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Prenatal Diagnosis of 4q Terminal Deletion and Review of the Literature

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Abstract
Terminal deletion of chromosome 4 (4q deletion syndrome) is a rare genetic condition that is characterized by a broad clinical spectrum and phenotypic variability. Diagnosis of the distinct condition can be identified by conventional chromosome analysis and small deletions by novel molecular cytogenetic methods such as microarray comparative genome hybridization (aCGH). Prenatal diagnosis is challenging; to date 10 cases have been described. We report a prenatally diagnosed case of de novo 4q deletion syndrome confirmed by conventional karyotyping and FISH due to an elevated combined risk for Down syndrome and prenatal ultrasound findings. aCGH validated the diagnosis and offered exact characterization of the disorder. Cytogenetic and microarray results described a 4q32.1qter terminal deletion of the fetus. Prenatal ultrasound detected multiple nonstructural findings (micrognathia, choroid plexus cysts, echogenic fetal bowel, short femur, and cardiac axis deviation). Pregnancy was terminated at 20 weeks. In addition to the index patient, we reviewed the 10 prenatally published cases of 4q deletion syndrome in the literature and compared these with our results. We summarize the patients' characteristics and prenatal clinical findings. Alterations of maternal serum biochemical factors, an elevated combined risk for trisomies, and distinct ultrasonographic findings can often be observed in cases of prenatal 4q deletion syndrome and may facilitate the otherwise difficult prenatal diagnosis.

4q deletion syndrome is a rare structural chromosomal abnormality caused by a partial interstitial or terminal deletion of the long arm of chromosome 4. The estimated incidence of the disease is 1 in 100,000 with a nearly equal ratio in male and female patients [Strehle et al., 2001]. The majority of the deletions occur de novo, but approximately 14% of the cases develop as a result of unbalanced segregation of parental reciprocal translocation. The overall mortality of the disease is 28% [Strehle and Bantock, 2003]. The term 4q deletion syndrome is applied to 2 different groups of deletions depending on the exact chromosomal breakpoint and the region of 4q that is deleted, classified as interstitial from the centromere to 4q31 and as terminal deletion with a loss from 4q31 to 4qter.

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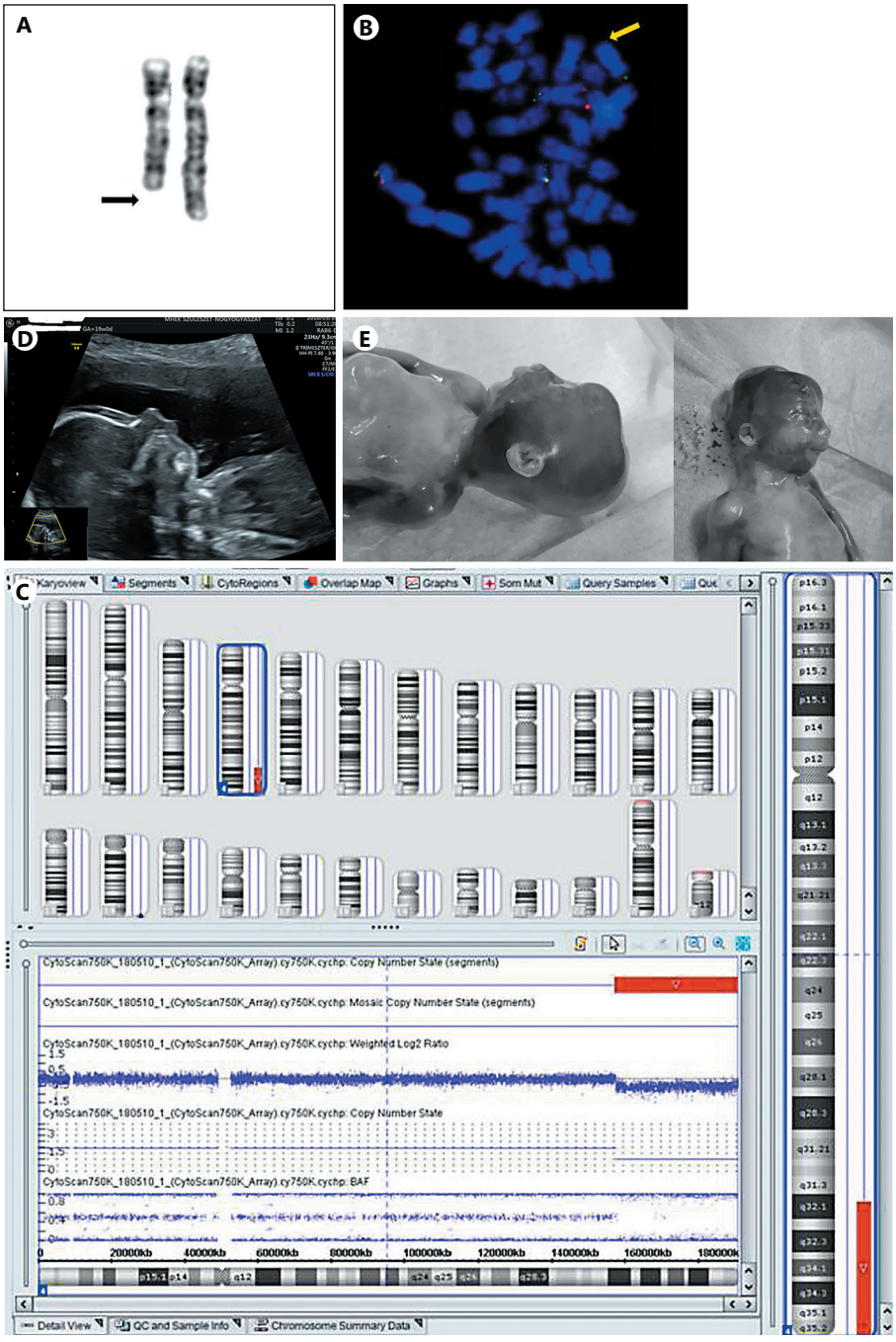

Fig. 1. A GTL-banded pair of chromosome 4 showing the 4q terminal deletion (arrow). **B** Metaphase spread (FISH) analysis using 4p (green) and 4q (red) subtelomeric probes. Note that the probe mixture also gives red and green signals to the chromosome 21q telomeric region. The arrow indicates the deletion in chromosome 4q. **C** aCGH analysis showing the deleted segment of 4q32.1qter with a loss of 33.5 Mb (chr4:157,455,107–190,957,460). **D** Ultrasonography of the fetal facial profile showing micro and retrognathia (week 18). **E** Images of the fetus at termination of pregnancy (week 20 + 5). Note the characteristic hypertelorism, low-set ears, micro- and retrognathia, small nose and depressed nasal bridge.

The main clinical features of the congenital multisystemic malformation syndrome are developmental delay, growth retardation, craniofacial dysmorphism (low-set ears, broad nasal bridge, short upturned nose, and micrognathia), digital dysmorphism, autism spectrum disorder, abnormalities of the cardiovascular, musculoskeletal, urogenital, and gastrointestinal system. Despite common characteristics of the distinct condition, the symptoms and severity of the disease vary greatly and depend on the size of the deleted region, the exact chromosomal breakpoint, and the genes that are located in the deleted region. Moreover, apart from the deleted region, other genes within a certain distance from the breakpoint and other genomic structures such as transposable elements can mediate genomic rearrangements and influence the phenotype [Vlaikou et al., 2014]. So far, a definite genotype-

phenotype correlation has not yet been determined. There are fewer than 200 cases of 4q deletion reported in the literature, the majority of the deletions were diagnosed postnatally [Strehle, 2011]. The number of prenatally diagnosed cases in the literature is small, not more than 10 cases described. Prenatal diagnosis of 4q deletion syndrome is challenging owing to the phenotypic variability of the disease and the lack of distinct ultrasonographic findings characteristic to the condition. A very limited number of cases was reported when invasive testing due to prenatally detected ultrasonographic soft markers [Manolakos et al, 2013] or severe generalized hydrops of the fetus [Russel et al., 2008] made it possible to set up the diagnosis of fetal 4q deletion syndrome. Classical cytogenetic techniques, even with commercially available FISH probes, are not sufficient diagnostic tools in most cases as deletion breakpoints cannot be specified correctly. The use of chromosomal microarrays enhances the detection rate and reduces the time which is of utmost importance in prenatal diagnosis.

In this report, we present a case of prenatally diagnosed 4q deletion syndrome based on high-risk combined screening test results and second trimester ultrasound soft markers by conventional and molecular genetic methods. We also provide a review of the literature on prenatal diagnosis of the multisystemic disease.

Case Report

A 38-year-old woman (gravida 2, para 1) was referred to our prenatal center at 13 weeks of gestation because of advanced maternal age and a high-risk combined test result for Down syndrome (DR: 1:83, βHCG: 2.63 MOM, PAPP-A: 0.5 MOM). A detailed ultrasound scan did not show fetal anomalies (crown rump length: 72 mm, nuchal translucency: 2.0 mm). Chorionic villus sampling was performed at 14 weeks of gestation, followed by amniocentesis at 16 weeks to rule out the possibility of confined placental mosaicism, as ultrasound examination still did not show any abnormalities. Ultrasound scan at 18w+3d was done according to standard second trimester protocols (GE Voluson® E8, GE Medical System Kretztechnik GmbH & Co OHG) and revealed multiple minor malformations: micrognathia, bilateral plexus choroid cysts, echogenic bowels, left axis deviation of the heart, and a short femur. The parents decided to terminate the pregnancy at 20 weeks and 5 days gestation. The postmortem examination found a male fetus of 325 g body weight. The fetal measurements corresponded to be slightly below the normal range. Fetal foot length (29 mm) appeared to be <10th percentile for the age of the pregnancy. An autopsy revealed distinct facial dysmorphism with hypertelorism, a small nose with depressed nasal bridge, low-set ears, micro- and retrognathia. The central nervous system and cardiac, urogenital, and skeletal anatomy were mostly normal. Besides facial dysmorphism, no definite other abnormality was detected.

Materials and Methods

Cytogenetic analyses were performed on direct and cultured chorionic villus samples, amniotic fluid cells, and postmortem fibroblast cultures. GTL-banding was done according to standard methods. FISH was carried out with probes specific to chromosome regions DXZ1, DYZ3, D18Z1, 13q14.2, and 21q22.13 using the FAST FISH Prenatal Enumeration Probe Kit (Cytocell) to exclude the most frequent aneuploidies. We performed FISH with probes specific to the 4q subtelomeric region and 4q35 (D4S2930) (TeloMark, Cytocell) on directly prepared chorionic metaphases to rule out a possible subtle translocation. All FISH reactions were done according to the manufacturer's instructions. Parental karyotypes from peripheral lymphocyte cultures were performed in line with the prenatal examinations.

SNP microarray analysis was performed using the Affymetrix Cytoscan 750K platform which uses 750,000 markers for copy number analysis, including 200,000 SNP and 550,000 non-polymorphic probes with the median spacing (gene and non-gene backbone) of 4,125 kb. 250 ng of total genomic DNA extracted from cell suspension was digested with *Nsp*I and then ligated to NSPI adaptors, respectively, and amplified using Titanium Taq with the Sensoquest PCR system. PCR products were purified using AMPure beads and quantified using Nanodrop ND1000. Purified DNA was fragmented, biotin labeled, and hybridized to the Affymetrix Cytoscan 750K GeneChip. Data were analyzed using the Chromosome Analysis Suite. The analysis is based on human genome version GRCh37/hg19 (Fig. 1).

Results

Parental karyotypes showed no alterations, so the fetal chromosome aberration was specified as de novo. On the basis of the cytogenetic and microarray results, the fetal karyotype was finally described as 46,XY,del(4)(q32).ish del(4)(q35)(D4S2930–).arr[GRCh37] 4q32.1q35.2(157455107_190957460)×1 dn.

The terminal deletion size in chromosome 4 was 33.5 Mb (chr4:157,455,107–190,957,460) and the number of the deleted genes was 148, respectively.

Discussion

Here, we report a case of prenatal diagnosis of a rare multisystemic malformation condition, 4q deletion syndrome. The parents decided to terminate the pregnancy at 20 weeks' gestation. The diagnosis was confirmed by conventional karyotyping and molecular cytogenetic methods.

Classical G-banding detects larger chromosomal deletions with an estimated maximum resolution of 5–10 Mbp, even with high-resolution banding techniques.

Table 1. Genotype-phenotype correlation of terminal 4q deletion

Author	No. of cases	Clinical features	Genes/loci	Regions
Keeling at al., 2001	1 (postnatal)	Cleft lip and palate Neurodevelopmental delay	–	4q33
Rossi et al., 2009	2 (prenatal) 6 literature (postnatal)	Cleft palate Congenital heart defects	*HAND2*	4q34.1q34.2 174–178 Mb
Strehle et al., 2012a	2 (postnatal) 1 (prenatal)	Cleft palate	–	4q33q35.1
Strehle et al., 2012b	20 (postnatal)	Cleft palate Congenital heart defect	*SORBS 2*	4q35.1
Xu et al., 2012	1 (postnatal) 101 literature (postnatal)	Congenital heart defect	*TLL1* *HPGD* *HAND2*	4q32.2q34.3
Hemmat et al., 2013	1 (postnatal) 10 literature (postnatal)	Congenital heart defect	*PDLIM3* *ArgBP2*	4q35.1
Vona et al., 2014a	1 (postnatal) 35 literature (postnatal)	Congenital heart defect 1	*TLL1* *HAND2*	chr4:160,717,000– 178,579,037
Vona et al., 2014a	1 (postnatal) 35 literature (postnatal)	Congenital heart defect 2	*SORBS2* *PDLIM3*	chr4:184,046,156– 186,997,806
Vona et al., 2014a	1 (postnatal) 35 literature (postnatal)	Autism spectrum disorder Hearing impairment	*MTNR1A* *FAT1* *F11* *DFNA24*	4q35.2 chr4:187,234,067– 188,424,639
Novo-Filho et al., 2016	4 (postnatal)	Neurodevelopmental delay	*HELT*	4q35.1

Number of cases, reports, and cases from the literature used for the comparison and analysis are indicated.

Novel molecular genetic examinations such as microarray comparative genome hybridization (aCGH) make it possible to scan the whole genome with high resolution and to determine the exact DNA breakpoints [Shao et al, 2008]. Recently, aCGH has been extensively used in the genetical analysis of children suffering from developmental delay [Manolakos et al., 2010]. Besides conventional G-banding techniques, introduction and application of high-resolution molecular cytogenetic methods have been of great importance, even in prenatal diagnosis [Friedman, 2009]. Employing these methods in prenatal diagnosis is especially important for the investigation of fetuses with abnormal ultrasound findings and normal conventional G-banding results, as microarray has a 2–10% diagnostic advantage over classical karyotyping [Kleeman et al., 2009; Wapner et al., 2012; Hillman et al., 2013; Srebniak et al., 2016].

Since the cytogenetic description of the deletion in the long arm of chromosome 4 in 1967 [Ockey et al., 1967], Townes et al. [1979] were the first to use the term 4q deletion syndrome for cases of chromosome deletions in 4q31 with a distinct characteristic phenotype. Considering the small number of diagnosed cases, several authors made an attempt to specify genotype-phenotype correlation and determine the critical region or exact genes presumably playing a critical role in the development of the disease (Table 1) [Strehle et al., 2012b]. Deletion in the chromosomal region 4q31q34 may be responsible for the majority of postnatal phenotypes. Some authors hypothesize 4q33 to have a critical role in syndrome-specific-manifestations [Keeling et al., 2001; Giuffrè et al., 2004]. In most reported cases of distal terminal 4q deletions (4q34q35), the phenotype can be characterized by slightly milder clinical symptoms and neurodevelopmental deficit [Caliebe et al., 1997].

We compared our case with other 4q deletion syndrome cases published so far in the literature, and we found 2 cases with proximal breakpoints at 4q32.1.

Tzschach et al. [2010] reported a case of a 22-year-old female patient characterized by moderate intellectual disability, obesity, psychosis, and facial dysmorphism (broad nasal root, sparse lateral eyebrows, thin upper lip, short philtrum, and micrognathia). Diagnosis setup by FISH and aCGH revealed a complex rearrangement comprising an interstitial deletion of approximately 10 Mb in 4q32.1q32.3 together with a balanced translocation between chromosomes 2 and 5 [t(2;5)(p22,q12,1)]. The proximal deletion, chr4:156,628,981–160,162,888, was 3.4 Mb in size, followed by a 5.4-Mb deleted segment between chr4:160,971,479 and chr4:166,358,059, and finally, a 0.11-Mb deletion flanked by chr4:166,628,236 and chr4:166,802,966. Thirty genes are located in the deleted region. The authors hypothesized the glutamate receptor coding gene, *GRIA2*, and the glycine receptor coding gene, *GLRB*, as candidates for intellectual disability and the neuropeptide Y receptor genes *NPY1R* and *NPY5R* for cognitive defects. Facial characteristics of the patient were very similar to our prenatal case. Although this case was characterized by a complex interstitial chromosomal rearrangement, in terms of breakpoint, its proximal breakpoint (156,628 kb) seems to be the closest to our case (157,455 kb) of all those found in the literature.

The other case was reported by Novo-Filho et al. [2016], who investigated patients suffering from developmental disability and congenital anomalies. Among the 105 patients examined, the authors found 4 patients with 4q terminal deletion, one female patient among them with a 4q32.1q35.2 deletion and a 5p15.2 duplication. Molecular testing was done by MLPA and was characterized using Illumina-CytoSNP array. Although the exact breakpoint, the patients' characteristics, and phenotype were not mentioned in the study, the deletion incorporated the *FSTL5* gene which corresponds to chr4: 161,383,892. Out of the 111 deleted genes, 2 genes were found by array analysis that could play a role in the development or the function of the nervous system: *CASP3* (in neurological system process and neuron apoptosis) and *CYP4V2* (in neurological system process). They also found 2 genes, *SLC25A4* and *HELT*, which were deleted in all patients affected by 4q deletion. The *HELT* gene was regarded to be expressed in undifferentiated neural progenitor cells in the developing mesencephalon and diencephalon. The authors suppose that the *HELT* gene may play a crucial role in developmental delay and intellectual disability. This case, in terms of proximal and distal chromosomal breakpoints and the size of the deletion, is most similar to the present case (161,38–190,9 and 157,46–190,9 kb, respectively). It is important to emphasize that the 2 abovementioned patients affected by 4q deletion are postnatal cases, while the present fetus was terminated at 20 weeks of pregnancy. Direct comparison of pre- and postnatal cases is difficult, whereas it is feasible to investigate craniofacial dysmorphism or certain structural malformations in both instances; features such as behavioral disturbances, developmental delay, sensorineural or intellectual deficit cannot be studied prenatally. None of the prenatally diagnosed cases in the literature had any similarity to our case regarding the breakpoints.

According to the OMIM database, the terminal region of chromosome 4, 4q32, incorporates 148 genes, a number of them with proven pathogenic phenotypes in humans by the DECIPHER v9.27 genome browser. A significant number of other genes in this region can be characterized by moderate or high pLI scores, meaning that the gene is intolerant for loss-of-function variations. Thus, a heterozygous mutation of the gene results in haploinsufficiency and makes the gene a candidate for morbidity (in detail in Fig. 2) [Lek et al., 2016]. Checking the deleted chromosomal region in the present case (4q32.1q35.2), we found a total of 14 genes with a pLI score ≥0.9, suggesting a loss-of-function intolerant gene. The OMIM database indicates that only 2 of the 14 genes with high pLI scores have known pathogenic consequences in humans. The *RAPGEF2* gene (4q32.1), which is a member of the RAS subfamily of GTPases, functions in GTP/GDP-regulated signal transduction pathways and represents the key link between cell surface receptors and RAS activation. High *RAPGEF2* expression can be detected in kidney, placenta, and liver, intermediate expression in ovary, and low expression in brain and lungs. Heterozygous expansion of the *RAPGEF2* gene was identified in a patient with familial adult myoclonic epilepsy-7 (OMIM 618075), suggesting that $TTTCA_n$ expansion can be responsible for the phenotype. The *TENM3* gene (teneurin transmembrane protein 3) is mapped to chromosome 4q34.3q35.1. Teneurins predominantly bind the lectin domain of latrophilins independent of calcium and presumably play a role in classic heterophilic intercellular adhesions. In mouse models, teneurin-3 is highly expressed in specific brain regions such as amygdala, caudate nucleus, hippocampus, and the medial entorhinal cortex. It is suggested that teneurin-3 may regulate the assembly of certain complex distributed circuits in the mammalian brain. In humans, homozygous splice mutations of *TENM3* were demonstrated in 2 families causing bilateral colobomatous microphthalmia (microphthalmia, isolated, with coloboma 9, OMIM 615145). Unaffected parents and relatives were identified as heterozy-

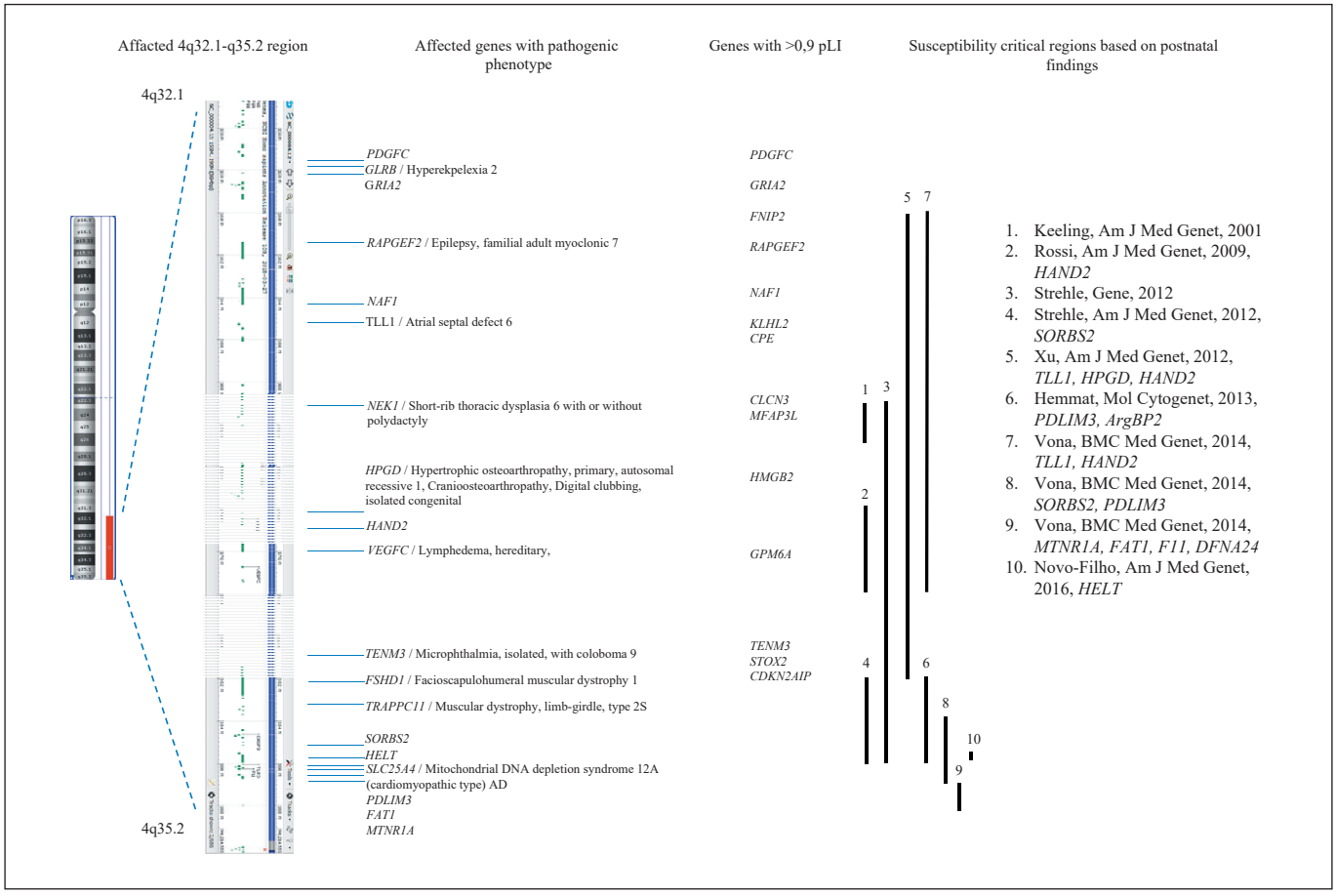

Fig. 2. Schematic representation of the deleted region 4q32qter in the present case (red bar). Proven pathogenic genes and consequent diseases according to DECIPHER database v9.27 and genes with a pLI score ≥0.9 are shown. Susceptibility regions, genes, and loci of genotype-phenotype correlation by different authors found in the literature are also listed.

gous for the mutation indicating that a heterozygous mutation of the gene itself is not responsible for the phenotype [Chassaing et al., 2016]. It may be important to note that 8 of the 14 genes with a pLI score >0.9 are confirmed as being expressed in the central nervous system in animal or human studies (*PDGFC*, *GRIA2*, *RAPGEF2*, *KLHL2*, *CLCN3*, *GPM6A*, *TENM3*, and *STOX2*). *GPM6A* (neuronal membrane glycoprotein M6A) is expressed in rodent neurons and has a significant role in neural cell adhesion and in some aspects of neurite growth; it is also found in human hippocampal cells. *CLCN3* (chloride channel 3) encodes an intracellular chloride channel mainly in the hippocampus and olfactory cortex in mice and in humans. Experimental null mice show signs of behavioral abnormalities and neuronal degeneration in the retina and hippocampus. *GRIA2* (glutamate receptor 2) gene products are ligand-activated cation channels that mediate the fast component of excitatory postsynaptic currents in neurons of the central nervous system. The majority of them are detected in the hippocampal cells of rat and human brain tissues. The functions of glutamate receptors are confirmed in synaptic plasticity and memory, formation of dendritic spines, spine growth, and stability and changes in excitatory postsynaptic current properties. In transgenic mice for *GluR2*, a motor neuron disease can develop later in life that resembles human amyotrophic lateral sclerosis. *KLHL2* (Keltch like 2) gene protein products' highest levels of expression were found in adult and fetal brain, primarily detected in the amygdala, caudate nucleus, corpus callosum, and hippocampus. The *STOX2* gene of unknown function was isolated from adult brain, heart, spleen, ovary, and fetal brain tissues. *PDGFC* (platelet derived growth factor C) is a major mitogen and stimulant of motility in mesenchymal cells,

such as fibroblasts and smooth muscle cells, and also acts on other cell types including capillary endothelial cells and neurons. Expression of *PDGFC* is the highest in prostate, testis, and the uterus, with less expression in the spleen, thymus, and small intestine as well as little expression in the colon and peripheral blood leukocytes. Animal studies suggest that the gene has a major role in the development of craniofacial structures, the neural tube, and mesodermal organs. *Pdgfc* null mice die due to feeding and respiratory difficulties associated with a complete cleft of the secondary palate. Some authors hypothesize that *PDGFC* signaling is a novel and independent mechanism that regulates palatogenesis in early embryonic development [Ding et al., 2004]. On the whole, many suspected loss-of-function-intolerant genes in the 4q32qter region are expressed in rodent or human brain tissues, particularly in the structures of the limbic system, which is connected mainly to memory, orientation, behavior, or mood. Heterozygous deletion of these genes may be related to those features that can often be observed in 4q deletion syndrome as intellectual disability, or developmental delay, whereas mutation or deletion of the *PDGLC* gene may lead to craniofacial dysmorphysm and palatal abnormalities.

Besides the abovementioned genes with high pLI score, many authors presume the pathogenic role of other genes in terms of genotype-phenotype correlation. Genes located in 4q32.2q34.3, such as the zinc-dependent metalloprotease gene *TLL1* (Tolloid-like protein1 gene) and basic helix-loop-helix family of the transcription factor gene *HAND2* (heart- and neural crest derivatives-expressed protein 2 gene), are major candidates for cardiac morphogenesis based on animal models and loss-of-function mutations in patients [Xu et al., 2012]. Some authors presume that the more distally positioned *SORB2* gene (sorbin and SH3 domain containing 2 gene, 4q35.1) may have a role in the formation of congenital heart disease (CHD) and cleft lip/palate. Mutation of certain genes (*HELT*, *MTNR1A*, and *FAT1)* possibly influence autism spectrum disorder and neurodevelopmental deficit [Vona et al., 2014a]; however none of them are mentioned in any of the genome browsers as pathogenic genes. An autosomal dominant deafness locus, *DFNA24* (deafness, neurosensory) was mapped to the terminal region of chromosome 4 (4q35qter), although most cases with terminal 4q deletion overlapping this region are not characterized by hearing impairment [Häfner et al., 2000; Vona et al., 2014b]. Figure 2 summarizes the morbid genes and the hypothesized critical regions in genotype-phenotype correlation in 4q deletion syndrome published in the literature.

In the present case, although a large deletion in the long arm of fetal chromosome 4 (4q32.1qter) eliminated the supposed critical chromosomal regions and potential morbid genes mentioned above, neither CHD nor cleft lip or palate was detected during prenatal and autopsy examinations. Fetopathological examination confirmed characteristic facial dysmorphism (small nose with a depressed nasal bridge, micro- and retrognathia, hypertelorism, and low-set ears). According to previous data, the exact critical region and susceptibility genes of the phenotype in 4q deletion syndrome are still elusive.

Prenatal diagnosis of the syndrome has been reported in a limited number so far. In the English medical literature, 10 prenatally diagnosed cases can be found, of which 3 describe interstitial and 7 terminal deletions (Table 2). The first prenatal case was diagnosed by amniocentesis due to elevated maternal serum AFP level in 1986 [Campbell et al., 1986]. Chronologically, the first 3 (interstitial) diagnoses were established by conventional G-banding via amniocentesis [Campbell et al., 1986; Koppitch et al., 1990; Hsu et al., 1998]. Starting from 2008, high-resolution FISH and, since then, FISH and aCGH were applied to determine chromosomal breakpoints. In 3 out of 10 cases, out of the normal range of serum level of maternal biochemical markers or increased risk for Down syndrome was the indication for invasive procedures [Campbell et al., 1986; Hsu et al., 1998; Strehle et al., 2012a]. In 5 cases that represent 50 percent of all prenatal cases, abnormal ultrasound findings were detected in the second or third trimester regarding fetal central nervous, cardiovascular or urogenital system [Rossi et al., 2009; Manolakos et al., 2013; Gonzalez et al., 2018]. In one case, severe generalized fetal hydrops and polyhydramnion [Russel et al., 2008] and in another case, complex fetal malformations (double bubble, facial dysmorphism, and cardiovascular defects) were observed in the third trimester [Rossi et al., 2009]. The fetopathological and postnatal examinations revealed that the most specific trait in 4q deletion is facial dysmorphism that was reported in 6 cases. Forty percent of all the cases were characterized by CHD and digital anomalies and 30% by cleft lip or palate; none of them were diagnosed prenatally. It is worth noting that the abovementioned abnormalities were equally detected in both interstitial and terminal deletions. Prenatally observed first trimester ultrasound findings such as increased nuchal translucency, facial dysmorphism, or CHD have not been detected in any of the reported cases. Rapid development of ultrasound technology and widespread practice of fetal echocardiology should increase the detection of prenatal malformations, especially in the

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Table 2. Patient characteristics and clinical findings in prenatally diagnosed cases of chromosome 4q deletion found in English literature

Author	Patient data	Deletion	Invasive procedure, method	Indication	Prenatal ultrasound findings	Pregnancy outcome	Postnatal/postabortum findings
Campbell et al., 1986	27 years Gravida: 0	4q21q27, interstitial	AC 17 weeks Karyotyping	Elevated serum AFP	–	TOP 21 weeks 360 g	Bilateral cleft lip/palate, digital dysmorphism CHD: aorta coarctation, double SVC
Koppitch et al., 1990	35 years	4q22q26, interstitial	AC Karyotyping	n.d.	–	TOP 22 weeks	Facial dysmorphism, anomalies of brain, trachea, liver, and kidneys
Hsu et al., 1998	31 years Gravida: 5 Para: 1	4q12q21.1, interstitial	AC 20 weeks Karyotyping	DR 1:67 (AFP: 0.51 MOM, βHCG:3.17 MOM)	2nd trimester IUGR Short femur	TOP 28 weeks 920 g, length 31.5 cm	Facial dysmorphism: low-set ears, depressed nasal bridge, micrognathia, exopthalmus, small mouth CHD: ASD
Russel et al., 2008	31 years	4q33qter, terminal	AC 32 weeks Karyotyping, FISH	US findings	3rd trimester Hydrops (ascites, pleural effusion) Macroglossia, polihydramnion	CS 34 weeks	Facial dysmorphism: small nose with narrow nasal bridge, micrognathia CHD: VSD, PDA
Rossi et al., 2009 Case 1	33 years	4q34.3qter, terminal 12 Mb chr4:178,470,008–191,121,285	CVS 13 weeks Karyotyping, FISH, aCGH	Maternal symptoms, known maternal 4q34.3qter deletion	2nd trimester Renal anomalies	Full-term pregnancy 3,490 g, length 53.5 cm	Facial dysmorphism: micrognathia, Iris coloboma CHD: VSD, PDA Bilateral hydronephrosis
Rossi et al., 2009 Case 2	n.d.	4q34.1qter, terminal 16.5 Mb chr4:174,685,919–191,121,195	AC weeks, n.d. Karyotyping, FISH, aCGH	n.d.	–	Full-term pregnancy	Facial dysmorphism: short nose with abnormal nasal bridge, micrognathia, hypertelorism Myopia Clinodactyly (toes)
Strehle et al., 2012a	43 years Gravida: 0	4q33qter, terminal	CVS weeks, n.d. Karyotyping, FISH	DR 1:143	–	TOP 18 weeks, 5 days	Cleft lip, small mandible, glossoptosis (Pierre-Robin sequence)
Manolakos et al., 2013 Case 1	36 years	4q35.1qter, terminal 8.18 Mb chr4:182,717,805–190,896,815	AC 19 weeks Karyotyping, aCGH	IVF Parental anxiety	–	TOP 22 weeks	Facial dysmorphism: depressed nasal bridge Macrocephaly Limb abnormalities Clinodactyly
Manolakos et al., 2013 Case 2	28 years	4q35.1qter, terminal 8.0 Mb chr4:182,920,816–190,896,815	AC 22 weeks Karyotyping, aCGH	US findings	3rd trimester Ventriculomegaly, oligohydramnion	TOP 24 weeks 692 g, length 22.5 cm	Facial dysmorphism: hypertelorism, beaked nose Macrocephaly Limb abnormalities Campodactyly
Gonzales et al., 2018	27 years Gravida: 2 Para: 1	4q34.3qter, terminal 4q11q21q25, duplication Mother: (4;11)(q34;q21), balanced translocation	AC 26 weeks Karyotyping, QF-PCR, aCGH	US findings	3rd trimester Exophthalmos, double bubble, persistent left SVC, 3rd and 4th ventricle dilatation, myocardial hypertrophy, agenesis of DV, IUGR	Delivery, 37 weeks 2,180 g	Pterygium coli, cleft palate, facial hemangiomas, digital dysmorphism, neurological disorders
Present case	38 years Gravida: 1 Para: 0	4q32.1qter, terminal 33.5 Mb chr4:157,455,107–190,957,460	CVS 14 weeks AC 16 weeks Karyotyping, FISH, aCGH	DR 1:83 (βHCG:2.63 MOM, PAPP-A 0.5 MOM) US findings	2nd trimester CPC, micrognathia, echogenic fetal bowel, short femur, left deviated heart axis	TOP 21 weeks	Facial dysmorphism: small nose with depressed nasal bridge, micrognathia, hypertelorism, low-set ears

AC, amniocentesis; aCGH, array comparative genomic hybridization; ASD, atrial septum defect; CHD, congenital heart disease; CPC, choroid plexus cyst; CS, cesarean section; CVS, chorionic villus sampling; DR, Down risk; DV, ductus venosus; IUGR, intrauterine growth restriction; IVF, in vitro fertilization; n.d., not determined; PDA, patent ductus arteriosus; QF-PCR, quantitative fluorescent polymerase chain reaction; SVC: superior vena cava; TOP, termination of pregnancy; US, ultrasonography; VSD, ventricular septal defect; –, absence.

field of CHD, and thus should enhance the detection rate of rare chromosomal aberrations.

In the present case, maternal age and an elevated risk for Down syndrome in the combined test (DR: 1:83, βHCG: 2.63 MOM, PAPP-A: 0.5 MOM) served as the indication for chorion villus sampling. The multiple soft markers we detected during the second trimester are more often seen in fetal aneuploidy and primarily increase the mathematical risk of trisomies such as Down syndrome. However, it deserves to be mentioned that according to our observations and based on literature data, certain soft markers and nonstructural ultrasound anomalies (e.g., facial dysmorphism, micrognathia, short femur or humerus, and echogenic fetal bowel) are definitely more prevalent in structural chromosomal aberrations and rare genetic syndromes [Tidrenczel et al., 2018]. An elevated biochemical risk for trisomies and bias in maternal serum level of PAPP-A, β-hCG or AFP are also more frequently perceived in non-trisomic chromosomal rearrangement (unpublished own data). In a study published recently by Bornstein et al. [2018], an abnormally low level of first trimester maternal serum free β-hCG (<0.45 MOM) was associated with an increased risk of fetal significant CNVs by aCGH in a retrospective cohort study of singleton pregnancies (n = 2,880). According to Scott et al. [2018] in a large prospective study, rare autosomal trisomies (RATs, chromosomes other than 13, 18, 21, X, and Y) were often accompanied by a low level of PAPP-A, especially in cases of intrauterine fetal growth restriction as a predictive factor for placental dysfunction. Abnormal maternal serum levels of proteins produced by the placenta (PAPP-A, HCG) may be indicators for the functional disruption of the feto-placental unit and consequently, early fetal intrauterine growth restriction as it is often observed in non-constitutional chromosomal abnormalities. This may be an independent mechanism of fetal growth restriction caused by the abnormal genotype itself and the gross number of affected genes concerning different aspects of fetal development. Although uniform typical ultrasound findings have not been detected in the so far published 10 cases of prenatally diagnosed 4q deletion syndrome, the characteristic facial features, CNS findings, short long bones, cardiac anomalies, and early fetal growth restriction have been reported in several cases and may draw attention to potential structural chromosomal disorders. However, the broad variability of the phenotype observed postnatally in patients makes the targeted prenatal ultrasound detection of the syndrome difficult. An increased risk of Down syndrome has been noticed in a total of 4 cases out of 11 in 4q deletion syndrome. As a result, variations in serum maternal biochemical factors can be indicative not only for common trisomies.

Cell-free fetal DNA tests [cffDNA, also called noninvasive prenatal test (NIPT)] based on next-generation sequencing (massive parallel sequencing or SNP analysis) have been recently applied in prenatal screening and diagnosis worldwide. Prenatal cffDNA tests have yielded high specificity and sensitivity results in the screening of common aneuploidies (trisomy 21, 18, and 13) with high accuracy and with a low false-positive and negative rate. A large body of evidence has shown NIPT to be an extraordinary noninvasive screening method for common trisomies. More recently, the spectrum of reportable chromosomal abnormalities by cffDNA has expanded with sex chromosome aneuploidies and copy number variants. Since 2016, a limited number of publications have reported that cffDNA tests can detect other chromosomal abnormalities (RAT, structural disorders) or monogenic diseases and could be a procedure for performing even noninvasive prenatal karyotyping of the fetus in the near future [Bianchi and Chiu, 2018]. However, at present we have only limited and inconclusive data about the exact performance and reliability of this method in the prenatal settings other than for common trisomies. At the moment, scientific organizations worldwide recommend the routine application of NIPT as a screening test only for common trisomies. Using the commercially available cffDNA test in the present case would have resulted in eventuated negative result for common trisomies and in spite of multiple ultrasonography findings would have mistakenly reassured the parents. The use of NIPTs in cases of structural or cumulated nonstructural fetal ultrasonography findings should be avoidable and performing invasive diagnostic procedures and methods with diagnostic accuracy should be the chosen practice.

Conclusion

In summary, we present a prenatally diagnosed case of de novo 4q deletion syndrome confirmed by molecular genetic testing. By review of the prenatally diagnosed cases, we suggest that abnormal levels of maternal serum biochemical factors may be indicative for non-numerical chromosome aberrations as well, and prenatal diagnosis of the disease can be facilitated by certain structural and nonstructural ultrasonography findings in the second trimester. The diagnostic method of choice is

aCGH due to its high resolution capability. The availability of novel molecular genetic methods and advanced ultrasound technique in prenatal settings should aim to prenatally diagnose more rare fetal disorders in the future.

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Disclosure Statements

The authors have no conflicts of interest to declare.

Author Contributions

Z. Tidrenczel performed ultrasound, genetic counseling, invasive procedures, and wrote the manuscript. E.P. Tardy was involved in genetic counseling, cytogenetic and data analysis, and writing the manuscript. I. Böjtös and E. Sarkadi did literature research and data analysis. H. Pikó carried out array CGH. A. Beke, J. Simon, J.P. Kósa, and J. Demeter revised the manuscript. All authors reviewed and approved the manuscript.

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