ELECTRONIC LETTER

Hereditary duplication of proximal chromosome 1q (q11q22) in a patient with T lymphoblastic lymphoma/leukaemia: a family study using G banding and comparative genomic hybridisation

N P H Chan, M H L Ng, S H Cheng, V Lee, K S Tsang, T T Lau, C K Li

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Aberrations of the long arm of chromosome 1 have been linked to many diseases, both in acquired somatic neoplastic conditions and constitutional genetic disorders. These abnormalities include structural rearrangements and various deletions or amplifications affecting whole chromosome arms or specific regions. Gain of chromosome 1q has been reported in many solid tumours, including invasive carcinomas of the breast, cervical cancers, renal cell carcinoma, sarcomas, and hepatocellular carcinomas.¹⁻⁶ Complete or partial trisomy of 1q has been reported in cases of acute myeloid leukaemia, myelodysplastic syndrome, myeloproliferative diseases, Fanconi anaemia, and B lineage lymphomas, where involvement of 1q21 is frequently observed.⁷⁻¹⁴

CASE REPORT

The index patient, born to unrelated parents, first presented at another hospital in 1998 at the age of 9 years with a two month history of left groin lymphadenopathy. A lymph node biopsy showed two populations of lymphoreticular neoplastic cells, with positive staining for CD3, Tdt, and myeloperoxidase. No mediastinal mass was identified. Bone marrow (BM) biopsy and cerebrospinal fluid (CSF) cytology were both negative. BM cytogenetic study was reported to be normal (46,XY (20)). He was diagnosed as having acute mixed phenotypic lymphoma/leukaemia. Complete remission was achieved with hybrid chemotherapy completed in August 2000. However, he presented again in January 2001 with a few days' history of fever, petechial rash, right eye proptosis, and diplopia. Clinically, he showed pallor, petechiae, hepatomegaly, and multiple right cranial nerves palsies (III, IV, V, and VI). Peripheral blood showed pancytopenia and the presence of blasts. BM examination confirmed T lymphoblastic lymphoma/leukaemia relapse. The blasts were negative for myeloperoxidase, Sudan Black B, chloroacetate esterase, and periodic acid-Schiff reaction. They expressed CD3, CD7, and Tdt, but no other B lineage markers (CD10, CD19, CD20, CD22, IgM) nor CD13, glycophorin A, or CD61. A repeat BM cytogenetic study showed that there were both diploid and tetraploid clones with structural abnormalities of chromosomes 7 and 17 (46,XY,-7,add(7)(q36),add(17)(p11),+mar[3]/92,XXYY,-7x2,add(7)(q36)x2,add(17)(p11)x2,+marx2[6]/46,XY[3]). Blasts were also present in the CSF. Tumour infiltrate at the right orbital apex and right cavernous sinus was shown on CT scan of the brain. As a result he was treated with chemotherapy for high risk acute lymphoblastic leukaemia. A second remission was obtained and he was then referred to us for further management by allogeneic peripheral blood stem cell transplantation with his HLA identical older brother as donor. BM examination and cytogenetic study was again performed as pretransplant workup. There was no evidence of residual leukaemia. He

Key points

- We report a case of inherited proximal duplication of chromosome 1q in an 11 year old boy presenting with T lymphoblastic lymphoma/leukaemia. The duplication of chromosome 1 (q11q22) was identified using G banding and confirmed with comparative genomic hybridisation.
- Cytogenetic studies performed in his family members showed the same chromosomal aberration in his mother and sister, but not in his older brother who had a normal karyotype. No dysmorphic syndromes, congenital malformations, or psychomotor retardation were observed in either the index patient or his mother or sister.
- Constitutional duplication of proximal 1q is rare with involvement of the region closely related to q11-22 being reported in six patients so far (q11-22, q12-23, q12-25, q12-21.3, q12-22, and q11-25). All these six cases are de novo constitutional disorders characterised by dysmorphic syndromes or psychomotor retardation.
- Thus, this is the first report to show inherited proximal chromosome 1q duplication in a Chinese family.

underwent allogeneic peripheral blood stem cell transplantation on 24 July 2001 using a conditioning regimen of fludarabine, cyclophosphamide, and anti-thymocyte globulin and methotrexate prophylaxis for acute graft versus host disease. Neutrophil engraftment was achieved on day 29 with no evidence of acute graft versus host disease.

He had neurological deterioration first noted on day 32 with elated emotion and abnormal behaviour followed by rapid neurological deterioration with spastic quadriplegia, blindness, and aphasia. Magnetic resonance imaging of the brain showed extensive grade II-III leucoencephalopathy. CSF study at the same time isolated HSV1 by the PCR method. He was treated with 14 days' of high dose intravenous acyclovir with no improvement at the time of this report.

The patient's past medical history was essentially unremarkable. He was the third child in the family with an uneventful birth history and normal neurodevelopment. He studied at ordinary school with satisfactory academic achievement. Both his older brother and sister enjoyed good health with normal intelligence and academic ability. His mother was a housewife. His father worked as a trader in mainland China. There was no family history of malignancy, blood diseases, mental or motor retardation, epilepsy, or presence of dysmorphic features.

Figure 1 (A) G banded karyotype of the patient's BM cells at leukaemia remission. It shows a male karyotype with direct duplication of chromosome 1q between region q11-22 indicated by arrows [46,XY,dup(1)(q11q22) [17]]. (B) CGH profile of patient's peripheral blood DNA showing gain of 1q21-22. A sex mismatched experiment was carried out as an internal control and the results validated by loss of the X chromosome as expected in a male. The grey line in the middle is the baseline ratio (1.0). The red/left (loss) and green/right (gain) lines indicate the threshold values of 0.85 and 1.15. The green bar (far right) indicates gain of study DNA in that region. The value in the bracket of each frame shows the number of chromosomes analysed. (C) Metaphase FISH analysis using 1q22 locus specific (BAC) probe showed two signals on chromosome 1 with the duplication whereas only one signal could be discerned on the other one.

MATERIALS AND METHODS

G banding cytogenetic analysis

Preservative free heparin anticoagulated BM cells within an hour of collection were processed for direct chromosomal

Figure 2 The family pedigree showing the duplication of chromosome 1q. The karyotypes of the index patient (indicated by an arrow and full shading) and his family members are shown. The patient's mother and sister show the same 1q duplication. Their carrier status is indicated by half shading. These findings confirm the hereditary nature of the karyotypic aberration.

preparation and three day short term culture with and without synchronisation using standard techniques, as described previously.¹⁵ The peripheral blood cells were subjected to phytohaemagglutinin stimulated culture for three days before metaphase harvest without synchronisation. Metaphase cells were obtained and G banding was performed as previously described.¹⁵ At least 15 well spread metaphases were analysed and karyotyped according to the criteria of ISCN (1995).¹⁶

Comparative genomic hybridisation (CGH)

Total genomic DNA was extracted from the buffy coat isolated from peripheral blood using standard SDS-proteinase K treatment, followed by phenol/chloroform/isoamylalcohol extraction. As described previously,¹⁷ patient and normal female reference DNA were differentially labelled with biotin-16-dUTP and digoxigenin (dig)-11-dUTP (Roche Diagnostics, Mannheim, Germany), respectively, by nick translations. Biotin labelled patient DNA and DIG labelled normal reference DNA (1 µg each) were precipitated together with 70 µg of Cot-1 DNA (Gibco BRL, Gaithersburg, MD). The mixed probes were then dissolved in 12 µl of hybridisation buffer and applied to slides with human metaphase chromosome spreads. Hybridisation was carried out for three days in a 37°C incubator. Patient and normal reference DNA were detected by avidin-fluorescein isothiocyanate (FITC) and rhodamine conjugated anti-digoxigenin, respectively. Image acquisition was performed with a Zeiss (Jena, Germany) fluorescence microscope equipped with a cooled charge coupled device (CCD) camera. Averaged fluorescence ratios along each chromosome were calculated with a digital imaging system (ISIS3; Metasystems, Sandhausen, Germany). Ratio values were averaged from at least 15 metaphase cells for each case. Ratio values above 1.15 and below 0.85 were considered to represent chromosomal gains and losses, respectively.

Metaphase fluorescence in situ hybridisation (FISH)

Metaphases were prepared from the patient's peripheral blood. FISH analysis was performed according to standard procedures using a BAC clone B930F5 (CEPH, Paris, France) specifically locating at 1q22. BAC DNA was labelled by nick translation using dig-11-dUTP.

RESULTS AND DISCUSSION

Cytogenetic investigation performed on bone marrow aspirate and peripheral blood samples of the patient at the time of disease remission showed a male karyotype with proximal duplication of the long arm of chromosome 1 in all of the metaphases analysed (46,XY,dup(1)(q11q22)) (fig 1A) confirmed that this abnormality was a constitutional chromosomal disorder. However, this finding was not observed in the two previous BM cytogenetic analyses at the referring hospital. As no normal clone was found in our cytogenetic analysis of the index patient and the duplication carrying family members, the failure to detect the proximal 1q duplication in the referring hospital could not be attributed to the presence of mosaicism, but it does suggest that it could be easily missed during routine assessment. With the wider application of comparative genomic hybridisation nowadays, a more specific detection of duplication can be achieved in suspicious G banding cases.

Because CGH involves the simultaneous cohybridisation of equimolar amounts of patient and control DNA to normal metaphase chromosomes, any gain of material is likely to represent additional euchromatin rather than a stable alteration of chromosome conformation.^{17 18} Since the G banding results suggested that the duplication extended to the heterochromatic region near 1q11, to confirm that this duplication of 1q (q11q22) as evident from G banding involved euchromatic region on chromosome 1, CGH analysis, with Cot-1 DNA blocking the pericentromeric heterochromatic sequences, was performed. There was chromosomal gain in 1q21-22 but no other concomitant chromosomal gains or losses were found (fig 1B). The chromosomal gain was also confirmed by the finding of two signals on the chromosome 1 with the duplication and one signal on the other one, in the metaphase fluorescence in situ hybridisation analysis using 1q22 locus specific probe (fig 1C). Consistent with the G banding results, an enlarged centromeric region in the chromosome 1 with the duplication (not shown) was also observed in C banding analysis. Taken together, these data suggest that the duplication spanned between 1q11-22, but euchromatic gain essentially involved 1q21-22.

A karyotypic study of the family showed that both the mother and sister of the index patient also had the dup(1)(q11q22), while the older brother, who was the donor for the stem cell transplantation of the patient, showed a normal karyotype (fig 2). As a constitutional genetic disorder, 1q duplications more commonly involve the distal half of the long arm and have been seen frequently in patients with multiple visceral malformations, dysmorphic features, and psychomotor retardation.^{19 20} The majority are believed to be the result of unbalanced segregation of inherited 1q translocations and about 20% are sole trisomy 1q, which appears incompatible with live birth.^{19 20}

A review of these cases suggests that duplication of 1q25-32 may signify a poor prognosis.²⁰ Proximal duplication of 1q is rare with involvement of regions closely related to q11-22 being reported in six patients (q11-22, q12-23, q12-25, q12-21.3, q12-22, q11-25) so far.²¹⁻²⁶ A majority showed mosaicism with the presence of variable proportions of the normal clone. All the six cases were characterised by multiple dysmorphic features, congenital malformations, or psychomotor retardation. The male to female ratio was 1:2 and age at diagnosis between birth and 18 years of age. All the parents of the patients were normal, healthy, and unrelated. The patients' mother and also their father if available for analysis showed a normal cytogenetic picture, confirming that they were de novo constitutional disorders.²¹⁻²⁶ However, no associ-

ation with malignancies was observed. By contrast, our current patient had a normal phenotype without any of the abnormal phenotypic features observed in previous cases of proximal 1q duplication but did have T lymphoblastic lymphoma/leukaemia, which has not been associated with proximal 1q duplications, constitutional or somatic.

It can be postulated that duplication of a chromosomal region may have two consequences, the amplification or over-expression of growth or physiological regulatory genes through gene dosage effects or disruption of the promoter or coding function of these genes at chromosomal breakage sites. In this family, there have been no manifestations of the underlying chromosomal duplications in terms of aberrations of growth and development. By contrast with heterochromatic variation, euchromatic imbalance usually has a phenotypic impact with few exceptions.^{21 22} Bortotto *et al*²⁷ reported a duplication of 1q42.11-q42.12 in a mother and her son, both of whom apparently had no phenotypic manifestations. Zaslav *et al*²⁸ also described the transmission of euchromatic gain in 1p in a family of a phenotypically normal mother and her male infant. Previously, it has been shown that the absence of phenotypic effect in euchromatic gain may be the result of imprinting, which is related to the type of parental transmission. More recent studies have also shown that increased copy numbers of pseudogenes or dosage insensitive genes may underlie euchromatic duplication or variation, thus providing a novel explanation for the absence of phenotypic effects in some chromosomal imbalances.^{18 29}

In our index patient, the association with T lymphoblastic lymphoma/leukaemia may be related to his duplication or just incidental. Disruption of 1q21 is very frequent in B lineage lymphoma and the *MUC1* oncogene has recently been implicated.^{14 30} However, only very limited data are available for T lymphoblastic lymphoma/leukaemia, where deletion of the short arm of chromosome 1 with rearrangement of the *TAL1* gene (1p34) is more common. Duplication of 1q or rearrangement of 1q21 has not been reported in T lymphoblastic lymphoma so far. Apart from the CD1 gene cluster on chromosome 1q, the recent identification of *SH2D2A* encoding the T cell specific adapter protein at 1q21 may be relevant as subsequent studies showed that this protein had a predominantly nuclear localisation and could function as a potential transcription control protein in T cells.^{31 32} Whether and how this could be related to T cell lymphomagenesis is questionable at this stage. Alternatively, the association of the constitutional proximal 1q duplication with the T lymphoblastic lymphoma in our patient is purely coincidental as both the patient's mother and sister, harbouring the same genetic abnormality, appear unaffected.

To our knowledge, this is the first published description of a family with proximal 1q duplication and may provide additional insight into the pathogenesis of the constitutional proximal 1q duplication syndromes.

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Authors' affiliations

N P H Chan, M H L Ng, S H Cheng, K S Tsang, T T Lau, Departments of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong SAR, China
V Lee, C K Li, Department of Paediatrics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong SAR, China

Correspondence to: Dr M H L Ng, Haematology Section, Department of Anatomical and Cellular Pathology, The CUHK, PWH, Shatin, NT, Hong Kong SAR, China; margareting@cuhk.edu.hk

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