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Case Report

Euchromatic variants of 8q21.2 in twins

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ABSTRACT

Objective: Euchromatic variants (EVs) of 8q21.2 are extremely rare chromosomal abnormalities. So, far there have only been two reports on EVs of 8q21.2. Here, we report an 8q21.2 EV detected in cultured amniotic-fluid cells of twins. It was later found to be inherited from the mother, who did not present with abnormal phenotypes.

Case Report: A pregnant woman underwent amniocentesis at 16 weeks of gestation because of advanced maternal age. This pregnancy was monozygotic twins conceived naturally. A cytogenetic analysis of cultured amniocytes revealed 46,XY,?dup(8)(q21.2). Chromosomal microarray revealed no abnormalities. C-banding and fluorescent *in situ* hybridization using chromosome 8 painting probe suggested euchromatic nature of the extra chromosomal band. Karyotyping of the parents showed that the EV was inherited from the mother.

Conclusion: Many, but not all, EVs are clinically innocuous. This is the first case of 8q21.2 EV reported in the ethnic Han. More cases are needed to clarify whether 8q21.2 duplication as a *bona fide* EV.

Introduction

Heteromorphism of chromosomes (variant chromosomes) is an example of genetic polymorphism. Structural rearrangements of heterochromatic material, satellite polymorphism, or fragile sites, are well-known examples of common chromosome variation [1]. But now, a new class of variants that does not fit in with the usual perception of heteromorphism involves the so-called euchromatic variants (EVs), regions that are C-band negative and not generally anticipated to be variable in size or staining because they presumably contain genetic material [2].

Most visible chromosome abnormalities concerning euchromatic regions have serious phenotypic consequences. By contrast, the absence of phenotypic effects was reported in some cases of euchromatic anomalies [1]. Barber [3] divided euchromatic anomalies into two categories: (1) unbalanced chromosome abnormalities, such as deletions or duplications, and (2) EVs, mostly because of variation in the copy number of pseudogenes.

Copy-number variation (CNV) means a segment of DNA 1 kb or larger and is present at a variable copy numbers in the population. CNVs may arise from insertions, deletions, or duplications. This definition also includes large-scale copy number variants, which are variants that involve segments of DNA $\geq$50 kb, allowing them to be detected by array CGH [4]. If the size of amplified regions is big enough to become visible with the light microscope, the CNVs become EVs [5]. The common EVs comprise 4p16, 8p23.1, 9p12, 9q12, 9q13, 15q11.2, and 16q11.2 [6]. Most of the EVs are clinically innocuous, but the 8p23.1 EV has been associated with a number of traits [7] and the 4p16.1 EV cosegregated with microtia [8].

EVs of 8q21.2 is an extremely rare chromosome abnormality, and it has been reported only twice. The previous reports seemed to indicate that patients with 8q21.2 EV have clinical or reproductive problems [9]. In our case, the cytogenetic analysis of cultured amniocytes of two fetuses revealed 46,XY,?dup(8)(q21.2) in a twin gestation. The same karyotype was displayed with their mother. Being different from previous reports, the gravida was phenotypically normal and healthy.

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Case Report

A 36-year-old Taiwanese woman, gravida 2 para 1, underwent amniotic fluid sampling at 16 weeks' gestation because of advanced maternal age. Her height was 158 cm and her weight was 60 kg. Her menarche occurred at the age of 13 years, and her menstrual cycle has been regular since puberty. This pregnancy was monozygotic twins conceived naturally. Cytogenetic analysis of cultured amniocytes revealed that both twins were 46,XY,?dup(8)(q21.2) (Figure 1A). The chromosomal microarray analysis using the Affymetrix 750k SNP array (Affymetrix Inc., Santa Clara, CA, USA) revealed no genomic changes {arr(1-22)*2(X)*1(Y)*1}. The couple underwent karyotyping using peripheral blood lymphocytes. The husband was found to have a normal male karyotype (46,XY), but the wife was found to have duplication of 8q21.2 [46XX,?dup(8)(q21.2)]. So the fetus had inherited the abnormal chromosome from their mother. We performed fluorescence *in situ* hybridization using the painting probe as well as C-banding to prove the nature of the duplicated region (Figure 1B and 1C). However, no evidence of structural aberration of chromosome 8 was found. The parents continued the pregnancy, and high-level ultrasound showed no fetal malformations at 20 weeks. The babies were delivered via the vaginal route smoothly at 37 weeks' gestation in July 2015. The Apgar score was 8 and 9, respectively. Their birth weight was 2530 g (twin A) and 2410 g (twin B). There was no obvious SGA (small for gestational age) and they developed normally.

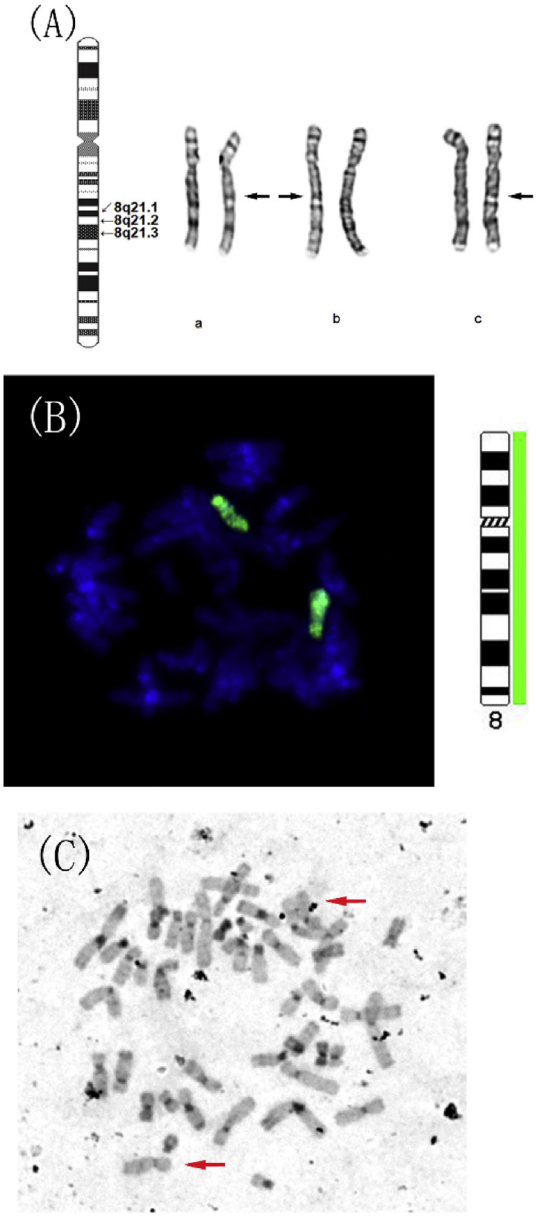

Figure 1. (A) Three pairs of chromosomes 8 (a and b, two fetuses; c, woman). Arrows indicate duplication of chromosome 8. (B) Fluorescence *in situ* hybridization (FISH) analysis. Of 10 metaphase cells examined, no evidence of structural aberration of chromosome 8 was found. FISH using the Aquarius Whole Chromosome Painting Probe (Cytocell, Inc.). Probe contains whole chromosome 8 painting probe. It was labeled with green fluorophore. (C) C-bands in addition to FISH analysis. C-bands by barium hydroxide using Giemsa was also performed to reveal constitutive heterochromatin. Red arrows indicate chromosome 8.

Discussion

Copy number variants visible with the light microscope of 8q21.2 have been reported only twice previously [9,10]. In one report, Manvelyan et al [10] described three patients of Middle European origin and Japanese descent who had this condition. In another report, Tyson et al [9] described the existence of this condition in Caucasian, Czech, and Palestinian patients. In this report, we found twins with this EV inherited from their mother, a Taiwanese Han. Our case represents the first case reported in the ethnic Han.

By analyzing patients with 8q21.2 EV using the probe BAC RP11-96G1 [11], Tyson et al [9] found that this 8q21.2 consisted of a sequence gap flanked by segmental duplications that contain the 12-kb components of one of the largest variable number tandem repeat arrays in the human genome. Using digital NanoString technology with a custom probe for the RNA exonuclease 1 homologue (*S. cerevisiae*)-like 1 (REXO1L1) gene within each 12-kb repeat, they observed significantly enhanced diploid copy numbers of 270 and 265 in an EV family and a median diploid copy number of 166 copies in 216 controls. They concluded that CNV at 8q21.2 contains tandemly repeated DNA families [12]. When the copy number is enough (microscopically visible), it predisposes to polymorphic inversions [13] and it has been associated with neocentromere formation [14]. Given that the *REXO1L1* gene is generally not included in the gene chip, all cases of 8q21.2 EV, including ours, are expected to have a normal chromosomal microarray analysis.

In order for a euchromatic deletion or duplication to be regarded as a normal variant, Jalal and Ketterling [15] proposed that the following criteria should be met. “It should have been: (1) reported in a relatively large number of individuals; (2) been passed on from parents to children; (3) associated with a normal phenotype; (4) the identity of the extra or missing chromatin confirmed by chromosome banding and/or molecular/molecular cytogenetic procedures.” In the past, most EVs were recognized as clinically innocuous, but now the 8p23.1 EV has been reported to be associated with a number of traits [7]. Gibbons et al [16] reported a patient and her two daughters with minimal dysmorphology, and Tsai et al [17] suggested that duplication 8p23.1 might also be the cause of serious clinical defects, including mental retardation, short stature, heart defects, or hypotonia, whereas Barber et al [18] suggested that it is in fact an EV without clinical effects. The 4p16.1 EV has been reported to be cosegregated with microtia [8], and 9p12, 9q12, 9q13, 15q11.2, and 16q11.2 are considered to have no clinical effects [1]. Interestingly, the EV region of 8q21 was already found to be involved in gene amplification in breast [19] and prostate cancer [20], as well as in aberrant methylation in osteosarcoma [21]. However, the impact of that has to be elucidated

in the future. For EVs 8q21.2, Tyson et al [9] reported that seven patients were ascertained to have congenital anomalies, autism, secondary amenorrhea, infertility, miscarriages, and a family history of intellectual difficulties and cystic fibrosis. The *REXO1L1* gene has no known disease association, and its product is a marker of hepatitis C virus infection; moreover, a possible association between REXO1L1 copy number and susceptibility to hepatitis C virus infection has been demonstrated [22,23]. They hypothesized that congenital anomalies, autism, or family history of intellectual difficulties and cystic fibrosis were incidental findings [9], but the possible association with infertility and/or miscarriage [12] would need to be tested in much larger cohorts and is more likely to reflect bias of ascertainment [24]. Considering the absence of reproductive or other health problems in our case, this case seems to support that EV of 8q21.2 is a benign finding. However, more cases are needed to clarify whether 8q21.2 duplication is a *bona fide* EV.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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