Letters to the Editor

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Breakpoint mapping by FISH in a Sotos patient with a constitutional translocation t(3;6)

EDITOR—Involvement of region 3p21 of chromosome 3 in the development of Sotos syndrome is suggested by two patients who have been described. One is a Sotos syndrome patient carrying an apparently balanced translocation t(3;6)(p21;p21). The other is a non-smoking female with Sotos syndrome who died of small cell lung cancer at 22 years of age. This type of cancer is characterised by a consistent loss of heterozygosity at 3p21.3. We were able to set up an EBV immortalised lymphoblastoid cell line from a blood sample from the patient with the t(3;6) and show by fluorescent in situ hybridisation that the translocation breakpoint on the short arm of chromosome 3 does not coincide with any of the regions on this chromosome arm that have been suggested to play a role in tumour development.

In 1964 Sotos *et al*[1] described five children with large body size and early accelerated growth, acromegaloid features, advanced bone age, and a non-progressive neurological disorder with mental retardation. Since this report, many cases have been described of what is now known as

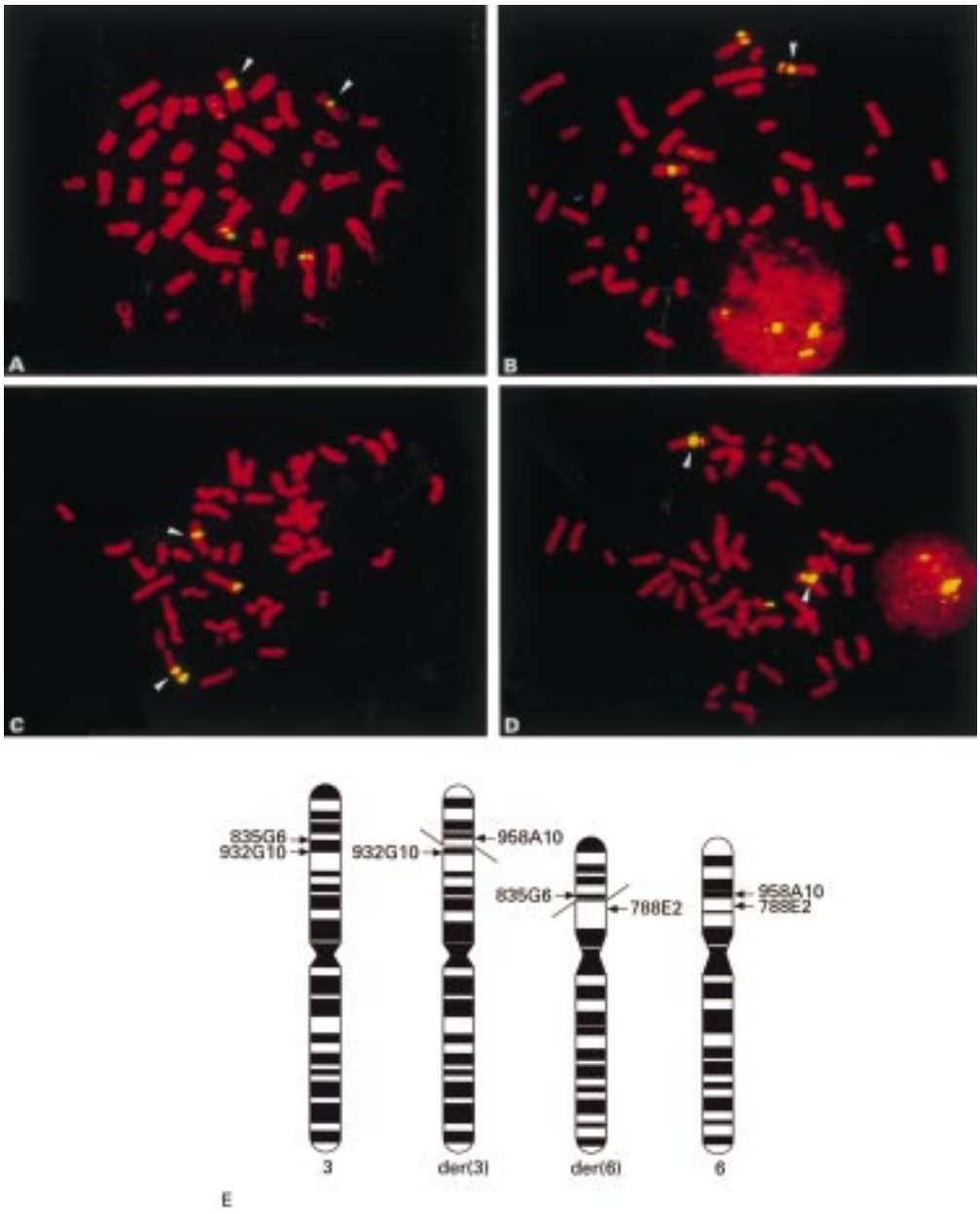

Figure 1 Fluorescent in situ hybridisation of metaphase spreads of the cell line derived from the patient with a chromosome 6 centromere probe, indicated by arrowheads, in combination with CEPH-YACs 932G10 (A) and 835G6 (B) from 3p22, and CEPH-YACs 958A10 (C) and 788E2(D) from 6p21. (E) Schematic representation of the translocation. The position of the flanking YACs is indicated.

Sotos syndrome or cerebral gigantism.[2] Most cases appear to be sporadic. There are some reports of familial clustering, including pedigrees compatible with an autosomal dominant mode of inheritance,[3 4] but a clear cut mode of inheritance has not yet been established. A number of Sotos patients have subsequently been diagnosed with fragile X, possibly owing to seemingly overlapping clinical characteristics of the two syndromes.[5] A Sotos-like phenotype can also be found in patients with acquired hypothalamic lesions. This has led to the assumption that Sotos syndrome can be regarded as an early onset overgrowth syndrome which can be caused by different aetiological factors. Therefore, the designation Sotos sequence might actually be more appropriate than Sotos syndrome.[6] Cytogenetic analysis has shown de novo chromosome abnormalities in some patients.[7 8] The chromosomal abnormalities appear to be inconsistent, and their association with Sotos syndrome may, therefore, be coincidental. Two case reports, however, suggested a possible involvement of the region p21 of chromosome 3. The first described a 6 year old boy with clinical features consistent with Sotos syndrome, such as mental retardation, postnatal overgrowth, and facial dysmorphism, with an apparently balanced translocation t(3;6)(p21;p21).[9] The other report described a non-smoking female with Sotos syndrome who died of small cell lung cancer (SCLC) at the unusually young age of 22.[10] This type of cancer is characterised by a consistent loss of heterozygosity at 3p21.3.[11 12] Therefore, it could not be excluded that a single mutation might be responsible for both the clinical features of Sotos syndrome and the development of SCLC in this patient.

We wanted to investigate whether the 3p21 breakpoint of the balanced translocation described by Schrander-Stumpel *et al*[9] coincided with any of the deletion regions on 3p reported for lung cancer.[12] We established an EBV transformed lymphoblastoid cell line from this patient and confirmed by cytogenetic analysis the presence of a balanced translocation between chromosomes 3 and 6. A precise determination of the chromosome 3 breakpoint was obtained by fluorescent in situ hybridisation of YACs from the region 3p21-3p25 to metaphase spreads of this cell line. CEPH-YACs were selected from several databases accessible through the internet. Their localisation and possible chimerism were determined on normal human metaphases. The positions of the chromosome 3 YACs eventually used in our breakpoint analysis are indicated in the left hand diagram of fig 1E.

FISH analysis with the chromosome 3 YAC 932G10 in combination with a probe specific for the centromere of chromosome 6 showed a fluorescent signal on two chromosomes that did not carry a centromeric signal (fig 1A). Consequently, these chromosomes represent the normal chromosome 3 homologue and the derivative chromosome 3. Thus, the breakpoint on 3p lies distal to this YAC. Similarly, YAC 835G6, also from chromosome 3, was shown to hybridise to the normal chromosome 3 and to the derivative chromosome 6, indicating that this YAC maps to the other side of the breakpoint (fig 1B). The more proximal YAC 932G10 contains marker D3S1277, localised to 3p22. It maps distal to both the critical 3p21 region assumed to be involved in the development of cancer[12] and the 800 kb homozygous deletion reported by Murata *et al*[13] to occur in a lung cancer cell line. The distal YAC 835G6 contains the marker D3S1266 positioned proximal to THRB, and most probably also proximal to RARB. Thus, the chromosome 3 breakpoint in this Sotos syndrome patient, now mapped at 3p22-p23, does not coincide with any of the regions suggested to be involved in the development of lung or any other type of cancer. We also localised the breakpoint on chromosome 6. The position of the chromosome 6 YACs used in our breakpoint analysis are indicated in the right hand diagram of fig 1E. FISH analysis indicated the chromosome 6 YAC 958A10 to be distal to the breakpoint (fig 1C) and YAC 788E2 to be proximal (fig 1D). YAC 958A10 contains marker D6S422 mapping in 6p22. YAC 788E2 contains marker D6S1944 mapping at 6p21.3, close to, or even within, the HLA gene region.

A schematic presentation of the translocation is shown in fig 1E. A possible role of the t(3;6) in the development of Sotos syndrome in this patient has to await cloning of the breakpoint regions and identification of possible genes affected by the translocation.

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