

CASE REPORT

Prenatal diagnosis of complex rearrangement of chromosome 21: The significance of interphase and metaphase fluorescence in situ hybridization and comparative genomic hybridization

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Introduction

Down syndrome is usually caused by the presence of an extra chromosome 21. Approximately 90% of Down syndrome cases are resulted from maternal meiotic error [1]. The duplication of the Down syndrome critical region (DSCR), localized in 21q22, has been postulated to be both necessary and sufficient to produce the phenotype of Down syndrome [2].

The second trimester maternal serum quadruple screening (MSS), which combines maternal age-associated risk of Down syndrome with the likelihood ratio calculated from the levels of four biochemical markers, is an appropriate screening test for Down syndrome. Although this test has been reported to be also helpful in identifying pregnancies with other chromosomal abnormalities, it is designed to detect neither structural chromosome abnormalities nor other genetic abnormalities [3, 4].

Interphase fluorescence in situ hybridization (I-FISH) on uncultured amniotic fluid cells is used for the rapid detection of the common aneuploidies involving 13, 18,

Key Clinical Message

Maternal serum screening–positive patient had prenatal diagnosis with amniotic fluid, which showed inconsistent results between interphase fluorescence in situ hybridization (three signals of 21q22.13–21q22.2) and G-banding analysis (46, XY). Further analyses proved that the fetus had extremely complex rearrangements of chromosome 21, including the interstitial duplication of Down syndrome critical region.

Keywords

aCGH, chromosome 21, Down syndrome, DSCR, interphase FISH, metaphase FISH.

21, X, and Y. The American Society of Human Genetics and The American College of Medical Genetics and Genomics announced that ~65–70% of cytogenetic abnormalities would be detected prenatally by this I-FISH. Its detection rate approached 80% for women aged 35 years or older [5]. However, this rapid testing cannot detect the cases with structural aberrations, mosaicism, or marker chromosomes [5].

Metaphase FISH (M-FISH) studies make the detection of submicroscopic chromosomal imbalances plausible. In M-FISH, labeled DNA probes are hybridized to metaphase chromosomes to detect the presence, number, and location of specific submicroscopic regions of chromosomes. On the other hand, microarray-based comparative genomic hybridization (aCGH) or single-nucleotide polymorphism array enable the genome-wide detection of chromosomal imbalances [6, 7].

Here, we describe a prenatal case of a complex rearrangement of chromosome 21, initially suspected by MSS, thereafter confirmed by I-FISH, M-FISH, conventional G-banded karyotyping, and aCGH.

Case History

A Japanese patient (36-year-old, *gravida 2, para 1*) was referred to our hospital for genetic counseling due to the positive MSS with an increased risk (1 in 4) for Down syndrome. After appropriate genetic counseling, the patient decided to have amniocentesis at 19 weeks of gestation. I-FISH with a set of DNA probes specific for 13q14, 21q22.13-21q22.2, and centromere regions of chromosome 18, X, and Y, all provided by AneuVysion[®] Assay Kit (Abbott Molecular Inc./Vysis[®], Downers Grove, IL), was performed. FISH analysis of 100 interphase nuclei using probes which are specific for 21q22.13-21q22.2 region including DSCR showed three hybridization signals, which is consistent with Down syndrome. On the other hand, standard G-banding chromosomal analysis of metaphase cells from 21 colonies from four separate cultures showed a 46,XY karyotype.

In order to resolve the inconsistent results, further examinations were performed. First, amniotic fluid cells in the original sample were reexamined by I-FISH, and again the analysis showed trisomy 21 pattern. In the second, M-FISH analysis was performed on the cultured amniotic fluid cells, which also showed the trisomy 21 cell pattern. Therefore, we concluded that this FISH analysis was not a specimen error.

Considering these results, the patient opted to terminate the pregnancy. Autopsy of the fetus identified no major structural malformations except the nuchal edema. I-FISH study on the fetal blood sample also showed three signals of the probe for 21q22.13-21q22.2.

In addition, further analysis examined the possibility of complex rearrangement. M-FISH was conducted on the cultured amniotic cells, and aCGH was conducted on the

umbilical cord. M-FISH analysis showed two hybridization signals of 21q22.13-21q22.2 within one chromosome 21 homologue, which was consistent with an interstitial duplication of DSCR within the long arm of one chromosome 21. The analysis of aCGH of the umbilical cord specimen, using a 135K feature whole-genome microarray (SignatureChip[®] Oligo Solution[™] version 2.0, custom designed by Signature Genomic Laboratories, LLC, made by Roche NimbleGen[®], Madison, WI) identified a complex rearrangement of 21q; six gains including DSCR and one loss, resulting in both partial trisomy 21q and partial monosomy 21q (Fig. 1). The proximal long arm of the abnormal chromosome 21 showed a 224 kb two-copy gain from 21q11.2, which contains at least three genes, immediately followed by a 2.7 Mb single-copy gain in 21q11.2-21q21.1 containing at least eight genes, followed by another 857 kb two-copy gain from 21q21.1 containing four genes. This was followed by a 132 Kb normal copy-number region, and a 1.35 Mb single-copy gain from 21q21.1 which contains at least two genes. The middle of the long arm showed a 3.77 Mb copy loss of 21q21.3-21q22.11 which contains 51 genes. The distal long arm showed a 5.2 Mb single-copy gain in 21q21.3-21q22.2 which contains 36 genes, including DSCR. This was followed by a 270Kb normal copy-number region, and a 5.9 Mb single-copy gain in 21q22.2-21qter which contains at least 101 genes. Based on the gene content and number, these alternations were expected to be clinically relevant, resulting in both partial trisomy/tetrasomy 21q and partial monosomy 21q.

Interphase fluorescence in situ hybridization and M-FISH analyses were performed using bacterial artificial chromosome clones from the duplicated, triplicated, and deleted regions of 21q to visualize the abnormalities.

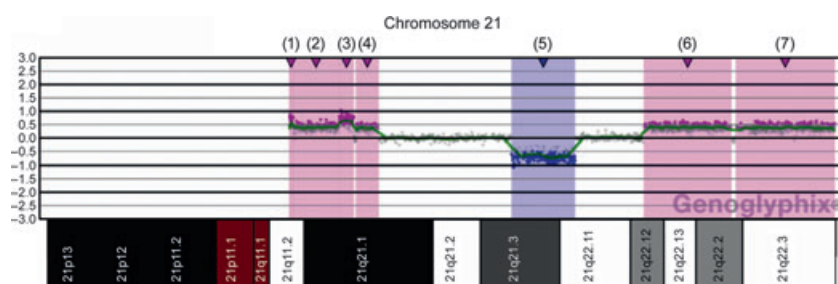


Figure 1. Microarray characterization of a complex rearrangement of chromosome 21. Microarray plot showing (1) a two-copy gain of 25 oligonucleotide probes from the long arm of chromosome 21 at 21q11.2, ~220 kb in size; immediately followed by (2) a single-copy gain of 285 oligonucleotide probes from 21q11.2-21q21.1, ~2.74 Mb in size; immediately followed by (3) a two-copy gain of 85 oligonucleotide probes from 21q21.1, ~860 kb in size. Also present are (4) a single-copy gain of 107 oligonucleotide probes from 21q21.1, ~1.35 Mb in size; (5) a single-copy loss of 153 oligonucleotide probes from 21q21.3q-21q22.11, ~3.77 Mb in size; (6) a single-copy gain of 483 oligonucleotide probes from 21q22.12.-21q22.2, ~5.21 Mb in size; and (7) a single-copy gain of 528 oligonucleotide probes from terminal 21q22.2-21q22.3, ~5.89 Mb in size. Probes are ordered on the x-axis according to physical mapping positions, with the most proximal q-arm probes to the left and the most distal q-arm probes to the right. Values along the y-axis represent log₂ ratios of patient:control signal intensities. Results are visualized using Genoglyphix[®] (Signature Genomics, Spokane WA).

These analyses confirmed that all alternations identified by aCGH are on the same homolog with the translocation of duplicated terminal segment from 21q22.2qter to the short arm of the chromosome (Fig. 2A, B). These results also confirmed the duplication of DSCR detected by cytogenetic analyses, and indicated that the interstitial duplications spanned the length of 21q in an extremely complex form.

G-banding analyses, I-FISH and M-FISH of the parents' blood specimens showed no abnormal findings, suggesting that the alterations identified in the fetus were apparently *de novo* in origin.

Discussion

Maternal serum quadruple screening is not designed to detect structural chromosome abnormalities. However, there have been some publications on the association between positive results of MSS and structurally abnormal chromosomes. For example, a 19-year-old woman with a positive MSS result (risk of Down syndrome at 1 in 83) was reported to have a fetus with isochromosome 21q by standard chromosome analysis [3]. In the second case, isochromosome 18q was identified after the positive MSS result (risk of trisomy 18 at 1 in 44) [4]. Our case showed a positive MSS result for Down syndrome and was identified to have extremely complex chromosome rearrangements of chromosome 21 by aCGH, including an interstitial duplication of DSCR which is responsible for Down syndrome phenotype [8].

The duplication of DSCR has been suggested to result in the phenotype of Down syndrome [2]. Familial duplication of DSCR was reported in the mother, her 8-year-old daughter, and fetus, all of whom had facial gestalt of

Down syndrome [9]. In another case, a 3-year-old girl with the full Down syndrome phenotype was reported to have dup(21) (q22.11q22.13) [10]. The change in the serum marker levels in our case might be attributed to the duplication of DSCR; however, additional reports would be necessary to prove it.

The interstitial duplication of DSCR was too small to be detected by standard cytogenetic analysis. Although I-FISH analysis used in the prenatal cases is not designed to detect structural chromosomal abnormalities which are undetectable by standard G-banding analysis, this case indicates the possibility of detecting structural abnormalities on the targeted regions with specially designed DNA probes. In our case, the inconsistent results between I-FISH study and G-banding analysis were observed. Molecular analysis with higher resolution such as aCGH identified not only the duplication of DSCR but also five gains and one loss, resulting in both partial trisomy/tetrasomy 21q and partial monosomy 21q.

This is the case of prenatal diagnosis of the duplication of DSCR with a positive MSS result for Down syndrome as a start. This case raises the possibility of missing the diagnosis of complex rearrangement by G-banding analysis alone. When the results of I-FISH and G-banding analysis are inconsistent, further examination using methods such as M-FISH and aCGH are essential not only to evaluate precisely the chromosome of the fetus but to also provide more appropriate genetic counseling to the family.

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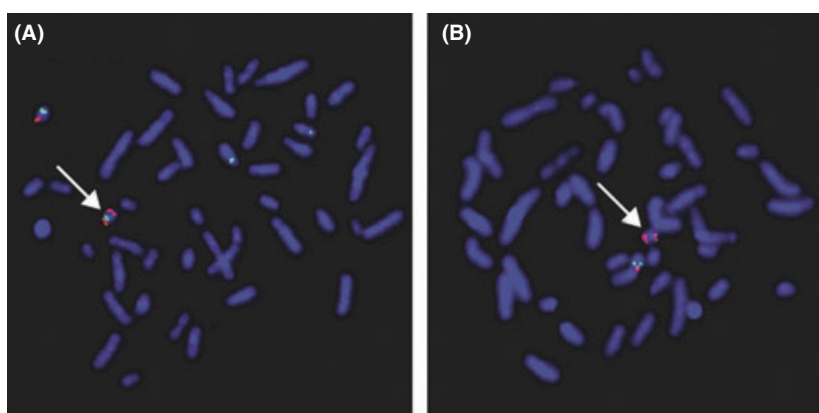


Figure 2. Fluorescence in situ hybridization (FISH) visualization of a complex rearrangement of chromosome 21. FISH showing translocation of 21q22.3 to 21p (A, B) and deletion at 21q21.3 (B). Bacterial artificial chromosome (BAC) clone RP11-71A7 from 21q22.3 is labeled in red. In (A) D13Z1/D21Z1 is labeled in green as a control, and in (B) BAC clone RP11-1113A5 from 21q21.3 is labeled in green. (A) The presence of two red signals on opposite ends of one homologue indicates translocation of the duplicated segment from 21q22.3 to 21p. (B) The absence of a green signal from that same homologue indicates deletion of 21q21.3 on the same der(21) (arrow).

Conflict of Interest

None declared.

References

1. Yoon, P. W., S. B. Freeman, S. L. Sherman, L. F. Taft, Y. Gu, D. Pettay, et al. 1996. Advanced maternal age and the risk of Down syndrome characterized by the meiotic stage of chromosomal error: a population-based study. *Am. J. Hum. Genet.* 58:628–633.
2. Arron, J. R., M. M. Winslow, A. Polleri, C. P. Chang, H. Wu, X. Gao, et al. 2006. NFAT dysregulation by increased dosage of DSCR1 and DYRK1A on chromosome 21. *Nature* 441:595–600.
3. Meyers, C. M., S. Ghorri, A. E. Roberson, M. G. Blitzer, and M. M. Cohen. 1997. 46, XY, i(21q) identified by maternal serum screening. *Md. Med. J.* 46:88–89.
4. Graf, M. D., P. Gill, M. Krew, S. Schwartz. 2002. Prenatal detection of structural abnormalities of chromosome 18: associations with interphase fluorescence in situ hybridization (FISH) and maternal serum screening. *Prenat. Diagn.* 22:645–648.
5. Test and Technology Transfer Committee. 2000. Technical and clinical assessment of fluorescence *in situ* hybridization: an ACMG/ASHG position statement. I. Technical considerations. *Genet. Med.* 2:356–361.
6. Pinkel, D., R. Seagraves, D. Sudar, S. Clark, I. Poole, D. Kowbel, et al. 1998. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat. Genet.* 20:207–211.
7. Rauch, A., F. Ruschendorf, J. Huang, U. Trautmann, C. Becker, C. Thiel, et al. 2004. Molecular karyotyping using an SNP array for genome-wide genotyping. *J. Med. Genet.* 41:916–922.
8. Korenberg, J. R., X. N. Chen, R. Schipper, Z. Sun, R. Gonsky, S. Gerwehr, et al. 1994. Down syndrome phenotypes: the consequences of chromosomal imbalance. *Proc. Natl. Acad. Sci. USA* 91:4997–5001.
9. Ronan, A., K. Fagan, L. Christie, J. Conroy, N. J. Nowak, G. Turner. 2007. Familial 4.3 Mb duplication of 21q22 sheds new light on the Down syndrome critical region. *J. Med. Genet.* 44:448–451.
10. Schinzel, A. 2001. Catalogue of unbalanced chromosome aberrations in man, 2nd ed. de Gruyter, Berlin, New York, NY.