Two sibs with Wolf-Hirschhorn and DiGeorge deletions resulting from an unbalanced chromosome rearrangement, 45,XX/XY, der(4)t(4;22)(p16.3;q11.2)mat,-22

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Abstract

A mother with an apparently balanced translocation between chromosomes 4 and 22 gave birth to two children (sib 1 and twin A) with 45,XX,der(4)t(4;22)(p16.3;q11.2)mat,-22 and 45,XY,der(4)t(4;22)(p16.3;q11.2)mat,-22 karyotypes. The mother was a slow learner and required special education. The imbalance in the sibs arose through a 3:1 malsegregation in the mother. The net result was deletions 4p16.3pter and 22q11.2pter. Deletion 4p is associated with Wolf-Hirschhorn syndrome (WHS). The 22q11.2 microdeletion is associated with a wide range of overlapping phenotypes including DiGeorge syndrome (DGS), velocardiofacial syndrome (VCFS), conotruncal facial abnormality, and sporadic or familial cardiac defect. Fluorescence in situ hybridisation (FISH) was performed. Cosmid probes D4S96, which maps to 4p16.3, and D22S75, which maps to 22q11.2, were used. In the mother, the translocation breakpoints were proximal to D4S96 on chromosome 4 and distal to D22S75 on chromosome 22. The two sibs had deletions of a D4S96 and a D22S75 probe loci. Sib 1, a 2½ year old girl, has multiple congenital abnormalities and profound developmental delay. The craniofacial features are generally of WHS. Hypoplasia of the thymus, hypocalcaemia, and seizures in early infancy, which are clinical features of DGS, were also observed. Twin A was one of a pair of dizygotic twins. He had multiple congenital abnormalities and died soon after birth.

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Key words: Wolf-Hirschhorn deletion; DiGeorge deletion; unbalanced rearrangement.

The simultaneous presentation of two deletion syndromes because of a chromosome rearrangement is extremely rare. This is the first report of WHS and DGS deletions in two sibs because of a 45,XX/XY,der(4)t(4;22)(p16.3;q11.2)mat,-22 karyotype. WHS is caused by a deletion of the region 4p16.3. The main clinical features of WHS are severe growth and mental retardation, microcephaly, "Greek helmet" facies, and closure defect (cleft lip or palate,

coloboma of the eye, and cardiac septal defect).

DiGeorge syndrome, velocardiofacial syndrome, conotruncal facial abnormality, and sporadic or isolated cardiac defects are clinically overlapping disorders associated with haploinsufficiency of chromosome region 22q11.2. The acronym CATCH 22¹ was coined for the main characteristics of these overlapping disorders and they are Cardiac defect, Abnormal facies, Thymic hypoplasia, Cleft palate, Hypocalcaemia, and 22q11.2 deletions. The incidence of this group of disorders is estimated to be greater than 1:5000.² In the majority (>85%) of patients, DGS results from a microdeletion of chromosome 22, del(22)(q11.2q11.23).^{3,4} The critical region of this microdeletion syndrome is about 1.5 Mb. This is considered to be a contiguous gene syndrome and it has been suggested that one or a few genes may play a major role in the aetio-pathogenesis. Recently a candidate gene *pf12-rnex40* (DGCR3) was cloned by Budarf *et al.*⁵

Case reports

MOTHER

An obese American black woman with hypertelorism was a slow learner and had an apparently balanced translocation, 46,XX,t(4;22)(p16;q11.2). Her clinical features were otherwise not remarkable. She has had three pregnancies with the same partner (fig 1). The first conception ended in a spontaneous abortion in the first trimester. During the second pregnancy, ultrasound examinations recorded delayed fetal growth of a symmetrical nature and severe oligohydramnios. The patient refused prenatal diagnosis. At 36 weeks, labour was induced with amniocentesis and the result was a normal spontaneous vaginal delivery of sib 1. The third pregnancy was a twin gestation. At 17 weeks ultrasound, twin A was found to have decreased amniotic fluid volume and growth discordance that increased with time. The patient again refused prenatal diagnosis. At 34 weeks a caesarian section delivery was performed and twins were delivered.

In the mother's flow cytometry marker study, there was a normal distribution of T cells and T cell subsets. Her T cell values were normal (B cell markers: CD19(B4)=18%, T cell markers: CD2(T11)=82%, CD3(T3)=73%, CD4(T4)=41%, CD5(Leu1)=74%, CD7(Leu9)=69%, CD8(T8)=28%, CD16/56=

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Figure 1 (A) Pedigree and partial karyotypes. The mother is a carrier of a balanced translocation $t(4;22)(p16.3;q11.2)$. Her partial karyotype shows the normal 4, the $der(4)$, normal 22, and $der(22)$. The arrows on the normal chromosomes 4 and 22 indicate the translocation breakpoints. The mother has had three pregnancies. The first resulted in a first trimester abortion, the second was sib 1, an abnormal girl, and the third were twins. Twin A was an abnormal boy and twin B a normal girl. Sib 1 and twin A have an unbalanced rearrangement, $45,XX/XY,der(4)t(4;22)(p16.3;q11.2)mat,-22$. Their partial karyotype contains a normal 4, a derivative 4, and a normal 22. (B) Mother and sib 1. (C) Sib 1.

9%). The parathyroid hormone and calcium values were within the normal range (PTH = 15.1 pg/ml and total calcium = 10.1 mg/dl).

SIB 1

Sib 1 was a 26 month old baby girl. She had multiple congenital abnormalities, profound developmental delay, and a $45,XX,der(4)t(4;22)(p16.3;q11.2)mat,-22$ karyotype. Her phenotypic features included microcephaly, flat forehead, prominent glabella, ocular hypertelorism, epicanthic folds, ptosis of the right eyelid, and a nose with a broad base. Her ears are large and low set with flat pinnae and preauricular pits. A posterior cleft palate and a downturned, fish-like mouth are present. The left leg is shorter than the right and the left fourth toe is prominently long and incurved. She has talipes equinovarus. A month after her birth the T lymphocyte count showed an absolute decrease in T8 suppressor cells to yield high H:S ratio. There were no discernible cardiac anomalies. Chest MRI indicated a small thymus. Parathyroid hormone levels were normal; however, hypocalcaemia and seizures were observed in early infancy. The neonatal ionised calcium level was 1.0. The left kidney was smaller than the right on ultrasound examination. At birth the lungs were hypoplastic.

The infant has growth deficiency (<3rd centile) and severe developmental delay.

Sib 1 was born at 36 weeks of gestation and weighed 1815 g. She was small for gestational age and had multiple congenital abnormalities. At 6 months of age, her development was severely delayed. She had questionable response to sound on the right and no response on the left. Increased tone in her shoulders and upper extremities and a slight increase in her trunk and lower extremities were noted. At 12 months of age, she displayed increased tone throughout and had a reflexive palmar grasp, but did not roll or sit. Six months later, at 18 months, she was tracking, could roll, and could grasp toys. However, at 2 years of age she had attained motor development of a 5 to 6 month level. Her language development was severely delayed. She was prone to severe and frequent upper respiratory tract infections.

TWIN A

Twin A was a male baby with a $45,XY,der(4)t(4;22)(p16.3;q11.2)mat,-22$ karyotype. At 34 weeks' gestation his birth weight was 1022 g, length was 34 cm, and head circumference was 27.5 cm (all below the 10th centile). At birth, his head appeared large because the torso was small with decreased sub-

Figure 2 FISH studies using D4S96 (4p16.3) WHS specific probe and chromosome 4 alpha satellite control probe (ONCOR). (A) The mother's chromosomes show two centromeric signals on chromosomes 4 and the arrows indicate one 4 with a D4S96 signal and the other D4S96 signal on the der(22). (B) The sibs show two chromosome 4 centromeric signals and only one D4S96 signal (arrow) on the normal 4. FISH studies, using D22S75 (22q11.2) DGS chromosome region probe with D22S39 (22q13.3) control probe (ONCOR). (C) The mother's chromosomes have four signals on three chromosomes, the normal 22 with the control and a DGS signal (arrow), a control signal on der(4), and the DGS signal on the der(22) shown by the arrow. (D) The sibs have three signals on two chromosomes, a control and a D22S75 signal on the normal 22 with an arrow, and a control signal on the der(4). They are missing one D22S75 probed locus.

cutaneous fat, and the neck was short. Widely spaced eyes, low set ears, flat nasal bridge, and micrognathia were also observed. He had widely spaced nipples. Club feet, short webbed fingers, and an incurved fifth finger on both hands were present. The cord had three vessels. The child was floppy and unresponsive and died soon after birth.

Twin B is a normal female infant.

Materials and methods

Peripheral blood cultures were synchronised with methotrexate (amethopterin 0.05 µg/ml, SIGMA#M6710) for 17 hours and released with thymidine (2.5 µg/ml; SIGMA#T5018) for 5.5 hours. The cultures were harvested following the addition of colcemid. Chromosomes were GTG banded; the karyotype was described according to the International System for Human Cytogenetic Nomenclature: ISCN (1995). FISH studies were initiated using the WHS specific probe D4S96 (4p16.3) with chromosome 4 alpha satellite control probe and DGS region probe D22S75 (22q11.2) with D22S39 (22q13.3) control probe (ONCOR). Slides were denatured for five minutes in 70% formamide/2 × SSC, pH 7, at 70°C followed by dehydration in cold (−20°C) 70%, 80%, and 95% ethanol for two minutes in each. Ten microlitres of digoxigenin

labelled probe was applied to the slide, coverslipped, sealed, and hybridised overnight in a 37°C humid chamber. The slides were washed in 2 × SSC at 72°C for five minutes and transferred to 1 × PBD (ONCOR) for two minutes. For hybridisation detection, 60 µl of rhodamine labelled antidigoxigenin was placed on the slide and incubated for 15 minutes in a humid chamber at 37°C. The coverslip was removed and the slides were washed three times for two minutes each in 1 × PBD. The cells were viewed with a triple pass filter (The Power Gene FISH system from Perspective Scientific Instruments Inc (PSI)). Metaphases were analysed and representative cells were captured electronically on an imaging system (PSI).

CYTOGENETIC AND FISH STUDIES

Peripheral blood from the mother, sib 1, and the twins were sent to our laboratory for chromosome analysis. GTG banded metaphases were analysed. The mother was found to have an apparently balanced translocation involving chromosomes 4 and 22. The karyotype was 46,XX,t(4;22)(p16.3;q11.2).

Sib 1 had a 45,XX,der(4)t(4;22)(p16.3;q11.2)mat,-22 karyotype. Twin A had a 45,XY,der(4)t(4;22)(p16.3;q11.2)mat,-22 karyotype and twin B a 46,XX karyotype (fig 1).

FISH studies using probes D4S96 and D22S75 (ONCOR) were carried out on the mother and the two abnormal sibs (fig 2). D4S96, which maps to 4p16.3, detects Wolf-Hirschhorn syndrome deletions. D22S75, which maps to 22q11.2, is deleted in the majority (>75%) of DiGeorge syndrome and velocardiofacial syndrome cases. In situ hybridisation was performed according to the manufacturer's (ONCOR) instructions. For D4S96 probe the mother had two signals, one on a normal chromosome 4 and another on the derivative 22 (fig 2). The sibs had only one signal on the normal chromosome 4. Therefore, in both sibs there was a deletion of D4S96 which maps to the Wolf-Hirschhorn region. Probe D22S75 gave two signals on the mother's metaphases, one on the normal chromosome 22 and other on the derivative 22 (fig 2). The breakpoint in the mother on chromosome 22 is distal to the cosmid probe D22S75. The two sibs had only one signal on the normal chromosome 22. Therefore, the probe D22S75 which maps to 22q11.2, the DGS region, was deleted. The key findings were that the mother had translocation breakpoints proximal to D4S96 on chromosome 4 and distal to D22S75 on chromosome 22. The two sibs had deletions of a D4S96 (WHS) and a D22S75 (DGS) probe loci.

Discussion

This is the first report of both WHS and DGS or VCFS deletions in two sibs. The two syndromes have come together because of an unbalanced translocation inherited from the mother, 45,XX/XY,der(4)t(4;22)(p16.3;q11.2)mat,-22. The mother has a balanced translocation t(4;22)(p16.3;q11.2). As a consequence of a probable maternal 3:1 meiotic segregation resulting in a tertiary monosomy for the der(22), two abnormal sibs were born. They received only the derivative 4 from the mother and the normal 4 and 22 from the father (fig 1). The clinical features in sib 1 appear to be a combination of both WHS and DGS. This patient has, in general, a WHS facies and some DGS features, such as hypoplasia of the thymus, hypocalcaemia, and seizures in infancy.

The deletion on 4p16.3 defining the WHS phenotype has been narrowed to 150 kb proximal to the telomere⁶ and encompassing 2.5 Mb with the breakpoint approximately 80 kb distal to D4S43.⁷ Further refinement of the minimum region involved in this syndrome was possible because of a subtle submicroscopic deletion

reported by Zackai *et al.*⁸ They have shown that loci D4S98 and FGFR3, within 100 kb of each other and ~300 kb distal to D4S43, were deleted. Therefore, deletion on chromosome 4 was proximal to D4S96, as was the breakpoint in the mother of the family we have studied.

There are reports of loss of 22q11.2 and DiGeorge syndrome owing to unbalanced translocations⁹⁻¹¹ and interstitial deletion.¹² However, the report by Augusseau *et al.*¹³ is the only balanced translocation associated with some features of DGS. The breakpoint in this patient is proximal to D22S75. In our study, we describe a balanced 4;22 translocation in the mother who is a slow learner. The translocation breakpoint, however, is distal to D22S75 loci.

In the mother, the WHS and DGS regions have been juxtaposed to form the derivative 22. Her learning disability and hypertelorism coincide with the clinical presentation of some DGS patients. The molecular characterisation of the breakpoint region on this chromosome would be interesting. It may involve the disruption of a gene responsible for learning disability in some CATCH 22 disorders.

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