Terminal 2q Deletion and Distal 15q Duplication: Prenatal Diagnosis by Array Comparative Genomic Hybridization Using Uncultured Amniocytes

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A 35-year-old, gravida 3, para 2, woman was referred to the hospital at 18 weeks of gestation for amniocentesis because of advanced maternal age and relatives with balanced and unbalanced chromosomal translocations. Her husband and her elder daughter had a balanced translocation of t(2;15)(q37.3;q24.3). Her younger daughter suffered from mental retardation and had an unbalanced translocation of der(2)t(2;15)(q37.3;q24.3)pat. The woman's karyotype was normal. Prenatal ultrasound during the current pregnancy revealed no structural abnormalities. Genetic amniocentesis was performed at 19 weeks of gestation, and 30 mL of amniotic fluid was aspirated, of which 10 mL was used for array comparative genomic hybridization (aCGH) using uncultured amniocytes and 20 mL was used for conventional cytogenetic analysis using cultured amniocytes. Within 3 days, bacterial artificial chromosome (BAC)-based aCGH demonstrated partial monosomy 2q and partial trisomy 15q [arr cgh 2q37.3q37.3 (RP11-299F2 → RP11-875C22) × 1, 15q25.1q26.3 (RP11-10K12 → RP11-530H6) × 3] (Figure 1). Conventional cytogenetic analysis revealed

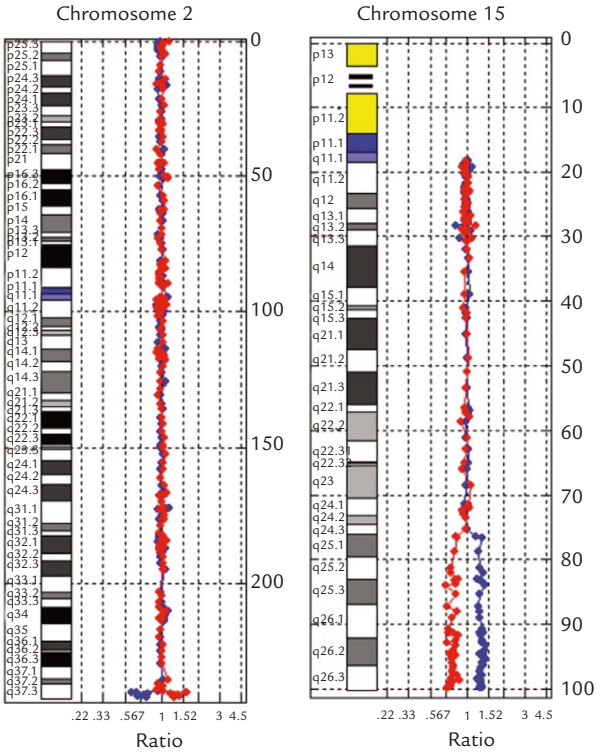

Figure 1. Bacterial artificial chromosome-based array comparative genomic hybridization using CMDX bacterial artificial chromosome array comparative genomic hybridization CA2500 chips (CMDX, Irvine, CA, USA) showed a deletion of terminal 2q [arr cgh 2q37.3q37.3 (RP11-299F2 → RP11-875C22) × 1] and a duplication of distal 15q [arr cgh 15q25.1q26.3 (RP11-10K12 → RP11-530H6) × 3].

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the karyotype 46,XX,der(2)t(2;15)(q37.3;q24.3)pat (Figure 2). Oligonucleotide-based aCGH further demonstrated partial monosomy 2q (2q37.3 → qter) and partial trisomy 15q (15q24.3 → qter) [arr cgh 2q37.3qter (240116312-242951149 bp) × 1, 15q24.3qter (75475172-96575460 bp) × 3] (Figure 3). The parents opted to terminate the pregnancy, and a 392-g fetus was delivered with facial dysmorphism, clenched hands, and malposition of the toes (Figures 4 and 5).

Comparative genomic hybridization (CGH) and BAC-based and oligonucleotide-based aCGH using cultured or uncultured amniocytes have been successfully applied for the prenatal diagnosis of chromosome abnormalities [1–11]. CGH and aCGH have the advantage of providing a rapid genome-wide study without the need for cell culture but the disadvantage of an inability to detect low-level mosaicism, balanced translocations, inversions, and polyploidy. Genome-wide association studies also provide insight into our understanding of diseases, as well as rapid diagnosis of uniparental disomy [12,13]. CGH and aCGH have been shown to be valuable alternatives to interphase fluorescence *in situ* hybridization for the rapid prenatal detection of unbalanced chromosomal abnormalities in uncultured amniocytes. Lapierre et al [1] first reported the clinical analysis of uncultured amniocytes by CGH in 71 amniotic fluid samples, of which 66 (93%) had informative results, five (7%) had uninformative results; seven trisomies (trisomy 13 [$n=1$], trisomy 18 [$n=1$], and trisomy 21 [$n=5$]) were detected and three inversions [inv(11)(p13q21), inv(9)(p11q13), and inv(Y) (p11.1q11.22)] were not detected. In a study of 30 uncultured prenatal samples from chorionic villi or 1–2 mL of uncultured amniotic fluid using aCGH, Rickman et al [3] found that 29 of 30 samples were correctly diagnosed, with the exception of one sample with triploidy. They also found that aCGH was able to detect aneuploidy in DNA isolated from as little as 1 mL of uncultured amniotic fluid. In a study of 98 pregnancies (56 amniotic fluid and 42 chorionic villus sampling specimens), Sahoo et al [4] demonstrated complete concordance between conventional karyotyping and BAC–aCGH results, including five positive cases with chromosomal abnormalities. BAC–aCGH results were available within an average of 6 days for uncultured cells and 16 days for cultured cells. In a study of 15 amniotic fluid samples from pregnancies of 15–22 weeks of gestation, Bi et al [7] extracted DNA from 5–10 mL of amniotic fluid for whole genome amplification using oligonucleotide-based aCGH and found that the results of aCGH were consistent with those of chromosome analysis. The authors obtained high-quality aCGH results using oligonucleotide-based aCGH in 86.7% (13/15) of the samples. They found that a shorter amniotic fluid storage time and a gestational age greater than 17 weeks yielded sufficient (> 300 ng) DNA from the uncultured amniocytes.

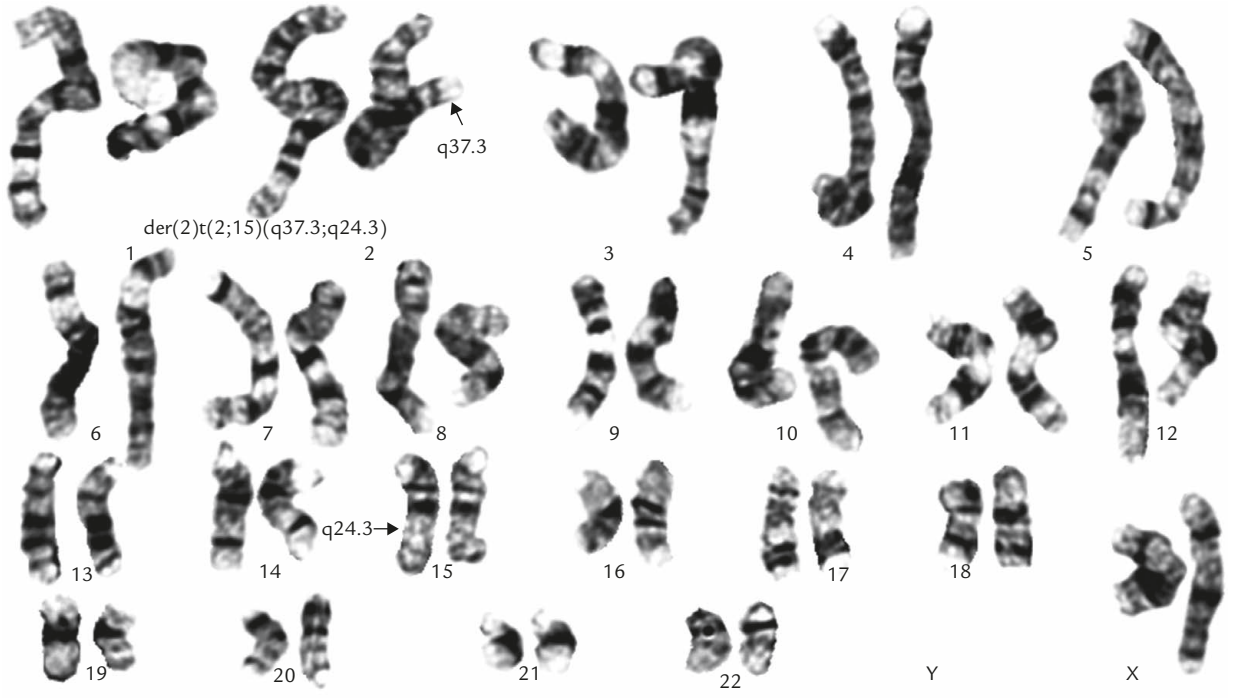

Figure 2. Karyotype 46,XX,der(2)t(2;15)(q37.3;q24.3) in the proband. The arrows indicate the breakpoints.

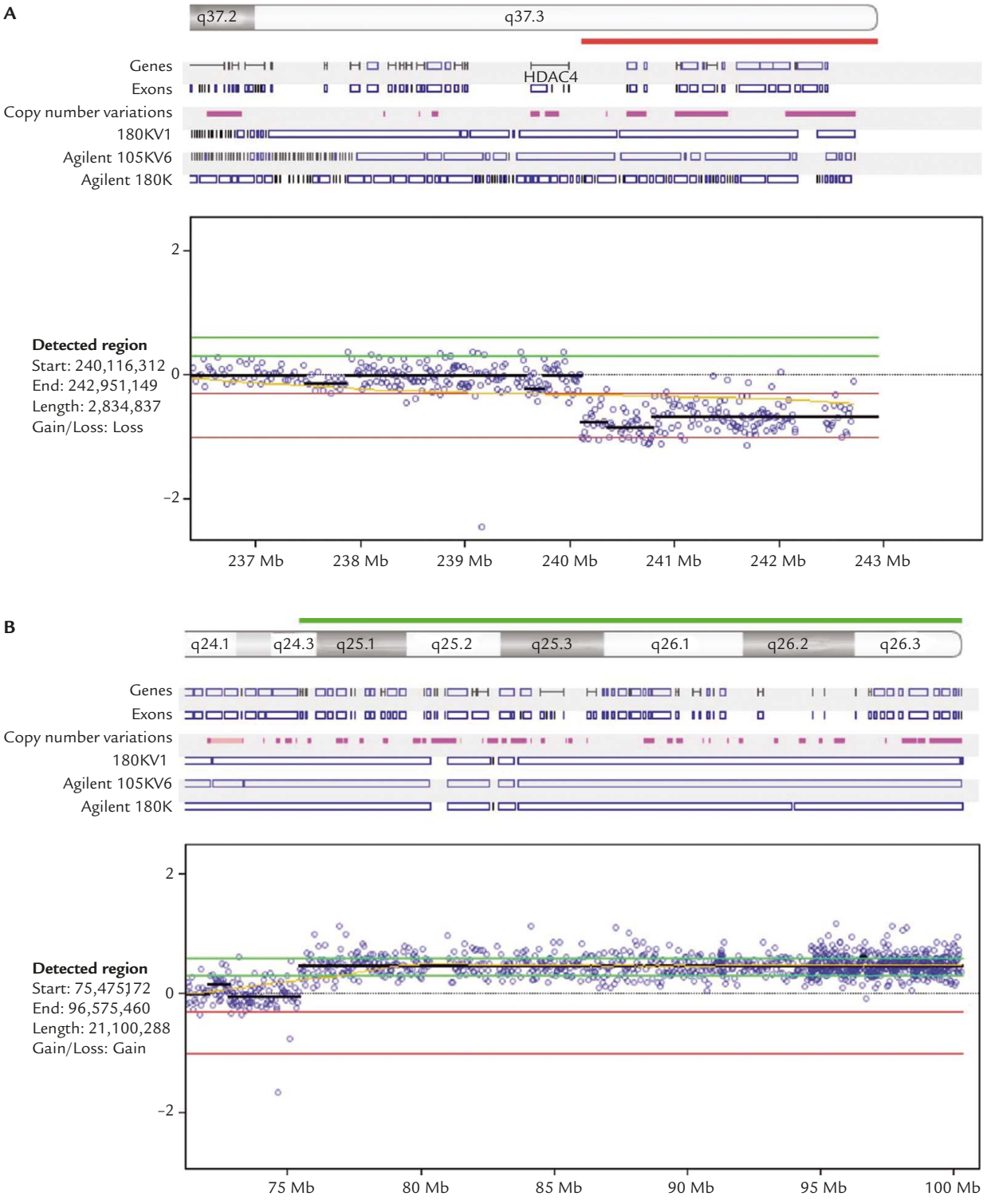

Figure 3. Oligonucleotide-based array comparative genomic hybridization using Oligo HD Scan (CMDX, Irvine, CA, USA) shows (A) a 2.8-Mb deletion in 2q37.3 → qter [arr cgh 2q37.3qter (240116312-242951149 bp)×1], and (B) a 21-Mb duplication of 15q24.3 → qter [arr cgh 15q24.3qter (75475172-96575460 bp)×3].

Circulating cell-free fetal DNA extracted from amniotic fluid supernatant has recently been investigated as a promising source of fetal DNA for prenatal diagnosis of chromosomal abnormalities [14–16]. Bianchi et al [14] demonstrated the presence of large quantities of cell-free fetal DNA in stored amniotic fluid supernatant samples, with 100 to 200 times more fetal DNA per milliliter of amniotic fluid supernatant compared with

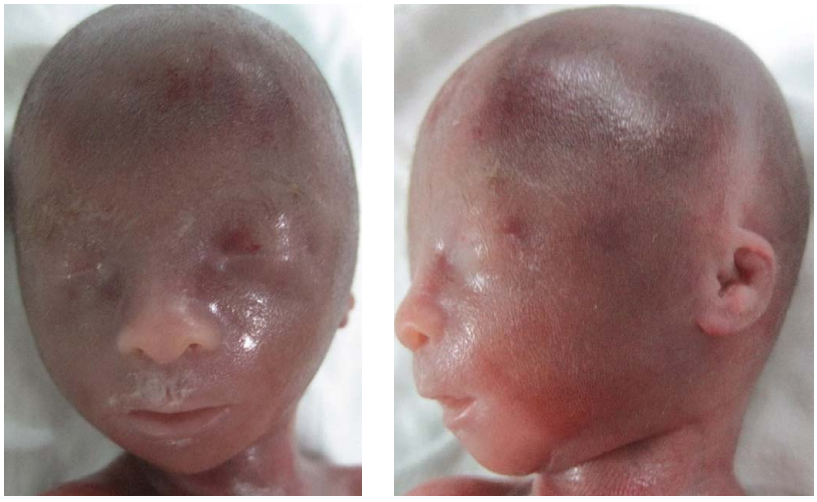

Figure 4. Anterior and lateral views of the fetus with a long thin face, dolichocephaly, hypertelorism, a prominent nasal bridge, a pointed chin, and upturned nares.

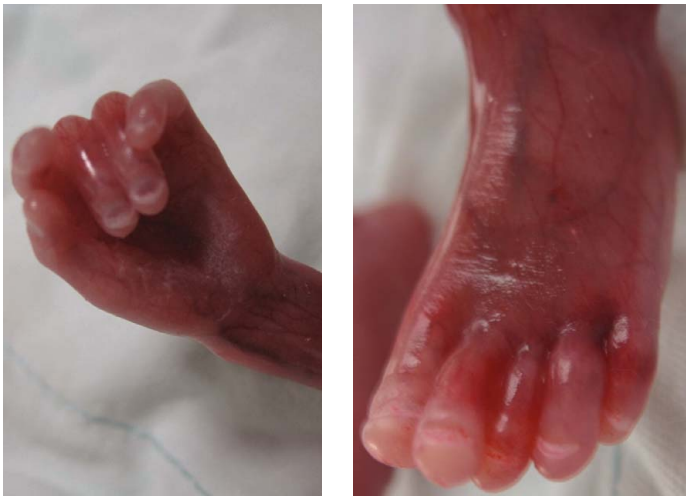

Figure 5. Long digits of the hand and foot.

maternal plasma. Larrabee et al [15] demonstrated that CGH microarray analysis using cell-free fetal DNA in amniotic fluid supernatant could correctly identify fetal sex and aneuploidies such as trisomy 21 ($n=3$) and 45,X ($n=1$). In a study of molecular karyotyping using BAC–aCGH and cell-free fetal DNA in 10 mL of amniotic fluid supernatant from nine test amniotic fluid samples, including trisomy 13 ($n=1$), trisomy 18 ($n=3$), trisomy 21 ($n=2$), trisomy 9 mosaicism (90%) ($n=1$), 69,*XXY* ($n=1$) and 45,X ($n=1$), Lapaire et al [16] found concordant results in eight of the nine cases, with the exception of one case with triploidy, and suggested that cell-free fetal DNA from amniotic fluid supernatant could be analyzed using aCGH to correctly identify human chromosome abnormalities.

In conclusion, we have presented the results of prenatal diagnosis of an unbalanced translocation by aCGH using uncultured amniocytes. This case demonstrates that aCGH can be used for the prenatal diagnosis of chromosomal abnormalities via a standard reproducible procedure that can produce reliable results in a short period of time.

Acknowledgments

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References

1. Lapierre JM, Cacheux V, Luton D, Collot N, Oury JF, Aurias A, Tachdjian G. Analysis of uncultured amniocytes

by comparative genomic hybridization: a prospective prenatal study. *Prenat Diagn* 2000;20:123–31.
2. Rickman L, Fiegler H, Carter NP, Bobrow M. Prenatal diagnosis by array-CGH. *Eur J Med Genet* 2005;48:232–40.
3. Rickman L, Fiegler H, Shaw-Smith C, et al. Prenatal detection of unbalanced chromosomal rearrangements by array CGH. *J Med Genet* 2006;43:353–61.
4. Sahoo T, Cheung SW, Ward P, et al. Prenatal diagnosis of chromosomal abnormalities using array-based comparative genomic hybridization. *Genet Med* 2006;8:719–27.
5. Van den Veyver IB, Beaudet AL. Comparative genomic hybridization and prenatal diagnosis. *Curr Opin Obstet Gynecol* 2006;18:185–91.
6. Shaffer LG, Bui TH. Molecular cytogenetic and rapid aneuploidy detection methods in prenatal diagnosis. *Am J Med Genet C Semin Med Genet* 2007;145C:87–98.
7. Bi W, Breman AM, Venable SF, et al. Rapid prenatal diagnosis using uncultured amniocytes and oligonucleotide array CGH. *Prenat Diagn* 2008;28:943–9.
8. Cain CC, Saul DO, Oehler E, Blakemore K, Stetten G. Prenatal detection of a subtle unbalanced chromosome rearrangement by karyotyping, FISH and array comparative genomic hybridization. *Fetal Diagn Ther* 2008;24:286–90.
9. Darilek S, Ward P, Pursley A, et al. Pre- and postnatal genetic testing by array-comparative genomic hybridization: genetic counseling perspectives. *Genet Med* 2008;10:13–8.
10. Kashork CD, Theisen A, Shaffer LG. Prenatal diagnosis using array CGH. *Methods Mol Biol* 2008;444:59–69.
11. Vialard F, Molina Gomes D, Leroy B, et al. Array comparative genomic hybridization in prenatal diagnosis: another experience. *Fetal Diagn Ther* 2009;25:277–84.
12. Soong YK, Wang TH, Lee YS, et al. Genome-wide detection of uniparental disomy in a fetus with intrauterine growth restriction using genotyping microarrays. *Taiwan J Obstet Gynecol* 2009;48:152–8.
13. Wang TH, Wang HS. A genome-wide association study primer for clinicians. *Taiwan J Obstet Gynecol* 2009;48:89–95.
14. Bianchi DW, LeShane ES, Cowan JM. Large amounts of cell-free fetal DNA are present in amniotic fluid. *Clin Chem* 2001; 47:1867–9.
15. Larrabee PB, Johnson KL, Pestova E, et al. Microarray analysis of cell-free fetal DNA in amniotic fluid: a prenatal molecular karyotype. *Am J Hum Genet* 2004;75:485–91.
16. Lapaire O, Lu XY, Johnson KL, et al. Array-CGH analysis of cell-free fetal DNA in 10 mL of amniotic fluid supernatant. *Prenat Diagn* 2007;27:616–21.