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First prenatal case of proximal 19p13.12 microdeletion syndrome: New insights and new delineation of the syndrome

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ABSTRACT

Proximal 19p13.12 microdeletion has been rarely reported. Only five postnatal cases with intellectual disability, facial dysmorphism, branchial arch defects and overlapping deletions involving proximal 19p13.12 have been documented. Two critical intervals were previously defined: a 700 kb for branchial arch defects and a 350 kb for hypertrichosis-synophrys-protruding front teeth. We describe the first prenatal case, a fetal death in utero at 39 weeks of gestation. Agilent 180K array-CGH analysis identified a heterozygous interstitial 745 kb deletion at 19p13.12 chromosome region, encompassing both previously reported critical intervals, including at least 6 functionally relevant genes: *NOTCH3*, *SYDE1*, *AKAP8*, *AKAP8L*, *WIZ* and *BRD4*. Quantitative PCR showed that the deletion occurred *de novo* with a median size of 753 kb. *NOTCH3* and *SYDE1* were candidate genes for placental pathology whilst *AKAP8*, *AKAP8L*, *WIZ* and *BRD4* were highly expressed in the branchial arches. Molecular characterization and sequencing of candidate genes for placental pathology and branchial arch defects were carried out in order to correlate the genotype-phenotype relationship and unravel the underlying mechanism of proximal 19p13.12 microdeletion syndrome. This case also contributes to define the novel critical interval and expand the clinical phenotype spectrum of proximal 19p13.12 microdeletion syndrome.

1. Introduction

Microdeletion of 19p13.12 has been rarely reported. Individuals with 19p13.12 deletion display variable clinical features including mild to severe developmental delay, ear malformations, hearing impairment, cardiac anomalies, brain malformations, facial dysmorphic features, synophrys and hypertrichosis. Moreover, no fetal phenotype of 19p13.12 microdeletion was reported to date. Patients with distal 19p13.12 microdeletion share a common 359 kb SRO encompassing six annotated genes: *LPHN1/CIRL1*, *CD97*, *DDX39*, *PKN1*, *PTGER1*, *GIPC1* and show typical clinical features including moderate to severe psychomotor impairment, language delay, hearing loss, facial dysmorphism, mild congenital cardiac anomalies and/or rhythm disturbance (Bonaglia et al., 2010). Recently, several postnatal cases involving proximal 19p13.12 submicroscopic rearrangements were reported. All previously reported patients had branchial arch defects including ear malformations, branchial pits and high palate (Table 1). As shown in Table 1, patients with proximal 19p13.12 microdeletion present distinctive facial dysmorphism, branchial arch defects with ear malformations, hearing loss, psychomotor and language delay. A 700 kb critical region for branchial arch defects in proximal 19p13.12 microdeletion was previously defined by Kosaki et al. (2011) while a 305 kb candidate interval for hypertrichosis-synophrys-protruding front teeth was documented by Jelsig et al. (2012).

We report a fetal death in utero at 39 weeks of gestation carrying the smallest 753 kb microdeletion of proximal 19p13.12. Having reviewed the literature data, the genotype-phenotype correlation in the present case has been delineated and the new critical region of proximal 19p13.12 microdeletion syndrome refined.

2. Clinical report

A healthy 33-year-old woman (gravida 2, para 1) went into spontaneous labor at 39 weeks of gestation after an uneventful pregnancy. The stillbirth was discovered with no fetal cardiac activity by electronic fetal heart rate monitoring just before the labor. Autopsy showed a mildly macerated female fetus presenting facial dysmorphism including square face, hypertelorism, downturned corners of the mouth, preauricular skin tag and cupped ears (Fig. 1A). Her weight, length and head circumference were respectively 3145 g (28^{th} centile), 53 cm (90^{th} centile), 34 cm (50^{th} centile). Internal examination revealed small kidneys (combined weight: 16.2 g; expected: 27 + 7.5 g) with normal cortico-medullary differentiation histology. Placenta was slightly hypertrophic (460 g; expected weight: 425 ± 5 g; foetoplacental ratio: 6.8; expected: 7.5 ± 0.1). The cord was marginally inserted. Placenta histology showed a global villous hypoplasia with massive perivillous fibrin depositions (MPVFD). Ischemic villous changes with increased syncytial knots were diffusely present (Fig. 2). The family history was unremarkable.

3. Materials and methods

3.1. Conventional cytogenetic analysis

Patient-index placental trophoblasts were cultured in AmnioMAXTM-C100 Supplement (ThermoFisher Scientific, France) and metaphase

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Table 1
Detailed phenotype in previously reported patients with 19p13.12 interstitial deletion. Note that all patients had branchial arch defects and distinctive dysmorphic features in previously reported cases.

		High forehead	Synophrys	Thick eyebrows	Epicanthal folds	Downslanting palpebral fissures	Hypertelorism	Clinical features		Low-set or malformed ears	Preauricular tags	Preauricular or branchial pits	High/ Cleft palate	Long philtrum	Micrognathia
								Dysmorphic features							
								Strabismus	Depressed nasal bridge						
Postnatal diagnosis cases	Kosaki et al. (2011)	-	–	+	+	+	+	-	+	+	+	+	-	-	+
	Jelsig et al. (2012)	-	+	+	-	+	–	+	-	+	–	–	+	-	-
	Van der Aa et al. (2010)	-	+	+	+	+	–	-	+	+	–	–	+	-	-
	Engels et al. (2007)	-	+	-	+	-	–	-	-	+	+	–	-	+	-
	Jensen et al. (2009)	+	–	-	+	+	+	+	+	+	–	+	-	+	+
	Bonaglia et al. (2010) (case 1)	+	+	-	–	–	–	–	+	+	–	–	+	+	–
	Bonaglia et al. (2010) (case 2)	–	+	-	+	–	–	–	+	+	+	–	–	+	–
	Decipher 255839	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Decipher 265764	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Decipher 249355	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fetal phenotype	Our case	-	–	–	-	-	+	NA	+	+	+	–	-	–	-

Other malformations											aCGH or MLPA result
Postnatal diagnosis cases		Microcephaly	Hearing loss	Intellectual disability or psychomotor delay	Language delay	Stenosis of the external auditory canal	Hypertrichosis	Congenital heart defect	Renal hypotrophia	Placental anomalies	Growth restriction	
	Kosaki et al. (2011)	+	+	+	+	+	-	-	–	ND	ND	arr[GRCh37] 19p13.12(15439339_16203271)x1 dn [0.76 Mb]
	Jelsig et al. (2012)	+	+	+	+	+	+	-	–	ND	ND	mlpa 19p13.12(14,382,780-15,583,)x1 dn [1.4 Mb]
	Van der Aa et al. (2010) [Decipher 255743]	+	ND	+	+	-	+	-	ND	ND	ND	mlpa[GRCh37] 19p13.12(14,382,780-15,492,848)x1 dn [1.11 Mb]

(*continued on next page*)

Table 1 (*continued*)

Other malformations											aCGH or MLPA result
Postnatal diagnosis cases		Microcephaly	Hearing loss	Intellectual disability or psychomotor delay	Language delay	Stenosis of the external auditory canal	Hypertrichosis	Congenital heart defect	Renal hypotrophia	Placental anomalies	Growth restriction	
	Engels et al. (2007)	+	+	+	+	-	-	+	ND	ND	+	arr cgh 19p13.12p13.12(RP11-959H20→CTB-55O6)x1 [2.06 Mb]
	Jensen et al. (2009)	ND	+	+	+	+	-	+	ND	ND	ND	arr cgh 19p13.12(13,930,000-16,360,000)x1 dn [2.52 Mb]
	Bonaglia et al. (2010) (case 1)	+	+	+	+	–	+	+	–	–	+	arr[GRCh37] 19p13.12(14274000_16192000)x1 [1.9 Mb]
	Bonaglia et al. (2010) (case 2)	+	+	+	+	–	–	+	–	–	+	arr[GRCh37] 19p13.12(14072000_16192000)x1 [2.1 Mb]
	Decipher 255839	+	ND	+	ND	ND	ND	ND	ND	ND	ND	chr19:15,188,235-16,624,583 [1.44 Mb] dn
	Decipher 265764	ND	+	+	ND	ND	ND	ND	ND	ND	ND	chr19:15,052,889-16,244,406 [1.19 Mb] dn
	Decipher 249355	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	chr19:14,661,584 15,655,570 [0.993 Mb]
Fetal phenotype	Our case	-	-	NA	NA	-	–	-	+	+	–	arr[GRCh37] 19p13.12(15124013_15959347)x1 dn [0.745 Mb]

+ Present clinical sign.
- Absent clinical sign.
ND Not Determined.
dn: *de novo*.
NA Not Applicable.

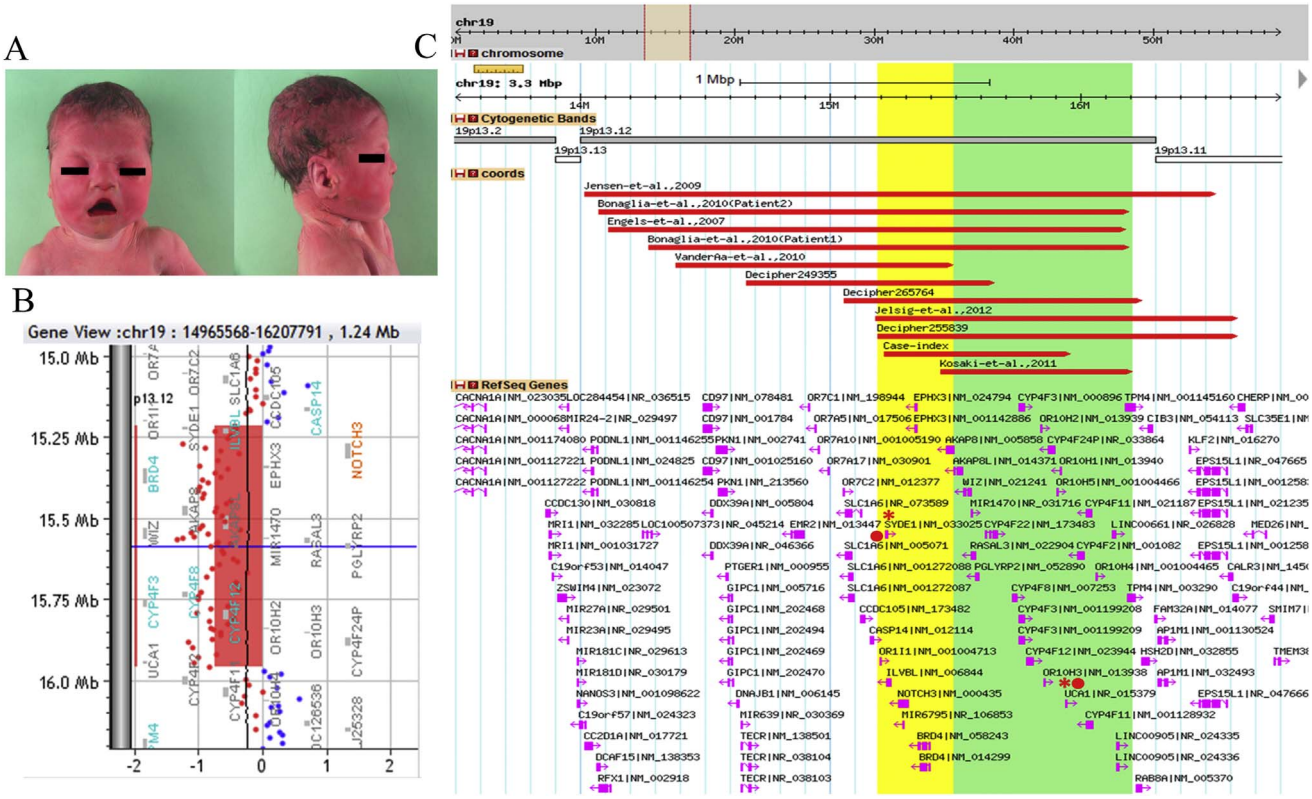

Fig. 1. A. Photographs of the fetal face and profile demonstrated mild dysmorphic features including square face with flat profile, hypertelorism, depressed nasal bridge and downturned corners of the mouth, right preauricular skin tag and malformed ear. B. Array-CGH identified a 745 kb interstitial deletion in 19p13.12 chromosomal region. C. Localization of overlapping deletions detected in the literature (Decipher Databases and all previously reported cases) and genes located within the 753 kb deletion of proximal 19p13.12 chromosome region. A 305 kb critical interval reported by Jelsig et al. (yellow highlighted) and a 700 kb critical interval documented by Kosaki et al. (green highlighted), respectively. The fetus-index carrying a 753 kb deletion defining a new critical interval for placental pathology and branchial arch defects. Quantitative PCR were performed with available primers in the intergenic regions, *SYDE1*, *UCA1* and *LINC01835* (red asterisk: deleted primers in *SYDE1*, *UCA1* and red circles: non deleted primers in intergenic regions). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

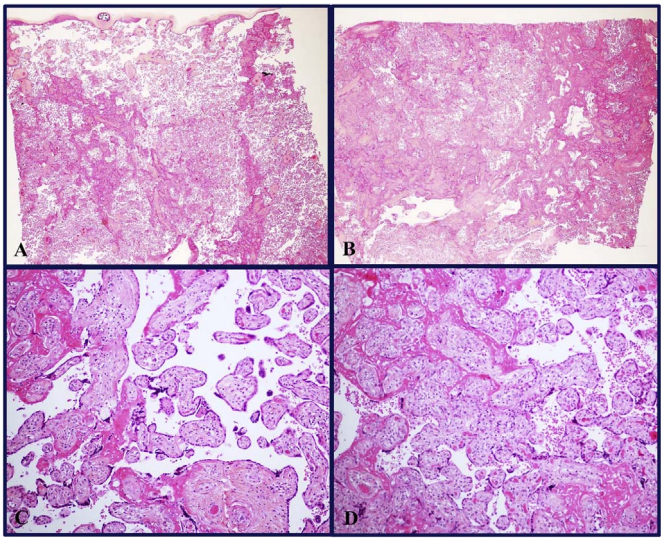

Fig. 2. Placenta histology (HES) in the fetus (39 weeks) showing: hypoplastic and ischemic tertiary villi with diffuse "patches" of massive perivillous fibrin deposition, either on para-central vertical section (A, C) or basal, horizontal section (B, D). Original magnification, X0,5 (A, B), X10 (C,D).

chromosomes were harvested according to the manufacturer's protocol. Cytogenetic analysis was performed on metaphases at 400 band resolution.

3.2. Fluorescence in situ hybridization (FISH)

FISH analysis was performed on paraffin-embedded fetal tissues (fetal lung) with two probes: RP11-79P23 (chr19:15,531,146-15,715,969) (red labeled probe) and RP11-81K14 (chr19:15,534,051-15,702,495) (red labeled probe) and a control subtelomeric 19p (green labeled probe) (genomic

coordinates GRCh37/hg19).

3.3. Array-CGH

180K array-CGH (Agilent technologies, Santa Clara, CA, USA) with 13 kb average probe spacing was carried out according to the manufacturer's protocol, chromosomal imbalances were detected considering a minimum of three consecutive probes with abnormal $\log_2$ ratio. After obtaining written consent, DNA was extracted from fetal thymus and array-CGH was performed using 4 × 180K oligo plateform (Agilent Technology, Santa Clara, CA) according to the manufacturer's protocol.

3.4. Quantitative PCR

Real-time PCR using SYBR® Green PCR Master Mix (Life Technologies, Woolston Warrington, UK) was carried out. All samples were run in triplicate. The dosage of each amplicon relative to control amplicon of *ALB* (gene ID213) and normalized to control DNA using the 2-ΔΔCT method as described (Yuan et al., 2008).

3.5. Microsatellite marker analysis

Markers for 19p13.12 microdeletion: D19S252, D19S588, D19S923 were amplified with available primers upon on request. The amplified products were separated by capillary electrophoresis using the ABI 3130xl Genetic Analyzer (Life Technologies).

3.6. Candidate gene sequencing analysis

Mutation analysis of candidate genes in placental pathology and branchial arch defects were performed. Primer sequences for amplifying the entire coding regions of *SYDE1*, *AKAP8*, *AKAP8L*, *BRD4* and *WIZ* were available upon on request. The amplified products were determined by gel electrophoresis (2% agarose). PCR products were purified and then sent to sequencing directly in an ABI 3130xl Genetic Analyzer (Life Technologies).

4. Results

Conventional cytogenetic analysis showed a normal result 46,XX. Array-CGH analysis showed a 745 kb interstitial deletion at chromosome band 19p13.12 (Fig. 1B): arr[GRCh37] 19p13.12(15124013_15959347)x1. No other CNV with clinical impact were present. FISH analysis demonstrated that the deletion occurred *de novo* in the fetus (Supplemental Fig. S1). Real-time PCR analysis showed that the deletion occurred *de novo* ranging from 15,206,761 to 15,959,347 with a median size of 753 kb and both deletion breakpoints were located in the intergenic regions, the position map of the proximal centromeric breakpoint was between 15,959,347-15,961,724 and the position map of the distal telomeric breakpoint located between 15,203,301-15,206,761. The hemizygous region contains 20 RefSeq genes (Fig. 1C). The maternal inheritance of the deletion was confirmed by microsatellite marker analysis with D19S252, D19S588 and D19S923 (Supplemental Fig. S2). Sequencing of candidate genes for placental pathology and branchial arch defects revealed no pathogenic mutations except for a rare homozygous intronic variant *BRD4*:c.285 + 44C > T (rs34855805, MAF = 1.45×10^{-5}). Another intronic variant *SYDE1*:c.1418-15A > G (rs2040858, MAF = 0.28) was informative in this family and confirmed the maternal contribution of the deletion (Supplemental Table S1).

5. Discussion

To the best of our knowledge, only five postnatal cases with overlapping deletions involving proximal 19p13.12 chromosome region have been recorded ([3,4], Decipher 249355, Decipher 265764, Decipher 255839) (Fig. 1C). Furthermore, no fetal phenotype of proximal 19p13.12 microdeletion has been reported. Overlapping interstitial deletions at 19p13.12 lead us to define a new critical candidate region for proximal 19p13.12 microdeletion syndrome, including at least 6 functionally relevant genes: *NOTCH3*, *SYDE1*, *AKAP8L*, *WIZ, BRD4* and *AKAP8*, encompassing both previously reported critical intervals. *NOTCH3* (MIM *600276) is a candidate gene for neurodevelopmental disorder, renal hypotrophy and abnormal placental development in the present fetus (Van der Aa et al., 2010). It is strongly expressed in central nervous system, renal blood vessels, glomerular tuft and placental cytotrophoblasts. *NOTCH3* encodes an evolutionarily conserved NOTCH receptor family with pleiotropic effects, protein partner of *JAG1*, a causative gene in autosomal dominant Alagille syndrome associated with the wide spectrum of renal pathology including renovascular disease, renal tubular acidosis, tubulointerstitial nephritis and renal dysplasia/hypolasia (Leimeister et al., 2003). *NOTCH3* mutations cause autosomal dominant CADASIL syndrome (cerebral ateriopathy with subcortical infarcts and leukoencephalopathy 1) characterized by migraine, ischemic strokes, white matter lesions and cognitive impairment in some patients. *NOTCH3* is also involved in the regulation of proliferation, maturation and survival of vascular smooth muscle cells and their abnormal breakdown would lead to small vessel infarct in CADASIL syndrome. Thus, we suggest that haploinsufficiency of *NOTCH3* might cause abnormal placental vasculature leading to placental ischemia in the present fetus-index. *SYDE1*, primarily expressed in placental trophoblasts, is another candidate gene for placental anomalies observed in this case. *Syde1* −/− KO mice placenta result in intra-uterine growth restriction and placentas with aberrant phenotype in the placental-yolk sac barrier, maternal-trophoblast interface and placental vascularization (Lo et al., 2017). In line with this idea, some patients with proximal 19p13.12 microdeletion display low birth weight that could be explained by placental pathology due to *SYDE1* and/or *NOTCH3* haploinsufficiency (Engels et al., 2007; Jensen et al., 2009). However, no placental phenotype was recorded in previously postnatal reported cases. Since the present fetus had a normal growth development, it is worth mentioning that other functionally redundant genes such as *SYDE1* homologue *SYDE2* and components of the renin-angiotensin system can compensate the *SYDE1* haploinsufficiency. Additionally, the present fetus had ear malformations with right preauricular skin tag and cupped ears. Of interest, one patient (case 2) carrying a 2.1 Mb microdeletion at 19p13.12 chromosome region presenting right preauricular tag associated with small and low-set ears has been reported (Bonaglia et al., 2010). Branchial arch defects are common clinical manifestations in patients with 19p13.12 microdeletion syndrome. Most patients exhibited preauricular skin tags, preauricular and branchial pits, malformed and low-set ears, stenosis of the external canal and high palate. A wide spectrum of clinical manifestations of branchial arch defects in patients with 19p13.12 microdeletion could

overlap the clinical features of common branchial arch syndromes such as Goldenhar syndrome, Treacher-Collins syndrome, Branchio-Oto-Renal syndrome and Nager Acrofacial Dysostosis syndrome. However, other specific associated diagnostic features of these syndromes could provide clues to the differential diagnosis. Of note, the fetus-index had facial dysmorphism including hypertelorism, depressed nasal bridge, macrostoma, right preauricular tag, malformed ears and a mild hypoplastic right side of the face that could resemble Goldenhar syndrome, one of the most common genetic disorder associated with the first and second branchial arch defects characterized by hemifacial hypoplasia, external and/or middle ear anomalies (microtia, preauricular tags/pits), ocular abnormalities, unilateral mandibular hypoplasia, cardiac, renal and vertebral anomalies. In addition to branchial arch defects, all reported patients with 19p13.12 microdeletion syndrome had intellectual disability/psychomotor and language delay, microcephaly and hearing loss are other frequently observed clinical features. Distinctive dysmorphic features may include high forehead, synophrys, thick eyebrows, hypertelorism, epicanthus, downslanting palpebral fissures, depressed nasal bridge, long philtrum and micrognathia (Table 1). Among 19 genes included within the 700 kb candidate interval for branchial arch defects reported by Kosaki et al., only *AKAP8L* and *WIZ* have an abundant expression in branchial arches. Furthermore, *BRD4* and *AKAP8* located within the 305 kb critical interval reported by Jelsig et al., are also involved in branchial arch development. *Brd4* heterozygous null mutation mice had a small mandible, suggesting that it fulfills an important function in the development of the first branchial arch.

In order to identify candidate genes for ear malformations and placental anomalies observed in the present fetus, sequencing of *AKAP8*, *AKAP8L*, *WIZ*, *BRD4* and *SYDE1* was performed but no pathogenic mutations were identified in these five candidate genes (Draaken et al., 2013). A rare homozygous intronic variant *BRD4*:c.285 + 44C > T (NM_058243.2) with unknown clinical significance was identified. This variant was located in the potential donor splicing site at the +38 position (motif GAGgtcgtc) predicted by Human Splicing Finder and could potentially affect the mRNA splicing. Additional functional studies might be carried out in order to clarify the potential effects of these rare variants on gene expression.

In light of other reported cases, we point out that patients with proximal 19p13.12 microdeletion have a wide clinical phenotypic spectrum, some clinical characteristic features of proximal 19p13.12 microdeletion become evident during postnatal development. Hypertrichosis-synophrys-protruding front teeth were not noted at birth and only reported during postnatal development in previous cases. Similarly, some common clinical features such as intellectual disability, developmental delay as well as hearing loss present in 19p13.12 microdeletion syndrome can only be diagnosed in the postnatal development, constituting a major challenge to prenatal diagnosis and genetic counseling of this novel contiguous gene deletion syndrome. Of note, since array-CGH has been widely used in prenatal diagnostic setting, many rare microdeletion syndromes without major ultrasound findings are early detected during the prenatal period. It is important to report all prenatal cases of these rare syndromes, especially in prenatal period in order to better define a genotype-phenotype correlation and to help counselors be able to adapt genetic counseling to parents. It is becoming increasingly apparent that clinical features of microdeletion syndromes are highly diverse and complex. Clinical expression of proximal 19p13.12 microdeletion can vary widely and be explained by haploinsufficiency of different, contiguous genes within this region. Since no pathogenic mutations were identified in any candidate genes, the phenotype is determined by haploinsufficiency of certain clinically and functionally relevant genes with major effect and influenced by a limited number of additional genetic and environmental factors with a small effect.

Taken together, the present prenatal report has defined a novel 0.75 Mb critical interval for branchial arch defects and placental pathology harboring six genes: *SYDE1*, *NOTCH3*, *AKAP8*, *AKAP8L*, *BRD4*, *WIZ* and extended the clinical spectrum with fetal phenotype of proximal 19p13.12 microdeletion syndrome including dysmorphic features, branchial arch defects, bilateral renal hypotrophy and placental anomalies. We also propose *SYDE1* as a good candidate gene for placental pathology as well as *AKAP8*, *AKAP8L*, *BRD4*, *WIZ* are potential candidates for branchial arch defects in proximal 19p13.12 microdeletion syndrome. Moreover, our study also highlights the important role of array-CGH in detection of unbalanced chromosomal abnormalities causing genomic disorders. Overlap of these clinically non-recurrent CNVs of different sizes would lead to the identification of common critical region in potentially new genetic syndromes.

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None.

Disclosures

Non declared.

Ethics statement

This work is not a clinical research and considered as routine clinical care.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmg.2018.01.009.

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