

Original Article

Open Access

Molecular study of a consanguineous family with autosomal recessive mental retardation and speech disorder

Syed Farhan Ahmad*¹, Abdul Hameed², Maryam Jehangir,³ Jabar Zaman Khan Khattak¹

- 1) Faculty of Basic and Applied Sciences, Department of Biotechnology and Bioinformatics, Islamic International University, New Campus, Sector H-10 Islamabad, Pakistan
- 2) Institute of Biomedical and Genetic Engineering KRL Islamabad, Pakistan
- 3) Department of Bioinformatics, Shaheed Benazir Bhutto Woman University, Peshawar, Pakistan

ABSTRACT

Mental retardation (MR) is one of the most frequently found major genetic disorders around the world, affecting 1-3% of the people in the general population. The recent advancement in molecular biology and cytogenetic study has made possible the identification of new genes for a variety of genetic disorders including autosomal recessive MR. Recessive genetic disorders are common in Pakistan due to the high rate of consanguinity. A central focus of the present study was to map and identify the disease causing gene in a mentally retarded consanguineous Pakistani family with speech disorder. The study comprises of 20 individuals including 10 patients. Genetic analysis of autosomal recessive MR and speech disorders was carried out for eight known fundamental loci sorted out on the basis of clinical features. These loci including 3p26.2, 3p21.3, 7q22, 8p22, 11p15, 14q11.2-q12, 19p13.12, and 22p13 were screened using polymorphic microsatellite markers. To identify the disease locus, genomic DNA from each individual was genotyped for homozygosity analysis. Microsatellite markers were amplified using PCR. The study provided valuable data to exclude linkage of the above mentioned loci. The absence of any linkage of the Pakistani family with autosomal recessive MR to the eight well known loci, confirms the genetic heterogeneity of MR. These results need to be verified and the candidate gene be found by a complete genome scan, which may help us in establishing the genotype-phenotype correlation of MR, improving genetic counseling, carrier screening, DNA based prenatal diagnosis and the opportunity to develop appropriate animal models to test new forms of cell or gene therapies.

Key words: Consanguinity, Linkage analysis, Mental retardation, Pakistan

* Address for correspondence: Faculty of Basic and Applied Sciences, Department of Biotechnology & Bioinformatics, Islamic International University, New Campus, Sector H-10 Islamabad, Pakistan. Fax: Phone: Email: zaid_khan68@yahoo.com

INTRODUCTION

With a frequency of almost 2%, mental retardation (MR) disorder is familiar, frequently resulting in severe disability all over the world, causing huge problems to the suffering families as well as the society [1]. In many countries cousin marriages are the first choice of people, due to cultural values; therefore, the people of those countries, including Pakistan, are at higher risk of being affected by MR and other congenital disorders. Both severe and mild MR is considered to be more common in the offspring of consanguineous marriages [2].

There are five main categories of MR; mild, moderate, severe, profound and unable to classify (DSM-IV). The basis of this classification is the intelligence quotient (IQ) level which decreases from mild to severe states. This disorder may be categorized into either syndromic MR (S-MR) or non syndromic MR (NS-MR).

Both genetic and environmental factors are responsible for causing MR but, in almost 60% of the cases, the actual cause could not be identified precisely [3]. Almost 25-50% of the MR cases are thought to be caused by genetic mutations, although this figure varies according to the severity of the disease [4]. In most cases of MR, the major cause was the variations in chromosome number and structure [3]. Numerical aberrations, such as the increase in the numbers of autosomal and sex chromosomes, nearly always effect a number of syndromic mentally retarded patients, as demonstrated by numerical chromosomal aberrations such as Down Syndrome (21st pair of chromosomes), which is an extremely common inherited type of MR [3]. The aim of this study was to search for families suffering from inherited disorders, particularly MR, and to find the defective genes responsible for the disorder.

MATERIALS AND METHODS

Materials/reagents used in the study: Biological specimens (blood samples), Vacuoliner tubes, Syringes, ethylenediaminetetraacetic acid (EDTA), $MgCl_2$, proteinase K, phenol, sodium dodecyl sulphate (SDS), microsatellite markers, primers, *Taq* DNA polymerase, Non-denaturing poly acrylamide gel, dNTPs, PCR buffer, Tris-HCl.

Clinical examination: A Pakistani family affected with an autosomal recessive trait was examined by KRL Hospital, Islamabad, Pakistan. Ten living members of the family were found to be affected by MR. Detailed clinical examination was performed on the available family members including a total of twenty one individuals, ten of whom being affected, and eleven being normal individuals and twenty were available for study. The affected individuals were between 2-23 years old and had shown typical signs and symptoms of autosomal MR including speech delay, abnormal gait and oral salivation. Diagnosis was performed on the basis of the following parameters as carried out previously by other investigators: 1) According to the family history there were no MR patients or any other type of inherited disorders recorded in earlier generations. 2) Considering the consanguinity, cousin marriages were common, starting from great grandparents to grandchildren. 3) Based on the disease condition, a total of 10 persons were affected and the severity of the disease was found to be different from one individual to another (Figures 1 and 2).



Figure 1: Clinical Features of MR with speech disorder. A Pakistani family collected from Swabi, Khyber Pakhtoon Khwa Pakistan, for the present study. Patient with typical characteristics of mental retardation: bulging eyes, no articulation in tongue, speech and language impairments, gait abnormality, mild microcephaly, and muscle weakness or myasthenia.

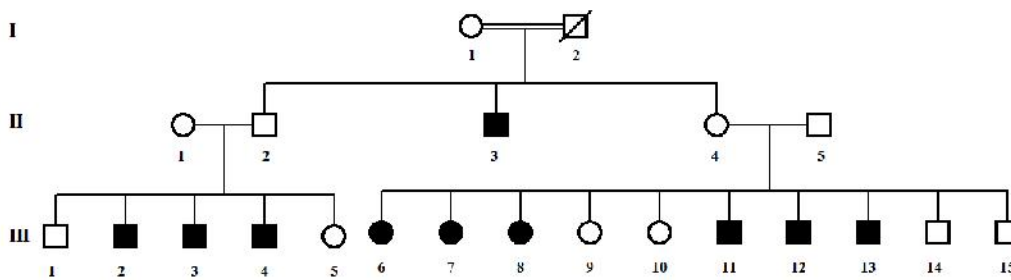


Figure 2: Pedigree of the Pakistani family suffering from Mental Retardation.

Crying, colour, weight, urination and meconium of the babies at birth were all normal. Most of the babies were born in hospitals but not vaccinated. Also, the babies' suckling ability was normal but due to low milk production of the mothers most of the children were fed by a feeder. The babies' eyes were found to be bugged out but eye movements were normal in all directions. Sitting was normal, but crawling, standing and walking were abnormal in some patients during childhood. Patients spoke their 1st word (naa, haaa) at the age of 10 years but their mental ability was found to be abnormal. Their limb structure was normal but seemed to be weak. No hypertelorism was detected and patients had less physical power and were normal statured. Palbarell fissure was detected in some patients and gait was abnormal. Language comprehension started at the age of 4 years. Speaking power improved with age and special common features were slanting out incisors and mild microcephaly. Dribbling saliva started from the age of crawling but decreased with the passage of time. Phonic was found to be negative i.e. vocal cords were functional but tongue articulation was positive which caused speech delay. Eating and drinking ability was not normal. To rule out the presence of autosomal recessive MR, unaffected parents and siblings were examined in detail, but no clinical signs and symptoms were revealed upon detailed examination.

Family history was taken during interviews with different family members and a family tree or pedigree was drawn to clarify the genetic relationships [5]. Pedigree drawing was performed using the Cyrillic (v2.10) program. Squares and circles in the pedigree represent male and female members of the family respectively. Normal individuals are symbolized with unfilled squares and circles while the affected ones with black-coloured filled squares and circles. Crossed squares and circles represent deceased individuals. Consanguineous marriages are shown with double lines between the partners.

Sample collection: Blood samples from patients and other clinically normal family members, including siblings and parents were collected with informed consent. About 5-10 mL of blood sample was collected in 10 mL vacutainer tubes (Becton Dickinson, Mountain View, CA.) containing acid citrate dextrose (ACD) or heparin from each individual.

Genomic DNA extraction, Microsatellite and linkage analysis: Genomic DNA was extracted from peripheral blood by the standard phenol-chloroform DNA extraction procedure [6]. This method is known as an organic method of DNA extraction that involves the digestion of cells with proteinase K in the presence of EDTA and a detergent such as SDS followed by extraction with phenol.

To identify the disease locus, genomic DNA from each individual was genotyped using microsatellite markers for homozygosity analysis [7]. Polymorphic microsatellite markers (Human Mapping Set, ver. 8: Research Genetics, Inchinnan, Scotland, UK) were amplified using polymerase chain reaction (PCR). Each reaction was carried out in a 10 μ L volume containing 1.5 mM MgCl₂, 0.6 μ M of each primer, 0.2mM dNTPs, 1U *Taq* DNA polymerase and PCR buffer (16 mM (NH₄)₂SO₄, 67mM Tris-HCl, pH 8.8, and 0.01% of the non-ionic detergent Tween-20) (Bio-line, London, UK). Amplification was performed under the following conditions. The PCR was performed according to methods previously published. Also, the PCR products were separated on 8% non-denaturing polyacrylamide gel electrophoresis [8]. Non-denaturing polyacrylamide gel electrophoresis was employed to distinguish the different alleles of the polymorphic microsatellite markers used in linkage/homozygosity analysis.

Results and Discussion

Detailed clinical examination by physicians confirmed that the Pakistani family was suffering from a rare autosomal recessive MR and speech disorder. After sample collection and DNA preparation through the organic method, as described above, the quality of all DNA samples was checked by measuring their optical densities at 260 nm which were found to be around 1.8 and by PCR amplification and subsequent running polyacrylamide gel electrophoresis. Also, linkage analysis studies were carried out and different sets of microsatellite markers were used for this analysis. The aim was to identify shared homozygous microsatellite DNA markers in the affected individuals. The family under examination consisted of ten affected individuals from two different extended branches who suffered from speech impairment with MR. Based on the questionnaire; the clinical information of the affected individuals is presented in Table 1.

Table 1. Clinical details of the mentally retarded Pakistani family

Clinical Findings	II-3	III-2	III-3	III-4	III-6	III-7	III-8	III-11	III-12	III-13
Sex	M	M	M	M	F	F	F	M	M	M
Age (Years)	22	7	8	5	13	17	4	9	19	3
Developmental delay	+	+	+	+	+	+	+	+	+	+
Dysmorphic feature	+	-	+	-	-	-	+	+	+	-
Skeletal Problem	-	-	-	-	-	-	-	-	-	-
Epilepsy	-	-	-	-	-	-	-	-	-	-
Speech disorders	+	+	+	+	+	+	+	+	+	+
Mental retardation	S	S	S	S	S	S	S	S	S	S
Growth	N	W	W	N	W	W	W	W	W	N
Schooling	-	-	-	-	-	-	-	-	-	-
Learning Disability	-	-	-	-	-	-	-	-	-	-
Self biting	-	-	-	-	-	-	-	-	-	-

Notes: Each column indicates the data of an individual and row presents the category of clinical symptoms. M=Male, F=Female; N=Normal, W=Weak growth; S=Severe mental retardation; - and + indicate absence and presence of a clinical symptoms.

Different reported loci for autosomal recessive MR were screened using STRs markers. The loci were selected for linkage analysis on the basis of closely related clinical symptoms of the patients (Table 2). To check for each locus these clinical features were compared with the work of researchers on each locus. PAGE experiment was performed for each available marker of the respective locus. Different STRs marker i.e. D19S586, D19S1034, D3S2387, D3S1304, GATA164B08, D11S2362, D11S1997, D11S1981, D14S742, D14S1280, D14S608, D14S599, D8S1130, D8S1106, D8S1145, D8S136, D22S685, D22S283, D22S423, D22S274, D3S1766, D3S4542, D3S1285, D3S2406, D7S1799, D7S3061, D7S1804, D7S2487 and D7S530 were run to find the defected gene in loci 8p22, 3p26.2, 11p15, 14q11.2-q12, 19p13.12, 7q22, 3p21.3 and 22p13. Some of the markers showed high polymorphism, providing information on the variation among the individuals. Some were not amplified and none of the markers was linked to any locus. Three markers D19S586, D19S1034 and D22S685 were run to identify the linkage in defected genes of *MRT3* and *PRODH*. The results showed no linkage for any marker (Figure 3, Table 3).

Table 2. List of different searched loci for autosomal recessive mental retardation

Chromosome	Gene Symbols	Markers	Available Markers
3p21.3	<i>AMT</i>	D3S1766 D3S4542 D3S1285, D3S2406	D3S1766 D3S2406 D3S4542
3p26.2	<i>MRT2</i>	D3S3525, D3S1560 D3S630 D3S1304	D3S2387, D3S1304 GATA164B08
7q22	<i>AUTS3</i>	D7S1799 D7S3061 D7S1804, D7S2487 D7S530	D7S1799, D7S3061 D7S1804, D7S2487 D7S530
8p22	<i>TUSC3</i> <i>MRT7</i>	rs.113990 rs.1537587	D8S1130, D8S1106 D8S1145 D8S136
11p15	<i>MRT17</i>	rs.10769544 rs.11040272	D11S2362 D11S1997, D11S1981
14q11.2-q12	<i>MRT26</i> <i>MRT9</i>	rs.1998463, rs.243286	D14S742, D14S1280 D14S608 D14S599
19p13.12	<i>MRT3</i>	D19S840, D19S547 D19S1165, D19S564 D19S547	D19S586, D19S1034
22p13	<i>PRODH</i>	D22S685, D22S283 D22S423, D22S274	D22S685, D22S283 D22S423, D22S274

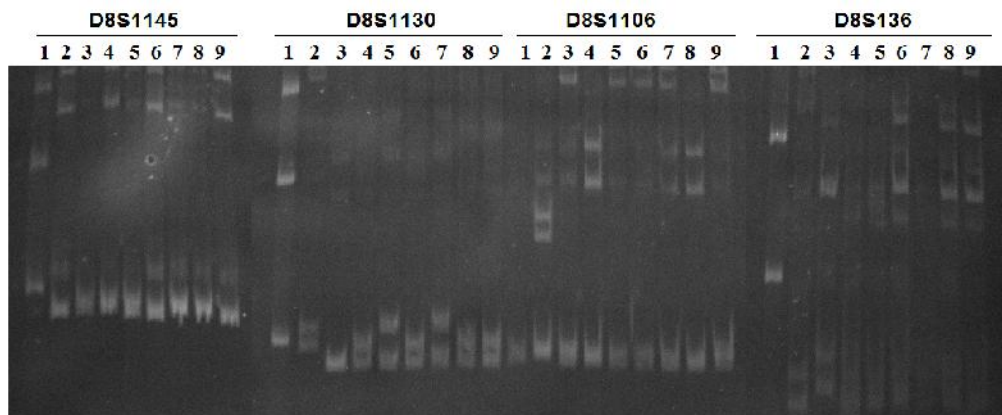


Figure 3: Polyacrylamide gel electrophoresis of 8p22 for detecting linkage in *MTR-7* and *TUSC3* genes: The marker D8S1145 results was satisfactory in seven patients and two normal persons because of same bands results, the bands of the remaining patients and normal were not matched so linkage was not found. At the same locus three other markers D8S1130, D1106 and D8S136 were run for the confirmation of linkage. No linkage was detected. From column 1-9 for each of the marker D8S1145, D8S1130, D8S1106 and D8S136 the samples used were II-4, II-5, III-6, III-7, III-9, III-11, III-12, III-14, respectively. Size range of the markers D8S1145, D8S1130, D8S1106 and D8S136 were 257-293, 132-156 and 74-80 bp, respectively.

Table 3. List of markers used for linkage analysis in current research

Markers	Probes	Positions	Product Size Range (bp)	Remarks
D7S1799	GATA23F05	115.6	175-187	Not amplified
D7S3061	GGAA6D03	132.2	114-154	Not linked (Hints)
D7S1804	GATA43C1	139.0	238-302	Not amplified
D7S2487	AFMb29d1	128.5	237-249	Not linked (Hints)
D7S530	AFM249xf9	135.2	106-118	Not linked (Hints)
D3S1766	GATA6F06	66.9	208-236	Not linked (Hints)
D3S4542	GATA1484	80.4	236-260	Not amplified
D3S1285	AFM191y5	81.1	232-242	Not linked
D3S2406	GGAT2G03	91.2	306-350	Not linked
D22S685	GATA6F05	42.2	172-208	Not linked
D22S283	AFM262vh5	43.6	128-152	Not linked
D22S423	AFM261xd9	44.2	215-235	Not linked
D22S274	AFM164th8	45.1	202-214	Not linked
D8S1130	GATA25C10	9.9	132-156	Not linked (Informative)
D8S1106	GATA23D06	12.2	127-159	Not linked (Informative)
D8S1145	GATA72C10	21.8	257-293	Not linked (Informative)
D8S136	cos140D4	25.5	74- 80	Not linked (Informative)
D3S2387	GATA22G12	0.3	167-215	Not linked (Informative)
D3S1304	AFM234tf4	2.4	253-269	Not linked
D11S236	ATA33B032	2.5	209-230	Not linked
D19S584	ATA19D12	12.6	124-145	Not linked

In developed countries, medical aspects of human genetic disorders have become more prominent as nonhereditary causes of ill health or early death, such as infectious diseases or nutritional deficiency, have declined [9]. Online Mendelian Inheritance in Man (OMIM), an authoritative database of inherited human conditions, identifies more than 4000 human genetic disorders and the number is increasing all the time as researchers learn more about our DNA [10]. Most of these disorders are severe and even life threatening, making them one of the big challenges for scientists and doctors all around the world. There is no proper treatment available to completely cure the inherited disorders. Therefore, to find out the disease causing genetic factors and procedures to cure them in future, several hundred research projects based on disease genetics are underway in different universities and research Institutes of the world.

Like the rest of the world, research on genetics has great potential in Pakistan. In this country, consanguineous marriages have occurred for centuries, resulting in various inherited disorders segregating in large multigenerational families. Such families require the attention of the molecular biologist to find the molecular bases and ways to treat these inherited disorders. Therefore, in the view of facts we collected from the consanguineous Pakistani family, in which MR and speech disorder is segregating as an autosomal recessive inherited disorder. MR is a devastating neuro-developmental disorder that has a serious impact on the affected individuals and their families, as well as health and social services. It is known to occur with a prevalence of ~1–3% within the population, and is frequently the result of genetic aberrations [11, 12]. Most of the members of the family studied during the current project also suffered severe MR due to the high frequency of cousin marriages throughout several generations. MR can be presented as the sole clinical feature, or it can be present with additional clinical or dysmorphological features (syndromic) [12]. Generally speaking, it is much more straightforward to identify the genetic cause of S-MR patients because the comorbid features frequently enable a medical geneticist to narrow down the suspected cause of a mutation in a short list of genes, or perhaps in a single

gene. However, for individuals with NS-MR, no secondary clues assist the molecular diagnosis. MR is significantly more frequent in males than females, and it was assumed that ~25% of the severe cases were X-linked; however, a recent review suggests that X-linked mutations contribute to no more than 10% of the cases, [13] and thus it is expected that there will be many more autosomal genes whose mutations cause NS-ID, both dominant and recessive. However, as a result of the high degree of genetic heterogeneity, mutations in only seven genes have been reported to cause non-syndromic autosomal recessive mental retardation (NS-ARMR), all of which being identified on the basis of mapping regions of autozygosity or homozygosity-by-descent (HBD) in multiplex consanguineous families [14]. The same is true for the patients of the current study in which males were more affected than females; in addition, their parents were not affected and did not show any phenotypic symptoms of MR, proving that the disease was autosomal recessive. The patients suffered from multiple symptoms such as tongue inarticulation, abnormal gait, muscular weakness and mild microcephaly. It can be concluded, therefore, that the disease was syndromic and multigenic. PCR-based linkage analysis was carried out to map the reported loci for autosomal MR and to find out any potential pathogenic mutation in the Pakistani MR family. This task was quite cumbersome because many loci had been reported for autosomal recessive MR. As more than 2000 genes might contribute to AR-MR and the mutation frequency of each single gene is presumably below 0.1%, each family in this study will most probably have a unique genetic defect, giving rise to the MR. Therefore, the overlap of several families with part of the *MRT7*, *MRT8* and *MRT10* loci, is unlikely to contain the causative genetic defect in all families [15].

Najmabadi *et al.* reported a consanguineous Iranian family (M100) in which 4 individuals had moderate NS-MR [16]. Linkage analysis identified a candidate locus on chromosome 8p, termed *MRT7*, with a maximum LOD score of 3.3. By autozygosity mapping, using microarray SNP analysis in a Pakistani family segregating NS-ARMR, a homozygous 6-Mb telomeric region on chromosome 11p15 between rs.10769544 and rs.11040272 was identified recently [17]. Linkage analysis yielded a maximum LOD score of 3.31. The locus was designated *MRT17*. A consanguineous Syrian family with NS-MR was also reported by Jamra *et al.* [18]. By homozygosity mapping of the Syrian family the researchers found linkage to a 9.1-Mb region on proximal chromosome 14q between rs.10132585 and rs.1278951 (LOD score of 3.85), referring to this locus as *MRT26* [18]. Furthermore, NS-MR has already been reported in 4 consanguineous families of Israeli-Arab origin with 10 affected and 24 unaffected members [19]. Also 5 additional families with NS-MR have been identified from the same village with the same family name, with a total of 16 affected individuals [20]. In all of the above mentioned families, a common homozygous disease-bearing haplotype for the polymorphic markers RFX1 and D19S840 was identified, defining a critical 0.9-Mb region between D19S564 and D19S547 on chromosome 19p13.12 [19, 20].

The simultaneous occurrence of autism and retinoblastoma in a patient with a deletion that extended from 13q12 to 13q14 has been reported previously [21]. A case of autism with a de novo deletion of 13q14-q22 was also described in previous research [22]. Moreover, the effect of incorporating language information and parental structural language phenotypes into the genome screening for autism have revealed 2 distinct peaks of linkage on 13q [23]. The same loci were screened in the present study but there was no defect found in the corresponding genes of the consanguineous Pakistani family. The results were probably controversial due to the presence of multiple clinical features in the family of MR under the current analysis. The manifestations were found to be in different combinations, unlike the other studies. Some of the symptoms were common between the patients of this family and others, yet no mutation in any gene was found.

The results of the current study showed deviation from those of previous works due to variation in biochemical techniques, as well as SNPs and microarray, which were not accessible in the present research. To our knowledge, no study has been conducted so far that has covered all the symptoms of this disease.

Most of the closely related reported loci for autosomal AR-MR were searched during the present study and no linkage was found. To our knowledge, there may be a novel mutation involved in causing the disease. Hence, a whole genome scan should be performed to point out the defected gene. Due to the limited period of the current research project and non availability of techniques like microarray, we were not able to perform a complete genome search. The most likely genetic cause of MR in patients with consanguineous parents is autosomal recessive conditions. Recent advancements in science and technology can uncover numbers of hidden facts and provide valuable information regarding genetic mutations involved in causing MR, protein functions and their versatile role in the body. The identification of the novel homozygous changes in the Pakistani family with MR may help us further understand the multi-functional role of protein in the development of the human brain and establish genotype-phenotype correlations. For individuals with MR, genetic discoveries allow for more accurate diagnosis, improved genetic counselling and carrier screening, feasibility of DNA based prenatal diagnosis and opportunity to develop appropriate animal models to test new forms of cell, protein or gene therapies. Further characterization of MR cell biology, therefore, is expected to provide fascinating new insight into its role in health and disease.

Acknowledgments

Islamic International University Islamabad, Pakistan is greatly acknowledged for supporting and financing this research. The contributions of the members of IB & GE Islamabad, Pakistan are highly appreciated. We also thank the affected family in Swabi, Pakistan, for providing us with the biological materials.

Conflict of Interest: There is no conflict of interest in this study.

REFERENCE

1. Ropers HH. X-linked mental retardation: many genes for a complex disorder. *Curr Opin Genet Dev* 2006;16:260-269.
2. Al-Ansari A. Etiology of mild mental retardation among Bahraini children: a community-based case control study. *Ment Retard* 1993;31:140-143.
3. Rauch A, Hoyer J, Guth S, Zweier C, Kraus C, Becker C, Zenker M, Hüffmeier U, Thiel C, Rüschenhoff F, Nürnberg P, Reis A, Trautmann U. Diagnostic yield of various genetic approaches in patients with unexplained developmental delay or mental retardation. *Am J Med Genet A* 2006;140:2063-2074.
4. McLaren J, Bryson SE. Review of recent epidemiological studies of mental retardation: prevalence, associated disorders, and etiology. *Am J Ment Retard* 1987;92:243-254.

5. Bennett RL, Steinhaus KA, Uhrich SB, O'Sullivan CK, Resta RG, Lochner-Doyle D, Markel DS, Vincent V, Hamanishi J. Recommendations for standardized human pedigree nomenclature. Pedigree standardization task force of the national society of genetic counselors. *Am J Hum Genet* 1995;56:745-752.
6. Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning: a laboratory manual*, 2nd ed. N.Y., Cold Spring Harbor Laboratory Press, 1995; p.1659.
7. Sayed HS, Aiysha A, Saba S, Shagufta K. Identification of *LIPH* gene mutation in a consanguineous family segregating the woolly hair/hypotrichosis phenotype. *J Pak Med Assoc* 2011;61:1060-1064.
8. Kryndushkin DS, Alexandrov IM, Ter-Avanesyan MD, Kushnirov VV. Yeast [PSI⁺] prion aggregates are formed by small Sup35 polymers fragmented. *J Biol Chem* 2003;278:49636-49643.
9. <http://www.ecomii.com/science/encyclopedia/human-genetics>
10. <http://www.ncbi.nlm.nih.gov/books/NBK21106>
11. Roeleveld N, Zielhuis GA, Gabreels F. The prevalence of mental retardation: a critical review of recent literature. *Dev Med Child Neurol* 1997;39:125-132.
12. Leonard H, Wen X. The epidemiology of mental retardation: Challenges and opportunities in the new millennium. *Ment Retard Dev Disabil Res Rev* 2002;8:117-134.
13. Ropers HH, Hamel BC. X-linked mental retardation. *Nat Rev Genet* 2005;6:46-57.
14. Kaufman L, Ayub M, Vincent JB. The genetic basis of non-syndromic intellectual disability: a review. *J Neurodev Disord* 2010;2:182-209.
15. Lencz T, Lambert C, DeRosse P, Burdick KE, Morgan TV, Kane JM, Kucherlapati R, Malhotra AK. Runs of homozygosity reveal highly penetrant recessive loci in schizophrenia. *Proc Natl Acad Sci USA* 2007;104:19942-19947.
16. Najmabadi H, Motazacker MM, Garshasbi M, Kahrizi K, Tzschach A, Chen W, Behjati F, Hadavi V, Nieh SE, Abedini SS, Vazifehmand R, Firouzabadi SG, Jamali P, Falah M, Seifati SM, Grüters A, Lenzner S, Jensen LR, Rüschenendorf F, Kuss AW, Ropers HH. Homozygosity mapping in consanguineous families reveals extreme heterogeneity of non-syndromic autosomal recessive mental retardation and identifies 8 novel gene loci. *Hum Genet* 2007; 121:43-48.
17. Rehman S, Baig SM, Eiberg H, Rehman S, Ahmad I, Malik NA, Tommerup N, Hansen L. Autozygosity mapping of a large consanguineous Pakistani family reveals a novel non-syndromic autosomal recessive mental retardation locus on 11p15-tel. *Neurogenetics* 2011; 12:247-251.
18. Jamra RA, Wohlfart S, Zweier M, Uebe S, Priebe L, Ekici A, Giesebrecht S, Abboud A, Al Khateeb MA, Fakher M, Hamdan S, Ismael A, Muhammad S, Nothen MM, Schumacher J, Reis A. Homozygosity mapping in 64 Syrian consanguineous families with non-specific intellectual disability reveals 11 novel loci and high heterogeneity. *Eur J Hum Genet* 2011; 19:1161-1166.
19. Basel-Vanagaite L, Alkelai A, Straussberg R, Magal N, Inbar D, Mahajna M, Shohat M. Mapping of a new locus for autosomal recessive non-syndromic mental retardation in the chromosomal region 19p13.12-p13.2: further genetic heterogeneity. *J Med Genet* 2003;40: 729-732.
20. Basel-Vanagaite L, Attia R, Yahav M, Ferland RJ, Anteki L, Walsh CA, Olender T, Straussberg R, Magal N, Taub E, Drasinover V, Alkelai A, Bercovich D, Rechavi G, Simon AJ, Shohat M. The *CC2D1A*, a member of a new gene family with C2 domains, is involved in autosomal recessive non-syndromic mental retardation. *J Med Genet* 2006;43:203-210.

21. Ritvo ER, Mason-Brothers A, Menkes JH, Sparkes RS. Association of autism, retinoblastoma and reduced esterase D activity. *Arch Gen Psychiatry* 1988;45:600.
22. Steele MM, Al-Adeimi M, Siu VM, Fan YS. A case of autism with interstitial deletion of chromosome 13. *J Autism Dev Disord* 2011;31:231-234.
23. Bradford Y, Haines J, Hutcheson H, Gardiner M, Braun T, Sheffield V, Cassavant T, Huang W, Wang K, Vieland V, Folstein S, Santangelo S, Piven J. Incorporating language phenotypes strengthens evidence of linkage to autism. *Am J Med Genet* 2001;105:539-547.