Directly transmitted 4.5-Mb triplication of 4q12-q13.1: Prenatal diagnosis and molecular cytogenetic characterization

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A 35-year-old primigravida woman underwent amniocentesis at 17 weeks of gestation because of advanced maternal age. Her husband was 37 years of age and healthy. There was no family history of attention deficit hyperactivity disorder (ADHD) and congenital anomalies. The twin pregnancy was conceived by in vitro fertilization and embryo transfer. Amniocentesis revealed a normal karyotype in one female co-twin and an aberrant chromosome 4 in the other male co-twin. The aberrant chromosome 4 contained cytogenetically detectable extra materials at 4q12-q13.1 (Fig. 1).

Level II ultrasound findings were unremarkable. Cytogenetic analysis of the parental bloods showed that the father carried the same aberrant chromosome 4. The unbalanced chromosome abnormality was directly transmitted to one of the fetuses. The maternal karyotype was normal. The chromosome aberration was characterized by array comparative genomic hybridization (aCGH) and fluorescence in situ hybridization (FISH). aCGH on the DNA extracted from the cultured amniocytes and paternal blood using NimbleGen ISCA Plus Cytogenetic Array (Roche NimbleGen, Madison, WI, USA) detected a 4.5-Mb triplication at 4q12-q13.1, or arr [hg 19] 4q12q13.1 (58,193,264–62,740,404) × 4 encompassing eight genes of P19, RPS12P9, and LPHN3, and none belong to OMIM genes (Fig. 2).

FISH analysis was applied on cultured amniocytes using a 4q13.1-specific bacterial artificial chromosome (BAC) clone probe RP11-478F6 (60,017,056–60,175,902, spectrum red) [hg 19] and a 4p16.3-specific BAC clone probe RP11-69L7 (752,790–506,783, spectrum green) [hg 19] as internal control. Metaphase FISH on cultured amniocytes showed three red signals and one green signal on the aberrant chromosome 4 and one red signal and one green signal on the normal chromosome 4 (Fig. 3). Interphase FISH on cultured amniocytes showed four red signals and two green signals on each cell, indicating triple segmental amplifications of 4q13.1 (Fig. 4). The karyotype of the father was 46,XY, trp(4)(q12q13.1). The karyotype of the male fetus was 46,XY,trp(4)(q12q13.1).pat. The parents decided to continue the pregnancy.

We previously reported a directly transmitted benign 4.4-Mb 4q12-q13.1 quadruplication or arr [hg 18] 4q12q13.1 (57,966,988–62,377,421) × 5 in a father and a son with no phenotypic abnormalities [1]. In this report, we additionally present a directly transmitted benign 4.5-Mb 4q12-q13.1 triplication or arr [hg 19] 4q12q13.1 (58,193,264–62,740,404) × 4 in a father and a son in another family. The two fathers from different families with no known kinship were born at the same district in northeast Taiwan, indicating a possible remote ancestral inheritance and transmission of duplication, triplication, or quadruplication of a 4.5-Mb region at 4q12-q13.1 in this geographic area.

Prenatal diagnosis of directly transmitted benign unbalanced chromosome abnormalities (UBCAs) should raise concerns, because phenotypic variation may exist in the family members with phenotypic abnormalities in the children, but a normal phenotype in the carrier parents [1–6]. In 130 families with directly transmitted UBCAs, Barber [1] found that 18% (23/130) had no phenotypic effect (Group 1), 23% (30/130) had affected probands and phenotypically normal family members (Group 2), and 59% (77/130) had consistent mild phenotypic
abnormalities (Group 3). Among the directly transmitted UBCAs involving chromosome 4, Barber [1] categorized del(4)(p15.2p16.1), del(4)(q33qter), del(4)(q33q35.1), del(4)(q32q33), dup(4)(q31.2q31.3), and dup(4)(q31.3q33) into Group 3, in which the phenotypically affected parents had the same UBCA as their affected children, and dup(4)(q31.3q33) into Group 2, in which the phenotypically unaffected parents had the same UBCA as their affected children. Rodríguez et al [5] reported a 3.3-Mb interstitial duplication

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\text{trp} = \text{triplication.}
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**Fig. 1.** Partial karyotype shows a normal chromosome 4 and an aberrant chromosome 4 of trp(4)(q12q13.1). trp = triplication.

**Fig. 2.** (A) and (B) array comparative genomic hybridization analysis of cultured amniocytes and paternal blood shows a 4.5-Mb triplication of 4q12-q13.1, or arr [hg 19] 4q12q13.1 (58,193,264-62,740,404) × 4.

**Fig. 3.** Metaphase fluorescence in situ hybridization (FISH) analysis of cultured amniocytes using the bacterial artificial chromosome (BAC) clone probes RP11-478F6 (4q13.1, spectrum red) and RP11-69L7 (4p16.3, spectrum green) shows triple segmental amplifications of the red signals (arrows) in the aberrant chromosome 4 of trp(4)(q12q13.1). trp = triplication.
of 4p16.1 in a phenotypically normal father and a healthy 5-year-old daughter. Wang et al [7] reported a 4.3-Mb triplication of 4q32.1-q32.2 in a family through two generations with phenotypic abnormalities in the affected parent and the affected children. Rodríguez et al [6] reported molecular cytogenetic characterization of a more complex chromosome abnormality responsible for phenotypic abnormalities in a child with directly transmitted benign UBCA and suggested that aCGH should be used to correctly categorize cryptic chromosome alternations. The present case demonstrates the usefulness of application of FISH and aCGH for molecular cytogenetic characterization of directly transmitted UBCAs and provides evidence for the benign nature of 4.5-Mb gene dosage increase at 4q12-q13.1 involving only RPS26P24, SRIP1, LOC100507160, LOC100421808, MIR548AG1, RPL17P19, RPS12P9, and LPHN3.

Acknowledgments

This work was supported by research grants NSC-99-2628-B-195-001-MY3 and NSC-101-2314-B-195-011-MY3 from the National Science Council and MMH-E-I02-04 from Mackay Memorial Hospital, Taipei, Taiwan.

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