RESEARCH PAPER

Improving the Diagnosis of Children with 22q11.2 Deletion Syndrome: A Single-center Experience from Serbia

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Objective: The incidence of the 22q11.2 microdeletion among children who have at least two out of five major clinical criteria for 22q11.2 deletion syndrome.

Design: Prospective study.

Setting: University Children's Hospital in Belgrade, Serbia between 2005 and 2014.

Participants: 57 patients with clinical characteristics of 22q11.2 deletion syndrome.

Methods: Standard G-banding cytogenetic analysis was performed in all children, and the 22q11.2 genomic region was examined using fluorescence *in situ* hybridization (FISH). For patients with no deletion detected by FISH, multiplex ligation-dependent probe amplification (MLPA) analysis was also done in order to detect cryptic deletions of this region and to analyze other genomic loci associated with phenotypes resembling the syndrome. A selected group of patients diagnosed to have 22q11.2 microdeletion by FISH underwent MLPA testing in order to characterize the size and position of deletion.

Outcome Measure: The frequency of 22q11.2 microdeletion among children with at least two of the five major characteristics of 22q11.2 deletion syndrome (heart malformations, facial dysmorphism, T-cell immunodeficiency, palatal clefts and hypocalcemia/hypoparathyroidism)

Results: Typical 22q11.2 microdeletion was detected in 42.1% of patients; heart malformation were identified in all of them, facial dysmorphism in 79.2%, immunological problems in 63.6%, hypocalcemia in 62.5% and cleft palate in 8.3%.

Conclusions: A higher detection rate compared to one-feature criterion is obtained when at least two major features of 22q11.2 deletion syndrome are taking into consideration. The criteria applied in this study could be considered by centers in low-income countries.

Keywords: *DiGeorge syndrome, fluorescence in situ hybridization; multiplex ligation-dependent probe amplification*.

22q11.2 deletion syndrome (22q11.2DS) is the most common microdeletion syndrome with an estimated incidence of approximately 1/4000 per live births [1]. More than 180 malformations are associated with 22q11.2 microdeletion; the most common are cardiac defects, a characteristic facial appearance, thymic hypoplasia, cleft palate/velopharyngeal insufficiency (VPI), hypoparathyroidism with hypocalcaemia, speech and language impairment and developmental delay [2]. Most patients (87%) have a deletion of 3 Mb; less frequently (10%) a loss of 1.5 Mb; while a few patients have unique deletions, translocations or point mutations of the *TBX1* gene [2,3]. Commonly used methods for detection of 22q11.2 microdeletion are FISH and MLPA [1].

Detection rate is an important issue to consider in terms of achieving a balance between patient coverage and costs. Previously, different recruitment criteria have been applied providing 22q11.2 microdeletion detection rates ranging from zero to 34.7% [4-15]. We present our 10-year experience with an approach, resulting in high detection rate, which could be beneficial for centers in low-income countries.

Methods

Our study included 57 Caucasian children (21 females, age 1 day-14 yr) (***Fig.* 1**), recruited at the University Children's Hospital during the period 2005 - 2014. We wished to assess the 22q11.2 microdeletion detection rate in a cohort of patients whose enrollment was based on the presence of at least two out of the five major characteristics of 22q11.2 deletion syndrome (heart malformations, facial dysmorphism, T-cell immunodeficiency, palatal clefts and hypocalcemia/hypoparathyroidism). Written informed consent was obtained from the patients' parents. The Ethical

Committee of the University Children's Hospital approved the study protocol.

In order to determine presence of the five major phenotypic features, all patients underwent dysmorphology assessment provided by a clinical geneticist, as well as further clinical examinations, including echocardiography, immunophenotyping of peripheral blood lymphocytes and measurement of serum calcium (if decreased, additional measurement of parathyroid hormone).

Standard G-banding cytogenetic analysis was performed on phytohemagglutinin-stimulated peripheral blood lymphocytes according to routine protocol for karyotyping. Flourescent *in situ* hybridization (FISH) analysis on metaphase spreads from cultivated lymphocytes and multiplex ligation-dependent probe amplification (MLPA) analysis using Kit P250-A1 DiGeorge (MRC-Holland, The Netherlands) were carried out as described in Cuturilo, *et al*. [16]. The applied high density MLPA kit enables detection of cryptic deletions of the 22q11.2 region and analysis of another five genomic loci associated with phenotypes resembling 22q11.2DS. All patients enrolled in the study were screened for hypocalcemia.

The Chi-square test was used to compare differences in the frequency of occurrence of the typical facial dysmorphism between patients with and without 22q11.2 microdeletion; a P value <0.05 was considered as statistically significant.

RESULTS

A normal karyotype was detected in 56 patients. In one case cytogenetic analysis identified a distal 4q deletion. In 27 out of 33 patients having no deletion detected by FISH, MLPA analysis was performed (***Fig.* 1**).

Among the 24 patients diagnosed to have 22q11.2 microdeletion by FISH (***Table* I**), 14 patients underwent MLPA testing in order to characterize the size and position of the deletion. This analysis revealed 3 Mb deletion in 13 cases, while in one patient a 1.5Mb deletion was detected.

The frequency of occurrence of different congenital heart malformation types are shown in ***Table* II**. Dysmorphic facial features were present in 51 (89.5%)

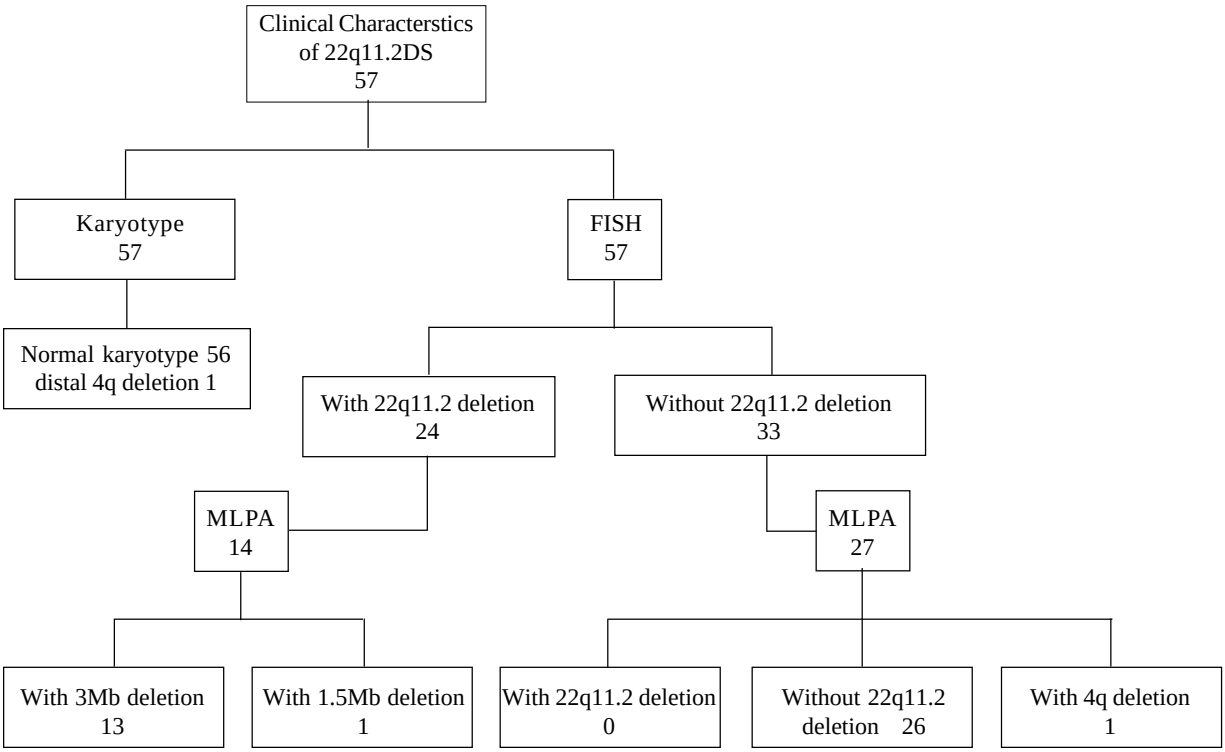

***Fig.* 1** *Flow of patients in the study.*

TABLE I MICRODELETION DETECTION IN CHILDREN WITH COMBINATION AT LEAST TWO OUT OF FIVE MAJOR CLINICAL CRITERIA FOR 22Q11.2 DELETION SYNDROME (*N*=24)

Heart Malformation with	*No.*
Facial dysmorpism	6
T-cell immunodeficiency	2
Hypocalcemia	2
Cleft palate	0
Facial dysmorpism and hypocalcemia	8
Hypocalcemia and T-cell immunodeficiency	1
Facial dysmorpism and T-cell immunodeficiency	1
Facial dysmorpism, T-cell immunodeficiency and hypocalcemia	2
Facial dysmorpism, cleft palate and hypocalcemia	2

TABLE II TYPE OF CONGENITAL HEART DISEASES AND 22Q11.2 MICRODELETION

Congenital heart disease	*22q11.2 microdeletion*	
	Present (no.)	*Absent (no.)*
Tetralogy of Fallot	6	16
Pulmonary artery atresia	4	6
Common arterial trunk	5	3
Interrupted aortic arch	6	1
Ventricular septal defect	2	3
Double outlet right ventricle	0	2
Aorto-pulmonary window	0	1
Mitral stenosis	1	0
Transposition of great arteries	0	1

participants. Typical facial dysmorphism (TFD) (short palpebral fissures, prominent nasal bridge and/or small mouth [17]) was observed in 20 patients (11 with the deletion). There was no difference in incidence of TFD between patients with and without 22q11.2 microdeletion (P=0.53). Non-specific facial dysmorphism (NSFD) was observed in 31 cases (8 with the deletion).

Decreased T-cell number was detected in seven patients with the 22q11.2 deletion. The incidence of overt cleft palate was 3/57 (5.3%), while microdeletion was disclosed only in one case. Furthermore, another patient with the 22q11.2 microdeletion and nasal speech was diagnosed with a submucous cleft palate.

Hypocalcemia was detected in 17 (29.8%) subjects among whom 15 had the microdeletion; hypoparathyroidism was confirmed in all but one of the patients with hypocalcemia.

DISCUSSION

Diagnosis of 22q11.2DS is directed towards early recognition and management, including multidisciplinary follow-up of the patients. Here we report our ten-year-experience in applying strict criteria for patient recruitment (presence of at least two out of the five major clinical characteristics of 22q11.2DS).

Many investigations of the 22q11.2 microdeletion detection rate in patients with a single phenotypic feature have found a very low rate, ranging from zero to 17.9% [5,7,9,10,12,15]. In contrast, other reports have suggested testing for microdeletion 22q11.2 only in patients with at least two 22q11.2DS features, with detection rates between 6.2 and 34.7% [4,6-8,10,11,13,14].

Our results provide additional support for using at least two of the five major clinical characteristics of 22q11.2DS for patient recruitment in order to achieve a satisfactory detection rate. Furthermore, the rate we obtained is higher than that of other studies. A possible explanation is detailed analysis of the phenotype of each patient by a clinical geneticist, implying that the detection rate for 22q11.2 microdeletion does not depend strictly on the number of anomalies, but also on a certain level of suspicion arising from the clinician's knowledge and experience.

The limitation of our approach is diminished coverage of patients, primarily those with a less typical 22q11.2DS phenotype. Furthermore, lack of MLPA testing of all patients diagnosed to have 22q11.2 microdeletion by FISH is another limitation of our study.

In conclusion, analyzing the patients with at least two major clinical features of 22q11.2DS we obtained a much higher detection rate for 22q11.2 microdeletion compared to the one-feature criterion. Furthermore, by applying both FISH and MLPA techniques, we could detect the typical 22q11.2 microdeletion and cryptic deletions of the 22q11.2 region. We could also analyze another five genomic loci associated with phenotypes resembling 22q11.2DS. Overall, this sets the basis for better care of children with 22q11.2DS in Serbia. Moreover, it could be interesting for other centers in low-income countries.

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Competing interests: None stated.

WHAT IS ALREADY KNOWN?

- Different criteria in patient recruitment for 22q11.2 microdeletion testing have been applied, providing a 22q11.2 microdeletion detection rate of 0 - 34.7%.

WHAT THIS STUDY ADDS?

- Recruitment based on the presence of at least two major phenotypic features enables a detection rate as high as 42.1%.

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