Recognition and reanalysis of a cell line from a manifesting female with X linked hypohidrotic ectodermal dysplasia and an X;autosome balanced translocation

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SUMMARY We have restudied a fibroblast cell line from a female with marked manifestations of X linked hypohidrotic ectodermal dysplasia (HED) and a balanced X;9 translocation. Chromosome analysis showed a karyotype of 46,X,t(X;9)(q13·1;p24) with an Xq breakpoint distal to the one previously reported. The significance of the cell line, previously unrecognised, for the mapping and eventual cloning of the HED locus is discussed.

The identification of females with X;autosome balanced translocations and marked manifestations of X linked disorders has assumed increasing importance over the past few years. It has become evident, as in the case of Duchenne muscular dystrophy (DMD), that such rare patients may not only supply a vital clue to the detailed localisation of the disorder, but in addition may assist in the actual cloning of the gene locus itself.^{1,2} It has recently been shown in DMD that the females with manifestations of the disorder have the breakpoints of their translocations within the disease locus.³ This is consistent with the theory that such translocations disrupt one normal allele in the formation of the derivative chromosomes and, with the usual preferential inactivation of the normal X chromosome in balanced X;autosome translocations, produces a phenotype similar to that of an affected hemizygous male.

Several groups have been involved in the regional mapping of the X linked disorder hypohidrotic ectodermal dysplasia.⁴⁻⁶ We have recently found, through linkage studies using restriction fragment length polymorphisms in 36 families, very close linkage of the disorder to three pericentromeric marker loci (*DXS159*, *PGK1*, and *DXS72*) physically localised to the Xq11-q21·1 region.⁷ However, we have been unable to order the disease and

marker loci because of a relatively limited degree of genetic recombination between the loci in the pericentromeric region. The fine subregional localisation of the disorder will depend on physical mapping of the disorder, assisted by the study of either affected males with cytogenetic or molecular deletions (none identified to date), or of manifesting females with X;autosome balanced translocations.

The sole X;autosome balanced translocation with manifestations of HED known to exist is a patient verbally reported at the first Human Gene Mapping Conference (1974) by the late Dr Peter Cook from the United Kingdom. The only published confirmation of this fact is in a footnote to the proceedings of the conference, but no clinical description of the patient was provided.⁸ The patient had been previously studied in the United States by Cohen *et al.*,⁹ ascertained at the age of six months because of failure to thrive without recognition of her manifestations of HED. She was reported as part of a study of the patterns of X chromosome inactivation in X;autosome translocations, with a karyotype of 46,X,t(9p+;Xq-).

Attempts have been made by our group and others to trace this patient, apparently lost to follow up, in order to establish a cell line for molecular studies, both for gene mapping purposes and for the eventual cloning of the gene itself. We wish to report the 'rediscovery' of a fibroblast cell line from this patient, to present relatively high resolu-

tion chromosome studies, and to draw attention to extremely useful published somatic cell gene mapping studies, which have used hybrids constructed from this cell line.

Methods

CYTOGENETIC ANALYSIS

Cytogenetic studies were undertaken on a fibroblast culture obtained from the NIGMS Human Genetic Cell Repository (GM0705),¹⁰ established from the patient originally described by Cohen *et al.*⁹ This cell line is also referred to as the AnLy line. Chromosome preparations were obtained using conventional techniques except that exposure to colcemid was restricted to 30 minutes in order to obtain chromosomes suitable for high resolution studies. GTG banding was accomplished using a modification of the method of Seabright.¹¹ Late replication studies were performed using a modification of the method of Perry and Wolff,¹² whereby BrdU (final concentration 100 µg/ml) was added for the last five hours of culture. Slides were stained in Hoechst 33258 (10 µg/ml deionised water), exposed to ultraviolet light for 15 minutes, and incubated in 2×SSC at 60°C for five minutes, before staining in 10% Giemsa.

Results

CYTOGENETIC STUDIES

Examination of the G banded preparations confirmed the presence of an X;9 balanced translocation. The breakpoints were at the junction of bands Xq12 and Xq13·1, or in the proximal region of Xq13·1 and at band 9p24, respectively (figs 1 and 2). There appears to be no reduction in the width of band Xq12 on the derivative X chromosome, nor is any dark band apparent at the junction of X and 9 chromosomal material on the derivative 9. The karyotype, according to conventional nomenclature,¹³ was thus designated: 46,X,t(X;9)(Xpter→Xq13·1::9p24→9pter;9qter→9p24::Xq13·1→Xqter). RBG banding showed the normal X chromosome to be late replicating in each of the 28 divisions examined. There was no evidence of late replication of either of the translocation products.

Discussion

The early clinical history of the patient initially reported by Cohen *et al.*⁹ is consistent with the type of clinical problems encountered in male infants with HED.¹⁴ During the first six months of life, she had frequent respiratory tract infections, feeding problems requiring a gastrostomy, and an unusual

FIG 1 GTG banded partial karyotypes from three cells showing the products of the balanced translocation $t(X;9)(q13·1;p24)$ and the normal homologous chromosomes.

facial appearance with frontal bossing and fine, sparse hair. It is not surprising that the correct diagnosis was not made at the time, since the diagnosis is frequently delayed in male infants when they are sporadic cases. Chromosome studies on her parents and sibs were normal. Recently, the past medical and dental records (including photographs) from the patient's childhood in the United Kingdom have been reviewed (K MacDermot, 1987, personal communication), and they confirm Cook's original observations of a female with marked manifestations of hypohidrotic ectodermal dysplasia.

Recently, one of the authors (JZ) noted, while reviewing the proceedings of the first Human Gene Mapping meeting, a report by Shows and Brown¹⁵

FIG 2 Diagram of the $t(X;9)(q13.1;p24)$ translocation showing the breakpoints (arrows) in the normal chromosomes and the translocation products.

on the localisation of several gene loci to the long arm of the X chromosome by the then relatively new method of somatic cell hybrid mapping. In this study, they constructed hybrid lines from the patient previously reported by Cohen *et al.*⁹ A check of the catalogue from the NIGMS Human Genetic Cell Repository showed that J A Brown had deposited a fibroblast line from this patient in 1974. The only clinical description of the patient listed by the repository (to the present day) is "failure to thrive".¹⁰ The first two letters of the first and last names of the patient from whom the cell line was established (AnLy) are identical to those of the patient originally diagnosed as having HED by Cook. In addition, the UK hospital records of this patient reiterate the patient's previous admissions to hospital and evaluations reported by Cohen *et al.*⁹ (K MacDermot, 1987, personal communication). These observations confirm that the AnLy cell line is from a female with severe manifestations of X linked hypohidrotic ectodermal dysplasia.

Shows and Brown^{15 16} originally placed the breakpoint of the translocation at the junction of the Xq11 and Xq12 bands, describing the karyotype as $46,X,t(Xq-;9p+)(q12;p24)$. Our reanalysis of the cell line, with higher resolution chromosome studies, defines a slightly more distal breakpoint, at the junction of the Xq12 and Xq13.1 bands, or in the proximal region of Xq13.1. We confirmed the earlier finding (as expected) of inactivation of the normal X chromosome in all cells examined, but did not observe evidence of additional late replication of the Xq portion of the derivative chromosome 9, as initially reported by Cohen *et al.*⁹ in 32% of cells.

A number of investigators have continued to use the AnLy cell line, without apparent recognition of the patient's true phenotype, in the construction of somatic cell hybrids to localise both expressed genes and anonymous DNA marker loci.¹⁷⁻²⁰ The wide use of somatic cell hybrids, derived from the AnLy line, by several groups physically mapping the pericentromeric region of the X chromosome, pro-

vides us with useful information on placing loci either proximal or distal to the translocation breakpoint.

Under the assumptions that the AnLy translocation physically disrupts the HED locus, and that there have not been any significant rearrangements in the somatic cell lines, we are now able to propose the order *cen-DXS159-HED-PGK1-DXS72* for HED and the tightly linked DNA marker loci.⁷ For example, we have found close linkage of *HED* and the *PGK1* locus ($\theta_{\max}=0.02$), but were unable to order the loci by multipoint analysis. However, two groups have localised the *PGK1* locus distal to the AnLy breakpoint; thus we would place the *HED* locus proximal to the *PGK1* locus.^{15, 17} Similarly, Arveiler *et al*²⁰ have mapped the *DXS159* locus proximal to and *DXS72* distal to the AnLy breakpoint. The ability to determine flanking markers is of crucial importance in the clinical application of linkage data for both carrier detection and prenatal diagnosis of X linked hypohidrotic ectodermal dysplasia.

The recognition of the full significance of the AnLy cell line should provide further opportunities for molecular studies. The location of the marker loci in relation to the translocation breakpoint, previously established by somatic cell studies, can be confirmed by in situ hybridisation studies on the fibroblast line, eliminating the problem of possible de novo chromosomal rearrangements in the somatic cell hybrids. Finally, although no simple task, the possibility now exists of isolating the junctional fragments from the derivative chromosomes, identifying RNA transcripts from these clones, and ultimately discovering the gene product necessary for normal ectodermal morphogenesis.

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