

Fine mapping of MRT9 locus through genome wide homozygosity mapping in a consanguineous Pakistani family

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Abstract

Intellectual disability (ID) or Mental Retardation (MR) is a broad term, which occupies several medical directions. It is extremely heterogeneous and has about reported 25,000 genes of which half of the genes expression have been found in the brain. Intellectual disability causes severe disability and has a worldwide prevalence of around 2% while autosomal recessive form of ID causes almost 25% of all non syndromic (NS) ID cases. A consanguineous family (who will be referred as) MR7 with phenotype of ID was sampled in Swat region of Pakistan. All affected individuals in the family were observed having a low IQ and cognitive mutilation with no sign of biochemical, skeletal or neurological abnormalities. Their deoxyribonucleic acid (DNA) was extracted and subjected to STS (Single tagged sequence) marker analyses which showed exclusion of all known non syndromic autosomal recessive (NS-AR) ID genes. In the family MR7, autozygosity mapping was performed by microarray single-nucleotide polymorphism analysis in all the collected samples, for a close examination of the homozygous region in all the affected however no homozygosity was observed for the normal parent. In this consanguineous family of Pakistan, autozygosity mapping showed linkage interval (chr14: 30,294,526-32,106,658) overlapping with already reported MRT9 locus (chr14:26,578,608-32,780,288) for NS- ARID.

Keywords: Intellectual disabilities (ID), NS-AR MR, Autozygosity mapping, Linkage interval, MRT9.

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Introduction

Intellectual disability (ID) or Mental Retardation (MR) is a complex and heterogenic term covering a number of clinical aspects. Crucial characteristics and description of ID comprises the overall intellectual performance to be

below normal, along with insufficiency in some of the skills required for self-survival. This is expressed particularly in childhood i.e. earlier than age of 18 years.¹ Two causes play a key role i.e. genetic and environmental, in rooting ID and can cause disturbance in the progress and operational performance of the central nervous system at any stage of prenatal, perinatal or postnatal.² Globally, 1-3% of the people suffer from phenotype of ID.³ Based on phenotypes, ID can be sub-categorised into syndromic ID with existence of additional distortion/s, altered descriptions or neurological deformities, and non-syndromic ID which occurs without any extra defects except ID. There is a high prevalence in males than females, supporting the idea of the involvement of several x-linked genes in non-syndromic ID.³ The total number of genes involved in NS-MR may exceed more than a thousand which is not surprising since 25,000 of the genes that have been reported, half have been found in the brain.⁴ Furthermore, it is probably similar to the autosomal forms of NS-MR which may be more frequent than X-linked NS-MR since the X chromosome only represents 4% of all the genes. In previous studies, 51 different loci for NS-ARID have been reported i.e. MRT1-MRT51,⁵ that have been described as loci having genes involved in the disease. Table-1 shows seven of these genes have the presence of disease causing mutation/s that have been recognised. Several other linkage regions have been also reported AR-NSMR.

Here we report a homozygous region showing an overlap with locus MRT9,⁶ that has been located in chromosome 14 q12-q13.1 by autozygosity mapping in the consanguineous Pakistani family with NS-ARMR that affected multiple members. Our reported homozygous region comprised 1.8 Mb of DNA and located in chromosome 14 q12-q13.1, spans about 1.8 Mb of DNA and narrowing down the reported locus from 6 Mb to 1.8 Mb.

Materials and Methods

Samples collection and clinical analysis

Figure-1 shows four members of the Pakistani consanguineous family (MR7) who were affected by Intellectual Disability which was determined after their

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Table-1: Showed the Published NSARMR loci.

Locus	Chromosomal arms position (P and Q)	Reported genes	Genomic position (physical) ^{a)}	Size (Mb)	OMIM	No. of mutations
MRT1	4q26	PRSS12	chr4:119421865 - 119493370	-	249500	1
MRT2	3p26 . 3	CRBN	chr3:3166696 - 3196390	-	607417	1
MRT3	19p13 . 12	CC2D1A	chr19:13878052 - 13902692	-	608443	1
MRT4	1p21.1 - p13.3	NR	chr1:105494413 - 112161992	6.7	611107	-
MRT5	5p15.3 - p15.2	NR	chr5:5144778 - 10786526	5.6	611091	-
MRT6	6q16 . 3	GRIK2	chr6:101953626 - 102624651	-	611092	1
MRT7	8p22	TUSC3	chr8:15442101 - 15666366	-	611093	1
MRT8	10q21.3 - q22.3	NR	chr10:71041135 - 80718164	9.7	611094	-
MRT9	14q12 - q13.1	NR	chr14:26578608 - 32780288	6.2	611095	-
MRT10	16p12.1 - q12.1	NR	chr16:22705103 - 48948637	26.3	611096	-
MRT11	19q13.2 - q13.32	NR	chr19:46843819 - 52292031	5.4	611097	-
MRT12	1p33 - p34.3	NR	chr1:37058812 - 46463958	9.4	611090	-
MRT13	8q24 . 3	TRAPPC9	chr8:140811770 - 141537860	-	613192	4
MRT14	2p25 . 2 - 2p25.3	NR	chr2:105224 - 4657839	4.6	-	-
MRT15	9q34 . 3	NR	chr9:137667484 - 139811354	2.1	-	-
MRT16	9p23 - p13.3	NR	chr9:12409772 - 35935361	23.5	-	-
MRT17	11p_ter	NR	chr11:0 - 5984667	6.0	-	-

^{a)} Genomic positions refers to hg18, NCBI build 36.1

*NR means not reported.

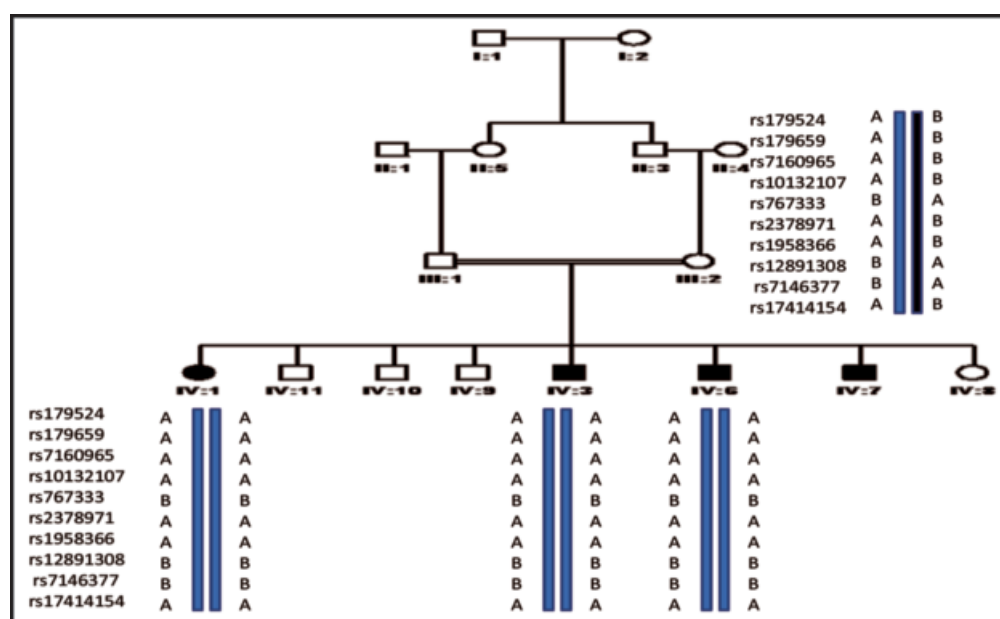


Figure-1A: Pedigree of four generations family (MR7) segregating autosomal recessive mental retardation (AR-NSMR). Haplotype for 14q12-q13.1 linkage region was constructed using informative SNP's heterozygous in the normal parent III:2 while homozygous in affected offsprings IV: 1, IV:3 and IV: 6.

samples were collected from District Swat, Khyber Pakhtunkhwa (KP), Pakistan. Prior permission and approval was taken for research purposes from the regional ethical committee in Denmark and from the Institutional Research Bioethics Committee, University

Science and Technology in Bannu, KPK.

A sampling trip was arranged to visit the family at their home for a descriptive clinical assessment, following by a verbal permission for venous blood samples that were collected from the affected persons, as well as the normal members of the family. Information was collected from the elders of the family to construct proper pedigrees. Standard questionnaire, photographs, interviews of parents and clinical histories were used for examination and assessment of all accessible

individuals in the family. Patients and parents were examined by psychologist for psychomotor and IQ assessment.

Molecular biology and DNA analysis

DNA was extracted from the blood collected in EDTA tubes by standard phenol chloroform: method. The homozygosity of known genes and loci (Table-2 and 3) of NS-ARMS was analysed by Short Tandem Sequence (STS) marker analysis. This was accomplished by using 3 primers method⁷ and for analysis ABI3100XLI sequencer and "GeneMapper" (Applied Biosystems, Carlsbad, California, USA) were used. Primer3 software was used for designing oligonucleotides.⁸ This was acquired from TAG Copenhagen (Copenhagen, Denmark). The genomic DNA was extracted from the blood of three affected and one healthy individual (AROS Applied Biotechnology, Århus, Denmark) and Genome wide SNP microarray analysis of 0.5µg DNA was performed by using SNP 6.0 software (Affymetrix, Santa Clara, USA). Chromosome Analysis Suite software (Affymetrix, Santa Clara, USA) and Genotyping Console were used for analysis of data. Homozygosity mapping by using STS marker showed linkage to the HECTD1 gene. Using bioinformatics tools and studding gene function, HECTD1 was selected as a candidate gene for sequencing. For the identification of pathogenic mutation/s, direct sequencing of HECTD1 gene was performed. This gene is located on chromosome 14 q12 - q13.1. Primer3 software was used for designing oligonucleotide primers, which is an online software. In two of the affected

individuals, the sequences of 5/ and 3/ UTR, coding and intron, exon boundaries were used for the analysis of mutation/s in a selected gene. When we compare these sequences to the reference sequences, no variants were identified.

Results and Discussion

MR7 is a four-generation family in which multiple members are affected because of consanguinity among first cousins. Blood samples were collected in EDTA tubes from both, normal individuals as well as affected individuals. Clinical examination was done of all individuals whose DNAs was extracted. Most of the

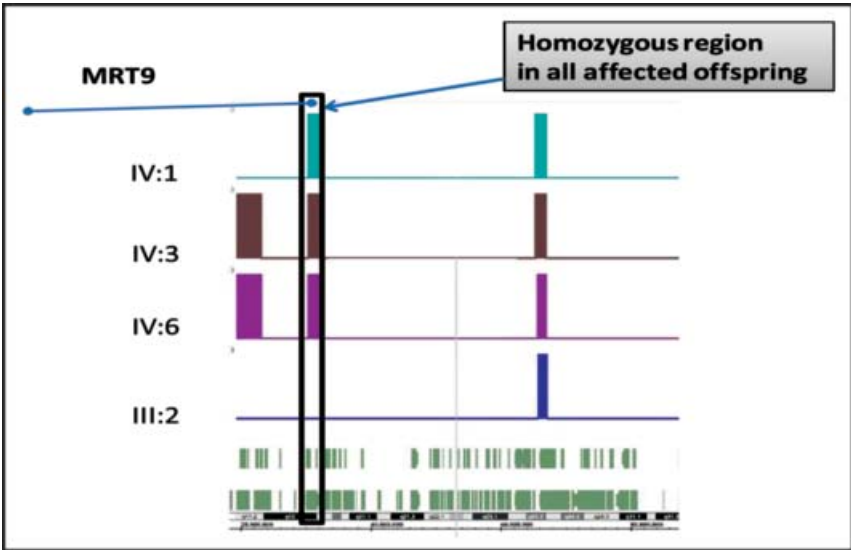


Figure-1B: Diagrammatic illustration obtained from Genome wide SNP 6.0 microarray analyses performed for three affected offsprings (IV:1, IV:3 and IV:6) and normal parent (III:2).

Table-2: Sequences of PCR primers for STS markers.

Markers	Forward primer	Reverse primer
D3S1297	TGACCGGCAGCAAAATTGTGCACATTAAGGAACAGGT	CATAATTGCTGCTTTGGAT
D3S3.578.995	TGACCGGCAGCAAAATTGTTCCAGCAATGCCTTGTC	GCTTGCATTAGTCTCAGCATC
D4S828	TGACCGGCAGCAAAATTGCAGCTCCATAATCATGTAAGCC	CTTATTTCACTTAGCATAATGTTTCA
D4S119.521.509	TGACCGGCAGCAAAATTGAAACAATCCTCCACGTGAG	AACACTGTAGCCCAGTGCAA
D4S402	TGACCGGCAGCAAAATTGCTTACTGTGTTGCCAAGGT	AGCTCTATGATTATTCAAGTTTG
D4S119.347	TGACCGGCAGCAAAATTGCCATTGCTTCATCTGTGTT	GGATTCAACAAGAAGCCACAG
D19S221	TGACCGGCAGCAAAATTGGCAAGACTCTGACTCAACAAAA	CATAGAGATCAATGGCATGAAA
D19S892	TGACCGGCAGCAAAATTGAGCTTGTGAAGGCGCAG	TTCCAGTCACACCAGGC
D8S1731	TGACCGGCAGCAAAATTGCCAAGCAATCATGGAAATC	AGCAAATTTATCCACAAGG
D8S549	TGACCGGCAGCAAAATTGAAATGAATCTGTATTAGCCAAC	TGAGAGCCAACCTATTCTACC
D4S2362	TGACCGGCAGCAAAATTGTAATATTGGTAGGATGAATGAATG	CCCATCCCGTTACCTCTTTA
D4S15.081	TGACCGGCAGCAAAATTGTCAGCACCAGCATGAAATC	CTGGTGCAATTGTTGATGCTC
D4S2960	TGACCGGCAGCAAAATTGAAGGCTTTATCATTAAGAATCCTA	TGAGGGTATAGTTACCATCTTTT
D6S1555	TGACCGGCAGCAAAATTGAGCCAATGCCCTTAAAAGAC	ATGAACAAAAGCTACAAATTGAA
D6S283	TGACCGGCAGCAAAATTGTTACAAAATCCTGTCCTGC	TTGATAGTTGCTTGATACAACAGA
Fluor_3fp	TGACCGGCAGCAAAATTG	

affected members share the same phenotype with some variation in the level of severity among the families, and was observed even within the family. Figure-1 shows third generation normal and healthy cousin marriage with normal pregnancies, while all the affected persons were the offspring. All affected individuals had delayed milestones and a low IQ level with delay psychomotor development. All the patients were found using signs and gestures for communication and were not capable of self-care. In all patients there were no clear abnormalities physically, but they were not developed socially.

Figure-1 (A, B) Cytogenetic analyses of the affected individuals were normal. The disease causing genes (Table-1) of NS-ARMR fragment analysed by polymorphic STS markers showed that these are not the genes for mutation (Table-2). The SNP microarray analysis for copy number variations in the family failed to identify large deletion or duplication in the genomes co-segregating with the NS-MR trait.

Genome wide SNP 6.0 microarray revealed linkage interval located on chromosome 14 q12-q13.1. For SNPs this region showed homozygosity with sample collected from three affected persons [IV: 1, IV3 and IV:6 (Figure-1)]. In this region the parent (III: 2) didn't show any homozygosity. The extremely polymorphic microsatellite markers such as D14S3.1 (TG), D14S3.06 (TG) and D14S1034 were used, which further confirmed the linkage region. SNPs with ID rs179524 and rs17414154 respectively cover the flanking region. Its DNA length is about 1.8Mb. The length of the contiguous markers is about expressed sequence tags (ESTs) and 12 genes which are well known. On chromosome 14 q12-q13.1 the homozygous region of the candidate showed overlapping with locus MRT9, which is already reported and is present on chromosome 14q12-q13.1. Its physical position is chr14:26,578,608-32,780,288.^{9,10} The length of MRT9 is about 6Mb, but in the region of the candidate it is about 1.8Mb in length of the DNA narrowing down the reported locus.

HECTD1 gene located on chromosome 14 q12-q13.1 in homozygous region. This gene was selected for sequencing as a candidate. It was sequenced due to the expected function of its product. In two affected persons, exon intron boundaries, coding sequence and 5' and 3' UTR sequences were analysed for mutation/s in the selected gene. When these were compared to the reference sequences, no variants were found. In future whole exome sequencing, new generation deep

sequencing and DNA capture technologies can be used for the analysis of this region. These will assist in the identification of disease causing mutation/s that caused the ARMAR phenotype in family MR7.

Conclusion

The homozygous region showed overlapping with non-syndromic autosomal recessive Intellectual disability MRT9 locus, which is already reported and narrowing down the reported locus from 6 Mb to 1.8 Mb of the DNA. This interval can be scrutinised by using candidate gene and high throughput sequencing of the candidate region, which will be helpful in the finding of the underlying gene.

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Conflict of Interest: None

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