Original Article

Genomic characterization of some Iranian children with idiopathic mental retardation using array comparative genomic hybridization

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BACKGROUND: Mental retardation (MR) has a prevalence of 1-3% and genetic causes are present in more than 50% of patients. Chromosomal abnormalities are one of the most common genetic causes of MR and are responsible for 4-28% of mental retardation. However, the smallest loss or gain of material visible by standard cytogenetic is about 4 Mb and for smaller abnormalities, molecular cytogenetic techniques such as array comparative genomic hybridization (array CGH) should be used. It has been shown that 15-25% of idiopathic MR (IMR) has submicroscopic rearrangements detectable by array CGH. In this project, the genomic abnormalities were investigated in 32 MR patients using this technique.

MATERIALS AND METHODS: Patients with IMR with dysmorphism were investigated in this study. Karyotype analysis, fragile X and metabolic tests were first carried out on the patients. The copy number variation was then assessed in a total of 32 patients with normal results for the mentioned tests using whole genome oligo array CGH. Multiple ligation probe amplification was carried out as a confirmation test.

RESULTS: In total, 19% of the patients showed genomic abnormalities. This is reduced to 12.5% once the two patients with abnormal karyotypes (upon re-evaluation) are removed.

CONCLUSION: The array CGH technique increased the detection rate of genomic imbalances in our patients by 12.5%. It is an accurate and reliable method for the determination of genomic imbalances in patients with IMR and dysmorphism.

Key words: Array comparative genomic hybridization, chromosome abnormality, dysmorphism, genomic variation, idiopathic mental retardation

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Introduction

In the general population, the incidence of mental retardation (MR) is 1-3%.[1] The etiology of MR is very heterogeneous and it can be caused by various environmental and/or genetic factors. However, for up to 60% of cases, there is no identifiable cause.[2] Genetic factors are the most common cause of severe MR and thought to be present in about 50% of cases.[3]

An important genetic factor has been shown to be due to chromosome abnormalities. Microscopic and submicroscopic chromosomal rearrangements account for nearly 25% of all patients.[4,5] Cryptic subtelomeric chromosomal imbalances are present in 5-20% of patients with idiopathic mental retardation (IMR).[6-10] Conventional cytogenetic can only detect abnormalities larger than 4 Mbp. Use of molecular cytogenetic techniques such as fluorescent *in situ* hybridization (FISH) and multiple ligation probe amplification (MLPA) techniques, as targeted techniques, can detect chromosome abnormalities localized in different regions

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with smaller resolution. Array comparative genomic hybridization (array CGH) is a powerful tool for detecting very small chromosomal imbalances and is used for genome-wide screening of chromosomal imbalances in patients with IMR. Using this technique, submicroscopic abnormalities have been detected in 15-25% of patients with IMR.[11,12]

In an attempt to evaluate the chromosome abnormality rate in children with IMR and dysmorphism, 32 patients were investigated with oligonucleotide array CGH. All the patients had normal karyotype, with no fragile X or metabolic disorder.

Materials and Methods

Patients

Patients were referred to the Genetics Research Center by different clinicians. All were clinically evaluated and a clinical evaluation form was completed for each patient. Children with IMR with or without dysmorphism and without any family history of MR were recruited. The IQ test used was Raven and the patients had an IQ < 70. The age of the patients was 4-18 years. The consent form was obtained from the guardian of the patients. Both heparinized and ethylenediaminetetraacetic blood samples were obtained.

Genetics tests

Karyotyping

Cytogenetic analysis was performed on cultured peripheral blood lymphocytes stimulated with phytohaemagglutinin M, using standard techniques.[4] Karyotype was determined in all patients by high resolution by high resolution Giemsa (G)-banding technique banding.

Metabolic tests

Patients were screened for 30 most common metabolic disorders using tandem mass spectroscopy. The tests included phenylketonuria, galactosemia, mucopolysaccharidosis, familial tyrosinemia, primary congenital hypothyroidism, classic galactosemia, Niemann-Pick disease, carnitine palmitoyltransferase 1 and medium-chain-acyl-coa-dehyrogenase-deficiency (MCAD).

Fragile X screening

Genomic deoxyribonucleic acid was isolated from peripheral blood leukocytes by standard salting out method. The initial analysis of the CGG repeat region of the FMR1 gene was performed by polymerase chain reaction (PCR) amplification using the primers FR1 and FR3. Amplification products were resolved by 8% polyacrylamide gel electrophoresis. To detect and to confirm the presence of a trinucleotide expansion in males or females, Southern blot analysis was performed.

Array CGH

Array CGH was performed using CytoChip International Standard Cytogenetic Array 4 × 44 k (v2.0) platform provided by blue genome. The array provides whole genome coverage with probe distance of 75 kbps and resolution of 300 kb, as well as targeted enriched gene regions with increased resolution of 150-200 kbs. All hybridized slides were scanned using Inno Scan 710. 25 of the patients were repeated on the same platform against different controls for confirmation of results. Slides were analyzed using BlueFuse Multi Software, Version 3.1 (BlueGnome Ltd. Cambridge CB21 5XE UK). All the data were controlled with University of California Santa Cruz human genome browser, ensemble, decipher and database of genomic variants. Polymorphic Copy number variations (CNVs) and those not containing genes were disregarded.

MLPA

The kits used were SALSA P070 and P036 human subtelomere test kits (MRC-Holland, Amsterdam, Netherlands: http://www.mrc-holland.com). The MLPA mix contained probes for all subtelomeric regions except the short arms of the acrocentric chromosomes (13p, 14p, 15p, 21p and 22p). MLPA analysis was carried out as suggested by the manufacturer. PCR amplification products were identified and quantified by a capillary electrophoresis using ABI 3100 genetic analyzer.

Results

The array CGH was performed on a total of 32 patients in whom the result of chromosome analysis, metabolic tests and fragile X screening were normal. Six patients

showed genomic abnormalities [Table 1]. The array CGH results for patients one and three are shown in Figures 1 and 2 as examples. The clinical manifestations of these patients are presented in Table 2. Two patients with normal karyotype results obtained from other centers (cases 34340, 1241) revealed large imbalances with array CGH and were consequently suspected to have chromosome abnormalities. Upon reevaluation, both patients showed chromosome rearrangements by microscope analysis [Table 1]. In both cases, mother was the carrier of a balanced reciprocal translocation.

The subtelomeric abnormalities in the patients were confirmed with MLPA test and the observed abnormality was investigated in the parents to determine its origin. Using MLPA, only in one patient (29660), the same abnormality was present in the patient's healthy mother and it is most probably a polymorphism. For four other patients, the parents were apparently normal and therefore the genomic imbalances in the patients are most probably pathogenic. The origin of the abnormality in the last patient (32000) was not determined.

Discussion

Many small chromosome abnormalities (less than 4 Mb) can be the causative reason for mental retardation, which cannot be detected using standard cytogenetic techniques. The reports on the chromosome abnormality rate in Iranian patients with IMR are sparse. Behjati *et al*.[13] reported the rate of chromosome abnormality in Iranian patients with an IMR with consanguineous parents as 1.24%. More advanced molecular Cytogenetics techniques like array CGH can be used to detect the chromosome abnormalities throughout the genome. The detection rate for chromosome abnormality has been increased using this technique. Different studies have shown an increase of 15-25% detection rate for submicroscopic abnormalities using array CGH in patients with normal karyotypes.[14,15] In this study, 32 children with IMR (IQ<70) and dysmorphism were investigated using whole genome oligo array CGH (blue genome). The karyotype analysis, fragile X and metabolic abnormalities were normal.

Table 1: The karyotype and array CGH results for six patients with abnormal results

Patient code	Sex	Age (years)	Consanguinity status	Karyotype	Array CGH result	Deletion size	Duplication size	Parental result (MLPA)
34340	M	3.5	Consanguineous	46, XY, der (18) t (6;18) (q25.3;q21.3) mat	arr 6q26q27 (161,591,994-170,316,535) x3, 18q21.33q23 (57,854,896-76,110,994) x1	18.2 Mb	8.7 Mb	N
1241	M	4	Non-consanguinous	46, XY, der (13) t (7;13) (q32;q32) mat	arr 7q33q36.3 (136,400-158,000) x3, 13q33.3q34 (106,404, 835-114,110,721) x1	7.7 Mb	22 Mb	N
29660	M	7	Consanguinous	46, XY	arr 3p26.3p26.3 (312,929-2,001,240) x1	1.68 Mb	-	Mother, the same as patient
30240	M	8	Consanguinous	46, XY	arr 15q11.2q13.1 (21,208,406-26,193,879) x1	4.9 Mb	-	N
34580	F	5	Consanguinous	46, XX	arr 1p36.33p36.33 (769,620-2,053,075) x1	1.2 Mb	-	N
32000	M	17	Non-consanguinous	46, XY	arr 15q24.1q24.2 (70,751,053-73,322,354) x1	2.6 Mb	-	ND

N: Normal, ND: Not determined, MLPA: Multiple ligation probe amplification, CGH: Comparative genomic hybridization, M: Male, F; Female

Table 2: The clinical features of six patients with abnormal array CGH results

Patient code	Clinical features								
	Dysmorphic facial features	Micrognatia	Other dysmorphic features	Hearing loss	Seizure	Autism	Unbalanced movement	Speech delay	FTT
34340	+	–	Microcephaly, CHD	+	+	–	–	+	+
1241	+	+	Brachycephaly, muscle atrophy Left hemiplegia	–	–	–	–	–	–
29660	+	+	Palmar transverse crease	–	+	–	+	–	–
30240	+	–	Skin and eye hypopigmentation	–	+	–	+	–	–
34580	+	–	–	–	–	–	+	–	–
32000	–	–	Macrocephaly	–	+	+	–	–	–

FTT: Failure to thrive, CHD: Congenital heart defect, CGH: Comparative genomic hybridization

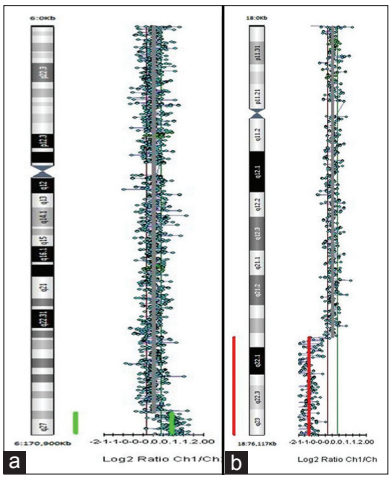

Figure 1: Array comparative genomic hybridization result for patient 34340 showing duplication of 6q (a) and deletion of 18q (b) as: arr 6q26q27 (161,591,994-170,316,535) x3, 18q21.33q23 (57,854,896-76,110,994) x1

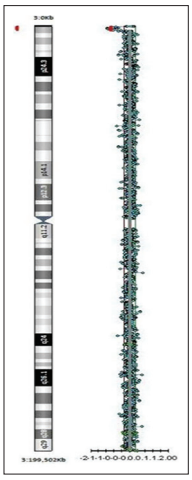

Figure 2: Array comparative genomic hybridization result for patient 29660 showing deletion in 3p as: arr 3p26.3p26.3 (312,929-2,001,240) x1

Six patients showed genomic abnormalities [Table 1]. Patients one and two both showed deletions/duplications, resulted from a maternal balanced translocation. Both patients had been reported to have normal karyotype by other laboratories. The karyotyping was repeated and showed abnormal chromosome complements confirming the array CGH results. In patient one, array CGH test showed abnormalities involving chromosomes 6 and 18. Most of the patient's clinical manifestations could be explained by partial monosomy 18q. However, partial trisomy 6q26q27 also has some specific features[16,17] which could be seen in our patient. These features include growth retardation, microcephaly, acrocephaly, hand and foot anomalies and dental decay. The partial deletion of 13q[18] and distal duplications of 7q[19] observed in-patient two are both well-known genetic disorders with a very wide spectrum of clinical phenotypes. Some of patient's clinical manifestation such as microcephaly and genital anomaly might be attributed to partial monosomy of 13q and others such as micrognatia to partial trisomy of 7q.[20]

Patients three, four and five all had subtelomeric deletions for the short arm of chromosome 3 (3p26.3p26.3) proximal region of long arm of chromosome 15(q11.2q13.1) and short arm of chromosome 1 (1p36.33p36.33) respectively [Table 1]. The MLPA test using subtelomeric SALSA MLPA KIT P036-E1 confirmed these abnormalities. The inheritance of these abnormalities was validated for patients 35 using MLPA and only the deleted 3p were inherited from the patient's mother. The size of 3p deletion was 1.68 Mb including *CHL1* and *CNTN6* genes. His healthy mother showed the same subtelomeric abnormality, which means it couldn't be the cause of clinical manifestations and most probably is inherited as a polymorphism. However, the inherited form of 3p36.3 deletion with a size less than 1 Mb (comprising only the gene *CHL1*) from a normal father to two affected children has been reported by Cuoco *et al.*[21] The two brothers had a mild MR without any other distinct features. This group suggested the *CHL1* gene was responsible for the mild phenotype symptoms observed in the patients.

The abnormality in patients four and five were apparently de novo and therefore pathogenic. Both abnormalities were consistent with previously known microdeletion syndromes. The abnormality in-patient four is consistent with Angelman syndrome compatible with the patient's clinical manifestation[22] and patient five genomic and clinical abnormality was consistent with the known 1p36 microdeletion syndrome.

Patient six had an interstitial deletion of 15q24.1q24.2. This is a known pathogenic abnormality as the 15q24 microdeletion syndrome was first described by Sharp *et al.* in 2007.[23] Phenotypic features include mild to moderate developmental delay, characteristic facial features, growth retardation, hypotonia, joint laxity, digital abnormalities and genital abnormalities. Smith *et al.* in 2008 also described a girl (previously been assessed by FISH[24]) with autism, developmental delay and mild dysmorphism containing a 15q24 deletion overlapping

the genomic region identified by Sharp *et al*. (Moyra Smith, personal communication), which has already been reported and is associated with intellectual disability and autism.[25]

In this study, six patients out of a total of 32 (19%) showed chromosome abnormalities using array CGH. However, two patients upon re-evaluation had gross chromosome abnormalities. Excluding these two patients, array CGH increased the chromosome abnormality detection rate by 12.5%. This is similar to some other reported cases.[15, 26-28]

Conclusion

The abnormality rate in patients with normal karyotype, using array CGH technique, was increased by 12.5%. The array CGH technique is an accurate and reliable method for the determination of genomic imbalances in patients with IMR and dysmorphism. However, in order to show the inheritance status of these abnormalities, FISH investigation is pending. In view of the fact that the chromosome abnormality had been missed by two laboratories, it is recommended that cytogenetic laboratories, which do not have enough expertise should refer patients with MR and dysmorphism for array CGH investigation.

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