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Research Letter

Prenatal diagnosis and molecular cytogenetic characterization of a proximal deletion of 22q (22q11.2→q11.21)

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A gravid 2, para 1, woman 34 years of age underwent amniocentesis at 18 weeks of gestation because of advanced maternal age. She had a healthy daughter 3 years of age, and there was no family history of congenital malformations. Amniocentesis revealed a karyotype of 45,XX,der(13)t(13;22)(q10;q11.2),-22 (Fig. 1). The father had a karyotype of 46,XY, the mother had a karyotype of 46,XX,t(13;22)(q10;q11.2) (Fig. 2), and the daughter had a karyotype of 46,XX. Prenatal ultrasonographic findings of the internal organs, including the heart, thymus, brain, and kidneys, were unremarkable. Oligonucleotide-based array comparative genomic hybridization (aCGH) analysis of cultured amniocytes using CytoChip Oligo array (BlueGnome, Cambridge, UK) revealed a 1.59-Mb deletion at chromosome 22q11.1-22q11.21 or arr 22q11.1q11.21 (17,397,528–18,984,490 bp) × 1 (National Center for Biotechnology Information (NCBI), Build 37; Fig. 3). The deleted region of proximal 22q was proximal to the DiGeorge syndrome (DGS) critical region. Fluorescence *in situ* hybridization (FISH) analysis of cultured amniocytes using Vysis DiGeorge region probe [Vysis, LSI TUPLE 1 (red spectrum)/LSI ARSA (green spectrum)] (Abbott Laboratories, Abbott Park, IL, USA) showed the presence of two red and two green signals, indicating no deletion of the DGS TUPLE 1 locus at 22q11.2 (Fig. 4). The woman underwent cord blood sampling at 24 weeks of gestation. The cord blood was analyzed by multiplex ligation-dependent probe amplification (MLPA) using the SALSA MLPA P250 DiGeorge probemix (MRC-Holland bv. Amsterdam, The Netherlands) to detect cat eye syndrome (22q11; CES), DiGeorge syndrome (22q11.2, LCR22-A∼G), velocardiofacial syndrome (22q11; VCFS) and the deletions of DiGeorge anomaly related regions such as 4q35, 8p23, 9q34.3, 10p14(DGS2), and 17p13. The SALSA MLPA P250 DiGeorge probemix showed haploinsufficiency of genes *IL17RA*, *SLC25A18*, *BID*, *MICAL3,* and *USP18* in the CES region but no deletion in the DGS critical region of *TBX1* or any other DiGeorge anomaly–related regions (Fig. 5). The parents decided to continue the pregnancy. At 39 weeks of gestation, a healthy 3,474-g female baby was delivered with no phenotypic abnormalities. At 3 months of age, the neonate was normal in growth and psychomotor development although long-term follow-up visits are required.

MLPA is a useful tool of rapid aneuploidy diagnosis without the need of cell culture. We previously reported the applications of the SALSA MLPA P095 aneuploidy kit for rapid aneuploidy diagnosis of chromosomes X, Y, 13, 18, and 21 in pregnancies with fetal congenital anomalies [1,2]. Here, we demonstrate the usefulness of the SALSA MLPA P250 DiGeorge probemix as well as FISH and aCGH for the diagnosis of proximal 22q deletion without involvement of the DGS critical region in a case with an unbalanced t(13;22) translocation involving chromosome 22q11.2. MLPA has

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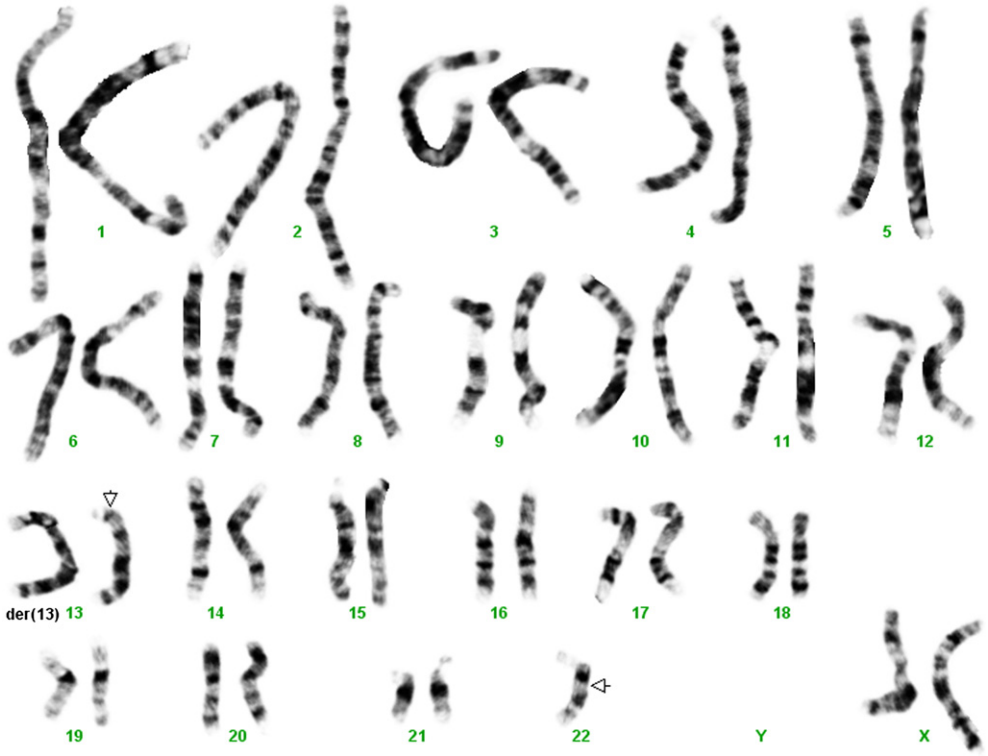

Fig. 1. A karyotype of 45,XX,der(13)t(13;22)(q10;q11.2) in the fetus.

proven to be a rapid, reliable, and sensitive tool for detecting copy number changes in the 22q11.2 region, including DGS, VCFS, conotruncal anomaly face syndrome, and CES [3–5]. The present case had a 1.59-Mb deletion at chromosome 22q11.1-22q11.21 encompassing the CES critical region genes of *CECR1* (OMIM 607575) (17,660,191–17,690,778 bp) and *CECR2* (OMIM 607576) (17,956,629–18,033,844 bp) but without involvement of the DGS critical region gene of *TBX1* (OMIM 602054) (19,744,225–19,771,115 bp) at 22q11.21.

CES (OMIM 115470) is characterized by coloboma of iris, anal atresia with fistula, preauricular tags and/or pits, downslanting palpebral fissures, cardiac and renal malformations, and normal of near- normal mental development. CES is frequently associated with partial tetrasomy 22q or a supernumerary chromosome of inv dup(22)(q11). Deletions of the juxtacentromeric region or chromosome 22 have been reported to be associated with phenotypic variability [6], ranging from phenotypically normal [7,8] to mild and relatively nonspecific

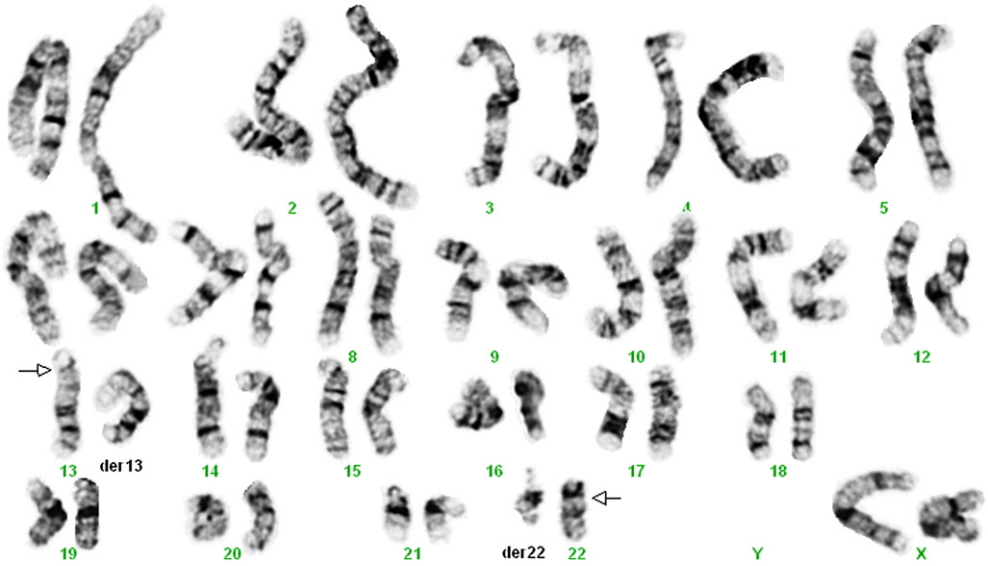

Fig. 2. A karyotype of 46,XX,t(13;22)(q10;q11.2) in the mother.

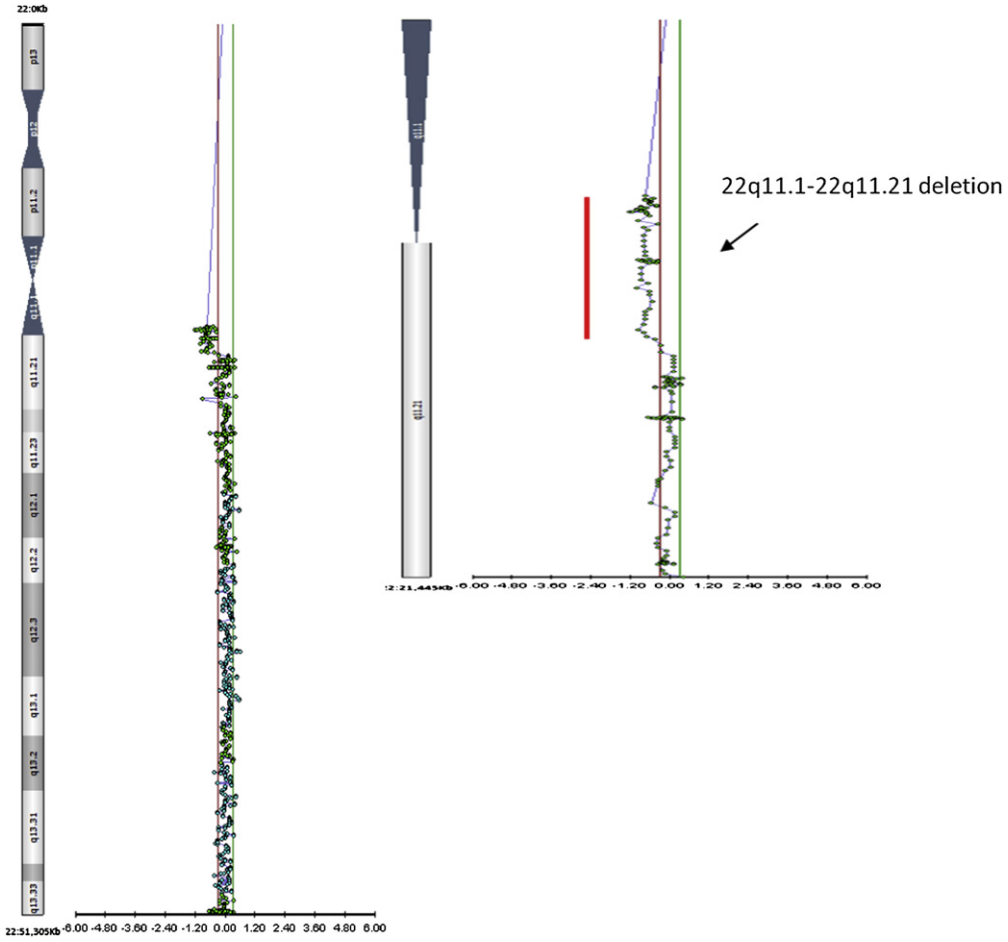

Fig. 3. Oligonucleotide-based array comparative genomic hybridization analysis of cultured amniocytes using CytoChip Oligo array (BlueGnome, Cambridge, UK) revealed a 1.59-Mb deletion at chromosome 22q11.1-22q11.21 or arr 22q11.1q11.21 (17,397,528–18,984,490 bp) ×1 (NCBI, Build 37).

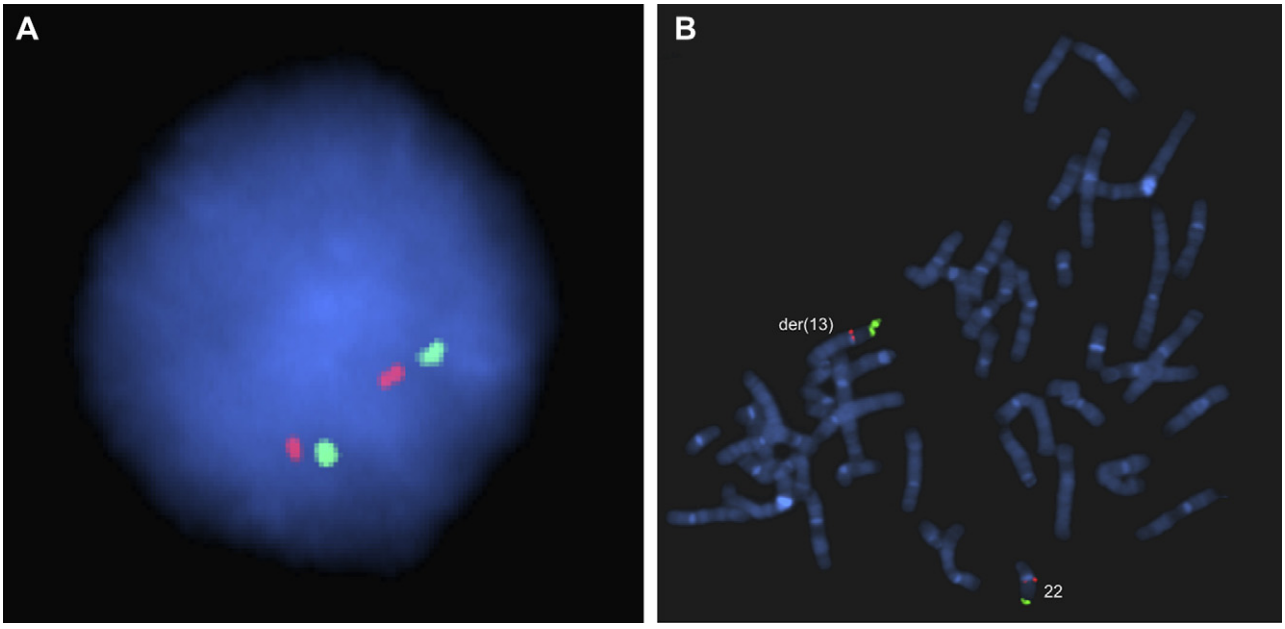

Fig. 4. (A) Fluorescence *in situ* hybridization analysis using the Vysis DiGeorge region probe [Vysis, LSI TUPLE 1 (red spectrum)/LSI ARSA (green spectrum)] (Abbott Laboratories, Abbott Park, IL, USA) showed the presence of two red signals and two green signals in interphase cultured amniocytes; and (B) metaphase cultured amniocytes, indicating no deletion of the DiGeorge syndrome TUPLE 1 locus at 22q11.2 in the fetus. der = derivative chromosome.

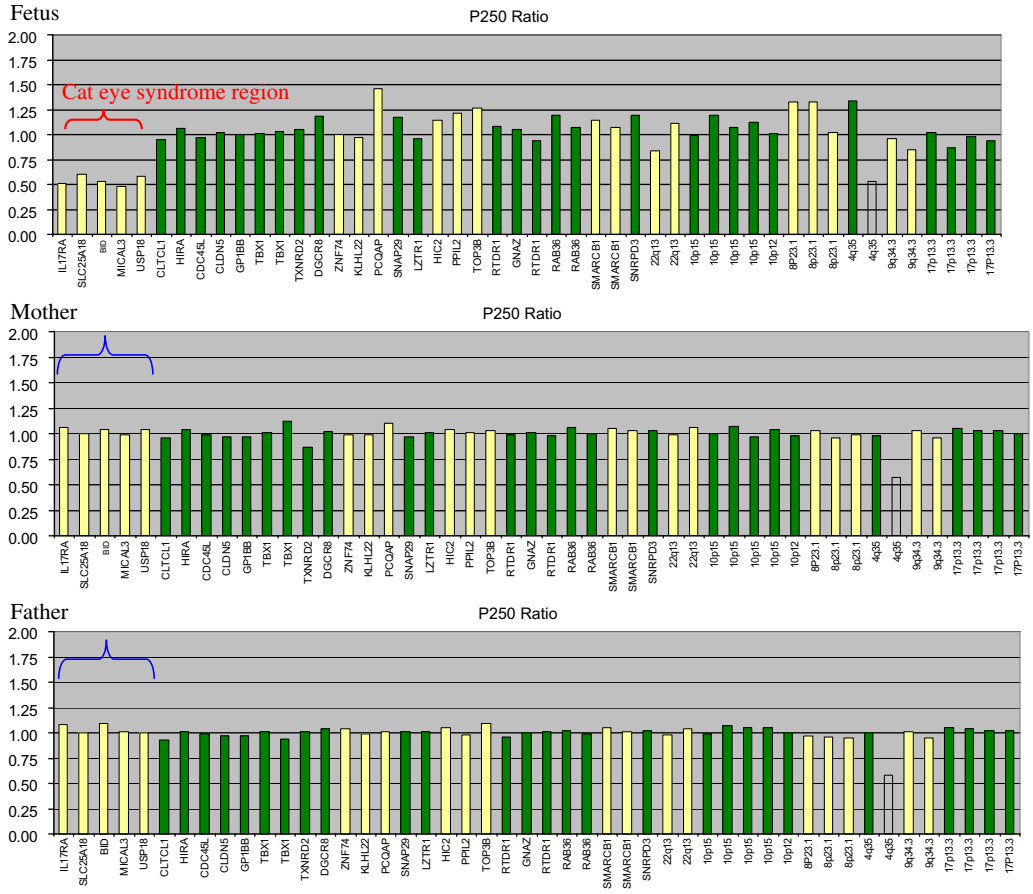

Fig. 5. The SALSA MLPA P250 DiGeorge probemix (MRC-Holland bv. Amsterdam, The Netherlands) analysis showed a hemizygous deletion in the cat eye syndrome region but no deletion in the DiGeorge syndrome critical region of *TBX1* and other DiGeorge anomaly related regions in fetal blood.

abnormalities and moderate mental retardation [9–11]. All the reported cases with familial or *de novo* unbalanced translocation of the long arm of chromosome 22 with the loss of the chromosome 22 centromere did not have molecular characterization of the involvement of DGS critical region; therefore, the observed genotype–phenotype correlation is not conclusive.

The chromosome 22q11.2 deletion syndrome, including DGS (OMIM 188400), VCFS (OMIM 192430), and conotruncal anomaly face syndrome (OMIM 217095), can be caused by a 1.5～3.0-Mb hemizygous deletion of chromosome 22q11.2 and is associated with a highly variable phenotype. DGS comprises hypocalcemia, parathyroid hypoplasia, thymic hypoplasia, and outflow tract defects of the heart. VCFS comprises velopharyngeal insufficiency with cleft palate, cardiac anomalies, typical faces, learning disabilities, microcephaly, mental retardation, short stature, auricular anomalies, and inguinal hernia. Conotruncal anomaly face syndrome comprises tetralogy of Fallot, truncus arteriosus, double-outlet right ventricle, interrupted aortic arch, and facial dysmorphisms of ocular hypertelorism, short palpebral fissures, bloated eyelids, a low nasal bridge, a small mouth, and minor ear lobe anomalies. Haploinsufficiency and point mutations of the *TBX1* gene are responsible for most of the physical malformations associated with the chromosome 22q11.2 deletion syndrome. TBX1 is required for inner ear morphogenesis and is expressed in otocyst development in the otic epithelium and in the periotic mesenchyme [12].

The probes of the SALSA MLPA P250 DiGeorge probemix were arranged according to specific chromosomal locations of 22q11, including the CES region, DGS region, and low-copy repeats chromosome 22 (LCR22)-A～G, 22q13, 10p14(DGS2), 8p23, 4q34-qter, 9q34.3, and 17p13.3. Features of DiGeorge anomaly have been observed in patients with chromosome deletions outside chromosome 22, e.g., chromosome 10p14 [13–15] and DGS/VCFS complex 2 (DGS2) (OMIM 601326), 8p23 [16], 4q34-qter [17], 9q34.3 [18], Kleefstra syndrome (OMIM 610253), and 17p13.3 [19].

In conclusion, prenatal diagnosis of proximal 22q deletion involving the 22q11.2 region should raise suspicion of DGS/VCFS. We suggest that aCGH, FISH, and MLPA are useful tools for rapid exclusion of DGS/VCFS under such circumstances.

Acknowledgments

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