ASCERTAINMENT, DIAGNOSTIC EVALUATION AND GENE MAPPING OF SOUTH AUSTRALIAN FAMILIES WITH POSSIBLE X-LINKED MENTAL RETARDATION

By

Zahiya Abdul Hameed Al Raisi (B.H.Sc, MD, DCH)

A thesis submitted for the degree of

Master in Clinical Medicine (by Research)

Department of Paediatrics

The University of Adelaide

November 2008


Appendices

Appendix 1  GOLD SA XLMR pedigrees  112
Appendix 2  List of tables  141
Appendix 3  List of Figures  142
Appendix 4  Consent to participation in the research  143
Appendix 5  Information sheet  146
Appendix 6  X linked mental retardation form  149
Appendix 7  GOLD SA 2007 newsletter  155
Appendix 1  GOLD SA XLMR pedigrees
Pedigree 3 – Family GOLD SA 3

- Normal male
- Normal female
- Affected male
- DNA available for study
- Deceased normal male
- Deceased normal female
- Deceased affected male
Pedigree 4 – Family GOLD SA 4
Pedigree 5 – Family GOLD SA 5

- Normal male
- Normal female
- Affected male
- Deceased normal male
- Deceased normal female
- Deceased affected male
- DNA available for study
Pedigree 6 – Family GOLD SA 6

Diagram of a family pedigree with symbols for different statuses:
- Normal male: □
- Normal female: ○
- Affected male: ■
- Deceased normal male: □
- Deceased normal female: ○
- Deceased affected male: ■
Pedigree 8 – Family GOLD SA 8

- Normal male
- Normal female
- Affected male
- DNA available for study
- Decreased normal male
- Decreased normal female
- Decreased affected male
Pedigree 10 – Family GOLD SA 10

- Normal male
- Normal female
- Affected male
- DNA available for study
- Deceased normal male
- Deceased normal female
- Deceased affected male
- Miscarriage
Pedigree 12 – Family GOLD SA 12

- Normal male
- Normal female
- Affected male
- DNA available for study
- Deceased normal male
- Deceased normal female
- Deceased affected male
- Miscarriage
Pedigree 13 – Family GOLD SA 13

Legend:
- Normal male
- Normal female
- Affected male
- DNA available for study
- Deceased normal male
- Deceased normal female
- Deceased affected male
Pedigree 14 – Family GOLD SA 14

Legend:
- Normal male
- Normal female
- Affected male
- Deceased normal male
- Deceased normal female
- Deceased affected male
Pedigree 15 – Family GOLD SA 15

- Normal male
- Normal female
- Affected male
- Deceased normal male
- Deceased normal female
- Deceased affected male

* DNA available for study
Pedigree 16 – Family GOLD SA 16

- Normal male
- Normal female
- Affected male
- DNA available for study
- Deceased normal male
- Deceased normal female
- Deceased affected male
Pedigree 17 – Family GOLD SA 17

- Normal male
- Normal female
- Affected male
- DNA available for study
- Deceased normal male
- Deceased normal female
- Deceased affected male
Pedigree 20 – Family GOLD SA 20

Diagram of the family pedigree with symbols indicating:
- Normal male
- Normal female
- Affected male
- Deceased normal male
- Deceased normal female
- Deceased affected male
- DNA available for study
Pedigree 21 – Family GOLD SA 21

I

II

Normal male
Normal female
Affected male
DNA available for study

Deceased normal male
Deceased normal female
Deceased affected male
Pedigree 22 – Family GOLD SA 22

<table>
<thead>
<tr>
<th>Diagram Legend</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Normal male</td>
</tr>
<tr>
<td>○ Normal female</td>
</tr>
<tr>
<td>■ Affected male</td>
</tr>
<tr>
<td>* DNA available for study</td>
</tr>
<tr>
<td>□ Deceased normal male</td>
</tr>
<tr>
<td>ℂ Deceased normal female</td>
</tr>
<tr>
<td>■ Deceased affected male</td>
</tr>
</tbody>
</table>
Pedigree 26 – Family GOLD SA 26

Legend:
- Normal male
- Normal female
- Affected male
- DNA available for study
- Deceased normal male
- Deceased normal female
- Deceased affected male
- Pregnancy
Appendix 2  List of tables

Table 1.1 Aetiology of intellectual disability (ID) based on the time and mechanism of the injury to the CNS. 7
Table 1.2 Causes of MR in literature surveys 8
Table 1.3 Benefits of evaluation of MR 16
Table 1.4 The effect of learning carrier status on the reproductive behaviour of women in XLMR families 17
Table 1.5 Summary of reported whole genome array genomic hybridisation studies for submicroscopic CNVs in idiopathic MR patients 22
Table 1.6 Overview of the diagnostic yield from investigation of MR 25
Table 1.7 Overview of diagnostic yield from cytogenetic, molecular genetic and clinical genetic assessment in intellectual disability 26
Table 1.8 Published recurrence risks for mental retardation by sex of proband 27
Table 2.1 The PCR components 39
Table 2.2 The PHF6 primers 43
Table 2.3 The UPF3B primers 44
Table 2.4 The UPF3B primers 45
Table 2.5 The GRIA3 primers 46
Table 3.1 Categories and numbers of XLMR families 48
Table 3.2 Availability of DNA samples from XLMR families 49
Table 3.3 Two point LOD scores with 48 markers spanning the X chromosome in Family GOLD SA 1 77
Table 3.4 All genes within the linkage interval of family GOLD SA 1 81
Table 3.5 Summary of the cardinal clinical features other than MR reported for the 9 XLMR genes from the minimal linkage interval of family GOLD SA 1 84
Table 3.6 Two point LOD scores with 48 markers spanning the X chromosome in Family GOLD SA 2 88
Appendix 3   List of Figures

Figure 1.1 Localization of all the currently known cloned XLMR genes                        13
Figure 1.2 The MRXS conditions                                                           14
Figure 1.3 The MRX conditions                                                             15
Figure 3.1 Order of the 48 microsatellite markers on the X                                 78
Figure 3.2 Boxed haplotype identifies the defined locus associated with the XLMR          79
Figure 3.3 The XLMR genes within the linkage interval of family GOLD SA 1                  80
Figure 3.4 The polymorphic change on Exon 1                                               90
Figure 3.5 The polymorphic change on Exon 6                                               90
Figure 3.6 The polymorphic change on Exon 9                                               90
Appendix 4  Consent to participation in the research
CONSENT TO PARTICIPATION IN RESEARCH

Name of Research Project:
Ascertainment, diagnostic evaluation and gene mapping of South Australian families with possible X-linked intellectual disability

Researchers:
Associate Professor Jozef Gecz
Dr Zahiya Al Raisi
Professor Eric Haan

I,………………………………………………………………………………………………………… consent to my/my child’s involvement in the above research project. I acknowledge that the nature, purpose and likely effects of the research project, especially as far as they affect me/my child, have been fully explained to my satisfaction by …………………………………………………………………... and my consent is given voluntarily.

1. I have been provided with an Information Sheet, and have had the opportunity to ask questions. I am satisfied with the explanations that I have been given and understand the information provided.

2. I have had the opportunity to discuss taking part in this research project with a family member or friend and /or have had the opportunity to have a family member or friend present whilst the research project was being explained by the researcher.

3. I consent to participate knowing that the aim of the research is to try to identify the gene change causing the intellectual disability in my family.

4. I understand that I/my child may not directly benefit by taking part in this study.

5. I acknowledge that the possible risks and /or side effects, discomforts and inconveniences, as outlined in the Information Sheet, have been explained to me.

6. I consent to the collection and storage of the health information that I will provide and of other relevant medical information about me/my child from medical records held by the SA Clinical Genetics Service, hospitals and other health professionals if necessary.

7. a) I consent to a blood specimen being collected from me/my child so that my/my child’s genetic material (DNA) can be obtained, stored and tested as part of the above project. I understand that no undertaking is made regarding the availability or suitability of the sample for subsequent use by me or my family.

b) I do/do not consent to the blood sample being used in any other research Project, provided the project has the approval of the Women’s & Children’s Hospital Research Ethics Committee.
8. I understand that the proposed genetic studies have the potential to confirm or exclude paternity and maternity, but that research participants will not be informed if non-paternity or non-maternity is found.

9. I understand that I can withdraw (my child) from the study at any stage and that this will not affect medical care or any other aspects of my/my child’s relationship with this hospital.

10. I understand that the researchers will inform me about the outcome of the research, whether or not it is successful in identifying the gene change causing the intellectual disability in my family.

11. I understand that I/my child be provided with the results of the studies done on me/my child.

12. I understand that if the research is successful, it may lead to other research in the future that may have implication for other family members, including my children.

13. I understand that while information gained in the study may be published, I/my child will not be identified and information will be confidential.

14. I am aware that I should retain a copy of the completed Consent Form and Information Sheet.

15. I understand there will be no payment to me/my child for taking part in this research.

Name of research participant .................................................. Name of witness ..................................................

Signature of research participant .................................................. Signature of witness ..................................................

Date .................................................. Date ..................................................

I certify that I have explained the study to the parent/patient and/or child and consider that he/she understands what is involved.

Signature .................................................. Date ..................................................

Status in project ........................................................................
Appendix 5  Information sheet
INFORMATION SHEET

Name of Research Project:
Ascertaining, diagnostic evaluation and gene mapping of South Australian families with possible X-linked intellectual disability

Researchers:
Associate Professor Jozef Gecz
Dr Zahiya Al Raisi
Professor Eric Haan

You are invited to take part in this research study because it is possible that there is a change in a gene on the X-chromosome that is responsible for intellectual disability in your family. The research will try to work out where the gene is on the X-chromosome and to identify the gene.

Your involvement in this study is entirely voluntary, and you are free to withdraw from the study at any time. You do not have to give any reason if you do not wish to participate or if you wish to withdraw. Your medical care will not be affected if you decide not to participate.

All affected and some unaffected family members will be invited to participate in the study.

Participation will involve providing health information and the collection of a blood sample.

Someone experienced in blood taking will collect the blood sample (20 ml, about four teaspoonfuls) from one of your veins. This may involve some mild discomfort or bruising. The blood sample can be taken at the Women’s & Children’s Hospital, through your general practitioner, or at one of the many blood collection centres throughout South Australia. Arrangements can be made for blood collection elsewhere in Australia if necessary.

The blood for the study will be sent to the Department of Genetic Medicine at the Women’s & Children’s Hospital, Adelaide.
- The genetic material (DNA) will be extracted from the blood and stored frozen until it is used for the research. Once frozen, the DNA will keep indefinitely.
- Part of some blood samples will be treated in a way that will allow the white cells to keep their ability to divide, and to provide more DNA in the future if needed.
- If initial studies are not successful in identifying the gene change, the stored DNA may be re-tested in the future to try again to identify it.
- Some DNA samples may be sent to our international collaborators to screen for the gene changes using the latest technologies.
- The amount of DNA obtained from each person’s blood sample should be sufficient for the whole research study.

The research may take a long time to complete (some studies like this one have taken several years) and may not be successful.
The research requires participation of several members of your family. We will not approach your relatives without your consent or the consent of another family member who is participating in the research. You may be asked to make the first contact with a relative to ask whether he or she agrees to be contacted by us. If consent is given, we will then make contact to explain the research in detail and to seek his or her consent to participation.

We will let you know the outcome of the attempt to map the gene, whether it is successful or not.

It is likely that most participants will not receive any direct personal benefit from the research.

For most participants, the research will not produce information about you that is not already known from your health history and how you are related to the other members of the family. However some family members who do not have intellectual disability and whose children do not have it may be shown to have a high chance of carrying the gene mutation even though it has not caused any problems. This would be unexpected information and could mean that unborn children or grand children could inherit the gene mutation and have intellectual disability.

If the research generates information about you that may be of relevance to the health of other family members, such as your children (e.g. that they might have inherited the intellectual disability gene mutation), your consent will be sought before offering to disclose such information to the family members concerned.

Non-paternity or non-maternity is the situation in which someone considered to be a person’s biological mother or father is not his or her real mother or father. Most genetic tests cannot determine this. However, when genetic tests are done on several members of one family, as in this research study, they might reveal non-paternity or non-maternity if it is present. If non-paternity or non-maternity is detected, we will not disclose it to anyone unless required to do so by law.

Any personal information we collect about you during the study and any results that come from the study will be kept confidential. No information that could lead to identification of any individual will be released without his or her written consent, unless we are required to do so by law. However, your DNA and health information will be used and stored in a way that allows to us to know whose DNA or health information it is – in other words, it will be possible to link your name and other personal details to your DNA sample and health information.

This study has been approved by the Research Ethics Committee of the Women's & Children's Hospital. Should you wish to discuss the study with someone not directly involved, particularly if you have any complaints or concerns, you should contact the Executive Secretary of the Committee, Ms Brenda Penny, at the Women’s & Children’s Hospital on 81616521.

If you have any questions or concerns about the study at anytime after reading this information sheet, you should contact Dr Zahiya Al Raisi or Professor Eric Haan on (08) 8161 7375 during working hours.
Appendix 6  X linked mental retardation form
X Linked Mental Retardation

Research Study Family No:

Personal Details:

Surname:

Given Names:

Sex: Male Female

Hospital URS: GF:

PK No: DNA No:

Address: DOB:

Post Code: Age: Years Months

Phone: Home Work Mobile

Family History:

Mother’s Details Father’s Details

Surname: Surname:

Given Names: Given Names:

Age: Years Months Age: Years Months

Race: Caucasian Aboriginal Race: Caucasian Aboriginal
Asian Other Asian Other

Consanguinity: Yes No

Plurality: Single Twin Triplet Quad Other

Pregnancy Outcomes: Live births Spontaneous abortions

23/03/2009
Still births
Terminations
Total

Family History of MR: None
  Sibling
  Parent
  Maternal Aunt/Uncle
  Maternal Cousin
  Maternal Grand parent
  Paternal Aunt/Uncle
  Paternal Cousin
  Paternal Grand parent
  Other

Family H/O Autism: Yes No
If yes, specify: .....................................................................................................................................

Family H/O Epilepsy: Yes No
If yes, specify: .....................................................................................................................................

Family H/O Other Neurological Problems: Yes No
If yes, specify: .....................................................................................................................................

CLINICAL INFORMATION OF THE AFFECTED MEMBER:

Birth wt gms
Birth Head Circumference cm
Birth Height cm
Neonatal Problems Yes No
If yes specify: .....................................................................................................................................

MR severity: Borderline Mild Moderate Severe Profound
IQ:

Specific delays or learning difficulties: Yes No

If yes,
specify..............................................................................................................................................

DD Assessment: - Clinical Yes No

If yes, Age: Years Months

Result..............................................................................................................................................

- Psychological Yes No

If yes, Age: Years Months

How?..............................................................................................................................................

Result..............................................................................................................................................

Pervasive developmental disorder: Yes No

If yes, specify: ......................................................................................................................................

Epilepsy: Yes No

If yes, specify: ......................................................................................................................................

Psychiatric Disorders: Yes No

If yes, specify: ......................................................................................................................................

Abnormal behavioural features Yes No

If yes, specify: ......................................................................................................................................

Birth Defects: Yes No

If yes, specify: ......................................................................................................................................

Vision: Normal Impaired

If impaired, specify: ............................................................................................................................

Hearing: Normal Impaired

If impaired, specify: ............................................................................................................................

Other Neurological Features: Yes No

If yes, specify: ......................................................................................................................................

Current Weight: Kg
Current Height:  .  cm

Current Head Circumference:  .  cm

Dysmorphic Facies  Yes  No

If yes, specify: ............................................................................................................ .
...................................................................................................................................

Other Dysmorphic Features  Yes  No

If yes, specify: ............................................................................................................ .
...................................................................................................................................

Malformations:  Yes  No

If yes, specify: ............................................................................................................ .

Neurological Signs:  Yes  No

If yes, specify: ............................................................................................................ .

Other Findings: ............................................................................................................ .

**Investigations:**

**Cytogenetics**

Routine cytogenetics before 1991

Routine cytogenetics after 1991

ST FISH

ST MLPA

Other FISH

**Molecular**

Fragile X (A) molecular test

Fragile X (A) cytogenetic test

Fragile X (E) molecular test

ARX

Microdeletion 17q21.31 (devries)

Copy number other imbalances (devries)

MECP2

PQBP1
Oligophrenin

Array-Affymetrix (SY)

Array-Illumina (SY)

Array-Nichol

Array-BAC/PAC tiling path (Belgium)

Array-Sequencing chip Affymetric (Germany)

Array-Comprehensive X-chromosome gene content (Sanger)

Linkage

Brain studies

CT brain

MRI brain

Ultrasound brain

EEG

Biochemistry

Urine AA/OA/MPS

TFT

CK

7-DHC

Lysosomal enzymes

Haemoglobin H bodies

Uric acid
Appendix 7  GOLD SA 2007 newsletter

NOTE:
This newsletter is included in the print copy of the thesis held in the University of Adelaide Library.