

Interstitial Deletion 13q31 Associated with Normal Phenotype: Cytogenetic Study of a Family with Concomitant Segregation of Reciprocal Translocation and Interstitial Deletion

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Gain or loss of a fragment in human chromosomes has been associated with abnormal phenotypes in numerous genetic disorders. However, it is also possible that lack or excess of a particular chromosomal segment is a neutral polymorphism among populations and thus does not cause obvious abnormal phenotype. In this study, conventional GTG-banded karyotyping and molecular cytogenetic analyses (including fluorescence *in situ* hybridization, spectral karyotyping and comparative genomic hybridization) were applied to study the genotype-phenotype correlation in a Taiwanese family, in which a concomitant segregation of del(13)(q31q31) interstitial deletion and t(13;18)(q32;p11.2) reciprocal translocation in a 2-year-old girl (the proband) was noticed. Two family members (the father and grandmother of the proband) who carried the del(13)(q31q31) but not the translocation t(13;18) both revealed a normal phenotype at adulthood. The finding, which appears novel, that interstitial deletion 13q31 could be associated with a normal phenotype, is therefore valuable in genetic counseling. [*J Formos Med Assoc* 2007;106(7):582-588]

Key Words: comparative genomic hybridization, fluorescence *in situ* hybridization, interstitial deletion 13q, spectral karyotyping

Human chromosome aberrations such as gain or loss of chromosomal segments have been shown to be the underlying factors resulting in numerous genetic disorders. In some cases, small fragment losses in human chromosomes cannot be detected in conventional GTG-banding at the 500-900-band level alone (while was able to be deciphered with feasible molecular or molecular cytogenetic tools), but they still have clinical significance, such as Di George syndrome (OMIM188400) and

Williams syndrome (OMIM194050), which are caused by microdeletions.^{1,2} Therefore, a significant lack or excess of a chromosomal segment is generally expected to be associated with phenotypic abnormalities.

However, this conviction contrasts with the nature of the chromosomal segment that is lacking or in excess.¹ Furthermore, chromosomal polymorphisms, or normal variants, have been noticed in human karyotype. These observations

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Received: May 18, 2006

Revised: September 12, 2006

Accepted: January 2, 2007

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are particularly important because a proper prediction of abnormal phenotype in a certain cytogenetic aberration is vital to give advice in genetic counseling.^{2,3} In this study, we describe a Taiwanese family segregated simultaneously with two rare chromosomal abnormalities, an interstitial deletion defined as a new chromosome polymorphism, and a reciprocal translocation between two autosomes.

Case Report

Clinical manifestations

A dysmorphic 2-year-old girl (i.e. the proband) was noticed to have developmental delay and multiple congenital anomalies including malformed ear, cleft lip, imperforate anus with rectocutaneous fistula, and dysplasia of the right hip (Figure 1). She received cutback procedure for imperforate anus soon after birth and lip reconstruction for cleft lip thereafter. The remaining family members revealed no abnormal phenotype, physically or mentally (Figure 2).

Cytogenetic studies

Cytogenetic analyses of the conventional GTG-banding were performed for the phytohemagglutinin-stimulated peripheral blood lymphocytes of: (1) the proband; (2) the mother of the proband; and (3) the father of the proband. In the proband, two apparently abnormal chromosome 13s were observed (Figure 3A). In the mother of the proband, a balanced reciprocal translocation between the long arm (q arm) of chromosome 13 and the short arm (p arm) of chromosome 18 was found (Figure 3B). In the father of the proband, an aberrant chromosome 13 with an apparent abnormality in the q arm was found (Figure 3C). According to the latest nomenclature ISCN2005, the karyotype of the proband was therefore formulated as 46,XX,der(13)t(13;18)(q32;p11.2)*mat*, *rea* (13q) *pat*. Notably, the aberrant chromosome 13 found in the proband's father was also found in the proband's grandmother (data not shown; but result is comparable with Figure 3C).

Several molecular cytogenetic methods were further used to delineate the size and composition of the aberrant chromosome 13 carried by the

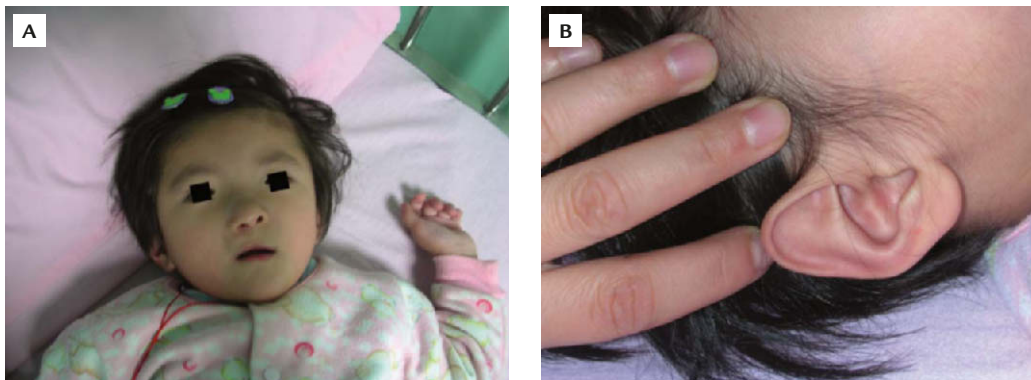


Figure 1. Phenotypes of the proband: (A) facial dysmorphism; (B) malformed ear.

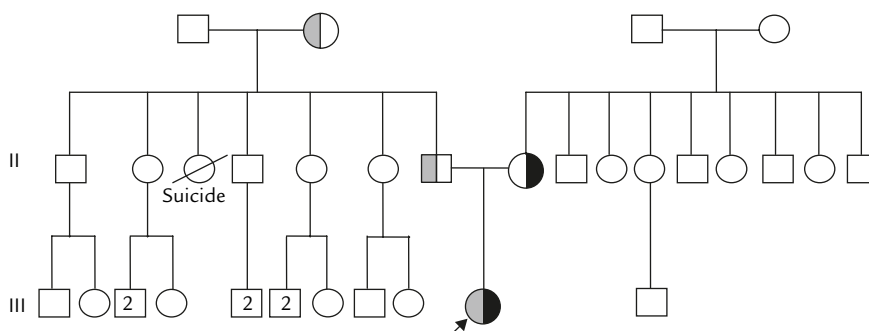
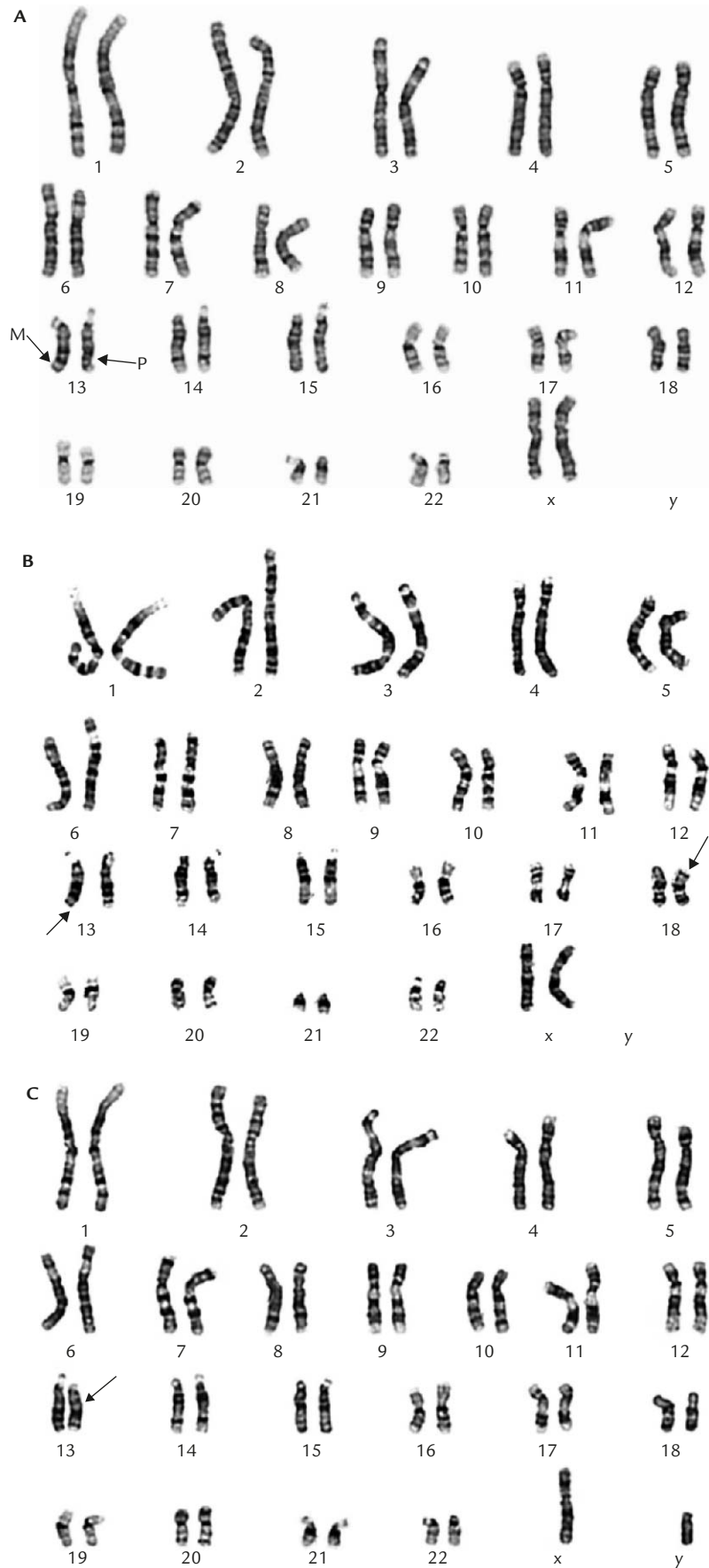


Figure 2. Pedigree of the proband's family: arrow indicates the proband who carries a $\text{del}(13)(\text{q}31\text{q}31)$ interstitial deletion inherited from her father (denoted as gray) and a $\text{t}(13;18)(\text{q}32;\text{p}11.2)$ balanced translocation inherited from her mother (denoted as black). The number "2" indicates two individuals.

Figure 3. (A) GTG-banded karyotype of the proband. The arrows mark the positions of the abnormalities in the pair of chromosome 13s. "M" denotes the maternally inherited aberrant chromosome 13 and "P" denotes the paternally inherited aberrant chromosome 13. (B) GTG-banded karyotype of the proband's mother. The arrows mark the positions of the reciprocal translocation between the long arm of chromosome 13 and the short arm of chromosome 18. (C) GTG-banded karyotype of the proband's father. The arrow marks the position of the abnormality in chromosome 13.



father and the grandmother of the proband. First, fluorescence *in situ* hybridization (FISH) was performed with the chromosome 13q-specific ToTelVysis Multi-color DNA Probe (13qter probe, D13S327, Spectrum Orange and Spectrum Green; Vysis Inc., Downer's Grove, IL, USA) in order to know whether the subtelomeric region of the q arm of this aberrant chromosome 13 was intact or deleted. Among the 20 metaphase cells scored, all had positive signals on each of the two chromosome 13 q arms (Figure 4). Then, spectral karyotyping (SKY) (Applied Spectral Imaging, Carlsbad, CA, USA) was performed with a set of multicolor-labeled painting probes (the SKY paint kit) to detect if an interchromosomal translocation had taken place in the aberrant chromosome 13. Of the 10 cells examined, no abnormal spectral karyotype was found (Figure 5). Finally, comparative genomic hybridization (CGH) was carried out to examine the integrity of the aberrant chromosome 13. As shown in Figure 6, an interstitial deletion spanning approximately 9 Mb was found at the band 13q31. Consequently, the cytogenetic aberration was denoted as *ish cgh del(13)(q31q31) and the karyotype of the proband was further delineated as 46,XX,der(13)t(13;18)(q32;p11.2) mat, del(13)(q31q31) pat.*

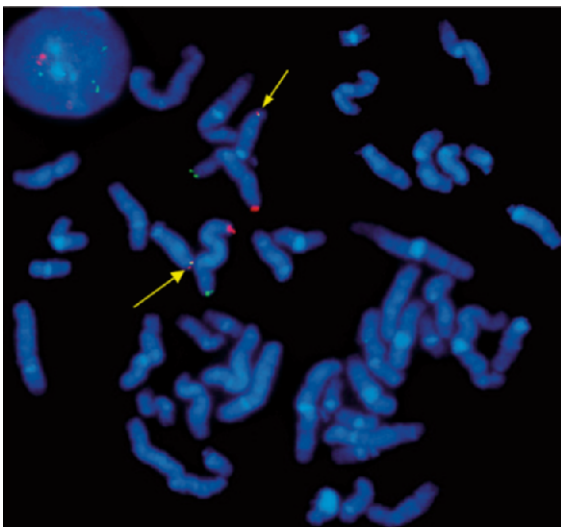


Figure 4. FISH analysis of the proband's father. Both 13q subtelomeres remained intact (yellow arrows).

Discussion

Variant chromosomes

Variant chromosomes do exist in forms such as variations in heterochromatin sizes/positions, acrocentric short-arm morphology, NOR translocation, fragile sites, and addition/deletion of G-bands. Research of normal variants is important for suitable and competent genetic counseling.³ Unbalanced autosome abnormalities, or segmental aneusomies, especially deletions, often cause an abnormal phenotype and mental deficiency in their carriers. Nevertheless, there are some exceptions to this rule since the distribution of functionally relevant genes is uneven in the human genome. The Table summarizes some of the exceptions reported in the English literature.⁴⁻¹⁴

Size of deletions

CGH is a powerful tool in determining the changes in chromosome copy numbers. The usual resolution of a metaphase CGH is about 2 Mb in deletions and 5–10 Mb in duplications.¹⁵ It is thus useful for detecting interstitial deletions and even segmental duplications.¹⁶

Interstitial deletions involving the long arm of chromosome 13

Reported phenotypes of interstitial deletion of 13q included postnatal short stature with micro-brachytrigonocephaly, mild to severe mental retardation,



Figure 5. Spectral karyotype of the proband's father. No obvious interchromosomal rearrangement was found.

developmental delay, encephalocele, agenesis of corpus callosum, hearing loss, hypertelorism, facial asymmetry, flat nasal bridge, protruding upper incisors, large and malformed ears, short neck, cardiac defects, anal anomalies, and minor anomalies of the distal limbs.¹⁷ All the previously reported interstitial deletions of 13q occurred *de novo* except in one case that was due to a complex unbalanced

translocation who was noticed to have borderline psychomotor retardation, speech delay, and mild facial anomalies.¹⁸

In addition, interstitial deletions of 13q were reported to be responsible for retinoblastoma. Several genes located within the segment of chromosome 13q31 → q32 may contribute to the development of retinoblastoma.¹⁹

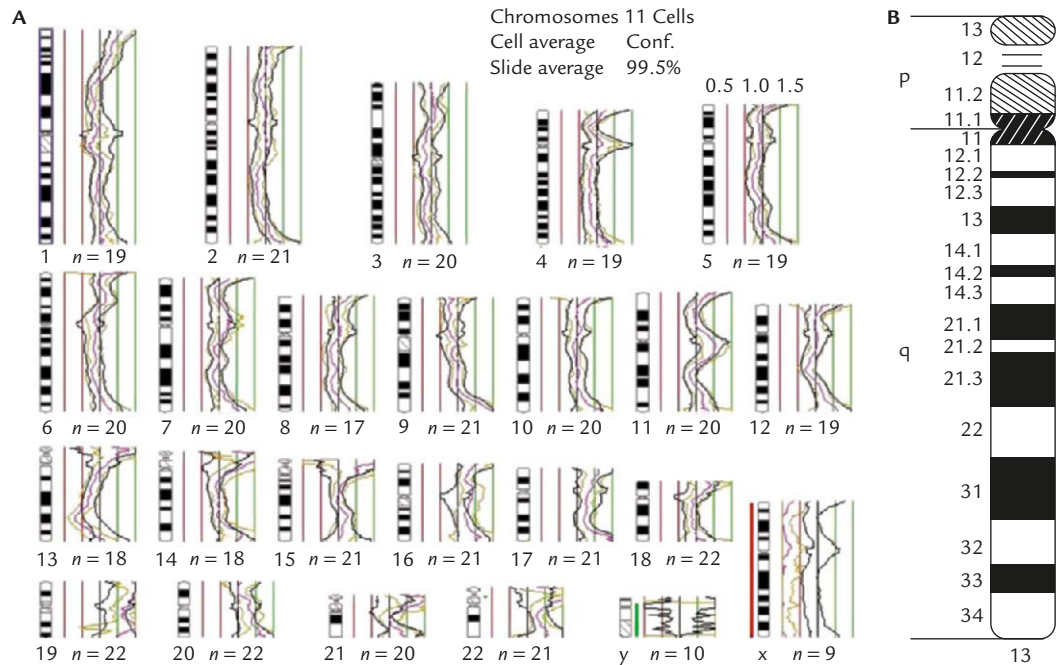


Figure 6. (A) Comparative genomic hybridization profiles of the proband's father. An interstitial deletion at band 13q31 was found (denoted as a red vertical bar beside chromosome 13). (B) An amplified ideogram of chromosome 13 with the deleted region marked by a red vertical bar on the right.

Table. Examples of interstitial deletions without apparent phenotypic abnormality

Deletion segment	Number of carriers	Number of generations	Reference
2q13q14.1	2	2	Sumption & Barber (2001)
3p25.3	2	2	Knight et al (1995)
5p14	8	5	Overhauser et al (1986) Hand et al (2000)
6q23.3q24.2	1	1	Kumar et al (1999)
11p12	3	2	Barber et al (1991)
11q14.3	1	4	Li et al (2002)
13q21	2	2	Couturier et al (1985)
13q31	2	3	This study
16q21	6	4	Witt et al (1988) Callen et al (1993) Hand et al (2000)
Xq26	2	2	Taysi et al (1983)

Notably, there are many disorders related to chromosome 13q31 segmental aneusomy. A haplotype analysis suggested that a possible gene defect in the chromosome region of 13q31-q32 might be associated with familial microcoria.²⁰

Furthermore, a familial vestibulocerebellar disorder was mapped to a locus on chromosome 13q31-q33.²¹ Mitral valve prolapse, an autosomal dominant disorder, was found to link with a new locus at chromosome 13q31.3-q32.1.²² Moreover, a possible candidate gene of the Gilles De La Tourette syndrome was suspected to be *Silt* and *Trk-like 1 (SLITRK1)* at the chromosome region 13q31.1.²³ Therefore, the normal phenotype noticed in our family is quite remarkable.

Possible explanations for a normal phenotype of del(13)(q31q31)

The case presented here concerns a large interstitial deletion without any detectable phenotypic abnormality. However, many possible explanations may apply. By the understanding of staining techniques used in cytogenetics, the deleted band was a G-dark band, and it has been observed that trisomies or monosomies of G-band segments have less severe phenotypic consequences than imbalances of R-band segments.¹ Consequently, it was assumed that G-bands (AT-rich regions) generally contain fewer genes than R-bands (GC-rich regions). It is well established that G-bands usually replicate during the second half of the S phase, that is, late replicating in the process of cell division and, thus, behave in a similar manner as heterochromatin.¹ The size of band 13q31 is approximately 9 Mb in human genome. Fifteen sequences with possible biological meaning were obtained from a search of the NCBI database (<http://www.ncbi.nlm.nih.gov/>) in band 13q31, whereas the number of such sequences with possible biological meaning was 14 in band 13q22 and 20 in band 13q32. No obvious difference was noticed in the number of genes buried in band 13q31 when compared with its neighboring band 13q22 and band 13q32. However, many other mechanisms regulating mammalian gene expression such as genomic imprinting, positional effect, chromatin

structuring, different extent of methylation/demethylation, and different status of histone acetylation/deacetylation may also contribute to the complex phenotypic variability. For example, Chochoska and colleagues once reported a highly variable phenotype in female patients carrying an interstitial deletion Xp22.2-22.3. The reasons were thought to be random inactivation of one X chromosome in carrier females.²⁴ The absence of pathologic consequences of genomic imbalances without apparent abnormal phenotype therefore may be explained by various theories including: (1) the region may contain few transcriptionally-active genetic materials; and (2) epigenetic mechanisms such as genomic imprinting, positional effect, chromatin structuring, or different extent of methylation/deacetylation were involved.²⁵ We conclude that our study is helpful for genetic counseling in future cases with similar interstitial deletions.

Acknowledgments

This study was partially funded by research grants from Changhua Christian Hospital (CCH9281 and CCH93008). The authors would like to thank all the colleagues of the Center for Medical Genetics, Changhua Christian Hospital, especially Ms Pao-Ying Wen and Ms Li-Wen Chen. The authors are also very grateful for the valuable advice offered by Professor Chyi-Chyang Lin (of China Medical University) during manuscript preparation.

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