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*Original scientific paper*

# SPEECH AND LANGUAGE ABILITIES OF CHILDREN WITH THE FAMILIAL FORM OF 22Q11.2 DELETION SYNDROME

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The 22q11.2 Deletion Syndrome (22q11.2DS), which encompasses Shprintzen syndrome, DiGeorge and velocardiofacial syndrome, is the most common microdeletion syndrome in humans with an estimated incidence of approximately 1/4000 per live births. After Down syndrome, it is the second most common genetic syndrome associated with congenital heart malformations. The mode of inheritance of the 22q11.2DS is autosomal dominant. In approximately 72 - 94% of the cases the deletion has occurred *de novo*, while in 6 to 28% of patients deletion was inherited from a parent. As a part of a multidisciplinary study we examined the speech and language abilities of members of two families with inherited form of 22q11.2DS. The presence of 22q11.2 microdeletion was revealed by fluorescence *in situ* hybridization (FISH) and/or multiplex ligation-dependent probe amplification (MLPA). In one family we detected 1.5 Mb 22q11.2 microdeletion, while in the other family we found 3Mb microdeletion. Patients from both families showed delays in cognitive, socio-emotional, speech and language development. Furthermore, we found considerable variability in the phenotypic characteristics of 22q11.2DS and the degree of speech-language pathology not only between different families with 22q11.2 deletion, but also among members of the same family. In addition, we detected no correlation between the phenotype and the size of 22q11.2 microdeletion.

*Keywords:* 22q11.2DS, speech and language, mode of inheritance, size of deletion

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## INTRODUCTION

The 22q11.2 Deletion Syndrome (22q11.2DS) is the most common microdeletion syndrome in humans with an estimated incidence of approximately 1/4000 per live births (FERNANDEZ *et al.*, 2005a). Moreover, it is the second most common genetic syndrome associated with congenital heart malformations after Down syndrome (BASSETT and CHOW, 1999; WIEHAHN *et al.*, 2004).

Chromosome 22q11.2 region is a well-characterized and literature data indicate that it is gene-rich and contains multiple region-specific low-copy repeats (LCRs) (MCDERMID and MORROW, 2002). It was demonstrated that LCRs mediate chromosomal rearrangements that result in genomic disorders (MCDERMID and MORROW, 2002; SHAIKH *et al.*, 2007). The majority (90%) of the patients with 22q11.2DS have a 3 Mb deletion; less common (8%) is a 1.5 Mb deletion; a few patients have atypical deletions of shorter length and in variable locations (BEAUJARD *et al.*, 2009). The mode of inheritance of the 22q11.2DS is autosomal dominant. In approximately 72% - 94% of the cases the deletion has occurred *de novo*, while in 6 to 28% of patients the deletion was inherited from a mildly affected or normal parent (CARELLE-CALMELS *et al.*, 2009; FERNANDEZ *et al.*, 2005b).

The clinical phenotype of the 22q11.2DS is characterized by variable expression and incomplete penetrance (RYAN *et al.*, 1997). It has been associated with over 180 clinical features (ROBIN and SHPRINTZEN, 2005; RYAN *et al.*, 1997) and the most common are conotruncal congenital heart defects (CHD), velopharyngeal anomalies, hypoparathyroidism, T-cell immunodeficiency, craniofacial features, cognitive deficits and high rates of psychiatric morbidity (e.g., schizophrenia, anxiety disorders and attention deficit hyperactivity disorder (ADHD) (GOTHELF *et al.*, 2009; BASSETT *et al.*, 2003; SHPRINTZEN *et al.*, 1981). In the literature it was revealed that most children with 22q11.2DS show both; developmental delay and learning difficulties and they often need a special approach in education (SOLOT *et al.*, 2000). It was demonstrated that they have learning disabilities in both, verbal (language, speech, articulation, reading, comprehension) and non-verbal skills (motor skills, maths, visuo-spatial organisation) (PERSSON *et al.*, 2006; SOLOT *et al.*, 2000).

In this study we analyzed speech and language abilities of two patients with inherited form of 22q11.2DS. We revealed that both patients have delays in speech and language development. Additionally, we found considerable variability in the clinical presentation and in the degree of speech-language pathology between different families with 22q11.2DS, as well as among the members of the same family. Patient 1 with 3Mb deletion has a milder form of speech-language impairment than her peer with inherited 1.5 Mb deletion. Furthermore, we found no correlation between the phenotype (clinical features, cognitive maturity, socio-emotional and speech-language development) and the size of 22q11.2 microdeletion.

## MATERIALS AND METHODS

### *Procedure*

As a part of a multidisciplinary study we examined the speech and language abilities of two patients with inherited form of 22q11.2DS, from two different families. Patient 1 was 7.66 years of age and Patient 2 was 8 years of age. Both patients were Caucasian.

Patients were evaluated by the medical team from the University Children's Hospital, Belgrade, Serbia and a speech and language therapist from the Institute for Experimental Phonetics

and Speech pathology (IEPSP), Belgrade, Serbia. Prior to the participation in the study, the consent was obtained from their parents. The ethical committee of the University Children's Hospital approved the study protocol.

***Genetic analysis***

The presence of 22q11.2 microdeletion was revealed by fluorescence *in situ* hybridization (FISH) and/or multiplex ligation-dependent probe amplification (MLPA). FISH on metaphase spreads from cultivated lymphocytes with the probe specific for the common deletion interval (TUPLE1, 22q11.2, SpectrumOrange) and the control probe (ARSA, 22q13.3, SpectrumGreen) (Vysis/Abbott) was performed as described by Cuturilo et al. (CUTURILO *et al.*, 2013). The MLPA was done using Kit P250-A1 DiGeorge (MRC-Holland, Amsterdam, The Netherlands). The kit was used according to the instructions of the manufacturer, with some modifications earlier described in Cuturilo et al. (CUTURILO *et al.*, 2013).

***Assessment of language abilities***

The tests applied in this study belong to IEPSP Test battery and are regularly used in speech and language clinical practice in Serbia. In the current study, these tests have been used in order to compare the language skills of the two children and not for the purposes of diagnosis.
The research was conducted according to the ethical guidelines following the Declaration of Helsinki. The Ethical committee of the University Children's Hospital in Belgrade approved the study protocol.

***A story generation test*** (KOSTIC and VLADISAVLJEVIC, 1983)

A story generation test is a spontaneous language test. The estimation is based on the evaluation of the child's capability to generate a story from a set of four pictures, which are specifically designed to be used for children. The pictures are placed in front of the child and the child is instructed to look at them carefully and describe what has happened in the pictures. If the child is not able to describe the pictures independently, the examiner asks a set of open ended questions (such as: ''What has happened here? What happened next?''). The spontaneous speech is recorded on a tape and transcribed orthographically. Total number of words, total number of sentences (clauses) (grammatical and ungrammatical) and the number of ungrammatical clauses were analyzed.

***Global articulation test*** (KOSTIC and VLADISAVLJEVIC, 1983*)*

This test is used to assess the quality of phoneme pronunciation in the Serbian language. It consists of 30 words: 25 disyllabic and 5 monosyllabic. All target phonemes are analyzed in the initial position in words, except vowels which are analyzed in the second position in words. Each stimulus – word should be repeated by the patient after the examiner pronunciation. Based on the auditive performance of acoustic features of spoken phonemes and the position of the examinee's speech organs at pronunciation, the examiner notices if any pathological pronunciation (type and degree) exists. The quality of the pronounced phonemes in the given word is marked from 1 to 7. The phonemes pronounced according to the standard norm for the Serbian language are marked 1, 2 or 3, while phonemes marked by 4 are classified as marginally pronounced. The distortion of phonemes is marked by 5 or 6, depending on the degree of deviation. The phonemes which are not

pronounced (omissions) or substituted with another phoneme (pathological substitutions) are marked 7.

***Test of oral praxis*** (STEVANKOVIĆ *et al.*, 1993)

The test of oral praxis examine whether the child can perform a set of movements and whether he/she adequately uses his/her speech organs (trachea, larynx, pharynx, mouth and nasal cavity, tongue, jaw). The test is consists of 31 items-oral movements that the child imitates involving tongue, soft palate, jaw and lip movements. Each item is scored (0) for an incorrect response or (1) for well-executed response. If there are more than 75% of well-executed oral movements, it represents a normal response.

It should be pointed out that none of the above mentioned language measures have been standardised on the Serbian population, however, all of them are regularly used in speech and language clinical practice in Serbia (VUKOVIC *et al.*, 2010).

***Assessment of intellectual ability***

Human Figure Drawing Test (GOODENOUGH and HARRIS, 1964) is an indicator of perceptual and conceptual development of children. This test is used for evaluating the intellectual status of children up to the age of puberty.

This test is used primarily as a screening device. The examiner asks the child to make a picture of a person. All the drawings that can be recognized as attempts to represent the human figure are scored plus or minus (gross, head and hand details, attachments, proportion, joints...).The stage in which the drawing cannot be recognized as a human figure is scored 0 (Aimless uncontrolled scribbling) or 1 (Lines somewhat controlled – approaches crude geometrical form). Scores are equivalents of mental age of the child (Table from Human Figure Drawing Test). The drawings of bright children more than 10 years old or those who have had drawing lessons will result in an invalid evaluation of the child's intellectual potential.

Based on the analysis of the children's drawings, the achieved levels of cognitive development are presented in terms of: cognitive development under chronological age (Below CA), cognitive development at chronological age (At CA) and cognitive development above chronological age (Above CA).

## RESULTS

***Detection of 22q11.2 microdeletion in patients with phenotypic features of 22q11.2DS***

Patient 1, a 7.66-year-old girl, received the diagnosis of ventricular septal defects (VSD). Additionally, facial dysmorphia with small mouth was noticed during examination. The observed clinical findings indicated the possibility of microdeletion 22q11.2. The MLPA analysis revealed 3-Mb deletion, spanning the 14-probe region, between proximal CLTCL1 and distal LZTR1 probe (Figure 1a). Her parents underwent genetic testing and the MLPA analysis revealed deletion of the same size and position at her mother (Figure 1b). No cardiac defect and facial dysmorphia was found at her mother.

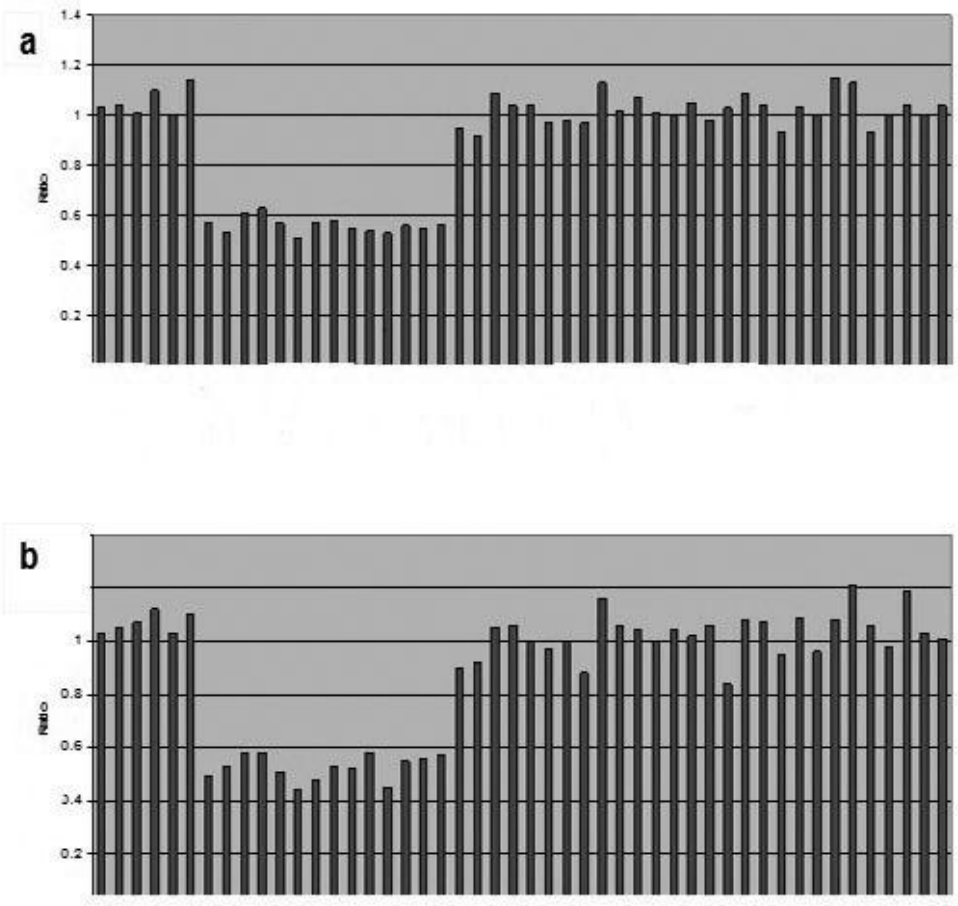


**Figure 1.** The diagrams of the results obtained by multiplex ligation-dependent probe amplification analysis of the 22q11.2 region in Patient 1(**a**) and her mother (**b**). The Results revealed a 3-megabase deletion spanning the 14-probe region, between proximal CLTCL1 and distal LZTR1 probe in Patient 1 and her mother. X-axis represents various probes, whereas Y-axis represents probe–height ratio.

Clinical presentation of Patient 2 was described in our previous report (CUTURILO *et al.*, 2008). He has a rare form of interrupted aortic arch type C and facial dysmophia with slightly deranged contour of the ear. The FISH analysis demonstrated 22q11.2 microdeletion in this Patient (CUTURILO *et al.*, 2008) (Figure 2a). His parents underwent genetic testing and, by applying the FISH, 22q11.2 microdeletion was detected in patient's mother (Figure 2b). Phenotypic characteristics associated with 22q11.2DS were not detected at his mother. To better

characterize the size and position of 22q11.2 microdeletion we performed the MLPA analysis (Figure 2). The results revealed 1,457 Mb deletion spanning the 9-probe region, between proximal CLTCL1 and distal DGCR8 probe in Patient 2 (Figure 2c) and his mother (Figure 2d).

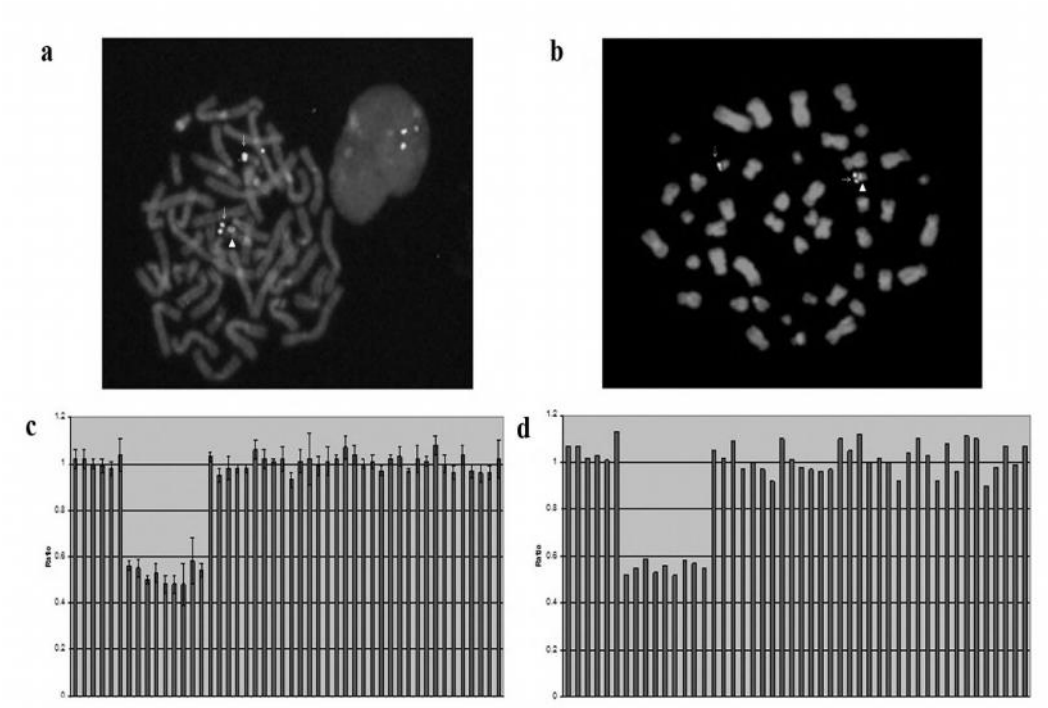


**Figure 2.** Fluorescence *in situ* hybridisation and multiplex ligation-dependent probe amplification analyses of the 22q11.2 region in Patient 2 and his mother.

Fluorescence *in situ* hybridisation with TUPLE1 probe (22q11.2) and ARSA control probe (22q13.3) revealed 22q11.2 deletion in Patient 2 (**a)** and his mother (**b**). The arrows designate the chromosome 22 (signal was detected for control probe), while triangles shown chromosome 22 without 22q11.2 microdeletion (signal was detected for probe specific for 22q11.2 region).

The diagrams of the results obtained by multiplex ligation-dependent probe amplification analysis. The Results revealed a 1.5-megabase deletion spanning the 14-probe region, between proximal CLTCL1 and distal DGCR8 probe in Patient 2 **(c**) and his mother (**d**). X-axis represents various probes, whereas Y-axis represents probe–height ratio.

### *Language abilities of children with inherited form of 22q11.2DS*

The spontaneous language abilities of Patients 1 and 2 were analyzed by applying the Story generation test (Figure 3). According to the norms of phonetics and syntax of the standard Serbian language, children with normal speech and language development, aged 7 and 8 years, should produce complex, grammatically correct sentences that describe causal relationships and events in the picture. A grammatically correct sentence involves the proper use of reports and proposals, verbs, pronouns, plural, singular, case, gender and number. The results obtained in this

study revealed that Patient 1 produced 16 words in 4 grammatical clauses to decribe events in the shown pictures. These clauses involved the proper use of 6 verbs, 2 nouns, 3 pronouns, 2 particle, 1 proposal. Contrary to Patient 1, Patient 2 showed lower score compared to those expected for their calendar age. He produced 12 words in 4 clauses (3 were grammatically incorrect) to describe the same picture from The story generation test. These clauses involved 4 verbs, 2 nouns, 3 pronouns, 1 particle, 2 conjunctions with no proper use in three clauses. Based on the obtained results we can conclude that there were no differences between them in the number of produced words, as well as in the number of total clauses produced spontaneously. However, we detected that Patient 2 produced higher number of ungrammatical clauses compared to Patient 1.

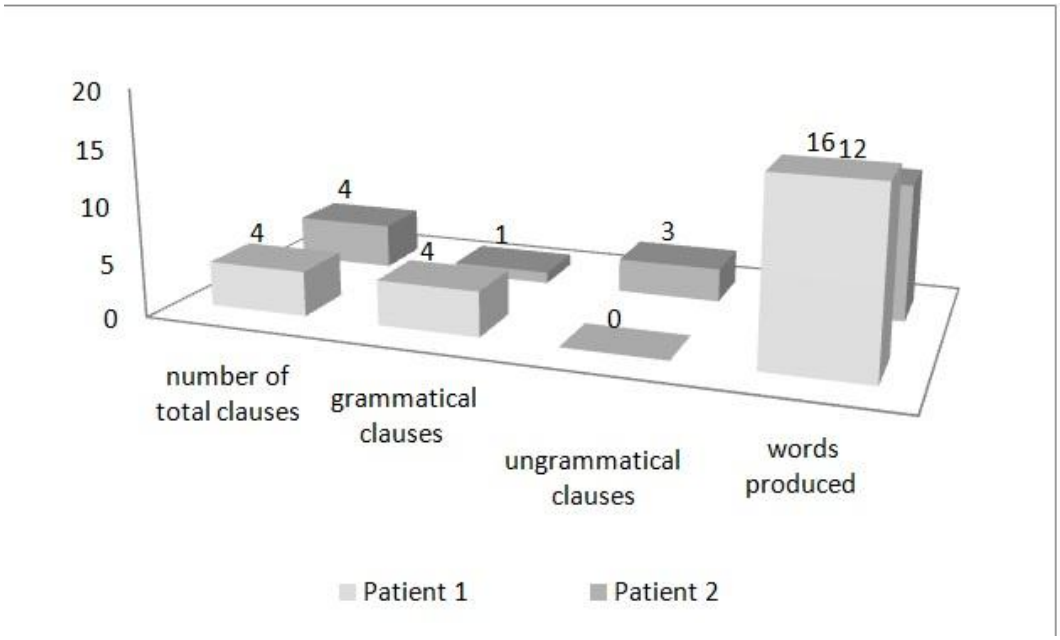


**Figure 3**. Analysis of the spontaneous language production. The results revealed that Patient 1 produced 16 words in 4 grammatical clauses to decribe events in the shown pictures, while Patient 2 produced 12 words in 4 clauses (3 were grammatically incorrect) to describe the same picture from The story generation test.

The articulation test revealed that both of our patients achieved significantly poorer performance (poor sequencing of sounds, poor respiratory support, vocal fatigue at the end of a phrase and impaired prosody (rhythm, intonation and stress patterns)) compared to those expected for 7-8- year-old child with normal speech and language development. In addition, we detected some differences in the articulation skills between these two Patients (Figure 4). Namely, we detected more correctly pronounced phonemes in Patient 1 (67%) compared to Patient 2 (20%). Furthermore, we found that Patient 2 had a higher number of misarticulated and omitted sounds compared to Patient 1. Specifically, our results revealed that distorted phonemes in Patient 1 were

the affricates (Č tʃ , Dž dʒ , Đ dʑ , S s , Z z ) and vibrant R r , while in Patient 2 distorted phonemes were the vocals (A a , E e ,I i , O o ,U u ) due to the nasal air emission. Substituted phonemes in Patient 1 were the affricates (C t͡ʂ , Ć t͡ɕ ), fricatives (Š ʃ , Ž ʒ ) and lateral (LJ ʎ ), while G g , F f , H x were substituted phonemes in Patient 2. There were no omitted phonemes in Patient 1. Omited plosives (T t , D d , K k , G g ), affricates (C t͡ʂ , Ć t͡ɕ ), Č tʃ , Dž dʒ , Đ dʑ ), fricatives (S s , Z z , Š ʃ , Ž ʒ ), vibrant R r and laterals (L l , Lj ʎ ) were observed in Patient 2. Interestingly, we found that Patient 2 had more difficulties in pronouncing the phonemes A a , E e , I i , O o , U u , T t , D d , K k , G g ,V v , H x and L z compared to Patient 1, which may have arisen due to the hypernasality and impacted intraoral air pressure, as already have been described in patients with 22q11.2DS (D'ANTONIO *et al.*, 2001; SCHERER *et al.*, 1999; SCHERER *et al.*, 2008).

Moreover, we detected differences in articulation skills between the patients and their mothers. While, Patient 1 demonstrated regularly the resonance of speech sounds, her mother showed pathological nasal speech sounds. In Patient 2 we observed the hypernasality and impacted intraoral air pressure, while his mother didn't have the nasal resonance of vowel sounds.

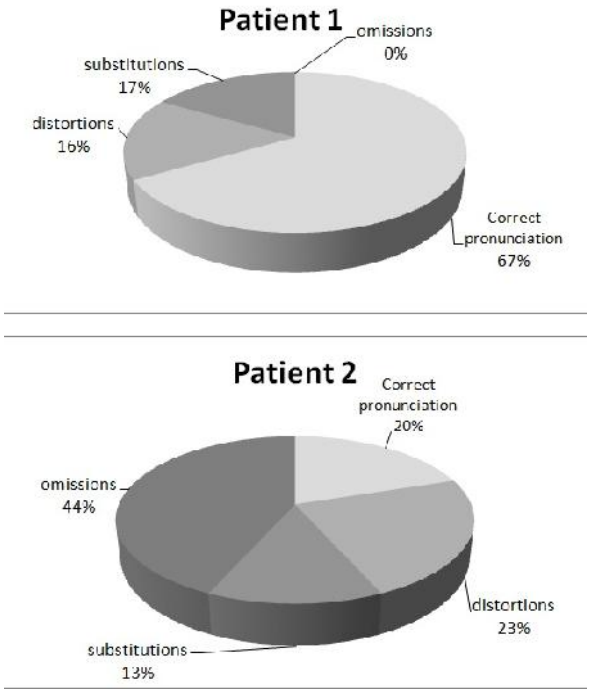


**Figure 4**. The quality of phoneme pronunciation. The articulation test revealed that both of our patients achieved a significantly poorer performance compared to those expected for 7-8 year-old child with normal speech and language development.

Children at the age of 8 are usually characterized by a good praxis of speech organs, which means that they adequately perform the specified motor models. The results of the oral praxis estimation in Patients 1 and 2 revealed that both of the studied Patients showed poorer performance compared to their peers. The features such as decreased facial animation, groping oral movements and poor imitation were observed in both of our Patients. Furthermore, we detected a lower number of correctly derived oral models in Patient 2, compared to Patient 1. Namely, Patient 1 correctly performed 22 of total 31 oral models, while Patient 2 correctly performed only 10 models of total 31 oral models, which indicated the difficulty in using the lips, tongue and jaw in Patient 2.

According to their medical history and a survey of the patients' parents, Patient 1 produced the first functional, spoken word at 14 months of age, which was in concordance with the average age of the first spoken word for children with typical speech and language development (BUGARSKI, 1996). On the other hand, Patient 2 produced the first functional, meaningful spoken word at 36 months of age.

***Intellectual ability (cognitive development)***

The assessment of cognitive development revealed that both Patients have not reached cognitive development that matches their calendar age. Additionally, Patient 1 (7.66 years of age) achieved lower levels of cognitive development compared to Patient 2 (8 years of age). Namely, Patient 1 achieved the results that correspond to the mental age of the child of 4 years and 9 months of age, while Patient 2 achieved the results that correspond to the mental age of the child of 6 years and 3 months of age.

## DISCUSSION

Here we analyzed the communication profile of two Patients with inherited form of 22q11.2DS from two different families. The MLPA analysis revealed 3 Mb deletion in Patient 1 and 1.5 Mb deletion in Patient 2. Literature data revealed that the majority of patients (90%) with 22q11.2DS have deletion of 3Mb (encompassing ~60 genes), while 8% of patients have a smaller 1.5 Mb deletion (~28 genes) (GONG *et al.*, 1996; MICHAELOVSKY *et al.*, 2012).

Literature data revealed that 1.5 Mb microdeletion is more frequent among the children with a familial form of 22q11.2 microdeletion (ADEYINKA *et al.*, 2004; FERNANDEZ *et al.*, 2005a; IASCONE *et al.*, 2002). ADEYINKA et al. assumed that one of the possible explanations for the ease of transmitting a 1.5 Mb deletion compared to a 3 Mb deletion would be that 3 Mb deletion results in haploinsufficiency of more modifier genes than the 1.5 Mb which could make a 3 Mb deletion more deleterious in embryos inherited this microdeletion (ADEYINKA *et al.*, 2004).

Both patients from our study inherited the deletion from their mothers. Our results are consistent with the previous literature data which has reported the preferential maternal transmission of 22q11.2 microdeletion (ADEYINKA *et al.*, 2004; BREWER *et al.*, 1999; CARLSON *et al.*, 1997; DIGILIO *et al.*, 2003; LEANA-COX *et al.*, 1996; MATSUOKA *et al.*, 1998; RYAN *et al.*, 1997). In the literature it was revealed that the possible explanation for preference for maternal transmission may be the reduced reproductive fitness of men with 22q11.2 microdeletion compared to affected females (COSTAIN *et al.*, 2011). Opposing, Leana-Cox et al. argue that there is no connection between fertility and 22q11.2 microdeletion, and they proposed that the female deletion carriers more likely transmit the deleted allele to their children (LEANA-COX *et al.*, 1996).

Additionally, it was proposed that the decreased survival of males with 22q11.2DS could be the cause for preferential maternal transmission (COHEN *et al.*, 1999).

When an inter- and intrafamilial comparison of the phenotypic characteristics was done, we found differences between families, as well as between Patients and their affected mothers. Several authors have also showed great inter- and intrafamilial variability (CIRILLO *et al.*, 2014; DIGILIO *et al.*, 2003; LEANA-COX *et al.*, 1996; MOTZKIN *et al.*, 1993; RAVNAN *et al.*, 1996). Namely, Leana-Cox J et al. defined the frequency of phenotypic abnormalities in 82 individuals from 32 families with 22q11.2 microdeletion (LEANA-COX *et al.*, 1996). They detected mental impairment in 97% of cases, abnormal face in 93% of cases, while cardiac malformations, thymic abnormalities, parathyroid abnormalities and cleft palate or velopharyngeal insufficiency were detected in 68%, 64%, 63% and 48%, respectively (LEANA-COX *et al.*, 1996). Regarding intrafamilial variability, higher prevalence of clinical features in the second generation of families with 22q11.2DS was detected by several authors (DIGILIO *et al.*, 1997; LEANA-COX *et al.*, 1996). They noted that affected subjects of the second generation usually showed a significantly higher number of clinical features compared to their parents. In particular, congenital heart defect, developmental delay, speech delay and calcium-phosphorus abnormalities were more represented in the second generation. In line with these data, our Patients also showed a more severe phenotype in comparison to those detected at their affected mothers. Namely, a significantly milder clinical picture was observed at the mother compared to her daughter in the first family, while the mother of Patient 2 is a completely phenotypically healthy woman. The detection of congenital heart defect (CHD) in both of our patients are in line with published data which revealed that the CHD are more often in the children than in parents (CIRILLO *et al.*, 2014; DIGILIO *et al.*, 1997; MCDONALD-MCGINN *et al.*, 2001). In the literature several hypotheses have been proposed to explain detected interfamilial and intrafamilial clinical variability. It has been postulated that different genetic and non-genetic factors such as genetic modifiers, mosaicism, unstable mutations, alterations of the chromatin folding code mediated by the deletion of low-copy-number DNA repeats, allelic variations at the haploid locus, a mechanism for self-repair, chance association and environmental factors may be involved in the interfamilial and intrafamilial phenotypic variability (CIRILLO *et al.*, 2014; DIGILIO *et al.*, 2003; IASCONE *et al.*, 2002; LINDSAY and BALDINI, 2001).

Speech impairment is one of the most common findings in children with 22q11.2DS, occurring in at least 70% of cases (SHPRINTZEN and GOLDING-KUSHNER, 2008). The majority of literature data focused on speech and language abilities of patients with 22q11.2DS, analyzed speech and language abilities of monolingual native speakers of the English language. To the best of our knowledge there are no published data of speech and language abilities of children with inherited form 22q11.2DS acquiring the Serbian language and no published data of language ability of children with familial form 22q11.2 acquiring South-Slavic languages.

Serbian is a language characterized by a rich verbal morphology system. Verbs are obligatorily inflected for person, number, tense and gender. It is also a language that allows a relatively free word order (in comparison with, e.g. English language). Additionally, Serbian morphology is stem based, as opposed to, for example, English and German morphologies that are word-based (CLAHSEN and DALALAKIS, 1999). Grammatically correct Serbian sentence involves proper use of reports and proposals, verbs, pronouns, plural, singular, case, gender and number. Both Patients analyzed in this study are monolingual native speakers of the Serbian language and we found that their communication profile is discordant for their age.

Our findings regarding the spontaneous language abilities of Patients 1 and 2 showed that Patient 1 produced complex, grammatically correct sentences while Patient 2 showed lower score compared to those expected for their calendar age. Namely, Patient 2 produced a significantly higher number of ungrammatical clauses compared to Patient 1. On the other hand, we did not detect significant differences between the Patients in the number of produced words, as well as in the number of total clauses produced spontaneously. The obtained findings are in line with previously published data. Namely, it was demonstrated that English-and Germany-speaking children with speech language impairament (SLI) more likely omit auxiliary verbs, especially when they attempt to produce sentences with greater argument structure (CLAHSEN, 1991; GRELA and LEONARD, 2000). Additionally, Hansson (HANSSON, 1997) reported that Swedish-speaking children with SLI tended to omit verbs more often than typical development children, but that this tendency decreased with age. In our study we found a higher average number of pathological distortions/substitutions and a lower number of correctly pronounced phonemes in both of our Patients. The pathological distortions, substitutions and omissions which we registered cannot be designated as developmental, because at 7 and 8 years old children all replacements are considered as pathological deviations (KAŠIC, 2003). At Patient 1 pathological distortions and substitutions were expressed mainly among the affricates and fricatives, while at Patient 2 pathological distortion, substitutions and omissions were expressed among these groups but also in the groups of plosives and vowels. In contrast to Patient 2, we did not detect any omissions at Patient 1. In Serbian language, only three consonants have nasal resonance; the /m/, the /n/ and the /nj/ sounds which means that they are produced with the sound or air coming out of the nose. In our study, hypernasality was noted at Patient 2 and at the mother of Patient 1. Hypernasality has already been described in patients with 22q11.2DS (D'ANTONIO *et al.*, 2001; SCHERER *et al.*, 1999; SCHERER *et al.*, 2008) and the pathological distortions of plosives and vowels detected at Patient 2 and at the mother of Patient 1 may have arisen due to the hypernasality and impacted intraoral air pressure.

We demonstrated that the most compromised sounds in the examined Patients with the inherited form of 22q11.2DS were fricatives and affricates. The glottal stop substitution pattern that occurs in both of our Patients, as well as in the majority of children with 22q11.2DS (SHPRINTZEN and GOLDING-KUSHNER, 2008), severely limits their intelligibility because it reduces the number and range of consonants produced during speech production. The hypothesis that lexical and phonological developments influence each other was confirmed in one study of young children with expressive language delay. This study demonstrated that the treatment focused on increasing a child's expressive vocabulary led to improvements in phonological diversity (GIROLAMETTO *et al.*, 1997).

Bearing in mind these data, we postulated that the articulation errors (substitution and omission) seen in our Patients, especially in Patient 2, could also be related to their vocabulary deficits. These deficits in our Patients are determined by the story generation task.

Our findings regarding differentiation of oral praxis showed a lower number of correctly repeated models in both of our Patients. Our observations are in concordance with previously published data which reported that the motor speech impairments, such as apraxia, are present at higher rates in children with 22q11.2DS (KUMMER *et al.*, 2007). Additionally, a much greater percentage of incorrectly derived oral models were seen in Patient 2 compared to Patient 1 who had more adequately performed the specified motor models.

One of the features of children with 22q11.2DS is the delay of acquisition of language milestones. Literature data demonstrated that approximately 90% of 2-year-olds with 22q11.2DS

were nonverbal or just using single words; 80% of 3-year-olds were nonverbal or just using words or simple phrases and about 30% of 4-years-old were still nonverbal or not yet speaking in whole sentences (SOLOT *et al*., 2001). The children with typical speech and language development will begin to produce their first real words around the age of 10 to 13 months (LYON *et al.*, 2012). We found that our Patient 2 was nonverbal until 3 years of age, while Patient 1 produced the first functional, spoken word at 14 months of age.

Cognitive development was assessed by the Test of Human Figure Drawing (GOODENOUGH and HARRIS, 1963). The assessment of cognitive development revealed that both Patients have not reached cognitive development that matches their calendar age. Interestingly, Patient 1 with better speech and language skills achieved lower levels of cognitive development compared to Patient 2. The variability in cognitive performance of children with VCFS has already been described (DE SMEDT *et al.*, 2007). Additionally, the obtained results contribute to previously published data (DE SMEDT *et al.*, 2007) which imply that the educational attainment level of the parents of children with inherited 22q11.2 could be a contributing factor for lower level of cognitive development. Namely, mother of Patient 1 has speech and language impairments (velopharyngeal insufficiency with nasal speech sounds) and borderline mental deficiency, while in mother of patient 2 we did not detected speech and language pathology. Considering these finding of the psychosocial deprivation (no stimulating environment and inadequate speech models for learning), it could be concluded that the presence of environmental intellectual disabilities may per se influence the mental development of the offspring.

This is the first study of speech and language abilities of monolingual native speakers of the Serbian language with inherited form of 22q11.2DS. Generally, our study revealed that Patient 1 with inherited 3Mb deletion, has a milder form of speech-language impairment than her peer with inherited, 1.5 Mb deletion. This observation indicates no correlation between the severity of phenotype (clinical features, cognitive maturity, socio-emotional and speech-language development) and the size of 22q11.2 microdeletion, which is in concordance with literature data (CARLSON *et al.*, 1997; GOLDMUNTZ, 2005; MCDONALD-MCGINN *et al.*, 1999; MCDONALD-MCGINN *et al.*, 2001). Additionally, we found considerable variability in terms of the degree of speech-language pathology and other phenotypic characteristics, not only between different families with 22q11.2 deletion, but also among the members of the same family.

Regarding the achievements of our Patients in relation to the phonetic and phonological development of the standard Serbian language for children aged 7 or 8, both Patients from our study showed significant delays in the field of cognitive, socio-emotional, speech and language development. So, it is very important to emphasize the need for the early stimulation of speech and language for all newborn members in families with 22q11.2DS. Also, early detection and early intensive therapy of any pathological forms of communication at these children are needed to improve speech and language skills of children with 22q11.2DS. Especially, phonological articulation therapy (shaping vocalizations into vowels and properly placed consonants) must be intensive and individualized. It should start at the first sign of a delay considering the fact that the development of early receptive and expressive language skills is critically important for the further cognitive development.

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REFERENCES

ADEYINKA, A., K. J. STOCKERO, H. C. FLYNN, C. P. LORENTZ, R. P. KETTERLING, S. M. JALAL (2004): Familial 22q11.2 deletions in DiGeorge/velocardiofacial syndrome are predominantly smaller than the commonly observed 3Mb. Genet Med., *6*: 517-520.

BASSETT, A. S. and E. W. CHOW (1999): 22q11 deletion syndrome: a genetic subtype of schizophrenia. Biol Psychiatry., *46*: 882-891.

BASSETT, A. S., E. W. CHOW, P. ABDELMALIK, M.GHEORGHIU, J. HUSTED, R.WEKSBERG (2003): The schizophrenia phenotype in 22q11 deletion syndrome. Am J Psychiatry., *160*: 1580-1586.

BEAUJARD, M. P., S. CHANTOT, M. DUBOIS, B. KEREN, W. CARPENTIER, P. MABBOUX, S. WHALEN, M. VODOVAR, J. P. SIFFROI, M. F. PORTNOI (2009): Atypical deletion of 22q11.2: detection using the FISH TBX1 probe and molecular characterization with high-density SNP arrays. Eur J Med Genet., *52*: 321-327.

BREWER, C., S. HOLLOWAY, P. ZAWALNYSKI, A. SCHINZEL, D. FITZPATRICK (1999): A chromosomal duplication map of malformations: regions of suspected haplo- and triplolethality--and tolerance of segmental aneuploidy--in humans. Am J Hum Genet., *64*: 1702-1708.

BUGARSKI, R. D. (1996): Jezik i lingvistika. Beograd, Čigoja štampa.

CARELLE-CALMELS, N., P. SAUGIER-VEBER, F. GIRARD-LEMAIRE, G. RUDOLF, B. DORAY, E. GUERIN, P. KUHN, M. ARRIVE, C. GILCH, E. SCHMITT, S. FEHRENBACH, A. SCHNEBELEN, T. FREBOURG, E. FLORI (2009): Genetic compensation in a human genomic disorder. N Engl J Med., *360*: 1211-1216.

CARLSON, C., H. SIROTKIN, R. PANDITA, R. GOLDBERG, J. MCKIE, R. WADEY, S. R. PATANJALI, S. M. WEISSMAN, K. ANYANE-YEBOA, D. WARBURTON, P. SCAMBLER, R. SHPRINTZEN, R. KUCHERLAPATI, B. E. MORROW (1997): Molecular definition of 22q11 deletions in 151 velo-cardio-facial syndrome patients. Am J Hum Genet., *61*: 620-629.

CIRILLO, E., G. GIARDINO, V. GALLO, P. PULIAFITO, C. AZZARI, R. BACCHETTA, F. CARDINALE, M. P. CICALESE, R. CONSOLINI, S. MARTINO, B. MARTIRE, C. MOLINATTO, A. PLEBANI, G. SCARANO, A. SORESINA, C. CANCRINI, P. ROSSI, M. C. DIGILIO, C. PIGNATA (2014): Intergenerational and intrafamilial phenotypic variability in 22q11.2 deletion syndrome subjects. BMC Med Genet., *15*: 1.

CLAHSEN, H. (1991): Child language and developmental dysphasia. Amsterdam, John Benjamins.

CLAHSEN, H. and J. DALALAKIS (1999): Tense and agreement in Greek SLI: A case study. Essex Research Reports in Linguistics, *24*: 1-25.

COHEN, E., E. W. CHOW, R. WEKSBERG, A. S. BASSETT (1999): Phenotype of adults with the 22q11 deletion syndrome: A review. Am J Med Genet., *86*: 359-365.

COSTAIN, G., E. W. CHOW, C. K. SILVERSIDES, A. S. BASSETT (2011): Sex differences in reproductive fitness contribute to preferential maternal transmission of 22q11.2 deletions. J Med Genet., *48*: 819-824.

CUTURILO, G., D. DRAKULIC, A. KRSTIC, M. GRADINAC, T. ILISIC, V. PAREZANOVIC, M. MILIVOJEVIC, M. STEVANOVIC, I. JOVANOVIC (2013): The role of modern imaging techniques in the diagnosis of malposition of the branch pulmonary arteries and possible association with microdeletion 22q11.2. Cardiol Young., *23*: 181-188.

CUTURILO, G., D. DRAKULIC, M. STEVANOVIC, I. JOVANOVIC, M. DJUKIC, S. MILETIC-GRKOVIC, M. ATANASKOVIC-MARKOVIC (2008): A rare association of interrupted aortic arch type C and microdeletion 22q11.2. Eur J Pediatr., *167*: 1195-1198.

D'ANTONIO, L. L., N. J. SCHERER, L. L. MILLER, J. H. KALBFLEISCH, J. A. BARTLEY (2001): Analysis of speech characteristics in children with velocardiofacial syndrome (VCFS) and children with phenotypic overlap without VCFS. Cleft Palate Craniofac J., *38*: 455-467.

DE SMEDT, B., K. DEVRIENDT, J. P. FRYNS, A. VOGELS, M. GEWILLIG, A. SWILLEN (2007): Intellectual abilities in a large sample of children with Velo-Cardio-Facial Syndrome: an update. J Intellect Disabil Res., *51*: 666-670.

DIGILIO, M. C., A. ANGIONI, M. DE SANTIS, A. LOMBARDO, A. GIANNOTTI, B. DALLAPICCOLA, B. MARINO (2003): Spectrum of clinical variability in familial deletion 22q11.2: from full manifestation to extremely mild clinical anomalies. Clin Genet., *63*: 308-313.

DIGILIO, M. C., B. MARINO, A. GIANNOTTI, B. DALLAPICCOLA (1997): Familial deletions of chromosome 22q11. Am J Med Genet., *73*: 95-96.

FERNANDEZ, L., P. LAPUNZINA, D. ARJONA, I. LOPEZ PAJARES, L. GARCIA-GUERETA, D. ELORZA, M. BURGUEROS, M. L. DE TORRES, M. A. MORI, M. PALOMARES, A. GARCIA-ALIX, A. DELICADO (2005a): Comparative study of three diagnostic approaches (FISH, STRs and MLPA) in 30 patients with 22q11.2 deletion syndrome. Clin Genet., *68*: 373-378.

FERNANDEZ, L., P. LAPUNZINA, I. L. PAJARES, G. R. CRIADO, L. GARCIA-GUERETA, J. PEREZ, J. QUERO, A. DELICADO (2005b): Higher frequency of uncommon 1.5-2 Mb deletions found in familial cases of 22q11.2 deletion syndrome. Am J Med Genet A., *136*: 71-75.

GIROLAMETTO, L., P. S. PEARCE, E. WEITZMAN (1997): Effects of lexical intervention on the phonology of late talkers. J Speech Lang Hear Res., *40*: 338-348.

GOLDMUNTZ, E. (2005): DiGeorge syndrome: new insights. Clin Perinatol., *32*: 963-978.

GONG, W., B. S. EMANUEL, J. COLLINS, D. H. KIM, Z. WANG, F. CHEN, G. ZHANG, B. ROE, M. L. BUDARF (1996): A transcription map of the DiGeorge and velo-cardio-facial syndrome minimal critical region on 22q11. Hum Mol Genet., *5*: 789-800.

GOODENOUGH, F. and D. B. HARRIS (1963): The Goodenough – Harris drawing test [Risunochnaya metodika Gudinaf-Harrisa]. New York, Harcourt, Brace and World.

GOTHELF, D., A. FRISCH, E. MICHAELOVSKY, A. WEIZMAN, R. J. SHPRINTZEN (2009): Velo-Cardio-Facial Syndrome. J Ment Health Res Intellect Disabil., *2*: 149-167.

GRELA, B. G. AND L. B. LEONARD (2000): The influence of argument-structure complexity on the use of auxiliary verbs by children with SLI. J Speech Lang Hear Res., *43*: 1115-1125.

HANSSON, K. (1997): Patterns of verb usage in Swedish children with SLI: an application of recent theories. First Language. *17*: 195–217.

IASCONE, M. R., S. VITTORINI, M. SACCHELLI, I. SPADONI, P. SIMI, S. GIUSTI (2002): Molecular characterization of 22q11 deletion in a three-generation family with maternal transmission. Am J Med Genet., *108*: 319-321.

KAŠIC, Z. (2003): Phonetics. Belgrade, Faculty of Special Education.

KOSTIC, D. and S. VLADISAVLJEVIC (1983): Testovi za ispitivanje govora i jezika [Tests for speech and language]. Beograd, Zavod za udzbenike i nastavna sredstva.

KUMMER, A. W., L. LEE, L. S. STUTZ, A. MARONEY, J. W. BRANDT (2007): The prevalence of apraxia characteristics in patients with velocardiofacial syndrome as compared with other cleft populations. Cleft Palate Craniofac J., *44*: 175-181.

LEANA-COX, J., S. PANGKANON, K. R. EANET, M. S. CURTIN, E. A. WULFSBERG (1996): Familial DiGeorge/velocardiofacial syndrome with deletions of chromosome area 22q11.2: report of five families with a review of the literature. Am J Med Genet., *65*: 309-316.

LINDSAY, E. A. and A. BALDINI (2001): Recovery from arterial growth delay reduces penetrance of cardiovascular defects in mice deleted for the DiGeorge syndrome region. Hum Mol Genet., *10*: 997-1002.

LYON, C., C. L. NEHANIV, J. SAUNDERS (2012): Interactive language learning by robots: the transition from babbling to word forms. PLoS One. *7*: e38236.

MATSUOKA, R., M. KIMURA, P. J. SCAMBLER, B. E. MORROW, S. IMAMURA, S. MINOSHIMA, N. SHIMIZU, H. YAMAGISHI, K. JOH-O, S. WATANABE, K. OYAMA, T. SAJI, M. ANDO, A. TAKAO, K. MOMMA (1998): Molecular and clinical study of 183 patients with conotruncal anomaly face syndrome. Hum Genet., *103*: 70-80.

MCDERMID, H. E. and B. E. MORROW (2002): Genomic disorders on 22q11. Am J Hum Genet., *70*: 1077-1088.

MCDONALD-MCGINN, D. M., R. KIRSCHNER, E. GOLDMUNTZ, K. SULLIVAN, P. EICHER, M. GERDES, E. MOSS, C. SOLOT, P. WANG, I. JACOBS, S. HANDLER, C. KNIGHTLY, K. HEHER, M. WILSON, J. E. MING, K. GRACE, D. DRISCOLL, P. PASQUARIELLO, P. RANDALL, D. LAROSSA, B. S. EMANUEL, E. H. ZACKAI (1999): The Philadelphia story: the 22q11.2 deletion: report on 250 patients. Genet Couns., *10*: 11-24.

MCDONALD-MCGINN, D. M., M. K. TONNESEN, A. LAUFER-CAHANA, B. FINUCANE, D. A. DRISCOLL, B. S. EMANUEL, E. H. ZACKAI (2001): Phenotype of the 22q11.2 deletion in individuals identified through an affected relative: cast a wide FISHing net! Genet Med., *3*: 23-29.

MICHAELOVSKY, E., A. FRISCH, M. CARMEL, M. PATYA, O. ZARCHI, T. GREEN, L. BASEL-VANAGAITE, A. WEIZMAN, D. GOTHELF (2012): Genotype-phenotype correlation in 22q11.2 deletion syndrome. BMC Med Genet., *13*: 122.

MOTZKIN, B., R. MARION, R. GOLDBERG, R. SHPRINTZEN, P. SAENGER (1993): Variable phenotypes in velocardiofacial syndrome with chromosomal deletion. J Pediatr., *123*: 406-410.

PERSSON, C., L. NIKLASSON, S. OSKARSDOTTIR, S. JOHANSSON, R. JONSSON, E. SODERPALM (2006): Language skills in 5-8-year-old children with 22q11 deletion syndrome. Int J Lang Commun Disord., *41*: 313-333.

RAVNAN, J. B., E. CHEN, M. GOLABI, R. V. LEBO (1996): Chromosome 22q11.2 microdeletions in velocardiofacial syndrome patients with widely variable manifestations. Am J Med Genet., *66*: 250-256.

ROBIN, N. H. and R. J. SHPRINTZEN (2005): Defining the clinical spectrum of deletion 22q11.2. J Pediatr., *147*: 90-96.

RYAN, A. K., J. A. GOODSHIP, D. I. WILSON, N. PHILIP, A. LEVY, H. SEIDEL, S. SCHUFFENHAUER, H. OECHSLER, B. BELOHRADSKY, M. PRIEUR, A. AURIAS, F. L. RAYMOND, J. CLAYTON-SMITH, E. HATCHWELL, C. MCKEOWN, F. A. BEEMER, B. DALLAPICCOLA, G. NOVELLI, J. A. HURST, J. IGNATIUS, A. J. GREEN, R. M. WINTER, L. BRUETON, K. BRONDUM-NIELSEN, P. J. SCAMBLER, et al. (1997): Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. J Med Genet., *34*: 798-804.

SCHERER, N. J., L. L. D'ANTONIO, J. H. KALBFLEISCH (1999): Early speech and language development in children with velocardiofacial syndrome. Am J Med Genet., *88*: 714-723.

SCHERER, N. J., L. L. D'ANTONIO, H. MCGAHEY (2008): Early intervention for speech impairment in children with cleft palate. Cleft Palate Craniofac J., *45*: 18-31.

SHAIKH, T. H., R. J. O'CONNOR, M. E. PIERPONT, J. MCGRATH, A. M. HACKER, M. NIMMAKAYALU, E. GEIGER, B. S. EMANUEL, S. C. SAITTA (2007): Low copy repeats mediate distal chromosome 22q11.2 deletions: sequence analysis predicts breakpoint mechanisms. Genome Res., *17*: 482-491.

SHPRINTZEN, R. J., R. B. GOLDBERG, D. YOUNG, L. WOLFORD (1981): The velo-cardio-facial syndrome: a clinical and genetic analysis. Pediatrics. *67*: 167-172.

SHPRINTZEN, R. J. and K. J. GOLDING-KUSHNER (2008): Velo-cardiofacial syndrome San Diego, CA: Plural.

SOLOT, C. B., M. GERDES, R. E. KIRSCHNER, D. M. MCDONALD-MCGINN, E. MOSS, M. WOODIN, D. ALEMAN, E. H. ZACKAI, P. P. WANG (2001): Communication issues in 22q11.2 deletion syndrome: children at risk. Genet Med., *3*: 67-71.

SOLOT, C. B., C. KNIGHTLY, S. D. HANDLER, M. GERDES, D. M. MCDONALD-MCGINN, E. MOSS, P. WANG, M. COHEN, P. RANDALL, D. LAROSSA, D. A. DRISCOLL (2000): Communication disorders in the 22Q11.2 microdeletion syndrome. J Commun Disord., *33*: 187-203; quiz 203-184.

STEVANKOVIĆ, M., V. RADIČEVIĆ, I. JULIUSB (1993): Primena testa za izdiferenciranost oralne praksije Defektološka teorija i praksa, *36*: 77-80.

VUKOVIC, M., I. VUKOVIC, V. STOJANOVIK (2010): Investigation of language and motor skills in Serbian speaking children with specific language impairment and in typically developing children. Res Dev Disabil., *31*: 1633-1644.

WIEHAHN, G. J., G. P. BOSCH, R. R. DU PREEZ, H. W. PRETORIUS, M. KARAYIORGOU, J. L. ROOS (2004): Assessment of the frequency of the 22q11 deletion in Afrikaner schizophrenic patients. Am J Med Genet B Neuropsychiatr Genet., *129B*: 20-22.

# GOVORNO JEZIČKE SPOSOBNOSTI DECE SA FAMILIJARNOM FORMOM SINDROMA DELECIJE 22Q11.2

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Izvod

Sindrom delecije 22q11.2 (22q11.2DS), spada u najčešće mikrodelecione sindrome kod ljudi sa incidencom rađanja od 1/4000 živorođene dece. To je drugi najčešći sindrom, posle Daunovog sindroma, povezan sa urođenim srčanim malformacijama. Način nasleđivanja 22q11.2DS je autozomno dominantni. Kod prosečno 72 - 94% slučajeva 22q11.2 mikrodelecija nastaje *de novo*, dok se kod 6 do 28% pacijenata nasleđuje od roditelja. Cilj ovog multidisciplinarnog istraživanja bio je ispitivanje govorno jezičke sposobnosti pacijenata sa familijarnom formom 22q11.2DS. Prisustvo 22q11.2 mikrodelecije detektovano je metodama fluorescentne *in situ* hibridizacije (FISH) i/ili metodom višestrukog umnožavanja proba koje je zavisno od ligacije (MLPA). Kod jednog pacijenta detektovana je delecija veličine 1.5 Mb, dok je kod drugog pacijenta detektovana delecija veličine 3Mb. Pacijenti iz obe porodice su pokazali značajna kašnjenja na planu kognitivnog, socio-emocionalnog i govorno-jezičkog razvoja. Takođe, detektovali smo i značajnu varijabilnost u fenotipskim karakteristikama i stepenu govorno-jezičke patologije, ne samo pri poređenju različitih porodica sa mikrodelecijom 22q11.2, nego i među članovima iste porodice. Pored toga, rezultati dobijeni u ovom istraživanju ukazuju da nema korelacije između fenotipa i veličine 22q11.2 mikrodelecije.

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