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MATERNAL-FETAL MEDICINE

Prenatal sonographic and cytogenetic/molecular findings of 22q11.2 microdeletion syndrome in 48 confirmed cases in a single tertiary center

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Abstract

Purpose We aimed to present the fetal ultrasound, cytogenetic/molecular testing and postmortem or postnatal clinical findings of cases with 22q11.2DS diagnosed prenatally.

Materials and methods A retrospective medical record review of 48 prenatal cases diagnosed with 22q11.2DS were evaluated in our institution. Detailed ultrasound examination was performed on all fetuses. Postmortem and postnatal examinations were evaluated. The microdeletions were detected by karyotyping or microarray, then confirmed by FISH. Descriptive statistical analysis was performed.

Results Demographic data of 48 prenatal cases including 46 singletons and 1 dichorionic diamniotic twin pregnancy were evaluated. The most common extracardiac anomaly was skeletal system anomalies (25%), in which PEV was the most frequent one (20.8%). Polyhydramnios rate was detected as 31%, in 6.6% as an isolated finding. Microdeletion has been detected by karyotyping in 13 cases (13/47, 27.7%) (including 2 unbalanced translocations), by FISH in 28 cases (28/48, 58.3%), by microarray/a-CGH testing in 7 cases. Microarray analysis showed that in one case with unbalanced translocation had two consecutive deletions; one was proximal and other one distal to critical region and not encompassing *TBX1* gene but *CRKL* and *LZTR1* genes.

Conclusion The current study demonstrates the whole spectrum of atypical phenotypic and genotypic variations of 22q11.2DS in the largest prenatal case series reported to date. Therefore, differential diagnosis should be considered not solely in CHD, but also in the presence of isolated clubfeet and polyhydramnios. Establishing the diagnosis in the prenatal period may allow a postnatal multidisciplinary approach, as well as affect the actual prevalence of the disease.

Keywords 22q11.2 microdeletion syndrome · DiGeorge syndrome · Array-based comparative genomic hybridization analysis · Prenatal diagnosis

Introduction

Deletion of 22q11.2 is the most common microdeletion with a prevalence 1 in 992 fetuses, 1 in 1500 miscarriages and 1:2500–1:4000 in newborns [1–4]. This microdeletion has a highly heterogeneous clinical spectrum, characterized by congenital heart defects (CHD), immune deficiency, hypoparathyroidism and various degrees of learning disorders. Due to the variable phenotype, this condition was named under different syndromes in the past, today, mostly, DiGeorge syndrome or 22q11.2 deletion syndrome (22q11.2DS) is used.

22q11.2DS is caused by a developmental field defect of the third and fourth pharyngeal pouches and the fourth aortic

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arch, which leads to multisystem manifestations including conotruncal CHD, velopharyngeal anomalies, craniofacial features, skeletal anomalies, gastrointestinal and renal anomalies, hypoparathyroidism, T-cell immunodeficiency, autoimmune disease, thrombocytopenia cognitive deficits and psychiatric disorders. The clinical variability in patients with the same breakpoints and size of deletion or within the same family or even in monozygotic twins is well known [2, 5]. Conotruncal CHDs are defined as defects of the outflow tracts or great arteries, like as tetralogy of Fallot (TOF), truncus arteriosus, double outlet right ventricle (DORV), transposition of the great arteries (TGA) and aortic arch abnormalities are the most common type of cardiac anomalies, which are detectable by fetal ultrasound (US) examination.

There are low copy repeats (LCRs) (from LCR22-A to LCR22-H) on 22q11.2 region, and the non-allelic unequal homologous crossover between these LCRs during the cell divisions causes to microdeletions and duplications [2, 6–8]. Most of the deletions (~ 85–90%) between LCR-A and LCR-D of 3 Mb are called as "typical deletion" encompassing ~ 60 genes occurring. Smaller "nested deletions" in size of 1.5 Mb between LCR-A and LCR-B encompassing ~ 28 genes, and also "atypical smaller deletions" are also reported [9–13]. The *TBX1* gene located between LCRA–B controls the cell proliferation in the pharyngeal arches and cardiac outflow tract and influences the formation of the organs and has the major role in the clinical manifestation of the syndrome, even the intragenic pathogenic variants of *TBX1* gene cause CHD's [14–17]. Nevertheless, some other genes (*CRKL, LZTR, MAPK1, VEGF* and others) in this region have also been described as having a modifier role in the phenotype [18]. Since it is the second frequent autosomal chromosomal anomaly following trisomy 21 in pregnancies, its prenatal diagnosis is important. In the presence of specific US findings in fetuses, specific genetic tests such as fluorescent in situ hybridization (FISH) or multiplex ligation-dependent probe amplification (MLPA) and/or microarray/array Comparative Genomic Hybridization (aCGH) techniques should be applied. In the absence of specific US finding, microdeletion may be missed in routine cytogenetic work-up.

In this study, we aimed to investigate the fetal US, cytogenetic, molecular cytogenetic, postmortem or postnatal clinical findings of prenatal cases diagnosed with 22q11.2DS. To the best of our knowledge, this is the largest case series from a single tertiary center.

Materials and methods

Institutional review board approval was obtained (Study number: 223011) and medical records of 48 prenatal cases with deletion of 22q11.2 from 47 unrelated families diagnosed between 2007 and 2019 in our Prenatal Diagnosis and Therapy Institution were evaluated. All medical records were analyzed retrospectively. In all cases, prenatal ultrasonography findings (including congenital heart defects) were defined before prenatal diagnostic tests. Descriptive statistical analysis was performed. Informed consent including anonymous data use for research purposes was obtained in all cases. All data and images were evaluated and reported anonymously. The following approach was applied when a congenital anomaly was detected in prenatal US and subsequent genetic management is described in detail below.

Ultrasound examination and invasive procedures

Prenatal US evaluation was performed with high-resolution equipment (Voluson 730 Expert; Voluson E6, GE Medical Systems, Zips, Austria) and transabdominal convex probes (AB 2–7; C 2–9, respectively). US images were recorded using an imaging archive system (Viewpoint®—GE—Healthcare Medical Systems, Penta, Turkey). Detailed systemic anatomical US examination was performed on all fetuses. All pathologic echocardiographic findings detected at prenatal ultrasonography were evaluated by five experienced perinatal physicians in the presence of a pediatric cardiologist based on International Society of Ultrasound in Obstetrics and Gynecology Guidelines. Sonographic evaluation of thymus was performed subjectively, without any predefined evaluation method. In the evaluation of polyhydramnios, Society for Maternal Fetal Medicine guideline was used. Polyhydramnios is categorized according to the deepest vertical pocket and is classified as mild when ≥ 8 cm, ≥ 12 cm as moderate, and ≥ 15 cm as severe polyhydramnios. Only moderate to severe polyhydramnios were described as 'polyhydramnios' in the present study. After genetic counseling, invasive procedures including 24 amniocentesis (AC), 22 fetal blood sampling (FBS) and 2 transabdominal chorion villus sampling (CVS) were performed by the same perinatal physicians.

Genetic investigation

In presence of specific ultrasound (US) finding/s, FISH for del22q11.2 on interphase nuclei of amniocytes or on cells or metaphases obtained by direct preparation of chorion villi material has been applied. Since 2010 aCGH/microarray was offered to all cases with specific or unspecific US findings including FISH positive cases. A total of 48 index cases, 35 couples and one affected mother and sibling have been investigated genetically. All index cases except one (due to the culture failure) were karyotyped by G banding at 500–600 band level. FISH technique was the gold standard in our laboratory and has been applied using one probe (N25/D22S75*, SHANK3,* Cytocell, Cambridge, UK). Microarray

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studies have been performed in 23 cases, by 180 K aCGH (n:15) (Agilent Technologies, Santa Clara, CA, USA) or by 250 or 750 K SNP arrays (n:8) (Affymetrix Inc., Santa Clara, CA, USA). All procedures were carried out according to the manufacturer's protocol. Copy number variations were determined using Feature Extraction software and Agilent CytoGenomics (v4.0.3.12 and v5.0.2.5 software) and Copy Number Analyzer for Affymetrics Gene Chip mapping software (version 2.0) or Affymetrix Chromosome Analysis Suite software was used according to the used platform. All results were evaluated using the UCSC human Genome Browser release of February 2009 (GRCh37/hg19). The deletions detected by karyotyping or microarray have been confirmed by FISH. All families have been counseled in details, a termination option has been offered after multidisciplinary prenatal consultation. Postmortem detailed physical exam and autopsy was routinely offered, and informed consent was obtained. When pregnancy resulted in live birth, infants underwent a detailed systemic examination.

Results

General characteristics

Demographic data of 48 prenatal cases including 46 singletons and one spontaneous dichorionic diamniotic twin pregnancy with del22q11.2 were evaluated (Table 1). The mean maternal age at diagnosis was 30 ± 5 (range 17–44); the mean time of diagnosis was 23 ± 4 (range 13–32) week of gestation, and 56.2% of the cases were diagnosed under 22 gestational weeks. The major indication for prenatal invasive diagnostic tests was abnormal US finding/s (92.8%) during second and early third trimester (Table 1). One fetus of the dichorionic diamniotic twin pregnancy (Table 5, *Case 26*) had abnormal US findings, whereas the co-twin was normal (Table 5, *Case 27*). Thirteen cases had additional indications, which were advanced maternal age ($n = 14$), pregnancies conceived by intracytoplasmic sperm injection (ICSI) ($n = 3$) and positive non-invasive prenatal testing (NIPT) for del22q11.2 ($n = 3$) (Table 1).

Table 1 The demographic characteristics for 47 pregnancies with 22q11.2 deletion syndrome

Characteristics	*N*
Maternal age [median ± std (range)]	30 ± 5.89 (17–44)
Gravida (median/min–max)	2 (1–6)
Para (median/min–max)	1 (0–5)
Number of fetuses	48
Single pregnancy (%)	46 (98)
Multiple pregnancy (twin) (%)	1(2)
Gestational age at diagnosis ort. ± std(range)	23 ± 4 (13–32)
Numbers of fetuses ≤ 22 gestational weeks n (%)	27 (56.2)
Prenatal invasive sample type *n* (%)	
Chorionic villus sampling	2 (4.6)
Amniocentesis	24 (50)
Cordocentesis	22 (45.4)
Etiology for prenatal invasive tests *n* (%)	
Pathological ultrasonographic findings (PUF)	47 (92.8)
PUF + positive non-invasive prenatal test results	3 (6.25)
Co-twin discordant fetal abnormality	1 (2.0)
All results for invasive tests by	48
Karyotyping (%)	11 (22.9)
FISH/aCGH (%)	37 (77.1)
Pregnancy outcomes (%)	
Termination of pregnancy	38 (79)
IUD	1
Live birth	10 (21)
Neonatal exitus	4
Inheritance (%)	
De novo	33 (91.7)
Familial	3 (8.3)
Unknown	12

FISH fluorescent in situ hybridization, *aCGH* array comparative genomic hybridization, *IUD* intrauterine demise

Genetic laboratory results

Microdeletion has been detected first; by karyotyping in 13 cases (13/47, 27.7%) (including two unbalanced translocations), by FISH in 28 cases (28/48, 58.3%), by microarray/a-CGH testing in 7 cases. All deletions were confirmed by FISH. In *Case 11* (Table 5) with atypical US presentation, some dysmorphic features of the mother and of 6 years old son have been noticed during the genetic counseling and DGS has been taken into account in the differential diagnosis. Microarray tests showed the same microdeletion in size and position in all three family members. The fetus died intrauterine at 35. gestational week (GW) and unfortunately family denied the physical examination and autopsy of the fetus. Discordant twin pair was investigated for 22q11.2DS due to the typical US findings of affected twin. Zygocity test using STR markers showed that they were monozygotic and parental studies showed that the deletion was de novo.

Microarray studies in 23 cases revealed that 20 deletions were greater than 2.5 Mb in size with common breakpoints encompassing *TBX1* gene. Out of three deletions smaller than 2.5 Mb, two were encompassing *TBX1* gene. *Case 17* (Table 5) with unbalanced translocation had two consecutive deletions; one was proximal and other one distal to critical region and not encompassing *TBX1* gene but *CRKL* and

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LZTR1 genes. *Case 25* (Table 5) was mosaic for two different deletions, first one with 2,796 Mb in size (breakpoints 19,004,070–21,800,471) and other one 16,559 Mb in size (breakpoints 16,888,899–33,448,136).

Family studies in 36 cases revealed that microdeletion was de novo in 33 (91.7%). Maternal inheritance (including one translocation case) has been found in two (8.3%) and paternal inheritance in one case (Table 1). Since Y chromosome was involved in the de novo unbalanced translocation (*Case 17*), it has been thought that the translocation might have occurred in spermatogenesis.

Ultrasound findings of heart and thymus

CHD was the most common US finding (45/48, 94%) (Table 2) and was isolated in 54% ($n=24/45$) (Table 5). The distribution of the anomalies constituted predominantly of conotruncal anomalies (54%), in which Fallot tetralogy was the most common one (44.4%) (Table 3), whereas transposition type double outlet right heart ventricle (DORV) and truncus arteriosus were less frequent (each 2.2%). Other heart anomalies were encountered as coarctation of the aorta (11.1%), right aortic arc (8.8%) and interrupted aortic arch with VSD (8.8%). Aberrant right subclavian artery (ARSA) as an isolated cardiac finding was encountered in 8.8% of all cardiac anomalies; as a single ultrasonographic finding in two cases (*Case 3* and *36*) (Tables 3, 5). Thymus was evaluated by ultrasound in 58.3% of the cases and hypoplasia was detected in 71% of them (Fig. 1). Postmortem autopsy was performed in only two cases, and thymus hypoplasia was confirmed in both (*Case 14* and *31*).

Extracardiac findings

The most common observed extracardiac anomaly was skeletal system anomalies (25%), in which pes equinovarus (PEV) was the most frequent one (20.8%) and was bilateral in 60% (Tables 2, 4; Fig. 2). In 20% of cases with PEV, CHD was not detected at presentation. Other observed skeletal anomalies were polydactyly, syndactyly and rocker-bottom feet. Moderate to severe polyhydramnios was detected in 31% of cases. In one case, tracheoesophageal fistula was the causing factor (Tables 2, 5).

Genitourinary system findings were encountered in 16.6% of cases and mild hydronephrosis was predominant. Other findings were hyperechogenic kidney, unilateral renal agenesis, unilateral multicystic dysplastic kidney. All these anomalies were presented with other system anomalies, mostly conotruncal CHDs (Fig. 3). Central nervous system findings were detected in 10% of cases, and mega cisterna magna was the most common one. A case with agenesis of cavum septum pellusidium (*Case 18*) had also ARSA and increased nuchal fold.

Table 2 Cardiac/extracardiac ultrasonographic findings in 48 fetuses with prenatal 22q11.2del syndrome

	N=48 (%)
Cardiac	45 (94)
Conotruncal	24 (54)
Others	21 (46)
Thymus (evaluated w US)	28
Normal	8 (32)
Hypoplasia/aplasia	20 (68)
Central nervous system	5 (10)
Agenesis of cavum septum pellusidium	1
Mega cisterna magna	3
Inferior vermian agenesis	1
Facial dysmorphism/oral cleft	5 (10)
Hypoplastic nasal bone	2
Bulbous nose	1
Micrognathia	1
Cleft lip	1
Gastrointestinal system	1 (2)
Tracheoesophageal fistula	1
Genitourinary system	8 (16.6)
Hyperechogenic kidney (*bilateral/unilateral*)	2
Multicystic dysplastic kidney (*unilateral*)	1
Renal agenesis (*unilateral*)	1
Hydronephrosis (*mild*)	4
Skeletal system	12 (25)
Pes equinovarus	10 (83)
Rocker-bottom foot	1
Polydactyly	2
Syndactyly	1
Polyhydramniosis	15 (31)
Other findings	11
Increased nuchal fold/nuchal translucency	3
Hyperechogenic intracardiac focus	4
Choroid plexus cyst	1
Hyperechogenic intestine	1
Single umbilical artery	1
Symmetrical IUGR	1
Umbilical vein varix	1

US ultrasound, *IUGR* intauterine growth restriction

Facial anomalies have been observed in 10% of cases, which included two hypoplastic nasal bone, one cleft lip and one micrognathia Fig. 4). All facial anomalies were associated with multiple anomalies, mostly conotruncal CHDs (Fig. 3; Table 2).

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Table 3 Cardiac anomalies in fetal echocardiography in prenatal 22q11.2 deletion

	N = 45 (%)
Conotruncal cardiac abnormalities	24 (54)
Tetralogy of Fallot (*absent pulmonary valve syndrome* = *2*)	20 (44.4)
DORV—transposition type	2 (4.4)
Truncus arteriosus	2 (4.4)
Other cardiac abnormalities	21 (46)
Right-sided aortic arch	4 (8.8)
Coarctation of aorta ± *muscular VSD*	5 (11.1)
Interrupted aortic arch + *perimembranous VSD, ARSA*	4 (8.8)
Pulmonary stenosis	1 (2.2)
AVSD (*incomplete*) (*with ARSA*)	1 (2.2)
Perimembranous VSD (*with ARSA*)	2 (4.4)
ARSA (*isolated*)	4(8.8)

DORV double-outlet right ventricle, *VSD* ventricular septal defect, *ARSA* aberrant right subclavian artery, *AVSD* atrioventricular septal defect

Pregnancy outcomes

Following the genetic counseling, 37 families (79%) opted for termination of the pregnancy (ToP) and 11 families decided to continue their pregnancies, of whom 10 (21%) ended with live births and one with intrauterine demise (IUD) (Table 5). The majority of the families gave permission only for a detailed postmortem physical examination (54%). Fifteen families refused the both exams based on religious beliefs.

The results of postmortem physical exam included predominantly dysmorphic face (hypertelorism, large-flat nose root, tubular/bulbous nose tip, etc.) in addition to the prenatal US findings (Table 5). Anomalies encountered at the postmortem physical examination which were undetected at the prenatal US included one case with PEV (*Case 9*), two fetuses with right hand type B polydactyly (*Case 27* and *42*) and a cleft soft palate in three cases (*Case 9*, *38* and *42*) (Table 5).

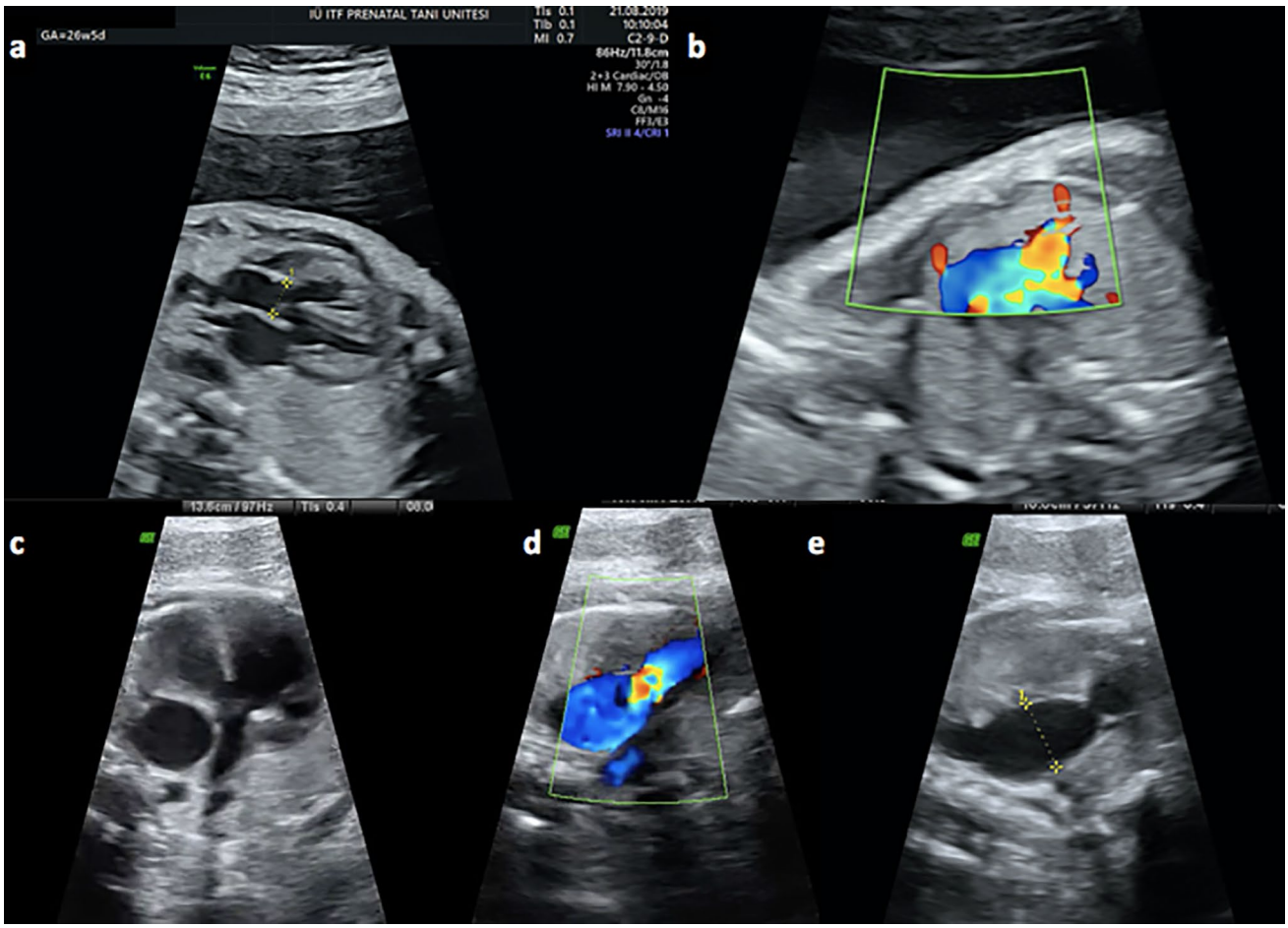


Fig. 1 Prenatal cardiac ultrasonographic findings detected in del22q11.2 syndrome: Tetralogy of Fallot (**a**), absent pulmonary valve syndrome (Case 13) (**c**–**e**). Thymus hypoplasia evaluated using 'Thy-box' method (**b**) in a fetus with tetralogy of Fallot (Case 46)

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Table 4 Features of prenatally diagnosed 22q11.2del fetuses with PEV and its association with accompanying anomalies

	Prenatal $N=10$ (%)	Postmortem $N=11$ (%)
Characteristic findings		
Unilateral	4 (40)	4 (36)
Isolated (with polyhydramniosis)	1	
Bilateral	6 (60)	7 (64)
Associated anomalies		
Cardiac defects	8 (80)	
Conotruncal	3	
Other heart defects	5	
No heart defects	2 (20)	
Extracardiac anomalies		
MCDK, hyperechogenic kidney	2	
MCM, inferior vermian agenesis	2	
CPC, hyperechogenic intestine	2	
Umbilical vein varix	1	

PEV pes equinovarus, *MCDK* multicystic dysplastic kidney, *MCM* mega cisterna magna, *CPC* choroid plexus cyst

Out of 10 live births, 4 died in the neonatal period due to the severe heart failure (Tables 1, 5), 6 were examined clinically and all demonstrated dysmorphic findings specific for 22q11.2DS, immunodeficiency, hypocalcemia and metabolic disorders and were operated for CHD.

Discussion

Despite the microdeletion of 22q11.2 is the most common encountered deletion in newborns, its prenatal diagnosis is challenging due to the wide phenotypic spectrum even among the monozygotic twins. The GW at the time of the diagnosis significantly impacts the counseling, pregnancy management and the neonatal care. In the French collaborative study including 272 cases from 37 cytogenetic laboratories, pregnancies without prior knowledge of parental status were diagnosed at a mean 26.3 weeks, whereas it was at 17.3 GW in cases with known parental carrier status, which constituted 9.2% of this series [19]. The mean time elapsed for the diagnosis was 0.5 weeks in CV samples for known carriers, whereas 5 weeks when the parental status was unknown [19]. In our series, none of the pregnancies were of known carriers. When all cases were considered, prenatal diagnosis was made at 22 weeks and earlier in 56.2% of cases. This is in contrast with the previous studies reporting later GWs at presentation, and may be attributed to the evaluation process involving experienced perinatologists at a tertiary referral center.

Major indication for invasive diagnosis in prenatal series was the detection of the CHD (76–100%) [10–22]. The most associated CHD is conotruncal anomalies and Fallot tetralogy ranks first (29–42%) [19, 21, 22]. Our results were in agreement with the published series (CHD 94%, conotruncal anomalies 54% and Fallot tetralogy 44.4%). In our cohort, interrupted aortic arch and truncus arteriosus were observed 8.8 and 4.4%, respectively. In the French study, the highest correlation between fetal ultrasonography and autopsy findings were reported for CHD [19]. However, rates of anomalies presented variations between the US and postmortem exams such as 28.5 vs 22.7% for Fallot tetralogy; 14.9 vs 22.7% truncus arteriosus and 8.3 vs 15.2% interrupted aortic arch, respectively [19]. It is possible that low postmortem investigation rate may have caused this discrepancy regarding the low incidence of interrupted aortic arch and truncus arteriosus anomalies in our series.

Thymus aplasia/hypoplasia leads to immunodeficiency in 75% of pediatric 22q11.2DS cases [23]. Although the relation between CHDs and thymus hypoplasia are well documented in the postnatal period, the prenatal US evaluation remains usually inadequate. Prenatal series reported



Fig. 2 Prenatal sonography (**a**) and postmortem examination images (**b**, **c**) of the Case 46 (**a**, **b**). Fallot Tetralogy, thymus hypoplasia and micrognathia (**c**) was also detected at 26 weeks of gestation in this case

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Table 5 Detailed ultrasound and postmortem examination findings of fetuses diagnosed with prenatal 22q11.2 microdeletion syndrome

Case	Gestational week	Multiple pregnancy	Inheritance	CHD	Thymus	CNS	FACIAL	GIS	Urinary tract	Skeleton	Polyhydramnios	Other findings	Outcome	Postmortem /postnatal physical examinations
1	21		De novo	Ao Coa, muscular VSD	N/A							EIF	TOP	Hypertelorism, broad nasal bridge, long phyltrum, tapering finger tips
2	20		De novo	RAA	N/A							EIF	TOP	N/A
3	28		Unknown	Fallot tetralogy	N/A			Tracheoesophageal fistula			Yes		Liveborn	Neonatal exitus, no autopsy
4	22		Unknown	Truncus arteriosus, PM VSD	N/A							SUA	TOP	Hypertelorism, broad nasal bridge, long phyltrum, blunted nose, small mouth and ears, long and thin fingers, positional anomaly in toes
5	22		De novo	RAA	Hypoplasia	MCM			Hydronephrosis				TOP	Hypertelorism, blunted nose, long phyltrum, high-inserted columella, anterverted nares, no uvula, long and tapering fingers, sacral dimple
6	22		De novo	RAA	N/A		Cleft lip			Right hand, polydactyly			TOP	N/A
7	22		De novo	Fallot tetralogy	Hypoplasia				Hydronephrosis				TOP	N/A

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Table 5 (continued)

Case	Gestational week	Multiple pregnancy	Inheritance	CHD	Thymus	CNS	FACIAL	GIS	Urinary tract	Skeleton	Polyhydramnios	Other findings	Outcome	Postmortem /postnatal physical examinations
8	31		De novo	Ao Coa, Muscular VSD	N/A								TOP	N/A
9	29		Familial (maternal)	DORV-Transposition type	Normal				Unilateral renal agenesis (R), unilateral hyperechogenic dysplastic kidney (L)		No (Anhydramniosis)	Symmetrical IUGR	TOP	Hypertelorism, bilateral epichantus, broad and wide nasal bridge, depressed supraorbital arches, **cleft soft palate**, narrow and high palate, micrognathia, low-set ears, redundant nuchal skin, turricephaly, bilateral D2 nad D5 clinodactyly, **bilateral Rocker-bottom foot and PEV**
10	22		Unknown	Fallot tetralogy	N/A				Hydronephrosis				TOP	N/A
11	30		Familial (maternal, brother)	Normal	N/A					Unilateral PEV, hyperextended toe	Yes		IUD	N/A
12	23		De novo	Fallot tetralogy	N/A								TOP	N/A

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Table 5 (continued)

Case	Gestational week	Multiple pregnancy	Inheritance	CHD	Thymus	CNS	FACIAL	GIS	Urinary tract	Skeleton	Polyhydramnios	Other findings	Outcome	Postmortem /postnatal physical examinations
13	20		Unknown	Fallot tetralogy w APVS	Hypoplasia								Liveborn	Hypertelorism, ectopic thymus, hypocalsemia, small mouth, thin lip, retrognathia, high palate, microglossia, prominent and folded antihelix, umbilical hernia, seperated nipples
14	18		De novo	RAA	Hypoplasia								TOP	*Fetal autopsy; Thymus hypoplastic, Right-sided aortic arch*
15	24		De novo	Fallot tetralogy	Normal						Yes	EIF	Liveborn	Hypertelorism, bilateral epichantus, broad nasal bridge, anteverted nares, long phyltrum, small mouth, tented upper lip, retrognathia, high palate, folded helix, umbilical hernia, sacral dimple, operated for CHD

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Table 5 (continued)

Case	Gestational week	Multiple pregnancy	Inheritance	CHD	Thymus	CNS	FACIAL	GIS	Urinary tract	Skeleton	Polyhydramnios	Other findings	Outcome	Postmortem /postnatal physical examinations
16	19		De novo	Fallot tetralogy	Normal		Nasal hypoplasia		Hydronephrosis				TOP	N/A
17	21		De novo	Fallot tetralogy	N/A				Unilateral MCDK	Unilateral PEV			TOP	Hypertelorism, prominent and broad nasal bridge, down turned corners of the mouth, tapering fingers, extension limition of the knee joint, unilateral PEV
18	25		Unknown	ARSA	N/A	Agenesis of the CSP						Increased NF	Liveborn	N/A
19	17		De novo	Fallot tetralogy	Hypoplasia						Yes		Liveborn	Neonatal exitus, no autopsy
20	21		De novo	Fallot tetralogy	Hypoplasia						Yes	EIF	TOP	N/A
21	30		Unknown	Ao Coa	Hypoplasia	MCM				Bilateral PEV, small toes	Yes	Umbilical vein varix	TOP	Bilateral PEV, plantar creases

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Table 5 (continued)

Case	Gestational week	Multiple pregnancy	Inheritance	CHD	Thymus	CNS	FACIAL	GIS	Urinary tract	Skeleton	Polyhydramnios	Other findings	Outcome	Postmortem /postnatal physical examinations
22	31		De novo	Truncus arteriosus	Hypoplasia								Liveborn	Hypertelorism, micrognathia, small-low set ears, bulbous nose, malar hypoplasia, small mouth, positional anomaly in the toes, operated for CHD, sacral dimple, immun deficiency
23	24		De novo	Fallot tetralogy	N/A						Yes		Liveborn	Hypertelorism, hypoplastic ala nasi, tubular nose, broad columella, brachicephalia, sparse eyebrow, operated for CHD
24	24		De novo	Fallot tetralogy	N/A								TOP	N/A
25	18		De novo	Pulmonary stenosis	N/A					Unilateral PEV		CPC	TOP	N/A

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Table 5 (continued)

Case	Gestational week	Multiple pregnancy	Inheritance	CHD	Thymus	CNS	FACIAL	GIS	Urinary tract	Skeleton	Polyhydramnios	Other findings	Outcome	Postmortem /postnatal physical examinations
26	21	Yes, fetus A	De novo	Ao Coa	Hypoplasia						Yes		TOP	Hypertelorism, depressed orbita, wide-depressed nasal bridge, bulbous nose, short columella, small mouth, bilateral small-low set ears, retrognathia
27	21	Yes, fetus B	De novo	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Yes	No	TOP	Hypertelorism, downslanted palpebral fissures, wide nasal bridge, depressed nasal tip, short columella, small mouth, high palate, bilateral small-low set ears, **postaxial polydactyly of the right hand (type B)**
28	23		De novo	ARSA	N/A						Yes	Increased NF	TOP	Hypertelorism, malar hypoplasia, high palate, redundant nuchal skin

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Table 5 (continued)

Case	Gestational week	Multiple pregnancy	Inheritance	CHD	Thymus	CNS	FACIAL	GIS	Urinary tract	Skeleton	Polyhydramnios	Other findings	Outcome	Postmortem /postnatal physical examinations
29	30		De novo	Fallot tetralogy w APVS	N/A								Liveborn	Neonatal exitus, not operated for CHD, no autopsy
30	32		De novo	Fallot tetralogy	N/A	MCM					Yes		TOP	N/A
31	22		De novo	Ao Coa	Hypoplasia								TOP	*Fetal autopsy; Thymus hypoplastic, VSD, Coarctation of the aorta*
32	20		De novo	Normal	N/A	Inferior vermian agenesis			Bilateral hyperechogenic kidney	L Rocker-Bottom foot, R PEV and postaxial polydactyly, R hand postaxial polydactyly, L syndactyly		HEI	TOP	N/A
33	22		Unknown	ARSA	Hypoplasia								TOP	N/A
34	20		Unknown	Fallot tetralogy	Normal								TOP	N/A
35	22		Unknown	Interrupted Ao, PM VSD, ARSA	Hypoplasia					Bilateral PEV	Yes		TOP	N/A
36	25		De novo	ARSA	Normal								TOP	N/A
37	13		De novo	Fallot tetralogy	N/A								TOP	Hypertelorism, retromicrognathia, long phyltrum, edematous nuchal skin

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Table 5 (continued)

Case	Gestational week	Multiple pregnancy	Inheritance	CHD	Thymus	CNS	FACIAL	GIS	Urinary tract	Skeleton	Polyhydramnios	Other findings	Outcome	Postmortem /postnatal physical examinations
38	24		De novo	ARSA, incomplete AVSD	Normal					Bilateral PEV			TOP	Hypertelorism, prominent nasal bridge, pinched nose, retromicrognathia, **cleft soft palate,** tapering fingers, clinodactyly of the right hand, bilateral PEV
39	21		Unknown	ARSA, PM VSD	Hypoplasia					Bilateral PEV			TOP	Hypertelorism, broad nasal bridge, depressed nasal tip, short columella, clinodactyly of right (2., 5.) and left (2.) hands, bilateral PEV and sandal gap
40	20		De novo	Fallot tetralogy	N/A					Bilateral PEV			TOP	N/A
41	20		De novo	DORV-Transposition type	Normal		Nasal hypoplasia						TOP	N/A

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Table 5 (continued)

Case	Gestational week	Multiple pregnancy	Inheritance	CHD	Thymus	CNS	FACIAL	GIS	Urinary tract	Skeleton	Polyhydramnios	Other findings	Outcome	Postmortem /postnatal physical examinations
42	19		De novo	Interrupted Ao, PM VSD	Hypoplasia							Increased NT	TOP	Hypertelorism, retromicrognathia, low set ears, cleft soft palate, **postaxial polydactyly type B of right hand**
43	22		Familial (paternal)	Interrupted Ao, PM VSD	Hypoplasia						Yes		Liveborn	Operated for CHD, thymus aplasia, immun deficiency, typical fascial dysmorphism
44	29		De novo	Fallot tetralogy	Hypoplasia		Bulbous nose				Yes		TOP	Hypertelorism, bulbose nose, midfascial hypoplasia
45	24		Unknown	ARSA, PM VSD	Hypoplasia								TOP	N/A
46	26		De novo	Fallot tetralogy	Hypoplasia		Micrognathia			Bilateral PEV	Yes		TOP	Hypertelorism, broad nasal bridge, long phyltrum, blunted nose, bilateral PEV and clinodactyly
47	26		Unknown	Fallot tetralogy	Hypoplasia								TOP	Hypertelorism, hypoplastic ala nasi, retromicrognathia, **T8 Hemivertebra**

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Table 5 (continued)

Case	Gestational week	Multiple pregnancy	Inheritance	CHD	Thymus	CNS	FACIAL	GIS	Urinary tract	Skeleton	Polyhydramnios	Other findings	Outcome	Postmortem /postnatal physical examinations
48	30		De novo	Interrupted Ao, PM VSD	Hypoplasia								Liveborn	Operated for CHD in postnatal 7. day, neonatal exitus in the postoperative 2nd day

Ao Coa aort coarctation, *VSD* ventricular septal defect, *DORV* double outlet right ventricules, *ARSA* abberrant right subclavian artery, *PM VSD* perimembranous ventricular septal defect, *APVS* absent pulmonary valve syndrome, *RAA* right aortic arch, *CHD* congenital heart disease, *EIF* echogenic intracardiac focus, *CPC* choroid plexus cyst, *MCM* mega cysterna magna, *MCDK* multicystic dysplastic kidney, *TOP* termiantion of pregnancy, *N/A* non-applicable

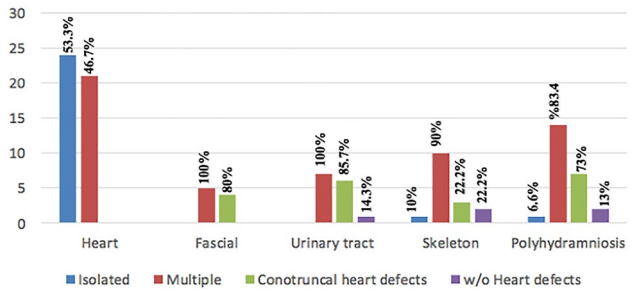


Fig. 3 Prenatal ultrasonographic findings' rates and concomitance

thymus aplasia/hypoplasia within a wide range from 3.7 to 26% in 22q11.2DS [19–21]. In the present cohort, thymus aplasia/hypoplasia was 68% of evaluated cases. However, the examination was arbitrary without any predefined criteria, and the lack of postmortem autopsy decreases the adequacy of investigation. Evaluation of thymus in both pre- and postnatal periods by means of ultrasonography is heterogeneous [24]. Chaoui et al. defined fetal thymus evaluation at > 15. GW at the "three vessel plane" [25]. By applying this methodology for detecting thymus aplasia/hypoplasia, they reported a positive predictive value of 81.8%, sensitivity 90% and specificity 98.5% for the diagnosis of 22q11.2DS especially with conotruncal anomalies [25]. Even though other methods including the thy-box and the TT-ratio (thymic-thoracic ratio) were developed thereafter to evaluate thymus hypoplasia during prenatal period, no standard of care methodology for thymus evaluation exists in clinical practice [26, 27]. However, in our opinion, accepting a reproducible method in prenatal thymus evaluation will increase the power of ultrasound in prenatal diagnosis, especially in diseases such as 22q11.2DS. Given this fact, it should also be kept in mind, that the association between conotruncal anomalies and thymus aplasia/hypoplasia are well documented, and it represents a major finding indicating for 22q11.2DS, which should be well evaluated in cases diagnosed with CHD in the prenatal period [20, 25, 28].

The indication for referral to prenatal diagnostic procedures based on these isolated findings other than CHD due to possible relation with this syndrome is controversial [19, 29]. In the French series, CHDs were reported as isolated in 62.5% of cases. Aplasia/hypoplasia of thymus was observed in 10%, polyhydramnios in 8% and facial anomalies in 6.2% of all cases as an isolated finding [19]. In this series, all genitourinary system anomalies were observed as associated with multiple anomalies, whereas no such an association was reported for skeletal system anomalies. In our series, CHD was observed in 40.5% of cases as an isolated finding, and in the non-isolated group, the most common detected associated findings were polyhydramnios and skeletal system

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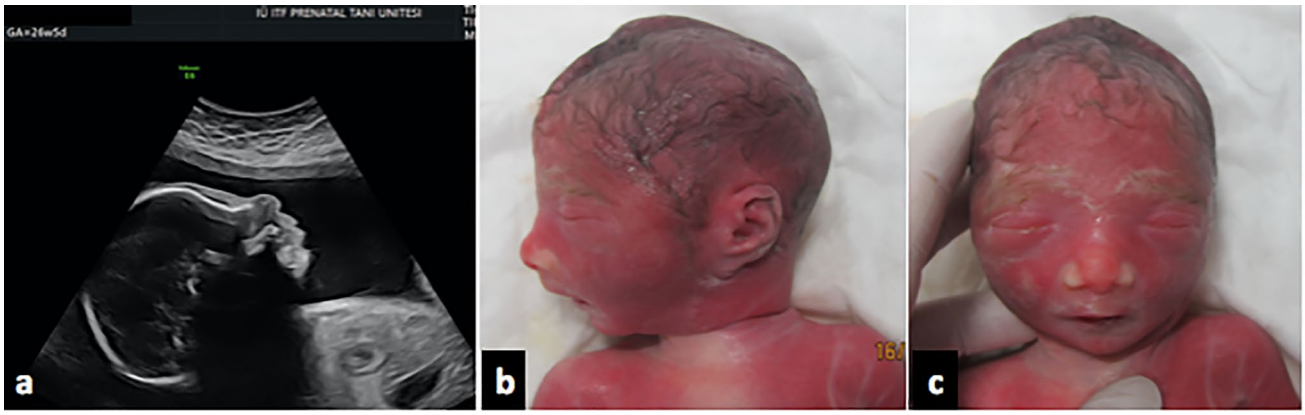


Fig. 4 The appearance of retromicrognathia in prenatal sonography (**a**) and postmortem examination (**b**) in the case with del22q11.2 detected at 24 weeks of gestation (*Case 38*). In most cases, wide nasal root (**c**) and pinched nose (**b**, **c**) may not be evaluated in prenatal examination

anomalies. The spectrum of genitourinary system anomalies was not different when compared to the literature. All were associated with additional anomalies, and conotruncal anomalies were the most commonly observed ones (85%) [19–21, 30].

In our DGS series, polyhydramnios rate was similar to the literature of order of 31%, and in 6.6% as an isolated finding. Polyhydramnios considered as a non-specific ultrasound finding for 22q11.2DS is assumed to be associated with velo-palatal insufficiency, when orofacial cleft or major gastrointestinal anomalies are absent [20, 30]. Sacca et al. studied 104 postnatal cases diagnosed with 22q11.2DS and found in 71% of cases airway anomalies including tracheomalasia, subglottic stenosis and laryngomalasia [31]. When the prenatal records of these anomalies were studied, associated polyhydramnios was observed in 16% of cases [31]. Lamouroux et al. reported polyhydramnios as the sole finding when congenital heart and other major anomalies were excluded from the series of fetuses with 22q11.2DS [29]. There is still no clear data directly suggesting amniocentesis in isolated polyhydramnios [32]. From our point of view, polyhydramnios should be categorized according to the current guidelines and described with an accurate sonographic manner. With such an approach, the prenatal diagnosis and management of isolated or severe polyhydramnios may be more appropriate. Also, it is known that genome-wide molecular karyotyping is more sensitive to detect the microdeletions and microduplications than conventional karyotyping [33]. Therefore, not only in moderate polyhydramnios related with structural malformations, we support the recommendation of aCGH after conventional karyotyping even in isolated cases of severe polyhydramnios without structural anomaly [34].

Orthopedic problems are reported within the phenotypic spectrum of the 22q11.2DS, although they are not as typical as CHD; hence, are occasionally overlooked at the time of diagnosis [35]. Virtually all patients demonstrate cervical vertebral anomalies, and scoliosis constitutes in 60% the most common problem [36]. PEV ranks at the top among other skeletal anomalies reported from series comprising early childhood and adulthood, though a prenatal diagnosis of which is possible. The association with 22q11.2DS had been reported of the order of 3.3–13% in published series [35, 37]. Schindewolf et al. presented a prenatal series of 42 cases, and skeletal system anomalies were reported in 18% of the cases, while 4.7% were PEV [20]. In our study, skeletal system anomalies were observed in 25% and PEV was the most common (20.8%) skeletal anomaly, and to the best of our knowledge, this is the highest rate reported in prenatal series. When all PEV cases are considered, 60% were bilateral, and 80% were associated with cardiac anomalies out of which only 37.5% were conotruncal anomalies. PEV was the only major structural anomaly in one case. Additionally, in *Case 9*, bilateral PEV could not be detected by prenatal US, because an optimal US evaluation was not possible due to anhydramnios caused by unilateral renal agenesis and MCDC in the contralateral kidney. In this case, we believe that the pes equinovarus may have developed secondary to deformation due to anhydramnios. As a result, PEV is one of the most common congenital anomalies, which can be detected isolated (50–70%) or complex (30–50%) during fetal life [38]. Apart from aneuploidies, it may also be associated with many genetic syndromes such as microdeletions. Therefore, based on the present study, 22q11.2DS should be taken into consideration in the differential diagnosis. When PEV is detected prenatally, it is reasonable to recommend karyotyping, even if the test result is predicted to be negative. If tests are negative, aCGH testing should be recommended, even if it is an isolated finding. In countries where aCGH testing is expensive, this stepwise method may be preferred, otherwise it may save time in terms of prenatal diagnosis to offer it concurrently with conventional karyotyping.

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The specific US findings guide to perform the rapid FISH testing for the microdeletion; however, atypical US findings influence the genetic laboratory workflow, delay the diagnosis and counseling [20, 30]. In our series, the microdeletion was detected by targeted high-resolution karyotyping only in 27.7% and the remaining cases by FISH or microarray/aCGH tests. Since microarray tests search the whole genome, it allows to determine the breakpoints, genes involved, and the other possible causative microdeletions and duplications in the genome elsewhere. For sure, it is a powerful and rapid technique; however, in our country, microarray test is about fivefold expensive than FISH and its cost should partially be covered by families in our institution. Therefore, molecular karyotyping was offered to all patients but microarray could not be applied in all cases. In our country, the gold standard prenatal diagnostic test for DGS is FISH. If 22q11.2DS was suspected due to the US findings, initially as rapid test I-FISH including common aneuploidies and 22q11.2DS was performed. When the result was normal, karyotyping and then microarray was performed, only in cases with late GWs in parallel.

The size of the microdeletions determined by microarray varied from 1.26 to 3.05 Mb. Cases with smallest one (1.26 Mb—*Case 28*) and greatest deletion (3.05 Mb—*Case 11*) had different phenotypes but not including CHDs (Table 5). The both deletions were covering *TBX1* gene but not *MAPK1* gene. *TBX1* gene is proposed the most important cause of the phenotype, since it regulates the development of cardiac, thymus, limb, lung, palate, parathyroid organs [30]. Haploinsufficiency of *CRKL* and *MAPK1* genes were also considered causative for cardiac anomalies [2, 39, 40]. Rump et al. reviewed a series including their 27 new postnatal patients and 52 cases from the literature with a microdeletion distal to classical region comprising the LCR22-B to LCR22-D region not encompassing *TBX1* gene but *CRKL* and *MAPK1* genes, which they called as "central 22q11.2 deletions" [12]. They observed that CHDs were less frequent in patients with central deletions and speculated that the haploinsufficiency of both *TBX1* and *CRKL* genes together, could be more deleterious than of either *TBX1* or *CRKL* gene alone. Our three cases *(Case 13, 25 and 32)* with a microdeletion covering *TBX1, MAPK1* and *CRKL* genes had variable manifestations. One of the fetus had typical conotruncal heart anomalies, one had pulmonary stenosis, but third one had no cardiac involvement. Our *Case 17* presented with full phenotype of the syndrome (Table 5) had two deletions, these deletions led to the haploinsufficiency of the genes *LZTR1* and *CRKL,* but not including *TBX1 or MAPK1* gene. *LZTR1* gene is important in the embryogenesis as a transcriptional regulator and its haploinsufficiency has been reported in 22q11.2DS and autosomal dominant Noonan10 syndrome (NS10) patients, where all had CHDs [41]. Heart malformations have also been found in Lztr1 ± mice [42]. It has been suggested that the deletions covering the both genes *CRKL* and *MAPK1* cause to higher penetrance of cardiovascular anomalies [43]. We suggest that the same mechanism, haploinsufficiency of both *CRKL* and *LZTR1* genes together can cause a more severe phenotype, too.

The involvement of modifier genes elsewhere in the genome may explain the phenotypic variability of the syndrome in cases with the same size and positions in unrelated or related individuals, like in our Case 11. The mice model study from Taddei et al., supported that genetic factors are major, but not sole determinants in the manifestation of 22q11.2DS in mice [44]. Voss et al. showed that lack of the histone acetyltransferase MOZ (MYST3/KAT6A) causes to DiGeorge syndrome like phenotype, and the MOZ complex occupies the Tbx1 locus, promoting its expression and histone 3 lysine 9 acetylation in mice [45]. However, hypothesis based on the effect of modifier genes cannot explain the variability of monozygotic twins. More discordant than concordant monozygotic twins are published because of the scientific interest [46–51]. Halder et al. reported a discordant monozygotic twin with different deletions in size [52]. Severe affected twin had 139,491 bp larger deletion including one extra gene (*GGT3P* gene) and its cardiac involvement is still unknown today.

Even though the major limitation of our study may have been its retrospective design, a relatively high number of cases from a single tertiary center may give us an opportunity to have a detailed idea about prenatal variable features of DGS. Although the parents were counseled to do so, we were unable to obtain postmortem physical exam and autopsy in the whole spectrum of pregnancy terminations in all cases, which would have enabled us to confirm prenatal findings and obtain valuable information regarding the association within anomalous organ systems. The detection rate of cleft palate is lower than cleft lip-palate, in general [53]. In our study, the frequency of cleft palate without cleft lip was found to be lower than in the literature (Table 5) [54]. In particular, the lower diagnostic rate of isolated cleft palate may be related to both the difficulty of prenatal diagnosis and the absence of postmortem examination of every case. Since there is no published standard and reproducible technique for cleft palate assessment in the literature, it is not recommended to be examined routinely [54]. However, careful evaluation of the high-risk population may increase the probability of prenatal diagnosis.

The current study demonstrates in the largest prenatal case series reporting the whole spectrum of atypical phenotypic and genotypic variations of 22q11.2DS. The knowledge regarding the distribution of prenatal findings within and associated with various organ systems may enable a rapid and precise diagnostic process during fetal development. This may improve the multidisciplinary prenatal

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counseling of the family, as well as postnatal prognosis. Prenatal counseling is important in this period, especially in the presence of conotruncal or aortic arch anomalies. The contribution of prenatal diagnosis of the syndrome was enormously important in the planning of postnatal care.

We believe that when all the phenotypic differences are taken into account, various anomalies including isolated polyhydramnios and PEV might be considered as an indication to search for deletion of 22q11.2 along with congenital heart diseases. All of these data will contribute to the establishment of the true prevalence of these anomalies and defects, and reveal the importance of the prenatal multidisciplinary counseling and the contribution of this condition to postnatal positive outcomes.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by TSS, UA, MK and BK. The manuscript was written by TSS, AY, RH and SB. TSS, IHK and RH analyzed and interpreted of the data.

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Availability of data and material Data will be made available by the authors upon request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The study protocol was approved by our institutional review board.

Consent to participate All subjects provided written informed consent.

Consent for publication All authors agree on the final manuscript and the order of authors.

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