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Uniparental Heterodisomy for Chromosome 14 in a Phenotypically Abnormal Familial Balanced 13/14 Robertsonian Translocation Carrier

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Summary

A 9-year-old mentally retarded girl with multiple congenital anomalies was found to carry a balanced 13/14 Robertsonian translocation [45,XX,t(13q14q)] which was also present in her father. Her mother carried a balanced reciprocal translocation between chromosomes 1 and 14 [46,XX,t(1;14) (q32;q32)]. Both of her parents were phenotypically normal. Molecular studies were carried out to determine the parental origin of chromosomes 1, 13, and 14 in the patient. Using probes for D14S13 and D14S22, we could show that the patient inherited both chromosomes 14 from her father and none from her mother. Similar studies using probes for chromosomes 1 (D1S76) and 13 (D13S37) loci showed the presence of both maternal and paternal alleles in the patient. Our findings indicate that paternal uniparental heterodisomy for chromosome 14 most likely accounts for the phenotypic abnormalities observed in our patient. It is suggested that uniparental disomy may be the basis for abnormal development in at least some phenotypically abnormal familial balanced-translocation carriers.

Introduction

Although the majority of individuals with familial balanced translocations are phenotypically normal, a small number of such individuals present clinically with varying abnormalities. These abnormalities are generally attributed to unknown causes independent of the translocation. Recently, genomic imprinting, or the differential expression of parental alleles, has gained recognition as a contributing mechanism for a number of human disease phenotypes (Hall 1990). In humans, uniparental disomy was first demonstrated for chromosome 7 in an individual with cystic fibrosis and short stature (Spence et al. 1988). Molecular studies revealed that both homologues of chromosome 7 in this patient were identical and were derived from the mother. Subsequently, uniparental disomy was demonstrated in a second patient with cystic fibrosis (Voss et al. 1989) and in two patients with nondeletion Prader-Willi syndrome (Nicholls et al. 1989). These observations prompted us to examine the possibility of uniparental disomy in a phenotypically abnormal girl who carried a paternally inherited balanced Robertsonian translocation.

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Subjects and Methods

Subjects

The patient, GR, born at 38 wk of gestation to a gravida 7 para 2 spontaneous abortion 5 mother, was initially evaluated in the newborn period because of multiple congenital anomalies including bilateral subarachnoid hygromas requiring a shunt, short neck with webbing, a small thoracic cage causing restrictive lung disease, marked angulation of the ribs, bilateral Simian creases, and facial dysmorphism including blepharophimosis, small ears, anteverted nares, and

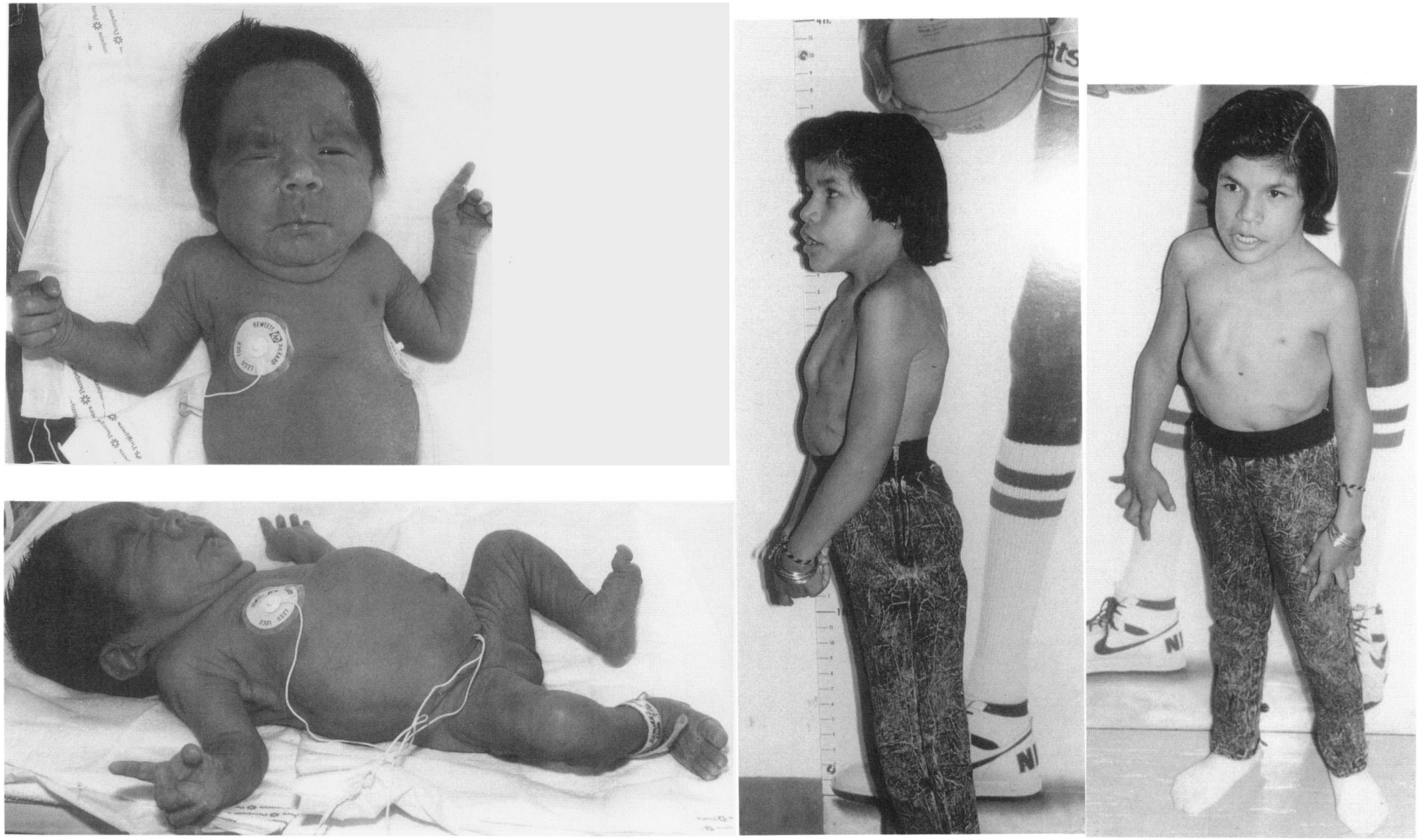

Figure 1 Clinical features of patient GR, as newborn and at age 9 years

protruding philtrum (fig. 1). Both birth weight and length were at the 10th percentile, while head circumference was at the 75th percentile. Chromosome studies at that time showed GR to be a carrier of a balanced 13/14 Robertsonian translocation [45,XX,t(13q14q)], which was also present in her father. Her mother was a carrier of a balanced reciprocal translocation between chromosomes 1 and 14 [46,XX,t(1;14) (q32;q32)]. Both parents were phenotypically normal. One brother was phenotypically and cytogenetically normal. GR was reevaluated several times over the years, but no etiological diagnosis has been made. At age 9 years she presented with severe mental retardation, severe kyphoscoliosis, a seizure disorder, and coarse facial features with frontal bossing and prominent maxilla and mandible (fig. 1).

Cell Culture and Cytogenetic Analysis

Lymphoblastoid cultures were established from GR, her parents, and her normal sib. A fibroblast culture was established from GR. Chromosome studies of peripheral blood lymphocytes, skin fibroblasts, and lymphoblastoid cell lines were performed by using standard techniques.

DNA Analysis

High-molecular-weight DNA was extracted from lymphoblastoid cell lines, was digested with appropriate restriction endonucleases under conditions recommended by the manufacturers, was electrophoresed on a 0.7% agarose gel, and was transferred to nylon filters by using the capillary method (Sambrook et al. 1989). DNA probes were nick-translated to a specific activity of $2–3 \times 10^8$ cpm/μg with both [^{32}P]dTTP and [^{32}P]dCTP (2,000–3,000 Ci/mmol; Amersham) and prehybridized for 30–45 min with 200 μg human placental DNA/ml in 5 × SSC, 1 × Denhardt's reagent, 10% dextran sulfate (Pharmacia), 250 μg heat-denatured salmon DNA/ml, 0.5% SDS. Hybridization was carried out at 65°C overnight, and the filters were washed once with 2 × SSC and then three times with 50 mM Tris, 1 mM EDTA, 1 × Denhardt's, 0.1% sodium pyrophosphate (pH 8.0) at 65°C over a 45-min period. The filters were exposed to prefogged Kodak XAR-5 film at −70°C with one DuPont Lightning Plus intensifier screen (Laskey 1980).

Probes

Genomic probes pCMM66(*D14S22*), pCMM101 (*D14S13*), pCMM12(*D1S76*), and pTHI62 (*D13S37*) were obtained from the American Type Culture Collection. They all detect VNTR polymorphisms (Nakamura et al. 1987, 1988*a*, 1988*b*). Restriction fragments CMM66–1.8, CMM12–4.3, and THI62–1.1 were further isolated from endonuclease-digested pCMM66 (*Eco*RI and *Hin*dIII), pCMM12 (*Eco*RI and *Hin*dIII), and pTHI62 (*Pst*I and *Bam*HI), respectively (Sambrook et al. 1989).

Results

Cytogenetic Studies

Partial karyotypes of the chromosomes of interest, from both GR and her parents, are shown in figure 2. The 13/14 Robertsonian translocation was present in GR as well as in her father, while the mother carried an apparently balanced 1/14 reciprocal translocation. At the time of reevaluation of GR at age 9 years, peripheral blood chromosome analysis was repeated, and lymphoblastoid cell lines were established from members of the family. At this time, uniparental disomy was considered as a basis for the clinical phenotype of GR, and chromosome polymorphisms (QFQ) were evaluated. The morphology of both the stalk region and satellites of the normal chromosome 14 present in GR was very similar to those in the father and different from those in the mother. These observations suggested that the normal chromosome 14 in GR was derived from her father and that she had paternal disomy for chromosome 14. Similar comparison of polymorphisms on chromosome 13 showed that they were consistent with a maternal origin of the normal chromosome 13 in GR. In 100 cells each of lymphocytes and fibroblasts, there was no evidence of mosaicism in GR. DNA studies were undertaken to verify the possible existence of uniparental disomy in GR.

DNA Studies

Two chromosome 14 probes, each detecting a VNTR polymorphism on the long arm, were used to determine the parental origin of the chromosomes 14 in GR. DNA digested with *Pst*I and probed with CMM66–1.8 yielded a 1.4-kb constant band in all individuals (fig. 3*A*). In addition, GR's brother inherited the paternal 6.1-kb allele and maternal 5.5-kb allele. GR, on the other hand, inherited the 15-kb and 6.1-kb allelic fragments from her father and had neither the 13-kb nor the 5.5-kb allele from the mother. This finding is consistent with paternal uniparental heterodisomy for chromosome 14 in GR. A sec-

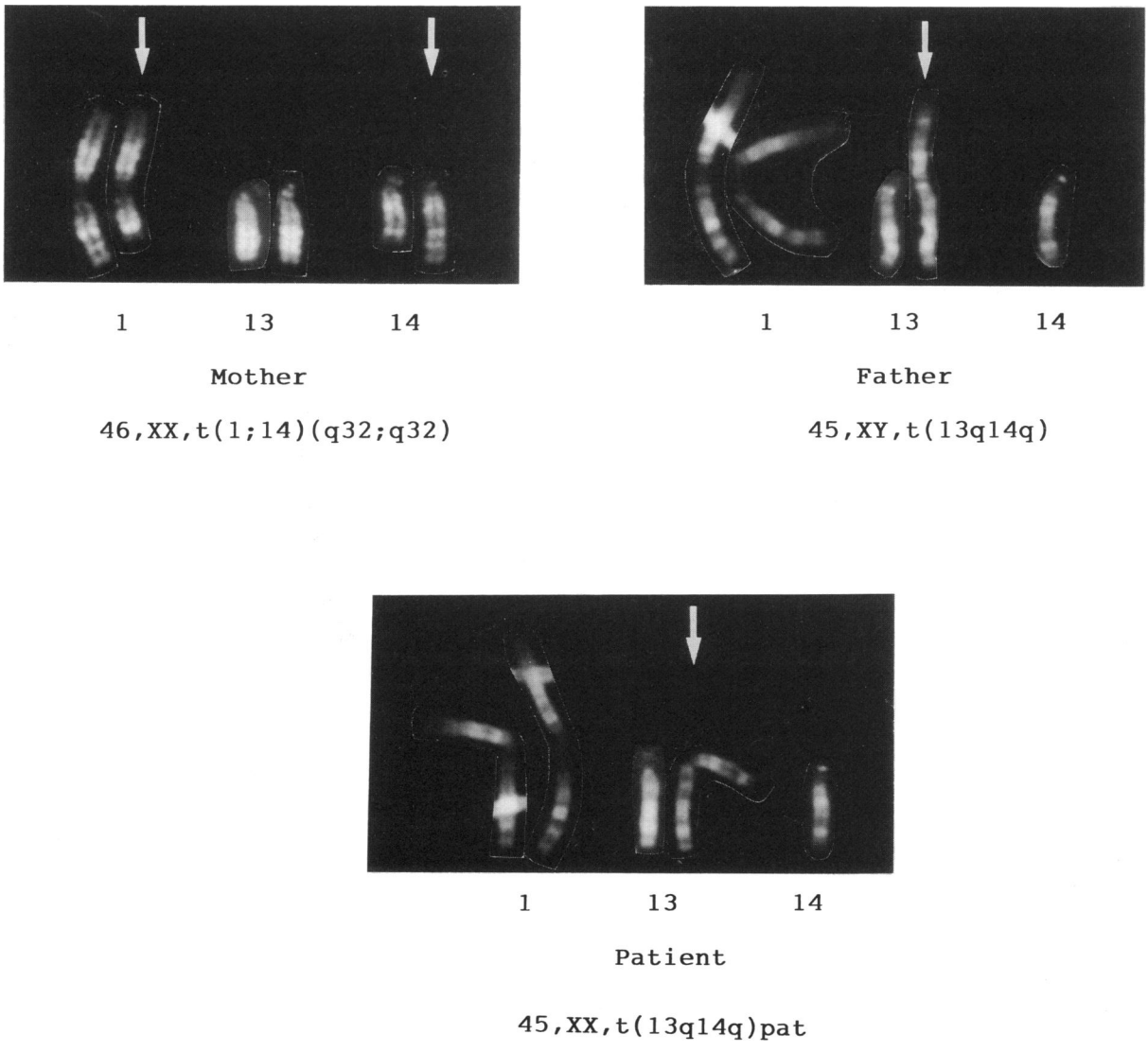

Figure 2 Partial karyotypes of chromosome pairs 1, 13, and 14 in patient and her parents. Chromosomes involved in translocation are denoted by an arrow.

ond probe pCMM101 specifying the *D14S13* locus at 14q32 was used to confirm this result (fig. 3*B*). GR's brother inherited a paternal 3.2-kb allele and a maternal 2.4-kb allele. Again, GR inherited both the paternal 3.2-kb allele and the paternal 8.0-/2.6-kb allele but neither the maternal 2.4-kb allele nor the maternal 6.4-/2.6-kb allele. Studies using probes CMM12–4.3 and THI62–1.1 for chromosomes 1 and 13, respectively, showed normal segregation, with the presence of both maternal and paternal alleles in both GR and her brother (data not shown).

Discussion

We have demonstrated by molecular methods that GR inherited both chromosomes 14 from her father and none from her mother. Thus GR has paternal heterodisomy for chromosome 14. The family reported here is unique in that both parents have translocations involving chromosome 14. As chromosomes involved in translocation are at an increased risk for aberrant segregation at meiosis, it is conceivable that malsegregation of chromosome 14 occurred in both

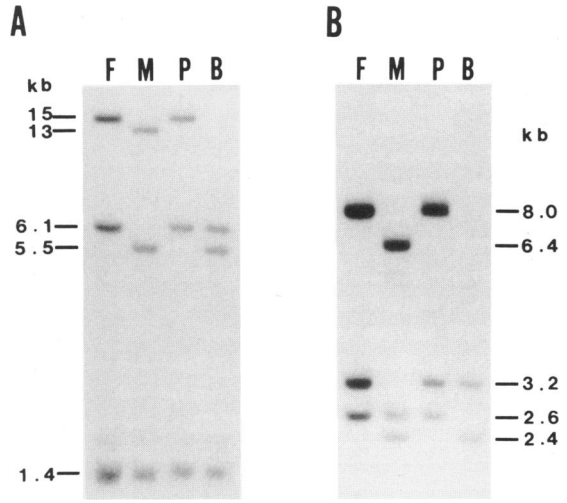

Figure 3 Southern blot analysis using chromosome 14 probes. The lanes are genomic DNA from the father (F), mother (M), patient (P), and brother (B). *A*, DNA digested with *PstI* and hybridized with probe CMM66–1.8. *B*, DNA digested with *MspI* and hybridized with probe pCMM101. The hybridization patterns are consistent with paternal uniparental heterodisomy for chromosome 14 in the patient. Molecular-weight standards were λ/*Hin*dIII and φX/*Hae*III.

parents. The most likely origin of the karyotype in GR is that an ovum missing a chromosome 14 because of a 3:1 segregation in the mother was fertilized by a sperm that had both the 13q14q and a normal chromosome 14 because of a 2:1 segregation in the father. In fact, maternal or paternal disomy can be readily produced in mice, with intercrosses between either Robertsonian or reciprocal translocation carriers (Cattanach 1986). Uniparental disomy can also occur for normal chromosomes, with both parents having normal chromosome complements, as exemplified by the nondeletion Prader-Willi syndrome patient reported by Nicholls et al. (1989). It has been suggested that these Prader-Willi patients were trisomic for chromosome 15 at conception but that viability was achieved by loss of a paternal chromosome 15 in a cell which was then able to outgrow the trisomy 15 cells (Hall 1990). Similarly, it is possible that GR started out with trisomy 14 after a 2:2 segregation in the mother and a 2:1 segregation in the father, with subsequent loss of maternal chromosome 14. However, we were unable, in either lymphocyte cultures (done on three occasions) or skin fibroblasts, to demonstrate the presence of mosaicism for trisomy 14 in GR.

Studies in mouse embryos by using nuclear-transplantation techniques demonstrate that both maternal and paternal genomes are required for normal development (McGrath and Solter 1984; Surani et al. 1984, 1986). It has also been shown that maternally and paternally derived transgenes are differentially methylated (Hadchouel et al. 1987; Reik et al. 1987; Sapienza et al. 1987; Swain et al. 1987) and in several instances the methylation patterns are reversible from generation to generation when there is a change in the parental origin. These observations further support the concept of genomic imprinting and suggest a possible mechanism for differential expression of maternally and paternally derived alleles. The involvement of genomic imprinting in human diseases is probably more widespread than has been demonstrated to date.

While an undetected low-level mosaicism for trisomy 14 remains a possibility, our findings suggest that paternal uniparental heterodisomy for chromosome 14 most likely accounts for the phenotypic abnormalities observed in our patient. It is suggested that uniparental disomy may be the basis of abnormal development in at least some phenotypically abnormal familial balanced-translocation carriers. DNA studies should therefore be carried out to rule out uniparental disomy in all cases of familial balanced translocations associated with abnormal phenotype when no other etiological factors can be identified. These studies will also be helpful in determining whether we should consider any changes in counseling when prenatal diagnostic studies find that a fetus has inherited a balanced translocation from one of the parents.

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