are normal individuals and any selection pressure against them is small. Whilst these might prove harmful in homozygotes, by analogy with known clinical effects of hemizygosity for the fragile site at Xq27, no such ho-

mozygote has been recorded. In view of the probable long period of elapsed time in generations between mutation and ascertainment, any relationship that might exist between PI phenotype and mutation to the autosomal folate sensitive fragile sites would be unlikely to be detected. Suprisingly, an association was found in the present study.

A third class of fragile site at 10q25 is a harmless polymorphism. Lack of selection against this fragile site implies that virtually all ascertainments are far removed in generations from the actual mutational events, and genetic drift might be responsible for its common occurrence. No association was found between PI phenotype and this fragile site as expected.

The molecular mechanism underlying fragile site expression is unclear. The chromosomal phenotype is probably associated with a mutant gene given the heritable nature of fragile sites. Linkage between the fragile sites at 10q23 and 10q25 (Sutherland, et al., 1982) and between the fragile site at 6p23 and HLA (Mulley, et al., 1983) confirms that the genes responsible are at the position of fragile site expression. They may effect either condensation of DNA, DNA repair or DNA synthesis. Penetrance and expressivity at the cellular level is largely dependent upon the culture environment.

The statistical association between mildly deficient PI phenotypes and carriers of the folate sensitive fragile sites may be only coincidental because the numbers analysed were low. This could be confirmed by an independent investigation of a separate series, but folate sensitive fragile sites are extremely rare and a separate series will not be available to the present investigators in the immediate future.

Another matter for speculation arising from the series studied concerns the apparent high rate of double ascertainment. The observed rate is a lower limit because 22 families known to have folate sensitive fragile sites were not examined with BrdU for the presence of the fragile site at 10q25. The detection of the fragile site at 16q22 can be difficult and in some patients requires the addition of distamycin A to lymphocyte cultures 24 hours prior to harvest (Schmid et al., 1980) or high (30 mg/l) levels of BrdU 6-8 hours prior to harvest (Croci, 1983). Consequently the significance of double ascertainment involving the site at 16q22 is unknown as culture conditions for expression of this fragile site were suboptimal in the great majority of individuals examined.

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ERRATA

Additions to the Exclusion Map of Man (Publication 6)

Ann. Génét. 1980, 23: 198-200

J.C. Mulley, G.D. Bryant and G.R. Sutherland

This paper reported the exclusion of the MNS blood group from (4)(q27->31). Whilst the paper was in press a blood grouping error was detected, and as a consequence this exclusion is retracted.

Jk should be deleted from paragraph 1, line 10, of the RESULTS section.

The first sentence of the DISCUSSION section states that only ACP1 had been assigned by deletion mapping. To this must be added GC (see Chapter 3, DISCUSSION section).

A word in the caption for Fig.2 should read karyotype, not caryotype.

A Search for Linkage in Families with Fragile Sites (Publication 10)

Hum. Genet. 1983, 65: 79-81.

J.C. Mulley, C Nicholls and G.R. Sutherland.

In the note added in proof (p.81) should also be added that the assignment of Lu to chromosome 19 has been confirmed.

Linkage Relationships of Fragile Sites (Publication 11)

In: Fragile Sites on Human Chromosomes, 1985. (Sutherland, G.R. and Hecht, F., Eds.), pp. 179-200.

Oxford University Press, New York.

J.C. Mulley

The reference Ferguson-Smith et al. (1973) on page 183, line 19, should be changed to Ferguson-Smith (1973).

P on line 3 of the table on page 196 should be PGD.

Contemporary terminology for Km given in Table 12.4 (p.196) is IGK.

A Transient Cathodal PI Phenotype in an Infant with Mild Persistent Jaundice (Publication 13).

Disease Markers 1983, 1: 55-57.

J.C. Mulley, G. Hill and A. McPhee.

Wherever MI appears in the caption to Fig.1 it should be substituted with M1.