

Pathologic Genomic Rearrangements in Idiopathic Mentally Retarded Patients from Iran

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Abstract

Background: Mental retardation is a relatively frequent disorder and occurs in 1-3% of the general population. Chromosomal rearrangements involving microdeletion and microduplication particularly in subtelomeric regions are involved as a significant cause of idiopathic mental retardation. This study was designed to detect these aberrations in the Iranian patients with idiopathic mental retardation.

Seventy six patients with unexplained MR, a normal conventional karyotype and negative for fragile X without a clinically recognizable syndrome were included in our study. We used a combination of MLPA kits procured from MRC Holland to increase the detection rate of chromosomal abnormalities in idiopathic MR patients. These kits included P036 and P070 for subtelomeric screening, and in addition P064 and P096 for the 16 common microdeletion syndromes.

Results: The MLPA analysis revealed chromosomal aberrations in 8 cases.

Conclusion: Using the combination of four MLPA kits the rate of chromosomal abnormality detection in our study was 10.52% which confirms the previous published findings.

Keywords: Intellectual Disability; Genomic rearrangement; Iran

Introduction

Mental retardation (MR) is a variable, heterogeneous manifestation of central nervous system dysfunctions, occurring in 1-3% of general population [1, 2]. MR etiology is very heterogeneous and unfortunately, in about more than half the cases the cause of MR is still elusive [3]. Causes of mental retardation are numerous and include genetic and environmental factors [4]. Anything that damages and interferes with the growth and maturation of the brain can lead to MR, and this might happen before, during or after the birth of the child [1, 3]. Though it is generally assumed that half of the cases are due to genetic factors, the cause of the handicap in up to 50% of patients remains unexplained [5]. Chromosomal aberrations are the most common cause of mental retardation and it is estimated that 4-36% of moderate to severe mental retardations are due to chromosomal aberrations [6]. The detection rate of chromosomal abnormalities in patients with MR and dysmorphic features has increased due to the improvements of molecular cytogenetic methods. Cytogenetic analysis by conventional G-banding is the standard method of investigating chromosomal abnormalities but this technique cannot detect small structural abnormalities (<4Mb). Smaller chromosomal abnormalities

can be identified with array techniques, fluorescent in situ hybridization (FISH) or multiplex ligation-dependent probe amplification (MLPA) techniques [7-10]. In this study we investigated further molecular basis of the Iranian patients with idiopathic MR who had normal karyotype and were negative for fragile X syndrome for subtelomeric rearrangements and common microdeletion/microduplication syndromes.

Materials and Methods

Patients:

A total of 76 unrelated individuals with unexplained congenital MR or developmental delay who were referred to Tehran Medical Genetics Laboratory by clinical neurologists were recruited to this study with written consent from their parents or guardians. The study was also approved by local university ethic committee. All but one of these patients was male. All were evaluated by a clinical geneticist and had a normal G banded karyotype at 550 band level, and did not have Fragile X and no clinical syndrome had been recognized initially.

DNA extraction, MLPA analysis, array CGH and FISH technique:

Genomic DNA was extracted from peripheral blood using salting out procedure. All patients were screened for subtelomeric rearrangements using P036, P070 SALSA MLPA kits and microdeletion/microduplication syndromes using P064 MR1 and P096 MR2 kits following the standard procedures recommended by the MRC Holland. ABI 3130 Genetic Analyzer was used for separation of amplification products and Gene marker software (version 1.85) was used for the data analysis.

One of the samples was previously tested by the array CGH using Agilent platform by Oxford Gene Technology (OGT) company and

Cytosure interpret software was used to analyze the data. MLPA was applied to confirm the findings in the patient and his parents.

For one patient, Fluorescence In Situ Hybridization (FISH) was performed using Prader-Willi/Angelmanregion probe and 15q subtelomere specific probe following the protocol recommended by Cytocell aquarius company.

Results

MLPA method revealed abnormalities in 8 out of 76 patients. This is 10.52% detection rate which is consistent with other investigations [11,12]. The results are summarized in Table 1, and in the following each case is described separately.

Patient	Age/ Sex	Karyotype	Subtelomeric MLPA kits		Syndromic MLPA kits	
			P036	P070	P064	P096
P1	11/M	Normal	Dup 4p and del 9p	Dup 4p and del 9p	Normal	Dup 4p16.3
P2	8/M	Normal	Del 18p and dup5p	Del 18p and dup5p	Normal	Dup5p15
P3	33/M	Normal	Del 22q	Del 22q	Normal	Normal
P4	32/M	Normal	Dup4q	Dup4q	Normal	Normal
P5	9/M	Normal	Del 15q-cen	Del 15q-cen	Del15q11.2	Normal
P6	7/M	Normal	Normal	Normal	Dup 17p11.2	Normal
P7	30/F	Normal	Dup 3p	Dup 3p	Normal	Normal
P8	14/M	Normal	Del 18p	Del 18p	Normal	Normal

Table 1: Overview of the detected abnormalities found in patients with four sets of MLPA kits P036, P070, P064, and P096.

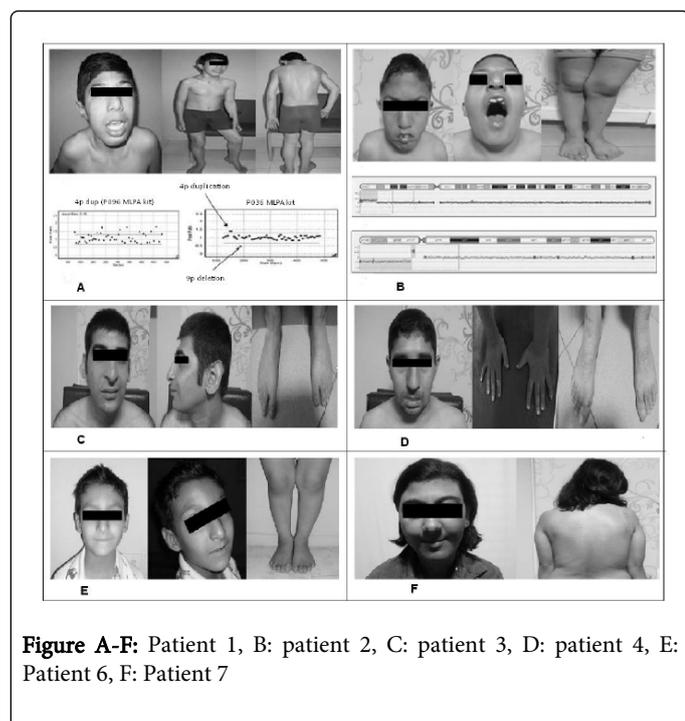


Figure A-F: Patient 1, B: patient 2, C: patient 3, D: patient 4, E: Patient 6, F: Patient 7

Patient 1

The patient had severe MR, seizure and facial dysmorphism, hypertelorism, microphthalmia, broad nasal bridge, short upper lip, down turned corners of the mouth, low hairline, high arched eyebrows, chest deformity and scoliosis (Figure A). MLPA testing detected duplication of 4p and deletion of 9p. Karyotypes and MLPA results of the patient's parents were normal. Therefore the observed structural rearrangements can be considered denovo.

Patient 2

He had MR, speech delay, dermatitis, macrocephaly, round face, low hair line, broad cheeks, prominent maxilla, hypertelorism, up-slanting palpebral fissures, retrognathia, epicanthic folds, glaucoma/buphthalmos, broad nasal bridge, abnormal nasal septum, small and downturned mouth, thick lips, high vaulted and narrow palate, tooth crown shape abnormality, oligodontia, dental caries, flat philtrum, posterior angulation of ears, anteverted/ bat ears, ear lobe abnormal size, short neck, umbilical hernia, inguinal hernia, scoliosis, lordosis, large hand, small penis, coxavara, coloboma of the eyelid, abnormally placed nipples, umbilical hernia, mild arachnodactyly, large foot, overlapping toes and knock knees- genu valgum (Figure B).

This patient was previously studied by the array CGH and three aberrations were observed (Figure B) which were suspicious to be pathogenic according to the databases such as DGV, CHOP CNV and decipher (<https://decipher.sanger.ac.uk/>). These aberrations and their

features are summarized in Table 2. There are some reported patients with chromosomal aberrations similar to this patient who are suffering from mental retardation and have other features some of which are in common with our patient

As a confirmatory test, MLPA was carried out for this patient and his parents. The first and second aberrations (Table 2) which are gain

and loss of large segments of chromosome 5p and 18p respectively were completely consistent with the MLPA results. The patient's parents were normal for these regions. Therefore these aberrations were de novo and most probably the cause of MR in this patient. We didn't have any MLPA kit to study the third aberration which was a small gain of chromosome 18p in more detail.

Aberration	1	2	3
Cytogenetic Location	5p15.2-5p15.33	18p11.21-18p11.32	18p11.21
Gain/Loss	Gain	Loss	Gain
Start-Stop	75149-10097255	139089-12869768	12974229-14071934
Size	10.022 Mb	12.731 Mb	1.098 Mb
# probes	231	231	46
Syndrome Region	Cri du Chat (5p deletion)	Holoprosencephaly 4	_____
# genes	69	108	12
# OMIM genes	6	2	1
OMIM genes	MTRR, SLC6A3, TERT, SLC6A19, NDUFS6, SDHA	NDUFV2, LPIN2	MC2R

Table 2: The aberrations found by Array CGH test in patient 2

Patient 3

He had severe MR and facial dismorphism, delayed speech, dolichocephaly, hypotelorism, wide eyebrow, long eyelashes, high arched palate, abnormal tooth position, large, prominent and posterior angulations of ear, broad thumb, sandal gap, large fleshy hands, cardiac defect and autism (Figure C). Deletion of 22q subtelomeric region was detected by MLPA method.

Patient 4

He had mild MR, structural asymmetry of face, prominent forehead, hypertelorism, beaked nose, high arched palate, short neck, scoliosis, mildly tapered fingers, proximally set thumb, gynaecomastia and broad thumb (Figure D). MLPA testing revealed duplication in the 4q subtelomeric region.

Patient 5

The DNA sample of this MR patient with a normal karyotype was referred to our laboratory. MLPA testing indicated heterozygous deletions in 15q11.2 which suggested the probability of the Angelman/Prader-Willi syndrome.

The FISH technique was performed for this patient using SNRPN/IC probe and a 15q subtelomere probe as a control which were applied on metaphase nuclei. Deletion of 15q was confirmed.

Patient 6

He had MR, hyperactivity, unusual onychotillomania, polyembolokoilamania, head banging, sleep apnea and autism. He had a characteristic craniofacial appearance and prominent forehead (Figure E). MLPA testing showed 17p11.2 duplication.

Patient

She had mild MR, squareface, broad cheeks, high cheek bones, hypotelorism, down -slanting palpebral fissures, epicanthic folds, flatend nose, deeply groovedphiltrum, narrow palate, deeply eruption of teeth, low set ears, abnormally placed nipples, hypoplastic nipples, kyphosis, lumbarlordosis, scoliosis, affected facial nerve, low hair line and epilepsy (Figure F). MLPA method displayed duplication in 3p subtelomeric region.

Patient

We could not examine the characteristic features of this MR patient. MLPA revealed 18p deletion in her genome.

Discussion

The detection rate of chromosomal abnormalities in idiopathic mentally retarded individuals has increased due to the improving resolution of molecular cytogenetic techniques [13]. Many studies have shown that subtelomeric aberrations are significant cause of 5 to 10% of idiopathic moderate to severe mental retardation [11, 12]. MLPA screening based on the SALSA P036, P070, P064 and P096 kits was performed in this study. We detected genomic abnormalities in 8 out of 76 (10.52%) patients with unexplained MR.

In patient 1, combination of symptoms of both 4p duplication and 9p deletion syndromes were present. Genes such as TACC3, LETM1 and FGFR3 which are involved in duplicated region may have roles in the patient phenotype such as Seizure and skeletal abnormalities [14-18]. As far as 9p deletion is concerned, patients with 9p deletion present with MR, craniofacial dysmorphic features and genital or gonadal disorders [19-21].

Although patient 2 had normal karyotype, the detected aberrations by array CGH approach revealed the rearrangement of large segments of chromosomal material. It can be argued that since the gained and lost segments were almost of equal sizes, an exchange of material in the germline has taken place between chromosomes 5p and 18p in the form of reciprocal translocation.

The 22q13 deletion syndrome (or Phelan-McDermid syndrome) is a microdeletion syndrome characterized by severe neonatal hypotonia and global developmental delay, normal to accelerated growth, absent to severely delayed speech and minor dysmorphic features. Of the more than 90 genes that may be deleted in 22q13.3 deletion syndrome (Phelan-McDermid syndrome), the majority of the neurological features are thought to be caused by haploinsufficiency of SHANK3 [22] which is deleted in patient 3 too.

4q duplication is a rare condition that usually presents with MR and multiple abnormalities, including microcephaly, malformed ears, flat nasal bridge, tooth and thumb anomalies [23, 24], most of which are shared by patient 4.

Patient 6 had 17p11.2 duplication (Potocki-Lupski syndrome). Deletion and duplication of the 3.7-Mb region in 17p11.2 result in two reciprocal syndromes, Smith-Magenis syndrome (SMS, OMIM #182290) and Potocki-Lupski syndrome (PTLS, OMIM #610883) respectively which arise through a faulty non allelic homologous recombination [25, 26]. The clinical features observed in patients with PTLS are distinct from those seen with SMS although cognitive and neurobehavioral abnormalities are present in both disorders. Clinically, PTLS presents as a milder syndrome than SMS, with distinct characteristics, though PTLS can be mistaken for SMS [25-28].

Patients 7 and 8 showed subtelomeric chromosomal rearrangements. These regions are gene-rich and a small deletion in these regions results in severe consequences such as moderate to severe MR and multiple congenital anomalies, dysmorphism, and abnormal growth or behavior [12].

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