

Prevalence of 22q11.2 microdeletion syndrome in Iranian patients with cleft palate

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Abstract

Background: 22q11.2 microdeletion syndrome is the most common multiple genetic disorder associated with learning disabilities, developmental delays, immune deficiency, hypocalcemia, and cleft palate. Finding some valid criteria for screening of 22q11.2 deletion syndromes in infants would be very helpful in early diagnosis and treatment.

Materials and Methods: Since 69% of individuals with 22q11.2 deletion have a palatal abnormality, we studied the prevalence of 22q11.2 deletion syndrome in 378 Iranian patients during a 5-year period, including 291 patients affected with cleft palate only without cleft lip (CPO) and 87 patients affected with velopharyngeal incompetence (VPI) and/or submucous cleft palate (SMCP). DNA copy number was analyzed with multiplex ligation-dependent probe amplification (MLPA) technique.

Results: In our study, 15/378 (3.97%) patients with palatal anomalies showed 22q11.2 deletion. Interestingly, this prevalence between syndromic patients was 15/104 (14.42%).

Conclusion: It seems that SMCP or VPI, in addition to one or more another features of 22q11.2 deletions, especially developmental delay, may be good criteria for molecular investigation of 22q11.2 region.

Key Words: Chromosome 22q11.2 deletion syndrome, cleft palate, developmental delay, DiGeorge syndrome, Iranian

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Received: 20.01.2014, Accepted: 05.07.2014

INTRODUCTION

Copy number changes of the 22q11 chromosomal region cause a variety of disorders, including DiGeorge syndrome (DGS; MIM 188400) or velocardiofacial syndrome (VCFS; MIM 192430), 22q11.2 duplication syndrome, and cat eye syndrome (CES; MIM 115470). 22q11.2 deletion syndrome (DGS/VCFS) is the most common microdeletion syndrome with a prevalence of 1 in 4000–6000 live births,^[1-3] as well as the most

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.192728

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How to cite this article: Nouri N, Memarzadeh M, Salehi M, Nouri N, Meamar R, Behnam M, *et al.* Prevalence of 22q11.2 microdeletion syndrome in Iranian patients with cleft palate. *Adv Biomed Res* 2016;5:201.

frequent syndrome associated with conotruncal heart anomalies^[4-6] and cleft palate.^[7-10] The presence of chromosome-specific, low-copy repeat (LCR) units A–D on the proximal part of the long arm of chromosome 22 predisposes this region to cytogenetic rearrangements. Unequal crossing over between LCRs usually results in a 3 Mb deletion on one of the chromosomes 22 and a reciprocal and similarly sized duplication on the other one.^[11] Over 30 genes are located on commonly deleted/duplicated 3 Mb regions. Most 22q11 deletion syndrome patients (95%) have a 3 Mb interstitial deletion with similar endpoints, but a minority have a somewhat smaller (1.5–2 Mb) deletion.^[12-14] 22q11.2 deletion syndrome is inherited as autosomal dominant condition, but in 90% of cases, it occurs by new mutation and mutations are not inherited from parents.

22q deletion syndrome has variable expressivity, even among affected members of the same family. However, the most common signs and symptoms include conotruncal congenital heart defects (CHD), palatal abnormalities, characteristic facial features, learning difficulties, developmental delays, immune deficiency, hypocalcemia, gastrointestinal anomalies, and renal anomalies.^[15]

Sixty-nine percent of patients with 22q11.2 deletion have a palatal abnormality (*Gene Reviews*), particularly velopharyngeal incompetence (VPI) (27%), submucous cleft palate (SMCP) (16%), overt cleft palate without cleft lip (CPO) (11%), bifid uvula (5%), cleft lip/cleft lip and palate (2%), and infantile VPI (8%).^[16]

Since delayed diagnosis of 22q11.2 deletion syndrome will postpone the accurate and timely interventions that may not be compensated in future, finding some valid criteria for 22q11.2 deletion screening in infants might be effective. Palatal abnormality is one of the most common symptoms in 22q deletion syndrome and it is usually more apparent at birth, compared to other features that may have lower incidence or late onset in 22q11.2 deletion syndrome. Therefore, it can be a good criterion for screening of 22q11.2 deletion. In order to estimate the suitability of CPO for 22q11.2 deletion screening, in this study, for the first time, we used multiplex ligation-dependent probe amplification (MLPA) as a highly sensitive and accurate tool for detecting copy number changes in the 22q11.2 region to measure the prevalence of 22q11.2 deletion in Iranian patients with cleft palate only without cleft lip, or affected with VPI or submucosal cleft palate.

MATERIALS AND METHODS

Sample set

A total of 378 Iranian patients from different province

of Iran were referred to cleft palate clinic of Isfahan Medical University during a 5-year period (2006–2011), including patients with overt CPO or affected with VPI or SMCP with/without VPI (213 males and 165 females; age: 18 months to 27 years). Of these, 165 patients had soft palate cleft, 3 had hard palate cleft, and 123 had both. In addition, 87 patients had VPI or SMCP with/without VPI. Structure of the soft palate was assessed by direct oral examination plus nasopharyngoscopy in all cases. Palatal anomalies were labeled as either normal, overt soft and/or hard cleft palate, submucous, and/or VPI.

Finally, the patients who were positive for deletion were investigated more for the most common features of the syndrome, including heart abnormalities, hormones, blood calcium, parathyroid and thyroid hormones, as well as learning difficulties, developmental delay, and hearing loss. The study was approved by the local ethical committee. The patients or their parents were informed of the aims of the study and their consent was obtained before genetics analysis.

Laboratory methods

Genomic DNA of the patients and their parents was isolated from peripheral ethylenediaminetetraacetic acid (EDTA)-treated blood cells by Qiagen DNA Mini kit (cat. No.: 51304). The MLPA reactions were performed according to the manufacturer's protocols P 250-B1 kit: DGS; MRC-Holland, Amsterdam, the Netherlands^[17] as follows: (1) Denaturing 200 ng of target DNA at 98°C for 5 min; (2) hybridization with the SALSA MLPA kit P 250-B1 at 60°C for 16 h; (3) ligation by Ligase-65 mix at 54°C for 15 min; and (4) ligase inactivation by incubation at 98°C for 5 min. Finally, multiplex polymerase chain reaction (PCR) was performed using the specific SALSA FAM PCR primers, dNTPs, PCR buffer, and polymerase for 30 cycles (95°C for 30 s, 60°C for 30 s, and 72°C for 1 min). The fragments were analyzed on a 3100 capillary sequencer (Applied Biosystems, USA) with a 36-cm capillary array and POP-4 polymer (Applied Biosystems) by mixing with 0.2 ml of the GeneScan500 ROX Size Standard (Applied Biosystems) and 10 ml of Hi-Diformamide (Applied Biosystems). The results (size and the peak area) were analyzed using Gene Marker software. The P 250-B1 kit contains PCR probes for 48 loci with amplification products between 130 and 487 nucleotides. We tested DNA in batches of 18–20 samples, including 5 normal control samples and 2 known positive 22q11 deletion syndrome samples in every run. Heterozygous deletions of recognition sequences should give a 35–50% reduced relative peak area of the amplification product of the probe (P 250-B1 kit: DGS; MRC-Holland).

RESULTS

Among 378 cases which were successfully analyzed, we found 15 (3.97%) patients with 22q11.2 deletions (8 males and 7 females). The patients had SMCP with/without VPI or cleft uvula or hard and/or soft cleft palate [Table 1]. Interestingly, one of these patients had cleft uvula with asymmetric pharyngeal movement and another one had SMCP with no movement of palate. However, none of the patients with 22q11.2 deletion had hard cleft palate, except one patient who had both soft and hard cleft palate anomaly. The type of palatal defect seen in most of the patients was SMCP with or without other palatal defects (10 patients). All these 15 patients with 22q11.2 deletion had at least two other symptoms and signs of the 22q11.2 deletion syndrome, including characteristic facial features (elongated face, short philtrum, hypertelorism, prominent nose, small ears, low-set ear, eye puffiness), congenital heart disease, immune deficiency, hypocalcemia, renal hypoplasia, hearing loss, hypocalcemia, hypoparathyroidism, hypoplastic thymus, polydactyly, gastrointestinal anomaly, tapering of finger, immaturity, social psychiatric illness, ear malformation, growth hormone deficiency, tong ankylosis, gastrointestinal abnormalities, and especially developmental

delay and learning difficulties that were seen in 12 patients.

Among these 378 patients, about 104 patients had at least one another defect or facial features of 22q deletion syndrome, in addition to cleft anomaly. Therefore, the prevalence of 22q11.2 deletions in patients with syndromic forms of cleft palate (overt, VPI, submucous) was 15/104 (14.42%).

DISCUSSION

Since delayed diagnosis of 22q11.2 deletion syndrome will postpone the accurate and timely interventions that may not be compensated in future, finding some valid criteria for 22q11.2 deletion screening in infants might be effective. Because of the high frequency of VPI and palatal deficiencies in VCFS/DGS syndrome, it is expected that many of these patients undergo surgical management of hypernasal speech or palatal deficiencies, and a severe problem in their surgery is that their internal carotid arteries are placed abnormally. The ectopic and medial placement of their arteries is a contraindication for pharyngeal flap surgery because it could result in carotid artery dissection during surgery.^[18,19] Therefore, operation approach in VCFS/DGS patients should be different

Table 1: Palatal findings and other symptoms and signs in 15 Iranian patients with 22q11.2 deletion syndrome

Patient ID	Sex	Age (year)	Cleft abnormalities	Symptoms and signs
P01	Male	11	Submucous cleft palate+cleft uvula with asymmetric pharyngeal movement+VPI	Characteristic facial features*, hypoparathyroidism, developmental delay
P02	Female	7	Submucous cleft palate+VPI	Characteristic facial features, developmental delay, learning difficulties, strabismus, umbilical hernia, astigmatism
P03	Male	3	Submucous cleft palate+VPI	Characteristic facial features, congenital heart disease, convulsion, diaphragmatic hernia, ankylosed tongue
P04	Male	17	Soft cleft palate+cleft uvula	Characteristic facial features, developmental delay, hemolytic anemia, ear malformation, right renal hypoplasia, hypoparathyroidism
P05	Male	8	Soft cleft palate	Characteristic facial features, developmental delay, joint hyper mobility, seizure, myopia, unilateral mild hearing loss
P06	Female	9	Submucous cleft palate+VPI	Characteristic facial features, developmental delay, learning difficulties, umbilical hernia, tapering fingers, hypoparathyroidism
P07	Female	5	Soft and hard cleft palate+submucous cleft	Characteristic facial feature, developmental delay, congenital heart disease, seizure, polydactyly of the hands, finger tapering, hypocalcemia
P08	Male	1.5	Soft cleft palate+cleft uvula	Characteristic facial feature, developmental delay, congenital heart disease, febrile seizure
P09	Female	12	Submucous cleft palate+VPI	Hypernasaly, congenital heart disease, finger tapering, depression in mood, learning difficulties
P10	Male	18	Submucous cleft palate with no movement of palate	Congenital heart disease, seizures, growth hormone deficiency, learning difficulties, deviation of the spine, severe depression in mood, club foot, hypocalcemia
P11	Female	3	Soft cleft palate+cleft uvula	Characteristic facial features, developmental delay
P12	Male	7	Submucous cleft palate+VPI	Characteristic facial features, congenital heart disease
P13	Male	10	Soft cleft palate	Characteristic facial features, hyperactivity, congenital heart disease, learning difficulties
P14	Female	2	Submucous cleft palate	Convulsion, hypocalcemia, finger tapering, developmental delay
P15	Female	5	Submucous cleft palate	Characteristic facial features, immune deficiency, finger tapering

VPI: Velopharyngeal incompetence. *Characteristic facial features include elongated face, short philtrum, hypertelorism, prominent nose, small ears, low-set ear, and eye puffiness

from that in other patients with cleft palate. In addition, the only phenotypic feature which is considered at birth in most of the infants affected with VCFS/DGS syndrome is cleft palate and/or heart disorders. However, patients may suffer from other clinical features in future, such as immunological disorders, learning disabilities, social psychiatric illness, and speech disorders. All these symptoms should be diagnosed early in childhood in order to start early interventions. Accurate diagnosis of this syndrome is essential to help patients in prognosis, treatment, avoiding adverse clinical effects, disease management, and determining inheritance pattern in the family and recurrence risk in next pregnancies. It is reported that 69% of patients with 22q11 deletion syndrome have palatal abnormalities,^[16] suggesting cleft palate as a suitable criterion for screening patients with 22q11 deletion syndrome. In the present study, the prevalence of 22q11.2 deletions in patients with syndromic CPO, VPI, and/or SMCP was 14.42% (15/104).

It seems that there are not enough data on the prevalence of 22q11.2 deletions in patients with CPO. In three studies on a total of 91 non-syndromic CPO patients, 22q11.2 deletion was detected only in 1 patient (1.1%) by metaphase fluorescence *in situ* hybridization (FISH) analysis.^[20-22] This led Ruiter *et al.* to suggest that 22q11.2 deletion screening is not warranted in infants with CPO without any other clinical anomalies, and it is in accordance with our results that all of our 15 patients with 22q11.2 deletion had more than three other symptoms and signs of the 22q11.2 deletion syndrome. In our investigation, among all 378 patients, about 104 patients had one or more defects or facial features of 22q. 11.2 deletion or duplication syndrome, in addition to cleft anomaly; therefore, the prevalence of 22q11.2 deletions between patients with syndromic overt CPO, VPI, and/or SMCP was 14.42% (15/104), which is considerable. Sivertsen *et al.* investigated the prevalence of duplications and deletions of 22q11.2 among 169 babies born with open CPO in Norway during a 5-year period (1996–2001).^[23] They found no 22q11.2 duplications, but three cases with 22q11.2 deletions. All three 22q11 deletion syndrome cases also had heart malformations. Therefore, the prevalence of 22q11.2 deletions was 3/169 (1.77%) in their population. In another recent study, Prabodha *et al.* evaluated 22q11.2 deletion in 162 patients with cleft palate in Sri Lanka; none of the patients had 22q11.2 deletion in their study population.^[24] Therefore, the prevalence of 22q11.2 deletions in two mentioned studies is much lower than the prevalence in our study [15/378 (3.97%)]. This may be because of lesser number of cases in the studies of Sivertsen *et al.* and Prabodha *et al.* in

comparison to our study. Also, Sivertsen *et al.* did not include VPI and/or SMCP cases in their study because submucous clefts are usually not diagnosed in the newborn. However, our results reveal that most of the patients (9 of 15) with 22q11.2 deletion have SMCP.

We used MLPA as it is a cost-effective, highly available, and higher sensitivity tool for diagnosis of 22q11 deletion syndrome, in comparison to array comparative genomic hybridization (array-CGH) method and standard FISH techniques. Although majority of the 22q11.2 deletions can be detected by FISH test by using an assay probing for TUPLE1 or N25 on 22q11.2 microdeletions, this test fails to detect deletions that are either proximal or distal to the FISH probes, as in Stachon *et al.*'s study where 12 patients were not detected by FISH but were diagnosed by MLPA.^[25] Additional advantages of MLPA testing are characterizing the size and position of deletion as well as 22q11.2 microduplications.

In conclusion, palate defects, especially SMCP and VPI, in addition to one or more other features of 22q11.2 deletions, especially developmental delay and learning difficulties, may be good criteria for molecular investigation of 22q11.2 region.

ACKNOWLEDGMENTS

This work was funded by the Deputy for Research, Isfahan University of Medical Sciences, Isfahan, Iran, with the grant number 190123. The authors wish to sincerely thank the patients and their families who assisted them in carrying out this research. There was no particular conflict of interest with any organization.

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Source of Support: This work was funded by the Deputy for Research, Isfahan University of Medical Sciences, Isfahan, Iran, with the grant number 190123. The authors are grateful to Cleft Lip and Palate Team in Isfahan University of Medical Sciences, who helped us in this study. **Conflict of Interest:** There are no conflict of interest.