

A Somatic Cell Hybrid Panel and DNA Probes for Physical Mapping of Human Chromosome 7p

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Received May 8, 1991; revised July 12, 1991

To identify by reverse genetics genes on the short arm of human chromosome 7 expected to be involved in the regulation of human craniofacial and limb development, we have set up a human mouse somatic cell hybrid panel that divides 7p into 9 fragments. The breakpoints are defined by deletions or translocations involving one chromosome 7 in the cells of the human cell fusion partners. Particularly densely covered with these cytogenetic anchor points is the proximal area of 7p within and around 7p13. The number of cytogenetic mapping points within proximal 7p could be increased by four, using two diploid human cell lines with small interstitial deletions in this region for dosage studies. We used Southern blots of this panel to assign to 7q or subregions of 7p more than 300 arbitrary DNA probes or genes that provide reference points for physical mapping of 7p. Three reciprocal translocations with one of the breakpoints in 7p13 mark the location of a gene involved in Greig cephalopolysyndactyly syndrome. To define an area in which we could identify candidates for this developmental gene, we established a macrorestriction map using probes flanking the putative gene region. The Greig translocations were found to be located within a 630-kb *NotI* restriction fragment. © 1991 Academic Press, Inc.

INTRODUCTION

Detailed physical and genetic maps are one prerequisite for the search for particular human genetic disease loci by reverse genetics. Unless microscopically visible mutations guide the way to the gene as was the case in DMD (Boyd *et al.*, 1986), several open reading frames in a chromosome region identified by linkage studies must be considered as candidate genes and

analyzed individually (Estivill *et al.*, 1987; Rommens *et al.*, 1989).

It has been suggested that the short arm of human chromosome 7 carries a gene cluster involved in craniofacial and limb development (Bructon *et al.*, 1988; Winter and Huson, 1988; Pohl *et al.*, 1990). Particularly rewarding for a reverse genetics search in this area is the gene underlying the Greig cephalopolysyndactyly syndrome (GCPS) because cytogenetic peculiarities, two translocations and three deletions, affecting band 7p13 have been associated with this syndrome (Tommerup and Nielsen, 1983; Krüger *et al.*, 1989; Wagner *et al.*, 1990; Pettigrew *et al.*, 1989). We had previously used cells from a Greig patient with a third translocation, t(6;7), involving a breakpoint in 7p13 for gene mapping with somatic cell hybrids (Johannsmann *et al.*, 1980). The syndrome consists of postaxial polydactyly of the hands, pre- and postaxial polysyndactyly of the feet, macrocephaly, prominent forehead, broad nasal bridge, and hypertelorism (Greig, 1926). Inheritance is autosomal dominant with complete penetrance but considerable intrafamilial variability (Temtamy and McKusick, 1969). A mouse model for the Greig syndrome may exist in the mutant extra-toes (*Xt*) (Johnson, 1967) and the probably allelic mutation anterior digit-pattern deformity (*add*) that has recently been described (Pohl *et al.*, 1990). In addition to a complex abnormality of embryonic development, a fundamental disturbance of the process of ossification may be associated with GCPS (Bructon *et al.*, 1988). Tommerup and Nielsen (1983) postulated a role of the gene(s) involved in this syndrome in neoplasia on the basis of the similarity of certain abnormalities to those that occur in the hereditary basal cell carcinoma syndrome.

Dominant mutations of a gene disturbing pattern formation in human and mouse and possibly predisposing to certain malignancies are of considerable gen-

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eral interest. Therefore in the course of our studies involving somatic cell hybrids as a means for physical mapping of 7p we have focused on the dissection of the 7p13 region. We describe a mapping panel for 7p, assign a large number of gene probes and arbitrary DNA fragments, and define a large restriction fragment that is likely to harbor the putative Greig gene region.

MATERIALS AND METHODS

Somatic Cell Hybrids

Somatic cell hybrids were constructed by fusing human fibroblasts containing translocations or deletions involving chromosome 7p with HPRT-negative RAG or A9 mouse cells using PEG 1640 (Koch Light) as described previously (Bender and Grzeschik, 1976). Clones containing chromosome 7 fragments were isolated by subcloning. The production of the primary hybrids from which the subclones IT A9 2-21-14, IT A9 2-18-9-7-11, Rag GN6, Ru Rag 4-13, and Ru Rag 6-19 were derived has been reported by Bauch *et al.* (1978).

The fibroblasts IT originate from a female Greig patient who was a carrier of a balanced translocation 46,XX,t(6;7)(q12;p13) (Johannsmann *et al.*, 1980). The fibroblasts RoH [46,XX,t(6;7)(q27;p13) (Krüger *et al.*, 1989)] and 1863 [46,XY,t(3;7)(p21.1;p13) (Tommerup and Nielsen, 1983)] were from Greig patients as well. These patients were members of large families in which the syndrome was associated with the translocation chromosomes involving breakpoints in 7p13. The lymphoblast cells 4IRB and 3BC were established from Greig patients with small interstitial deletions in one of their chromosomes 7: 4IRB had a deletion (7p11.2-7p13) due to a *de novo* translocation 7;20, and 3BC had a *de novo* deletion (7p12.3-7p13) (Wagner *et al.*, 1990). Fibroblasts GM 1696 [46,X,t(X;7)(q21;p22)], GM 0044 [46,XY,del(7)(p21.2p22.1),t(7;10)(7qter-7p21.2::10q11.21-10qter;10pter-10q11.21::7p22.1-7pter)], GM 1356 [46,XX,t(1;7)(p34;p13)], and the lymphoblast cell lines GM 3162 and GM 3105 were obtained from the NIGMS Human Genetic Mutant Cell Repository (Camden, NJ). Hybrid 5387 3cl10 was a gift from Dr. C. Croce (Croce and Koprowski, 1974).

DNA Probes

The majority of DNA probes (series pFS) were isolated from a flow-sorted human chromosome 7 library in phage Charon 21A (LA07NS01) (Deaven *et al.*, 1986). Plaques were screened according to Benton and Davis (1977) with either total genomic human or total genomic hamster DNA labeled with ³²P by random primed DNA labeling. Plaques that showed hy-

bridization with human DNA only were picked and DNA was prepared from plate lysates (Helms *et al.*, 1985). ³²P-labeled phage inserts were used as probes for hybridization to genomic Southern blots of hybrid or lymphocyte DNA after prehybridization with an excess of unlabeled human placental DNA (Litt and White, 1985). Inserts that mapped to chromosome 7p13 were subcloned into pBluescript SK⁻ (Stratagene Inc.).

The origin of probes 4-18, 6-20, 6-31, 7-13, 9-2, 5-23, 7-2, and 7-6 has been described previously (Jobs *et al.*, 1990). Clones pA89, pA116, pA117, pB4, pB36, pB69, pC105, pC111U, pC140, pE146L, pG179, SF4, TG38, TG121, TM60, TM66U, TM102L, TN34, TN122, TN157, TN200, TS93, and TS194 were gifts from Dr. L. C. Tsui, Toronto (Rommens *et al.*, 1988). Additional probes were kindly provided by Collaborative Research Inc. (CRI-P137, CRI-pS207, CRI-R944: Donis-Keller *et al.*, 1987), Dr. M. Cohen-Solal, Paris (pGAM 14: Mattei *et al.*, 1989), Dr. N. Shimizu, Tokyo (pERP8: Kawasaki *et al.*, 1988), or K. Kontula, Helsinki (pp12: Julkunen *et al.*, 1988) or were obtained from the ATCC (pHERA64-1, EGFR, No. 57484 and phage 6, D7S11, No. 57030).

Southern Blot Analysis

Genomic DNA was isolated according to standard procedures, digested with restriction enzymes (3 U/ μ g) as recommended by the supplier (Boehringer Mannheim), and separated on 0.8% agarose gels. For PFGE, DNA was prepared from freshly harvested lymphoblasts or fibroblasts embedded in agarose blocks according to Smith *et al.* (1987). Blocks containing 1×10^6 cells were digested with rare-cutter enzymes as described by Van Ommen and Verkerk (1986). Southern blots were prepared using Biodyne A nylon membranes (Pall, Dreieich, Germany) and a vacublot transfer system (Pharmacia).

Blots for gene dosage studies were carefully controlled for loading of equal amounts of DNA, and autoradiograms were independently evaluated by two persons.

Hybridization was carried out in 5 \times SSC, 5 \times Denhardt's, 0.5% SDS, 7% dextran sulfate, and 100 μ g/ml HSDNA at 65°C overnight. Filters were washed three times for 20 min in 0.2 \times SSC, 0.1% SDS at 55-60°C and exposed for 1-5 days at -70°C with intensifying screens.

Chromosome Walking

Long insert phage clones for the 5-23 locus were isolated from a human *Mbo*I partial digest library in EMBL3 (provided by S. Orkin) as described previously (Gessler and Bruns, 1988). A *Not*I site which was detected within cloned DNA is also cut in geno-

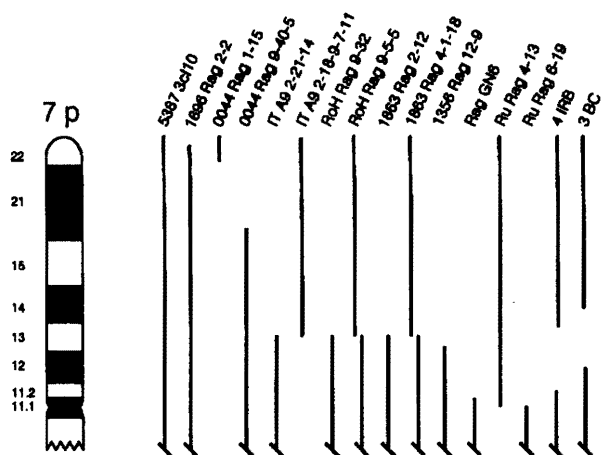


FIG. 1. Schematic representation of the chromosome 7p somatic cell hybrid mapping panel. The chromosome 7 content of each hybrid is indicated by the vertical bars. Hybrid 5387 3cl10 contains only a single human chromosome. The bars labeled 4IRB and 3BC represent hemizygous interstitial deletions of chromosome 7p material in lymphoblasts.

mic DNA, and a 2-kb *HindIII/NotI* fragment (p5-23ES6HN2) identified the adjacent *NotI* fragment upon PFGE analysis.

RESULTS

Development of Somatic Cell Hybrids

The somatic cell hybrid mapping panel used for these studies is shown schematically in Fig. 1. The chromosome 7p material present in each hybrid is represented by the vertical lines. 5387 3cl10 is used as a control because it contains an intact chromosome 7 as the only human material. For hybrids carrying translocations, available complementary clones were included (IT, Ru). In the fibroblasts, GM 0044 material from 7p21.1-p22.1 was lost completely due to the translocation event. The two complementary clones therefore define three regions (pter-p22.1, p22.1-p21.1, p21.1-qter). From the RoH and 1863 hybrids clones containing both translocation fragments but no intact chromosome 7 were included in order to detect minor deletions resulting from the translocation process. Clone Rag GN6 was the product of a spontaneous deletion during the cell fusion process. The deleted chromosomes of the lymphoblastoid lines 4IRB and 3BC are included in Fig. 1 although they were not separated from their intact counterparts by cell hybrid formation. Assignment to the deleted area was achieved by double hybridization of both probe 5-36 (D7S28) known to be localized on 7q (Jobs *et al.*, 1990) and various 7p test probes to genomic Southern blots of 3BC and 4IRB DNA and DNA from diploid controls with two intact chromosomes 7 (Fig. 2). The

relative intensities of the 7q and 7p bands were compared. Bands with half the intensity of the disomic controls indicated a localization in the monosomic region.

Regional Mapping on 7p

Of the 300 probes from the pFS series hybridized to the panel, 113 of them were localized on various regions of 7p (Fig. 3). The others were assigned to 7q (data not shown). Other probes previously assigned to 7p by Jobs *et al.* (1990) and Rommens *et al.* (1988) were ordered in a detailed map of 7p (Fig. 3). Particularly interesting with respect to the position of the Greig locus are TG38 and 5-23 immediately flanking the translocation breakpoints.

Wagner *et al.* (1990) assigned the locus for the muscle-specific form of phosphoglycerate mutase (PGAM2) missing in both deletions 4IRB and 3BC to the region 7p12.3-p13, and the locus for the epidermal growth factor receptor (EGFR) between the proximal breakpoints of 3BC and 4IRB. Drabkin *et al.* (1989) prepared a hybrid clone from the same Greig patient's cells as 1863 Rag 2-12 carrying the complementary fragment. Their clone was used to assign CRI-P137 and CRI-pS207 distal and CRI-R944 and EGFR proximal to the translocation breakpoint of fibroblasts 1863 in 7p13. Our results provide a definitive order for the genes in this region, with EGFR located most centromeric between breakpoint Rag GN6 and 3BC (proximal); then PGAM and IBP1, an insulin-like growth factor binding protein detected by probe pp12 in the next interval up to 1356 Rag 12-9; followed by CRI-R944 between this breakpoint and the Greig translocation region. Probes CRI-P137 and CRI-pS207 map distal to this region separated by the distal deletion boundary of 4IRB. Our assignment of CRI-pS207, a probe linked within 160 kb to the

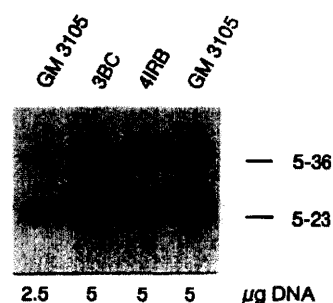


FIG. 2. Gene dosage analysis localizes probe 5-23 within the GCPS deletion of 4IRB and 3BC. A Southern blot filter containing *EcoRI*-digested DNA of the two GCPS deletion cell lines and a normal human control cell line (GM 3105) was simultaneously hybridized with probes 5-23 and 5-36. Probe 5-36 derived from 7q was used as an internal control. The lane with 2.5 µg of GM 3105 DNA provided a 50% intensity signal for comparison.

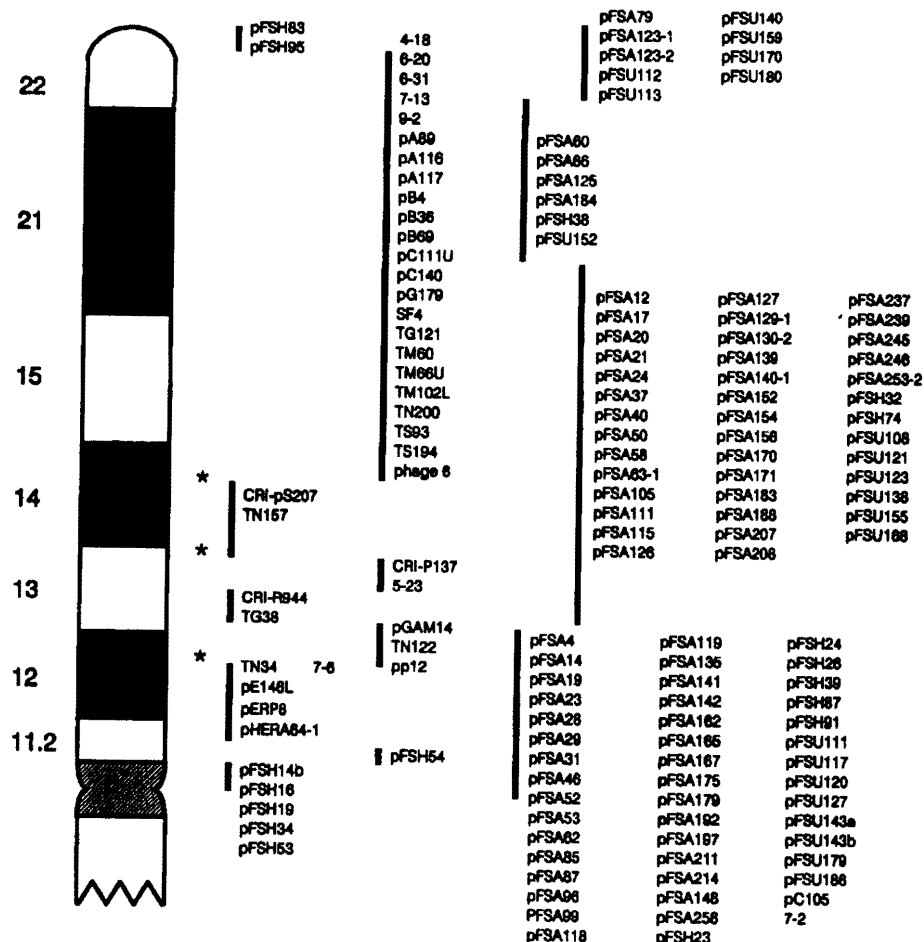


FIG. 3. Localization of DNA probes on 7p. The regions indicated by vertical bars are defined by the 7p breakpoints in the cells of the panel shown in Fig. 1. Asterisks indicate deletion breakpoints analyzed by dosage blot analysis.

TCRG region (Drabkin *et al.*, 1989), is in concordance with the results of Wagner *et al.* (1990). None of the probes described here mapped in between the three translocation breakpoints in Greig patients.

Pulsed-Field Analysis

From the segregation data on our hybrid clones, CRI-P137 and 5-23 were found immediately distal and CRI-R944 and TG38 proximal to the putative Greig locus. Since the CRI probes mapped genetically at a distance of 0 cM (Donis-Keller *et al.*, 1987) we attempted to span the Greig region with a macrorestriction map. Drabkin *et al.* (1989) did not detect any comigrating pulsed-field fragments by hybridization with CRI-P137 and CRI-R944. We also did not find any common fragments detected by these probes in digests of the lymphoblastoid cells GM 3162 with the rare-cutter enzymes *Bss*HIII, *Ksp*I, *Mlu*I, and *Not*I. The proximal probes CRI-R944 and TG38 share common fragments with all four enzymes. The 308-kb

*Ksp*I fragment is partially cut into two fragments of 220 and 88 kb recognized by CRI-R944 and TG38, respectively. The order and relative position of the individual fragments (Fig. 4b) were established by double digests and combinations with more frequently cutting enzymes like *Sal*I and *Sfi*I (data not shown). CRI-P137 and 5-23 detect a common 1420-kb *Not*I/*Mlu*I partial digest fragment, but are located at least 850 kb from each other (data not shown). To facilitate PFGE mapping we scanned the vicinity of the four probes for the presence of CpG islands. Probe 5-23 turned out to be located less than 20 kb from a *Not*I site. By chromosome walking in a human genomic EMBL3 library, probe 5-23ES6HN2, a 2-kb *Not*I/*Hind*III subfragment that detects the adjacent genomic *Not*I fragment, was isolated. This probe and CRI-R944/TG38 share a common 630-kb *Not*I fragment in fibroblast (GM 0044) and lymphoblast (GM 3162) DNA (Fig. 4a). Its position within a 1120-kb *Bss*HIII fragment is shown in Fig. 4b. As these probes are located on opposite sides of all three GCPS trans-

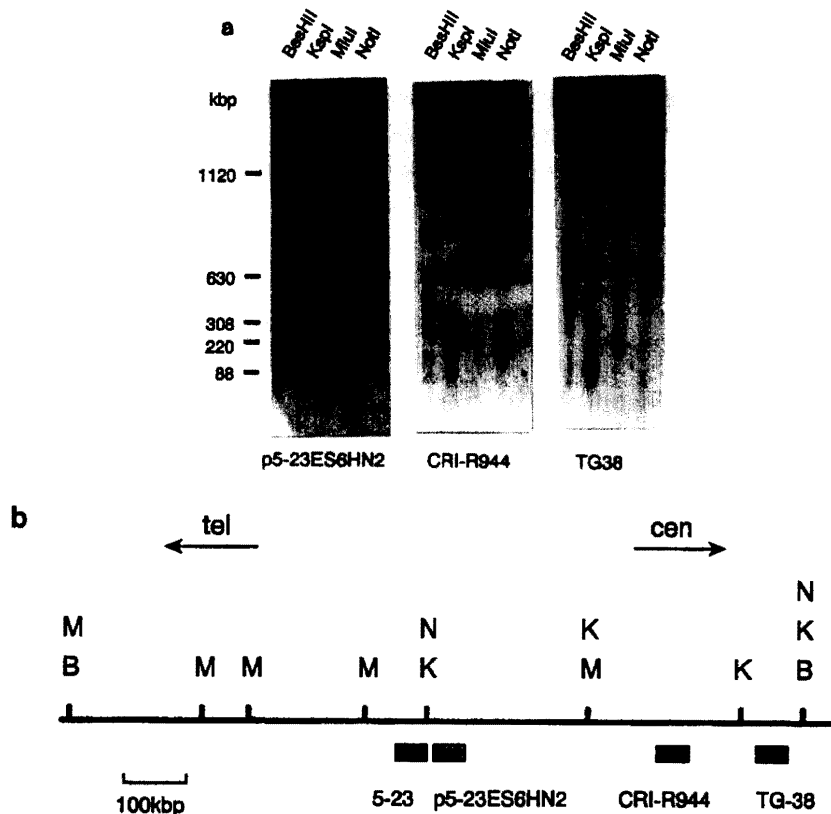


FIG. 4. Probes flanking the Greig translocation breakpoints are located within 630 kb. (a) Southern blot analysis of *Bss*HII-, *Ksp*I-, *Mlu*I-, and *Not*I-digested DNA isolated from lymphoblastoid cell line GM 3162 and hybridized to p5-23ES6HN2, CRI-R944, and TG38, respectively. The gel was run at 200 V with pulse time ramping 60–90 s for 24 h at 14°C. Size markers used were *S. cerevisiae* chromosomes and EMBL 3A concatemers. (b) Restriction map of the region covering the putative GCPS locus in 7p13 flanked by probes 5-23 and CRI-R944/TG38. The precise positions of CRI-R944 and TG38 within their respective restriction fragments have not been determined. B, *Bss*HII; K, *Ksp*I; M, *Mlu*I; N, *Not*I. Arrows point toward centromere and telomere.

locations, the chromosomal breakpoints must fall within this *Not*I fragment. This conclusion could be confirmed by PFGE analysis of fibroblast DNA from GCPS patients. In addition to the 630-kb fragment, probe CRI-R944 detected altered *Not*I fragments of 750 and 900 kb in RoH and 1863, respectively, whereas p5-23ES6HN2 hybridized to additional 580- and 370-kb *Not*I fragments (Fig. 5). We could not detect an altered *Not*I fragment in IT. Such a fragment, however, could be too large to be resolved under our PFGE conditions.

DISCUSSION

The development of the mapping panel described in this report serves two purposes: (i) it allows compartmentalization of 7p into regions approximately equivalent in size to low-resolution cytogenetic bands; (ii) it dissects band 7p13 into six fragments. Our strategy toward the construction of a physical map of 7p is to fill the individual compartments with about 50 arbitrary seed probes each from sorted libraries as foci from which YAC contigs can grow. The probes

mapped in this study are an important step toward this end. Furthermore, these probes will be valuable tools for the characterization of additional genes on chromosome 7p such as the gene for craniosynostosis postulated in 7p21 (Motegi *et al.*, 1985).

The region around the GCPS locus on 7p13 is densely covered with cytogenetic breakpoints that allow a fine resolution of probe order. Probes CRI-R944 and 5-23ES6HN2 are the closest flanking markers for the GCPS region, less than 630 kb from each other. They will be used for the isolation of genomic clones that can be screened for expressed sequences. Other disorders that should be considered in these studies are the acrocallosal syndrome, which is considered a recessive allele of GCPS, and the mouse mutant extra toes because of the similar limb and craniofacial malformations (Schinzel, 1982; Hendriks *et al.*, 1990).

TCRG and the gene for biliverdin reductase (BLVR) flank the Greig region in human 7p13 (Peters *et al.*, 1989). BLVR has previously been assigned to the region between the breakpoint in the IT cells (in 7p13) and the centromere (Meera Khan *et al.*, 1983). The breakpoint had been indicated as 7p14 due

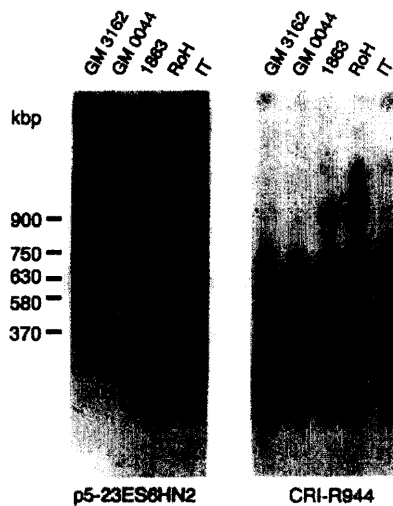


FIG. 5. Probes flanking the Greig locus detect different additional bands (arrowheads) in Southern blot analysis of *NotI*-digested DNA isolated from fibroblasts of Greig translocation carriers 1863 and RoH. GM 3162 is a lymphoblastoid cell line, GM 0044 a fibroblast control. The gel was run at 200 V with pulse time ramping 60–90 s for 24 h at 14°C.

to inadequate resolution of cytogenetic preparations. In mouse the *Tcrg* locus is linked on chromosome 13 to extra toes (*Xt*), which is most probably the mouse homologue of the Greig syndrome (Winter and Huson, 1988). The gene for the mouse biliverdin reductase, *Bivr*, however, is located on chromosome 2, closely linked to small eye (*Sey*) and limb deformity (*ld*) (Peters *et al.*, 1989). From these observations Peters and co-workers (1989) suggested that there was a cluster of genes for limb and craniofacial development in a common ancestor of human and mouse linked to TCRG and BLVR that has become broken up in the mouse. This would imply that the synteny of TCRG, GCPS, and BLVR in human represented the ancestral cluster. The human homologue to *Sey*, however, has recently been shown to be aniridia on chromosome 11p, and the human homologue to limb deformity is expected to be on chromosome 15 near the β_2 -microglobulin gene which is closely linked to *ld* also in the mouse (Glaser *et al.*, 1990; van der Meer-De Jong *et al.*, 1990).

Tommerup and Nielsen (1983) suggested that the bony abnormalities shared by the Greig syndrome and the hereditary basal cell carcinoma syndrome (BCNS) indicate a common pathogenic mechanism for these disorders. The development of medulloblastoma in one family member with the 3;7 translocation is discussed by these authors in view of the occurrence of medulloblastoma in BCNS.

The possibility of a close neighborhood of a series of genes involved in human development and neoplasia in 7p13 suggests that it is worthwhile to attempt a

complete map of all expressed sequences in the approximately 5 Mb of this region. Our panel and the new probes described here will be very helpful in this enterprise.

ACKNOWLEDGMENTS

We thank L.-C. Tsui, A. Jobs, Collaborative Research Inc., M. Cohen-Solal, N. Shimizu, and K. Kontula for DNA probes; C. Croce for hybrid cells; and S. Orkin for an aliquot of the human EMBL3 library. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Gr 373/14-1). N.T. is supported by the Danish Cancer Society and the Danish Human Genome Program.

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