UNIVERSITY OF ADELAIDE LIBRARY
30.7.03

Identification and Characterisation of the Gene for Börjeson-Forssman-Lehmann Syndrome

By

Karen Marie Lower

A thesis submitted in total fulfilment of the requirement

for the Degree of Doctor of Philosophy

April 2003

Department of Laboratory Genetics,

Women's and Children's Hospital, Adelaide,

South Australia

and

Department of Paediatrics,

The University of Adelaide, Adelaide

South Australia

TABLE OF CONTENTS

Tables

Thesis Summary

Mental retardation (MR) affects approximately 2-3% of the population. A high proportion of cases is due to genetic factors, with estimates of approximately 25% of MR being caused by genes on the X chromosome.

One of the earliest X-linked forms of MR described was Börjeson-Forssman-Lehmann syndrome (BFLS; MIM 301900). Affected males display a phenotype of mild to severe MR, gynecomastia, hypoplastic external genitalia, obesity, deep set eyes, visual problems, "heavy" face, long ears (specifically earlobes), shortened toes and tapered fingers, with variable features including epilepsy, microcephaly and short stature.

The gene for BFLS was known to map to a large region on Xq26-q27; however, the molecular basis of BFLS remained unknown. This research project refined the localisation of the BFLS gene, identified the gene, and completed preliminary analysis of the cellular function of the protein.

The critical genetic interval was reduced from 24.6 cM to approximately 8 cM, and an *in silico* physical and transcriptional map of this reduced minimal BFLS region was constructed. Of the 62 identified genes, 19 were screened for mutations.

Mutations associated with BFLS were identified in a novel PHD-like zinc finger gene, which has since been named *PHF6*. The full genomic structure, expression analysis in both human tissues and mouse brain, and cellular localisation of the protein was analysed. Eight different missense and truncation mutations were identified in seven familial and two sporadic cases of BFLS. *PHF6* is a widely expressed gene, present in nearly all adult tissues studied, with specific developmental stage expression in mouse brain. Transient transfection studies with tagged PHF6 protein showed diffuse nuclear staining with prominent nucleolar

accumulation. Such localisation, combined with the presence of two PHD-like zinc fingers, is suggestive of a role for PHF6 in transcription.

This work facilitates precise and early diagnosis of individuals affected with BFLS. Families will benefit from a direct DNA test of carrier status for females, and with the aid of counselling will have the ability to make informed reproductive choices. The identification of this gene also provides wider insight into the cellular pathways that are integral for normal cognitive function and physical development.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

This thesis is in the form of PhD by Publication during Candidature, as described in Rule 1.2.1 of The Code of Practice for Maintaining and Monitoring Academic Quality and Standards in Higher Degrees, The University of Adelaide, Australia.

19th April 2003

Karen Marie Lower Date

Acknowledgments

This work was undertaken in the Department of Laboratory Genetics, Women's and Children's Hospital, Adelaide, and was primarily supported by an NHMRC Program Grant. I gratefully acknowledge the University of Adelaide for scholarship support.

I would like to express my appreciation to Prof. Grant Sutherland and Dr. Sui Yu, for enabling me to carry out my research within the department. Thankyou also to Prof. Don Roberton for his assistance with university matters.

I would like to thank my principal supervisor, Dr. Jozef Gécz, for spending so much of his valuable time giving me advice and direction, and for his strong encouragement for me to write papers, and my co-supervisor, Assoc. Prof. John Mulley for his support, advice and help when ever needed.

I would like to thank Ági Gedeon for the family linkage information, Gillian Turner and Michael Partington for invaluable clinical advice, Paul Thomas and Shelley Ross for the mouse embryo *in situ* work, and Cathy Derwas and Sarah McDonald for assistance with cell lines.

A special thankyou to all members of the Department of Laboratory Genetics, and in particular Marie Shaw, Marie Mangelsdorf, Lynne Hobson, Merran Finnis, Catherine Everett, Kathie Friend, Rachael Bennett, and Kathy Cox, for invaluable help and good friendships.

Thanks to my family and friends, for all of their support over the past 29 years (but specifically the last 3).

A special thanks to my husband Michael, for being there for me through the whole time (and still wanting to marry me), for his love and support, and his tireless enthusiasm and optimism.

It's all swings and roundabouts

List of Abbreviations

AGTR2	angiotensin II receptor, type 2
ARHGEF6	Rho guanine nucleotide exchange factor 6
ARX	aristaless-related homeobox, X-linked
ATR-X	alpha-thalassemia/mental retardation syndrome, X-linked
BFLS	Börjeson-Forssman-Lehmann Syndrome
cM	centimorgans
FGD1	faciogenital dysplasia gene 1
FHF2	fibroblast growth factor homologous factor 2
FMR1	fragile X mental retardation 1
FMR2	fragile X mental retardation 2
GEF	guanine nucleotide exchange factor
HGP	human genome project
IQ	intelligence quotient
Mb	megabase
MECP2	methyl-CpG-binding protein 2
MIM	mendelian inheritance in man
MR	mental retardation
MRX	non-syndromic X-linked mental retardation
MRXS	syndromic X-linked mental retardation
OMIM	on-line mendelian inheritance in man
PHD	plant homeodomain
PHF6	PHD finger protein 6
RSK2	ribosomal protein S6 kinase
SD	standard deviation
SOX3	SRY (sex determining region Y)-box 3
XLMR	X-linked mental retardation
XNP	X-linked nuclear protein