of cytochrome b_{-245}: it has a dissimilar amino-acid composition from the β subunit[9] and its predicted M_r of 54K is more than twice that of the α subunit. It could be another redox component of the oxidase system, although it does not have an obvious haem[20] or nucleotide[21] binding site, or an associated structural protein. If, as they claim, the gene they have cloned is the site of the primary lesion in X-linked CGD, the identification of the gene product in question will be required in order to understand its relationship to the phenotypic absence of the protein subunits of cytochrome b_{-245} described here.

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Linkage of an X-chromosome cleft palate gene

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Many congenital malformations, such as cleft palate and neural tube defects, have a multifactorial origin involving both environmental and genetic factors. Conditions such as these may be exclusively monogenic, polygenic or environmental, but in most cases both genetic and environmental factors are involved[1]. This study describes the sub-chromosomal localization of a single gene defect causing cleft palate and ankyloglossia (tongue-tied) in a large Icelandic family. This defect is a model for the analysis of other neural-crest malformations that show a more complex multifactorial inheritance pattern.

Human secondary cleft palate (CP) occurs during the seventh to ninth weeks of embryological development when the lateral palatine shelves fail to fuse[2]. This malformation shows little variation between populations; the incidence is ~1 in 1,500[3]. CP is more prevalent in females than in males[4], which may be because the palatine processes fuse one week later in females[5], allowing more time for teratogenic agents to play a part in the potential for malformation in females.

The analysis of single gene mutations using restriction fragment length polymorphisms (RFLPs) for linkage studies has been successful in determining the chromosomal location of several common inherited disorders. Linkage to within 5 centimorgans has been detected, both in cases where the affected chromosome is known, as for the sex-linked disorders Duchenne muscular dystrophy[6] and chronic granulomatous disease[7], and when neither the autosome nor the biochemical defect is identified, as for Huntington's disease[8] and cystic fibrosis[9-11]. However, the analysis of polygenic disorders by linkage studies with RFLP markers is at present more complex both theoretically and practically.

One way to learn more about the aetiology of disorders with complex combinations of genetic and environmental factors is to use as a model a family in which the phenotype is due to a single gene defect, but displays the same features as more common multifactorial sporadic cases. Such a model for midline congenital defects has been found in a large Icelandic family

Table 1 X-chromosome probes that show linkage to the CP+A locus: their localization, polymorphism and lod scores at various recombination fractions (θ).

Probe	X-chromosome location	Polymorphism-enzyme	lod at θ value 0	0.01	0.05	0.10	0.20	0.30	0.40
L1.28	p11.3	*Taq*I	0.38	0.37	0.36	0.33	0.27	0.19	0.10
p58.1	p11-cen	*Msp*I	0.65	0.65	0.64	0.62	0.52	0.38	0.21
cpX203	p1.1	*Bgl*II	$-\infty$	−0.33	0.22	0.40	0.46	0.37	0.21
pDP34	q13-q21	*Taq*I	3.07	3.02	2.81	2.53	1.92	1.28	0.62
X65H7	q13-q22	*Hin*dIII	$-\infty$	−0.03	0.92	1.06	0.97	0.71	0.38
7b	q13-q22	*Pst*I	$-\infty$	−0.53	0.18	0.37	0.44	0.36	0.21

All likelihoods and lod scores were calculated with the LINKAGE computer program package[20]. Individuals with either cleft palate and/or ankyloglossia were considered to be uniformly affected (that is both phenotypes combined to one 'affection status') and the maximal likelihood estimate for the penetrance (t) of this single gene for 'carrier females' was calculated to be 82%. The lod scores (Z) were calculated according to Ott[21]: $Z(\theta) = \log \text{likelihood}\ (\theta, t) - \log \text{likelihood}\ (1/2, t)$, where $t = 0.82$. The frequency for the CP+A mutant allele was taken as 0.0001. Likelihoods calculated at a range of penetrances, RFLP and CP+A allele frequencies made no significant difference to the quoted lod scores (data available on request). Respectively 3, 6, 4, 8, 6 and 5 meioses contributed most of the linkage data for probes L1.28, p58.1, cpX203, pDP34, X65H7 and 7b (for probe localization see ref. 22).

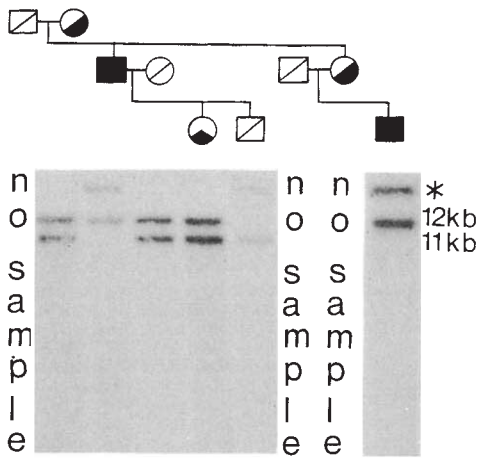

Fig. 1 *Taq*I-digested DNA samples from the pedigree are shown hybridized to the anonymous linked pDP34 marker. Section of the Icelandic family showing the X-linked inheritance of CP and A (⊠ or ⊘, unaffected ♂ and ♀; ■, affected ♂ CP+A; ◕, affected ♀, A alone; ◒, obligatory carrier; *, Y-specific band).
Method. Genomic DNA was prepared from 10 ml of whole blood[23]. DNA (4 μg) was digested with the relevant restriction enzyme revealing the polymorphism for each X-chromosome probe. The digested DNA was fractionated by electrophoresis on 1% agarose gels and transferred to Hybond-N membranes (Amersham)[24]. The probes used were labelled to a specific activity of 1×10^9 d.p.m. μg^{-1} by synthesis using random oligonucleotide primers. The filters were washed down to a final salt concentration of 0.1×SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate) in the presence of 0.1% SDS at 65 °C. Autoradiography was for 24 h at −70 °C with an intensifying screen.

(over 200 members) showing mendelian inheritance of X-linked secondary cleft palate and ankyloglossia ('tongue-tied') (CP+A). Both the large size of this pedigree and the availability of many defined X-chromosome probes has made it possible to localize this defect sub-chromosomally.

A section of the pedigree of 293 individuals in the family studied is shown in Fig. 1; three of us (A.B., A.A. and O.J.) have personally investigated 182 individuals in four living generations, and information on other family members was gained either from written or family records[12]. (Full pedigree data are available from the authors on request.) Blood was collected from 82 individuals for RFLP genotyping, including 9 males with secondary cleft palate and ankyloglossia (CP+A) and 10 females with ankyloglossia alone (A). One female had CP alone and one male had a patent but high-vaulted palate (HVP) characteristic of those affected. For purposes of linkage analysis each family member was designated affected if they had any of the features diagnostic for CP, A or HVP.

Of the 14 matings where a CP+A father has the opportunity to pass a mutant gene to his son, the son is never affected. This suggests that this gene is sex-linked. Occasionally, an unaffected female has affected sons and daughters, and is postulated to be a carrier; the maximal likelihood estimate for the penetrance of the mutation is calculated to be 82% (Table 1).

The analysis of inheritance of RFLPs was carried out using standard techniques (Fig. 1). Linkage at a lod score of 3.07 has been demonstrated between the CP+A locus and the locus *DXYS1* of the anonymous DNA probe pDP34, which maps to Xq13-Xq21[13] (Table 1). Further linkage analysis of the loci defined by an additional 14 informative probes has allowed exclusion of most of the rest of the X chromosome.

Localization of the mutation causing cleft palate in this family is a first step in understanding the genetic component of congenital neural tube and crest defects. This region of the X chromosome contains an X-Y homologous region[14]; the availability of many X-Y homologous probes should permit the fine mapping of the defect. As the limits of genetic mapping in this family are approached the techniques of cosmid walking and jumping and pulsed-field gel electrophoresis[15,16] will allow the gap between the genetic and physical map to be bridged.

The isolation of the gene and studies of its spatial and temporal expression during development of the secondary palate should lead us to a greater understanding of mechanisms which may lead to the failure of closure of the neural crest. This mechanism can be compared with those which may cause sporadic cases in humans, or in animal models. There are also families known in which an X-linked form of spina bifida and anencephaly occur[17]; any homology in linkage between these families and the Icelandic family would be of interest.

Many disorders have both genetic and environmental elements. Polygenic and/or multifactorial disorders are complex to analyse, as has been elegantly shown for the role of LDL receptor defects in atherosclerosis[18]. Eventual cloning of the 'cleft palate' gene could be used as a starting point for the analysis of the genetic element of this developmental abnormality. For example, this gene may be a member of a multigene family[19] involved in the embryonic development of other tissues. Analysis of CP+A as a sex-linked single gene could provide a model for identifying other genes regulating processes in embryonic development whose expression is usually hidden in phenotypic, polygenic complexity.

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