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Rearrangement in 22q11 implicated in Iranian patients with mental retardation

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ABSTRACT

Chromosome 22, particularly 22q11.2 region, is predisposed to rearrangements due to misalignments of low-copy repeats (LCRs). DiGeorge/velo-cardio-facial syndrome is a common disorder resulting from microdeletion within the same band. Although both deletion and duplication in this region are expected to occur in equal proportions as reciprocal events caused by LCR-mediated rearrangements, very few microduplications have been identified. The phenotype of these patients with microduplications is extremely diverse, ranging from normal to behavioral abnormalities to multiple defects, only some of which are reminiscent of the 22q11.2 deletion syndrome. The aim of this study was to investigate 22q11.2 microdeletion and microduplication among Iranian patients with mental retardation. For this purpose, 46 mental retarded patients who were tested negative for fragile X syndrome were involved in this study. The samples were assessed for 22q11.2 microduplication and microdeletions by Semi-Quantitative Multiplex Polymerase chain reaction (SQMPCR). MLPA was carried out to confirm the findings and to rule out other abnormalities in subtelomeric region. We found three patients with microdeletion and one with microduplication and one with 10p deletion syndrome. These findings proved evidence that microdeletion and microduplication of 22q11.2 can be a reason of mental retardation in Iranian population with unknown causes.

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1. Introduction

Chromosomal rearrangements can cause imbalanced gene dosage and lead to genetic disorders that are usually associated with congenital malformations and mental retardation. [1]. They are the results of deletions, duplications and unbalanced translocations of part of a chromosome. Some parts are more susceptible to chromosomal rearrangement because of having low copy repeats (LCR), the human 22q11.2 region is an example of these regions that can lead to four different syndromes [1]. The most well recognized disorder is velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS [MIM #192349 or MIM #188400]), associated with a recurrent 3-Mb hemizygous deletion [2–4]. Developmental disorder occurs in individuals with a duplication of the same region that is deleted and is one of the main manifestations of this submicroscopic abnormality [5].

The human 22q11.2 region contains three breakpoint regions, each one containing an LCR called LCR22. The most frequent deletions result from non allelic homologous recombination (NAHR) events between two 240-kb LCRs that are 3 Mb apart from each other termed LCR22-2 and LCR22-4 [4,6,7]. NAHR is one of the major mechanisms for chromosomal rearrangements. Duplications and deletions are generated when NAHR happens between directly mediated LCRs. Inversions are generated when NAHR is mediated by inversely oriented LCRs. Different complex rearrangements are generated depending on the combination of LCRs that participate in the NAHR event [7]. Approximately 7% of 22q11DS patients have a nested distal deletion endpoint in LCR22-3a which is lying between LCR22-2 and LCR22-4. This rearrangement results in a 1.5-Mb deletion [7]. In addition to deletions and duplications, translocations also occur in 22q11.2, by NAHR between repeats on different chromosomes, some within LCR22s. The constitutional t (11; 22) translocation is the most common non-Robertsonian recurrent translocation in humans and the breakpoint on 22q11.2 is in LCR22-3a [8]. The 22q11.2 deletion syndrome represents the most commonly discovered deletion syndrome in humans with prevalence estimated at 1/4000–6000 live births [9]. Features of the 22q11.2 deletion syndrome include cleft palate, velopharyngeal

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insufficiency, hypernasal speech, characteristic facial features, heart defects, learning disabilities, and behavioral disorders [10].

Since both deletion and duplication happens as a result of NAHR caused by unequal crossover of LCRs, they are expected to have equal frequency. Surprisingly, there are only few reports regarding the detection of microduplications [5,11,12]. Patients with 22q11.2 duplication may be undetected due to having less-different, unpredictable, or milder phenotype which can cause ascertainment problems and also technical difficulties involved in identifying microduplications. Thus, recently the microduplication 22q11.2 syndrome has been identified as a new syndrome. The patients with 22q11.2 microduplications had a variable phenotype, including mental retardation, heart defects, hearing loss, velopharyngeal insufficiency with and without cleft palate, cognitive deficits, learning disabilities, growth delay, behavioral problems, motor delay, and mild dysmorphic features. However, the phenotype of some individuals with 22q11.2 microduplication may overlap with that of individuals with the 22q11.2 deletion syndrome and Fragile-X syndrome, this overlap may represent only one part of this syndrome's phenotypic spectrum [5,13].

The purpose of this study was to investigate possible 22q11.2 microduplications and microdeletions with the use of semi quantitative multiplex PCR (SQMPCR), among forty six mental retarded patients as a cause of their abnormality.

2. Materials and methods

2.1. Patient selection

Forty six patients were recruited base on the presence of mild to severe mental retardation. Since microdeletion and microduplication of 22q11.2 and fragile-X syndrome have phenotypic overlapping and patients with microdeletion and microduplication can be detected as a fragile-X candidate mistakenly, at first they were screened for fragile-X and those that were negative were examined for microduplication and microdeletions in the region of 22q11. Ten samples from normal people were also taken as a control group.

3. DNA extraction

Genomic DNA was extracted from fresh peripheral blood of the patients and controls using salting out method as described previously [14].

3.1. Marker and primer selection and semi quantitative multiplex PCR set up

Five primer sets were used in this study (Table 1). Three of the primer sets were established for the sequence tag site (STS) markers. Two of them are target markers and are located in the 3 Mb typically deleted region (TDR) (D22S944, D22S936). D22S636 is located outside of TDR (control marker) between LCR-4 and LCR-5. The other two primer sets amplify HPRT and P155675. HPRT is used as an internal control and P155675 is a target marker situated in 5′ flanking region of 3Mb TDR (Fig. 1). Each multiplex PCRs were set up in a way that one control marker and one target marker in duplication regions were included. PCR reactions were performed in 25 μl total volume containing: 0.25 unit of DNA Taq polymerase, 2 μM of dNTP mix, 1X PCR buffer and 10 μM of each primer. PCR products were analyzed on a 7.5% polyacrylamide gel stained with EtBr (ethidium bromide) and visualized under ultraviolet light by gel document system in order to confirm expected PCR amplifications and quantify the specific intensity of the patient's band and their matched controls. The density ratio of the target DNA bands over control fragments was calculated for patients and compared to the normal control. PCR product quantitation was performed in the log phase at three PCR cycles (22, 25, 28 cycles) using Total Lab TL100 software (http://www.totallab.com).

Table 1
List of primers used in this study.

Primers names	Sequence	Amplicon size (bp)	*Tm*
D22S936-F	CAATCTTGGCAGCCAGTTTAG	180	59.5
D22S936-R	CAGCATCTTCCTGGTGGCC		64.1
D22S944-F	CATGTGAAAGATGCTACTTCC	166	55
D22S944-R	ATCCCATGCTCCTCCCCAT		63.5
D22S636-F[a]	AACCTTCTGATGGCTCCTCT	123	57.9
D22S636-R	CATGGAGCTGACACTGAGTG		57.9
P15567-F	GCCAGAGGATAGGGAGTGC	119	59.8
P15567-R	GTGGAAGCAGTCAAACAG AAC		57.4
HPRT-F[a]	ACGTCTTGCTCGAGATGTGA	98	57.3
HPRT-R	CCAGCAGGTCAGCAAAGAAT		57.3

[a] D22S636 and HPRT markers were used as internal control markers.

3.2. Multiplex ligation probe amplification

Multiplex ligation dependent probe amplification (MLPA) with the SALSA P023B MLPA DiGeorge Syndrome Test Kit (MRC Holland, Amsterdam, Netherlands) was used to confirm the results of Semi-Quantitative Multiplex PCR in samples with microdeletion or microdeplication. Probe sets 36 and 70 were used to check subtelomeric regions. MLPA with P023, P36 and P70 probe sets was performed according to manufacturer's recommendations (www.mrc-holland.com).

This commercial kit consists of 39 cloned probes covering different chromosomal regions associated to 22q11.2 Microduplication/Microdeletion Syndrome and Cat Eye Syndrome. In the latter syndrome, the microduplication region was covered by seven specific probes and a subtelomeric 22q13 control probe. All of our MLPA experiments included the concordant positive controls (DNA from individuals previously indicated as carriers of 22q11.2 duplications or deletions) to ensure the reliable detection of copy number changes. The MLPA reactions were carried out as described previously [15].

Briefly, DNA samples were diluted with TE to 5 μl and heated at 98 °C for 5 min. After cooling the samples to 25 °C, 1.5 μl of probe mix)containing 1 fmol of each probe) and 1.5 μl of MLPA hybridization buffer were added, and the solution was denatured

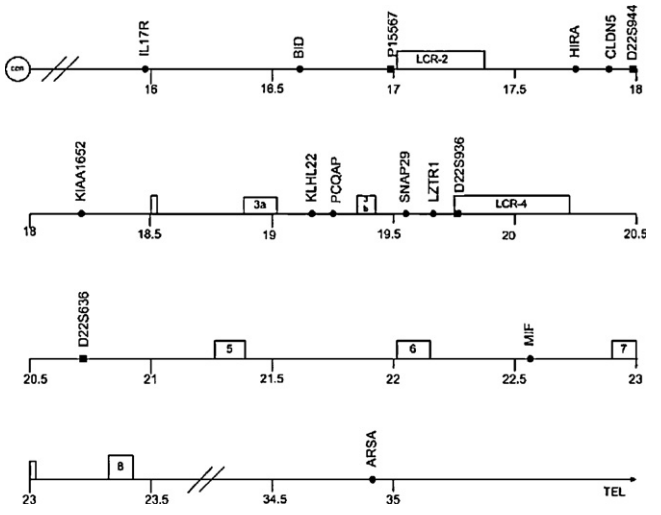

Fig. 1. Approximate location of markers used in this study as well as the other markers present in this region.

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