Diamond-Blackfan anaemia in a girl with a de novo balanced reciprocal X;19 translocation

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Abstract
A 7 year old girl is described with congenital hypoplastic anaemia (Diamond-Blackfan anaemia, DBA) and an apparently balanced reciprocal translocation, 46,XX,t(X;19)(p21;q13). The girl has associated features including short stature, unilateral kidney hypoplasia, and a branchial cyst. Fluorescent in situ hybridisation (FISH) studies with 19q specific cosmids showed that the chromosome 19 breakpoint is located between the RYR1 and the XRCC1 loci spanning a physical region of 5 Mb. There is no family history of DBA and the parents and two healthy sibs have normal karyotypes. This is the first report of a balanced translocation associated with DBA and we suggest that the distinct phenotype has resulted from a de novo disruption of a functional gene. DBA can be inherited as an autosomal trait and our observation may indicate a candidate gene for the disorder in the 19q13 region.

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Congenital hypoplastic anaemia or Diamond-Blackfan anaemia (DBA, McKusick 205900) is a rare macrocytic anaemia of unknown aetiology and pathogenesis. It is characterised by subnormal erythropoiesis with absent erythroid precursors and reticulocytopenia.[1-4] The differentiation and proliferation of granulocytes, macrophages, and megakaryocytes are usually normal. More than 90% of the patients are anaemic before the age of 1 year. The diagnostic criteria (European Working Group on DBA, personal communication) are diagnosis before the age of 2 years, low reticulocyte counts, and selective deficiency of red cell precursors in the bone marrow (<5% of nucleated cells). Additional features which are often observed include macrocytosis, raised fetal haemoglobin, and increased red cell adenosine deaminase (ADA). Sister chromatid breakage should be normal which excludes Fanconi anaemia. Familial cases support the diagnosis.

About 25% of patients with DBA present with associated congenital physical anomalies.[5] The most common single feature is malformations of the thumb.[2] Other frequent defects include atrial or ventricular septal defects, anomalies of the forearm, webbed neck, cleft lip and palate, urogenital anomalies, strabismus, cataract, and short stature.[2-7]

The majority of cases are sporadic, with an equal sex ratio, but at least 10% have a positive family history for the disorder. Both autosomal dominant[8-12] and autosomal recessive inheritance have been reported.[1,13,14] In addition, studies of several families indicate dominant inheritance with reduced penetrance.[15-18]

The molecular basis for the disease is unknown but successful bone marrow transplantation of DBA patients has unambiguously shown that the functional defect(s) is related to a haematopoietic progenitor cell or its derivatives.[19,20] Attempts have been made to find inhibitors of erythropoiesis and claims have been made both for and against the presence of T cell inhibitors in erythropoiesis.[21-24] Several investigators have tried to clarify the effect of haematopoietic growth factors on erythroid progenitors from patients with DBA. However, the in vitro response to growth factors, such as erythropoietin (EPO), IL-3, IL-6, GM-CSF, stem cell factor, and EPA, has yielded conflicting results.[25-28] Molecular analyses of candidate genes, such as the EPO receptor, have also been undertaken, but no mutations have been identified.[29]

We describe a girl with a de novo balanced X;19 reciprocal translocation in whom the haematological and clinical features were consistent with DBA.

Materials and methods
Standard chromosome preparations were made from bone marrow aspirate and from PHA stimulated peripheral blood derived from the proband. The parents' chromosomes were analysed from blood only. Conventional cytogenetic analysis of G banded chromosomes was carried out on 500 band metaphases. Analysis of sister chromatid breakage was performed after the induction of mitomycin C. The chromosome 19 cosmids used (Lawrence Livermore National Laboratory) and their relative order from the centromere to the telomere is: 16848, 10084, 9476, 8764, 22135, 16250, and 15567.[30,31]

Fluorescent in situ hybridisation was done essentially as previously described.[32,33] Cosmid DNA was labelled with biotin-11-dUTP or digoxigenin-16-dUTP (Boehringer Mannheim), using nick translation. Labelled DNA (50 ng) was combined with sonicated herring sperm DNA and 5 µg Cot-1 DNA (GIBCO BRL), ethanol precipitated, air dried, and then dissolved in a hybridisation buffer (50%

formamide, 2 × SSC, 10% dextran sulphate, and 50 mmol/l phosphate buffer, pH 7.0). Probes were denatured at 70°C for 10 minutes, followed by preannealing with Cot-1 DNA for 30 minutes at 37°C.

Chromosome preparations were denatured using 70% formamide/2 × SSC at 70°C. After two minutes denaturation, the slides were incubated in an ice cold ethanol series (70%, 85%, and 99%) and then air dried. Hybridisation was carried out at 37°C overnight in a moist chamber.

After hybridisation, the slides were washed three times for five minutes in 50% formamide/1 × SSC at 42°C and two 0.5 × SSC washes at 60°C. Detection of probe hybridisation was achieved by the application of a single layer of avidin-fluorescein isothiocyanate, FITC (Vector), or rhodamine labelled antidigoxigenin (Boehringer Mannheim) for 30 minutes. The slides were then washed in 2 × SSC, 0.05% tween for 15 minutes and in an ethanol series.

Chromosomes were counterstained with 0.05 µg/ml 4',6-diamidino-2-phenylindole, DAPI (Serva), and mounted in antifade solution (Vectashield). The slides were analysed with a Zeiss Axiophot epifluorescence microscope with a filter set for visualisation of rhodamine, FITC, and DAPI fluorescence. Images were merged using a CCD camera (Photometrics) and the IPlab software (Vysis). Photographs were taken directly from the computer monitor using Kodak 100 Ektachrome colour film.

Case report
The patient was born at term after an uneventful pregnancy (birth weight 3400 g) to unrelated parents. The parents and two older sibs are all healthy. At the age of 1 month, the proband developed fatigue, pallor, and dyspnoea. Examination showed anaemia with a haemoglobin concentration of 5.5 g/dl. The MCV and MCHC were within the normal ranges and reticulocyte counts were low or in the lower normal range (0-0.5%). Leucocyte and thrombocyte counts were normal. A bone marrow aspirate showed a selective decrease in erythroid precursors and reduced erythropoiesis. The proportion of erythrocyte progenitors was <5% of nucleated bone marrow cells whereas myelopoiesis and the megakaryopoiesis were found to be normal. The heart and skeleton were normal and the only associated malformations were a branchial cyst on the right side and hypoplasia of the left kidney. Treatment with methylprednisolone has not resulted in an increase in Hb concentration and from the age of 6 weeks she has been dependent on blood transfusions. Her mean haemoglobin concentration at the age of 7 is approximately 7.0 g/dl, of which 5% represents fetal haemoglobin. At the age of 7 she is 106 cm tall (-3 SD) and her weight is 31.5 kg (+2.5 SD) (fig 1).

Cytogenetic and FISH results
Conventional cytogenetic studies showed an apparently balanced reciprocal translocation

between the long arm of chromosome 19 and the short arm of the X chromosome, with the resulting karyotype 46,X,t(X;19)(p21;q13) (fig 2). The karyotypes of the parents and sibs are normal. FISH analyses showed that cosmid LLNL9476 was present on the der(19), whereas the LLNL8764 cosmid was located on the der(X) (fig 3). The cosmid LLNL9476 contains the RYR1 gene and the LLNL8764 cosmid contains the XRCC1 gene.[31] The two cosmids/genes are separated by 5 Mb. The in situ hybridisation results can be summarised as 46,X, t(X;19)(p21;q13).ish t(X;19)(9476; 8764+). The frequency of sister chromatid breakage was found to be normal (<3%) which excluded Fanconi anaemia.

Discussion
The introduction of molecular cytogenetic techniques has led to the identification of a number of submicroscopic translocations and deletions associated with clinically defined phenotypes.[34] In such cases, it is assumed that the chromosome breakage occurs within, and disrupts, a functional gene. In our case, a de novo balanced reciprocal X;19 translocation showed a distinctive haematopathology and associated anomalies consistent with DBA. This is the first balanced translocation reported in a case of DBA. Two previous cases with DBA and cytogenetic aberrations, both affecting chromosome 1, have been reported. One patient showed an achromatic region in chromosome 1p.[35] The second had a pericentric inversion of chromosome 1.[36] The association between chromosome 1 aberrations and DBA remains unclear and has not, as yet, been
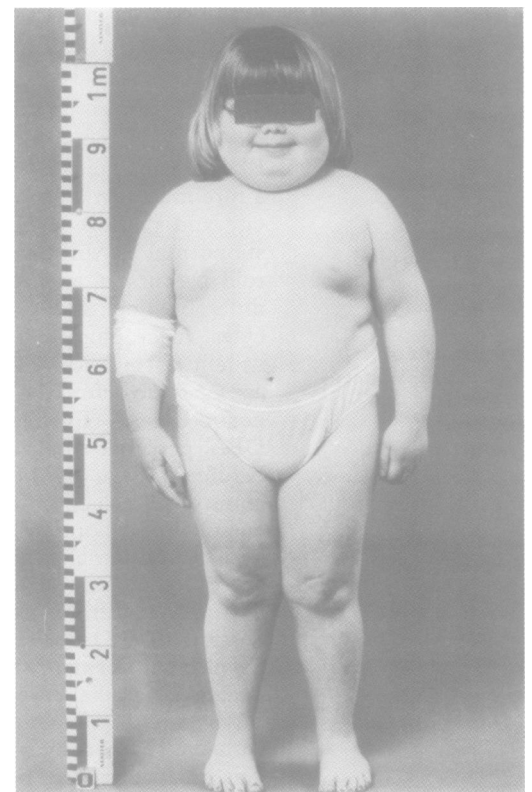

Figure 1   The proband aged 7 years showing short stature (-3 SD) and a Cushingoid habitus owing to steroid treatment. (Photograph reproduced with permission.)

Figure 2 Partial karyotype of G banded chromosomes showing the normal and derived chromosomes X and 19. The normal 19 and the der(19) (19pter-19q13::Xp21-Xpter) are on the left and the normal X and the der(X) (Xqter-p21::19q13-19qter) are on the right.

substantiated by other methods, such as linkage analysis on families segregating for DBA.

There are no previous reports of constitutional monosomy for the 19q13 region corresponding to the site of the translocation breakpoint reported here. One report described a balanced 6p;19q translocation in a patient presenting at necropsy with bilateral multicystic dysplasia and clinodactyly.[37] In our case left sided renal dysplasia was present and various anomalies of the hand and fingers are also an associated finding in DBA. Several acquired 19q rearrangements have been reported in malignancy.[38] Translocations involving 19q have been described in chronic B cell lymphoproliferative disorders[39] and a putative glioma suppressor gene has been mapped to the 19q region between APOC2 and HRC.[40 41] An increased incidence of leukaemias but not gliomas or other solid tumours[7] has been reported in cases with DBA.

In this report, we suggest that the DBA phenotype in the female patient is caused by disruption of the chromatin domain that causes loss of function of an otherwise normal gene. In approximately 10-20% of cases with Diamond-Blackfan anaemia, the inheritance is autosomal and apparently monogenic. The sex ratio for the disorder is 1:1 and no families have been described with an X linked pattern of inherit-

ance. In the absence of an X chromosome linked form of the disorder, our case may be the result of functional haploinsufficiency for a gene at 19q13. This is based on the assumption that the mutation can act in a dominant way, which has been shown previously.[8-12] The complex clinical and genetic pattern in DBA has hampered the identification of the basic defect(s) underlying this disease and there has been speculation about genetic heterogeneity in the disorder.[25 28] Although DBA may be caused by several genes, our observation may indicate one candidate locus for the disease.

The cosmids used for FISH analysis restrict the chromosomal breakpoint to a 5 Mb interval, which has recently been mapped in detail.[31] At least 14 genes are already assigned to the region. Defective erythroid progenitor cell maturation has been suggested as the origin of DBA. One candidate gene in the region is the transforming growth factor beta (TGFB1) gene, known to control proliferation and differentiation in many cell types.[42] The region also contains three gene families with tandemly repeated and related genes. One family is the carcinoembryonic antigen (CEA) gene cluster which includes the subfamily encoding pregnancy specific beta-1-glycoprotein (PSG). The PSG genes are highly expressed in the placenta which argues for their importance during development.[43]

Further FISH analyses with additional chromosome 19 derived cosmids will enable detailed mapping of the breakpoint and may precisely indicate candidate genes. Meanwhile, linkage analysis with polymorphic 19q markers in families segregating for DBA is in progress and will clarify the significance of the DBA candidate region.

Note added in proof

Since submission of this manuscript, we have performed linkage analysis in familial cases with DBA. The results show a significant two point lod score (Z=7.08) and our combined findings confirm a locus for DBA on chromosome 19q13.
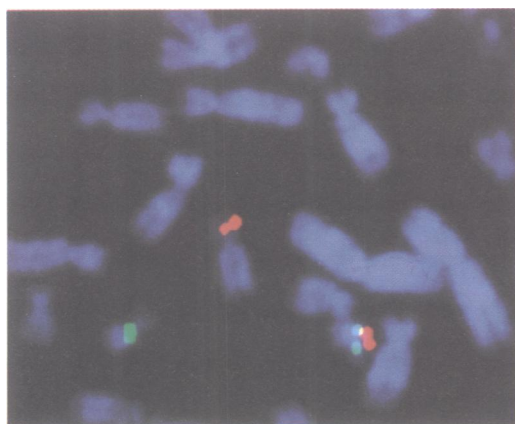
Figure 3   Fluorescent in situ hybridisation with the chromosome 19q specific cosmids 9476 and 8764 (Lawrence Livermore National Laboratory). The two cosmids are separated by 5 Mb which spans the breakpoint. The centromeric cosmid 9476 signal (green) was detected on the chromosome Xpter-p21:19q13-pter derivative and on the normal chromosome 19q. The more telomeric cosmid 8764 (red) shows a signal on the chromosome Xqter-p21:19q13-qter derivative and on the normal chromosome 19q. The yellow signal is the result of interference of the red and green signals on the normal chromosome 19.

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