*Am. J. Hum. Genet. 48:274–280, 1991*

# Association of Treacher Collins Syndrome and Translocation 6p21.31/16p13.11: Exclusion of the Locus from These Candidate Regions

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## Summary

**Treacher Collins syndrome (TCS) is an autosomal dominant defect of craniofacial development which has not been chromosomally localized. We have identified a mother and two children who have TCS and also a balanced translocation t(6;16)(p21.31;p13.11), which suggested the possibility that the TCS locus might be located at one of the translocation breakpoints. These were defined by in-situ hybridization as 6p21.31 (by using loci in the HLA complex defined by the probes p45.1DPbeta003/HLA-DPB2 and pRS5.10/HLA class I chain) and 16p13.11 (by using probes pACHF1.3.2/D16S8 and VK45/D16S131). Pairwise and multipoint linkage analysis using localized chromosome 6 probes and chromosome 16 probes in 12 unrelated TCS families with multiple affected siblings excluded the TCS locus from proximity to both translocation breakpoints. These data were confirmed when a third affected child, who did not exhibit the translocation, was born to the mother.**

## Introduction

Treacher Collins syndrome (TCS) is an autosomal dominant defect of craniofacial development (Rovin et al. 1964; Frazen et al. 1967) which affects approximately 1/50,000 live births. Sixty percent of cases arise as new mutations. The features of TCS include (1) abnormalities of the pinnae which are frequently associated with atresia of the external auditory canals and anomalies of the middle-ear ossicles (bilateral conductive deafness is therefore common) (Phelps et al. 1981), (2) hypoplasia of the facial bones, particularly the mandible and the zygomatic complex, (3) antimongoloid slanting of the eyes, with colobomata of the lower eyelids and a paucity of lid lashes medial to the defect, and (4) cleft palate. There is usually a reasonable degree of bilateral symmetry in these features. The expression of the gene is variable, and occasionally some individuals are so mildly affected that it is difficult to diagnose TCS. However, the gene is rarely nonpenetrant.

Since the tissues affected by TCS arise from the first and second branchial arches during early embryonic development, it has been proposed that the condition may result from a neural crest cell abnormality. However, the underlying genetic defect is unknown.

Genetic linkage analysis has previously proved successful in identifying the chromosomal location of other craniofacial anomalies in which the underlying biochemical defect is not known (Moore et al. 1987; Brueton et al. 1988; Murray et al. 1990). Cytogenetic abnormalities have been useful in directing attention to "candidate regions" in such studies (Tommerup and Nielson 1983; Bocian and Walker 1987).

We report here the identification of a mother and two children who exhibit concordance between TCS and a cytogenetically balanced translocation t(6;16)-(p21.31;p13.11). This suggests that the TCS locus

Received July 5, 1990; revision received September 21, 1990.

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0002-9297/91/4802-0012$02.00

might be at either of these chromosomal locations. Linkage analysis, however, excluded the TCS locus from both candidate regions. These findings were subsequently confirmed by the birth of a third child, who, despite being affected by TCS, did not exhibit the translocation.

## Subjects and Methods

### *Families*

The translocation family was identified by one of us (E. H.). The 12 families used for linkage analysis were identified by writing to consultant clinical geneticists throughout the United Kingdom and United States. All patients were examined by experienced clinicians and scored as affected if they presented with the clinical signs noted above. Venous blood samples for DNA preparation were taken from 100 individuals, 56 of whom were affected.

### *Cytogenetic and In-Situ Hybridization Analysis*

Short-term lymphocyte cultures were established and synchronized using a thymidine block and deoxycytidine release for karyotyping (Wheater and Roberts 1987). Probes p45.1DPbeta003 (ATCC), a 1.7-kb unique fragment in pcDV1-pL2; pRS5.10 (supplied by Dr. J. Pan), a 700-bp unique fragment in pBR322; VK45, a 21.3-kb insert in lambda phage, and pACHF-1.3.2, a 2.2-kb unique fragment in pSP64, were tritium labeled to a specific activity of approximately $3.5 \times 10^8$ cpm/μg according to a method described elsewhere (Callen et al. 1988).

Each of the four probes was hybridized to metaphase chromosomes from the mother (II.6), at concentrations ranging from 0.05 to 0.2 μg/ml for periods of as long as 3 wk. Probe VK45 contained repeat elements and was preassociated to an excess of unlabeled DNA before hybridization (Callen et al. 1988). All silver grains touching the chromosomes were counted to determine the pattern of hybridization of each probe.

### *DNA Analysis*

Genomic DNA was prepared from peripheral blood leucocytes (Kunkel et al. 1977). Five micrograms of DNA was digested with the restriction enzyme revealing the RFLP for the probe in question (table 1), according to the conditions specified by the manufacturer. The digested DNA samples were fractionated by agarose gel electrophoresis in tris/acetate buffer by using 0.7%–1.0% gels, were transferred to Hybond-N+ membrane (Amersham) by standard methods (Sambrook et al. 1989), and were hybridized with DNA probes radiolabeled by the random primer method (Feinberg and Vogelstein 1983) at 65°C. Final membrane washes were in 0.2 × SSC at 65°C for 30 min. Autoradiography was performed at −70°C with double intensifying screens for 1–4 d by using Fuji RX film.

**Table 1**

**Description of Linkage Markers Ordered pter-cen**

| Probe | Locus[a] | Polymorphic Enzyme(s) | Map Position | Reference |
|---|---|---|---|---|
| Chromosome 6: | | | | |
| F13a | F13A1 | *Bam*HI, *Bcl*I | 6p24-25 | Zoghbi et al. 1988 |
| HLA DQα | pDCH1 | *Taq*I | 6p21.3 | Auffray et al. 1982 |
| Chromosome 16: | | | | |
| D16S8 | ACHF1.1A6 | *Pvu*II | 16p13.13-13.12 | Kidd et al. 1989 |
| D16S96 | VK20A | *Taq*I, *Msp*I | 16p13.12-13.11 | Kidd et al. 1989 |
| D16S96 | VK20B | *Msp*I | 16p13.12-13.11 | Kidd et al. 1989 |
| D16S79 | 36.1 | *Taq*I, *Xmn*I, *Hin*cII, *Bcl*I | 16p13.12-13.11 | Kidd et al. 1989<br>Gedeon et al. 1989 |
| D16S131 | VK45C6 | *Taq*I | 16p13.11-12.3 | Kidd et al. 1989 |
| D16S75 | R99.6 | *Hin*dIII | 16p13.12.3 | Kidd et al. 1989 |

[a] As detailed in the references, with the exceptions of R99.6 (D16S75), which is a 2-kb single-copy fragment of the CRI-R99 probe listed by Kidd et al. (1989) that is subcloned into the *Eco*RI/*Hin*dIII site of pBR322, and ACHF1.1A6, a 1.2-kb *Eco*RI/*Hin*dIII fragment of the phage ACHF1 listed by Kidd et al. (1989) that is subcloned into pUC18 (a 600-bp *Bst*NI-*Bst*NI single-copy fragment of the insert was used as the probe).

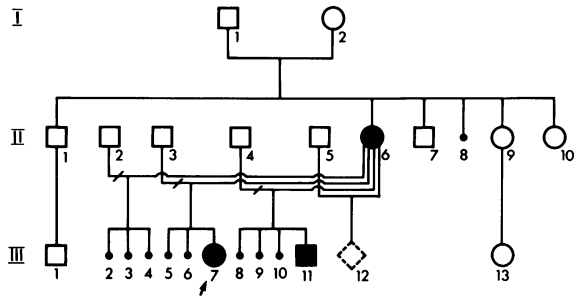

**Figure 1** Pedigree of family. Individuals II.6, III.7, and III.11 have both Treacher Collins syndrome and a cytogenetically balanced translocation 6;16.

### *Linkage Analysis*

RFLPs were scored, and the data were coded in linkage format. Pairwise and multipoint linkage analyses were performed using the LINKAGE program (Lathrop et al. 1984). A gene frequency of .0001 was assumed for the mutant allele. Penetrance was taken to be 99%. Standard significance cutoff points were used. In the case of the chromosome 6 loci the results of the pairwise analyses were graphically interpolated, and the genetic distances corresponding to a lod score of $-2.0$ were taken to be the extent of exclusion of the TCS mutation(s) around each locus.

## Results

The pedigree of the family exhibiting the translocation is shown in figure 1. The proband, III.7 (figs. 2*B* and 2*E*), was diagnosed as having TCS at 6 wk of age. Her karyotype was 46,XX,t(6;16)(p21.31;p13.11). The proband's half-brother, III.11 (figs. 2*C* and 2*F*), was also diagnosed as being affected and exhibited the same translocation. The children's mother, II.6 (figs. 2*A* and 2*D*) appeared to have no features of TCS on clinical examination. However, orthopantomogram and occipitomental radiographs revealed hypoplasia of the zygomatic arches and prominent antegonial notching of the mandible (figs. 2*G* and 2*H*). Her karyotype, determined following the discovery of the translocation in her daughter, was also 46,XX,t-(6;16)(p21.31:13.11)(fig. 3*A*). Both the mother's parents, as confirmed by genetic testing, were unaffected and had normal karyotypes. It was not possible to contact either of the fathers of the affected children; however, from photographs both appeared clinically normal.

The translocation breakpoints at 6p21.31 and 16p13.11 were further localized by in-situ hybridization to metaphase chromosomes by using probes known to map to these two regions (table 2). The probe concentrations and times of exposure were chosen to maximize the signal on the translocated chromosomes, and this resulted in a high number of background grains on the remainder of the karyotype.

To define the breakpoint on chromosome 6 two probes were used: p45.1DPbeta003, which defines a class II DP chain at the proximal extremity of the HLA complex (Spence et al. 1989), and pRS5.10, which defines a class I chain at the proximal extremity of the HLA complex (Srivastava et al. 1987). Results from in-situ hybridizations with the p45.1DPbeta003 probe showed an excess of grains on the normal short arm of chromosome 6 and on the short arm of the der(6) t(6;16), while the number of grains on 16p and on the short arm of the der(16)t(6;16) were consistent with the background distribution of grains on the remainder of the chromosome complement. Results with probe pRS5.10 showed an excess of grains on the short arm of the normal chromosome 6 and on the der(16); that is, this probe was translocated to the derived chromosome 16. It can be concluded that the breakpoint on 6p lies between the chromosomal regions defined by these two probes, proximal to pRS5.10 but distal to p45.1DPbeta003.

To define the breakpoint on chromosome 16 two probes were used, VK45 (D16S131), which was not relocated by the translocation but remained on chromosome 16, and pACHF1.3.2 (D16S8) which was relocated onto chromosome 6 by the translocation. The breakpoint on chromosome 16 therefore lay between the chromosomal regions defined by these two probes.

Pairwise lod scores from families with TCS are summarized in table 3. The data in table 3*A* exclude the site of the TCS mutation from approximately 13 cM around the clotting factor 13A1 locus and from approximately 22 cM around the HLADQα locus. As these probes are within detectable linkage distance (25–35 cM) of one another, and as the length of the short arm of chromosome 6 is estimated at approximately 55 cM (Zoghbi et al. 1988), our data further exclude the TCS locus from most of 6p except for the telomere.

Similarly, we were able to exclude the TCS locus from close proximity to markers at 16p13.11 (table 3*B*). Multipoint linkage analysis was used to further exclude the TCS locus from approximately 30 cM

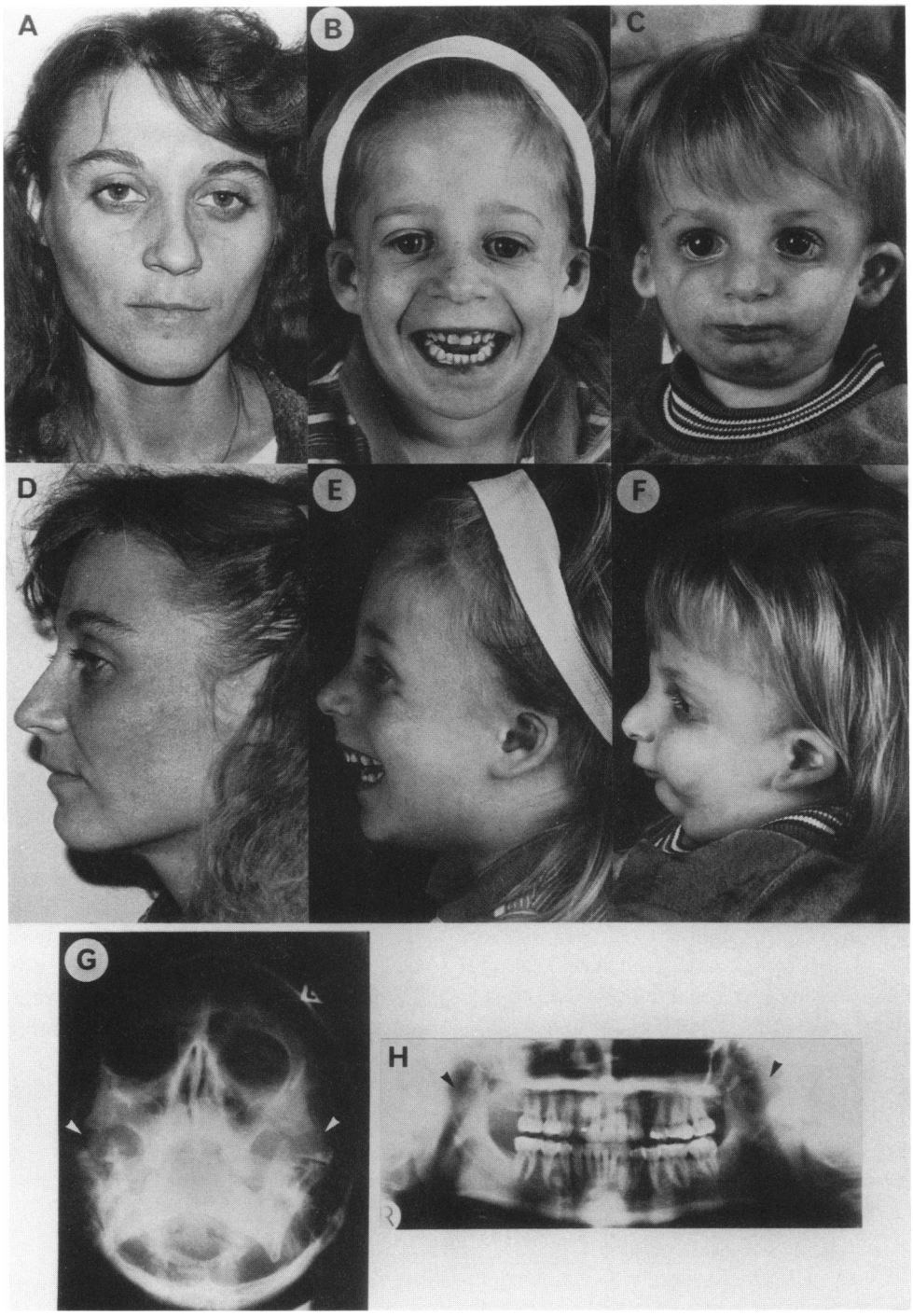


**Figure 2** Photographs of family (*A–F*) and radiographs showing absence of zygomatic arches (arrowed) in mother, II.6 (*G* and *H*).

around the translocation breakpoint at 16p13.11 (fig. 4). The anchor map was constructed using chromosome 16 RFLP typing from the CEPH reference pedigree panel. The linkage data were subsequently confirmed when the translocation mother gave birth to an affected child who was cytogenetically normal.

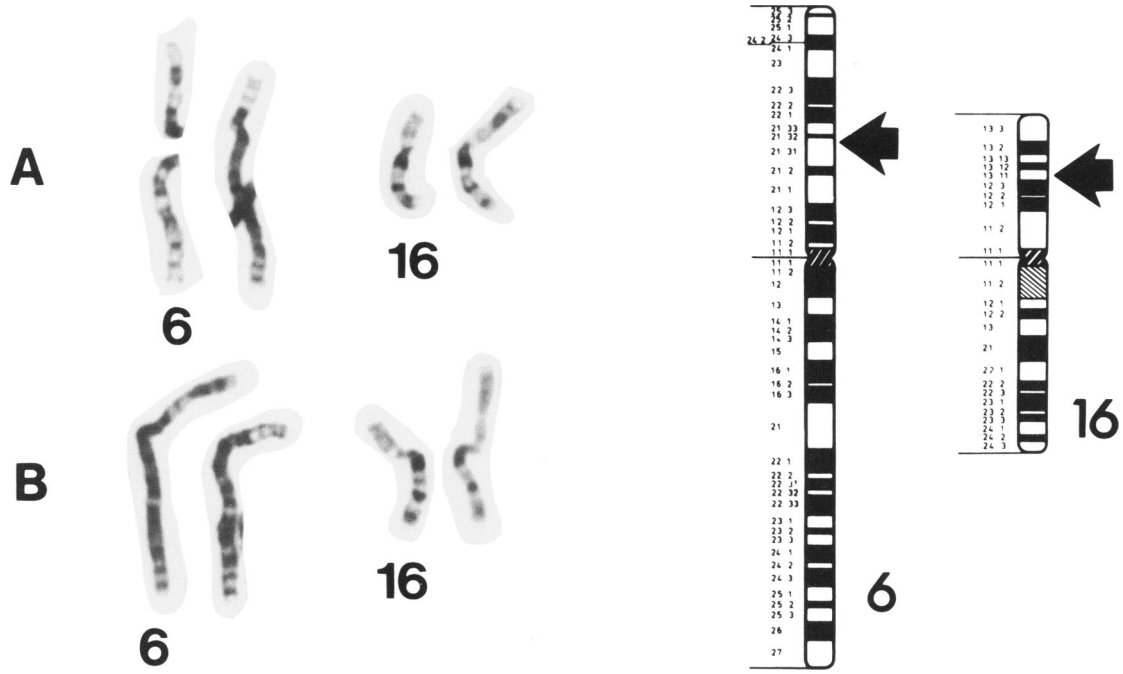


**Figure 3** Partial karyotype of translocation. Shown are two partial karyotypes (*A* and *B*) which, from left to right, show normal 6, der(6), normal 16, and der(16). The ideogram is of the two normal chromosomes with breakpoints indicated by arrows.

## Discussion

The genetic mutation(s) responsible for TCS is extremely variable in expression. Some individuals are so mildly affected that detection of obligate carriers is at times difficult. Rarely, the defective gene is nonpenetrant, although in the great majority of cases careful examination of the obligate carrier will reveal minor stigmata of TCS. This was the case for the translocation mother, who on routine clinical examination did not appear to exhibit any features of this disorder. Radiographic examination, by revealing bilateral hypoplasia of the zygomatic arches, permitted the mother to be correctly diagnosed as affected. This demonstrated concordance, at that time, between TCS and the cytogenetically balanced translocation between the short arms of chromosomes 6 and 16.

The translocation breakpoints were defined, cytogenetically and by in-situ hybridization, as 6p21.31

**Table 2**

**In-Situ Hybridization of Probes to t(6:16)**

| | Total No. of Grains | | | | | |
|---|---|---|---|---|---|---|
| | Chromosomal Region | | | | | |
| Probe | 6p[a] | der6p[b] | 16p[c] | der16p[d] | Remainder of Component[e] | No. of Metaphases |
| p45.1 DPbeta003 ..... | 20 | 19 | 5 | 4 | 500 | 52 |
| pRS5.10 ..... | 21 | 7 | 5 | 25 | 465 | 79 |
| VK45..... | 10 | 6 | 26 | 44 | 852 | 37 |
| pACHF1.3.2 ..... | 2 | 12 | 10 | 1 | 184 | 17 |

[a] Short arm of chromosome 6.
[b] 16pter-16p13.11::6p21.31-6cen (der6p).
[c] Short arm of chromosome 16.
[d] 6pter-6p21.31::16p13.11-16cen (der16p).
[e] Totaled over all metaphases scored.

**Table 3**

**TCS Linkage Values**

| A. TCS Linkage with 6p Markers | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Recombination Fraction θ | | | | | | | |
| Locus | .00 | .01 | .05 | .10 | .15 | .20 | .30 | .40 |
| F13A1..... | −19.15 | −9.49 | −4.69 | −2.69 | −1.65 | −1.02 | −.36 | −.10 |
| HLADQα ..... | −32.52 | −16.90 | −8.82 | −5.37 | −3.55 | −2.41 | −1.13 | −.47 |
| **B. TCS Linkage with 16p Markers** | | | | | | | | |
| | Recombination Fraction θ | | | | | | | |
| Locus | .00 | .01 | .05 | .10 | .15 | .20 | .30 | .40 |
| D16S8..... | −18.73 | −11.12 | −5.97 | −3.65 | −2.38 | −1.56 | −.63 | −.18 |
| D16S96..... | −17.74 | −9.98 | −4.80 | −2.57 | −1.44 | −.78 | −.15 | .03 |
| D16S79..... | −25.99 | −13.81 | −6.95 | −3.88 | −2.31 | −1.39 | −.49 | −.20 |
| D16S131 ..... | −1.49 | −.92 | −.13 | .18 | .29 | .32 | .24 | .11 |
| D16S75..... | −13.93 | −5.84 | −2.49 | −1.17 | −.55 | −.23 | −.01 | −.01 |

and 16p13.11. On chromosome 6 the p45.1DPbeta003 probe (HLA-DPB2) and the pRS5.10 probe (HLA class I chain) are at the opposite extremities of the HLA complex and are separated by approximately 4,000 kb. The chromosome 6 breakpoint must lie within this genetic distance (Spence et al. 1989).

We obtained DNA from an additional 12 families with TCS, none of whom had a visible chromosomal abnormality. Using the pDCH1 probe which defines a class II DQ alpha chain within the MHC complex, we were able to exclude the TCS mutation from the translocation breakpoint at 6p21.31. Moreover, as the factor 13A1 locus is within approximately 25–35 cM of the HLA complex, and as the length of 6p is estimated at 55 cM (Zoghbi et al. 1988), our data further exclude the TCS locus from most of 6p except for the telomere.

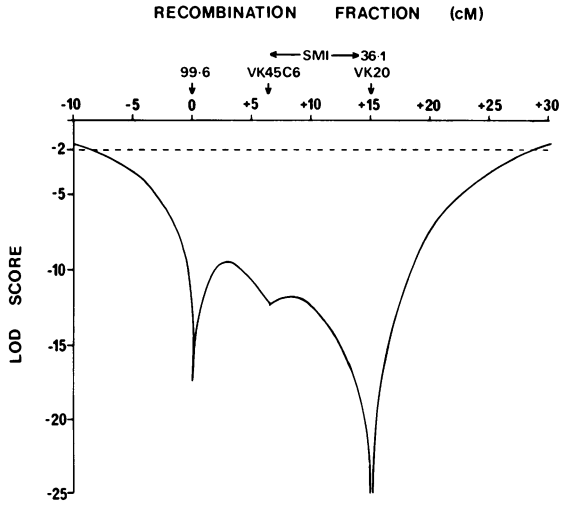


**Figure 4** Location map of chromosome 16p probes, summarizing lod scores (log 10 odds ratio) calculated for TCS locus at various map positions in fixed marker map of R99.6 (D16S75), VK45C6 (D16S131), 36.1 (D16S79), and VK20 (D16S96). The relative genetic position of R99.6 (D16S75) has arbitrarily been placed at 0. The interval into which the 16p13.11 translocation breakpoint maps is indicated by SMI.

The probes on chromosome 16, pACHF1.1A6 (D16S8) and VK45C6 (D16S131) have been located in the vicinity of 16p13.11-p12.3 by a combination of in-situ hybridization and Southern analysis of mouse/human hybrid cell panels (Callen et al. 1989; Hyland et al. 1989). The chromosome 16 breakpoint was defined as lying between these probes. Both pairwise and multipoint linkage analysis in the additional 12 families excluded the site of the TCS mutation from this second candidate region.

We initially thought that there was a possibility that our data supported genetic heterogeneity, with a mutation at one of the chromosomal breakpoints causing TCS in the translocation family and with mutations at other loci being responsible in the other families. However, our exclusion data do not support this conclusion; no evidence of linkage was detected in any of the informative families studied (data not shown). The subsequent birth of a third child to the translocation mother, a child who, despite being affected, did not exhibit the translocation, confirmed the linkage findings.

We are now constructing a complete exclusion map for TCS by using highly informative markers and by

continuing to study alternative candidate locations for the TCS locus, such as that at 5q11 (Balestrazzi et al. 1983). We would welcome knowledge of other TCS families from which multiple affected individuals are available for study.

## Acknowledgments

We thank the Treacher Collins families and the National Deaf Children's Society for their interest and cooperation, without which the present study would not have been possible. We should also like to thank those clinicians who collected samples on our behalf, particularly Drs. D. Donnai, A. Colley, and C. Benjamin (St. Mary's Hospital, Manchester), Dr. T. Hulse (Maidstone Hospital), and Dr. J. Raeburn (Western General Hospital, Edinburgh). Dr. J. Pan (pRS5.10) and Dr. U. Grundmann (F13a) supplied probes. The financial support of the Wellcome Trust, Medical Research Council, The Hearing Research Trust, The Royal Society, The Nuffield Foundation, and the Australian Cranio-Maxillo-Facial Foundation is gratefully acknowledged.

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