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Deletion of *GLI3* supports the homology of the human Greig cephalopolysyndactyly syndrome (GCPS) and the mouse mutant extra toes (*Xt*)

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The dominant mouse mutant extra toes (*Xt*) is characterized by preaxial and postaxial polydactyly of the feet and a white belly spot. An interfrontal bone is present in the skull in 90% of heterozygotes compared with 50% of normal mice. Homozygous *Xt*/*Xt* embryos exhibit multiple skeletal defects, extreme polydactyly in both fore- and hindlimbs, and malformations of the brain and the eye (Johnson 1967; Franz and Besecke 1991). Depending on the genetic background, *Xt* homozygotes die prenatally or perinatally (Johnson 1967). An allelic but recessive syndrome is the anterior digit pattern deformity mutation (*add*), which is the result of a transgene integration (Pohl et al. 1990). In this case the malformations of the mutants are restricted to the forelimbs. Using DNA probes spanning the *add* transgene integration site, Pohl and coworkers (1990) could show that at least 80 kbp of surrounding DNA are deleted in *Xt* mice.

On the basis of the similarity of the phenotype, *Xt* is considered the mouse homolog of the human autosomal dominant Greig cephalopolysyndactyly syndrome, GCPS (Winter and Huson 1988). The latter is characterized by polysyndactyly of hands and feet and mild craniofacial abnormalities (Gollop and Fontes 1985). The gene locus has been pinpointed to human Chromosome (Chr) 7p13 by different translocations and deletions associated with the disorder (Tommerup and Nielsen 1983; Krüger et al. 1989; Wagner et al. 1990; Pettigrew et al. 1991; Vortkamp et al. 1991b).

Using six hybrids from three GCPS translocation patients, we recently have demonstrated that the zinc finger gene *GLI3* (Ruppert et al. 1988, 1990) is disrupted in the first third of its coding region by two of the GCPS translocations (Vortkamp et al. 1991a). The third translocation breakpoint was shown to be located approximately 10 kbp downstream of the expressed sequence. Thus, mutations of *GLI3* resulting in a reduced gene dosage are probably responsible for the development of GCPS.

To characterize the *GLI3* gene in *Xt* mutant mice, we isolated different *GLI3* cDNA clones from an 8.5-day fetal mouse cDNA library (Fahrner et al. 1987), using segments of the human *GLI3* cDNA as probes. The position of the mouse clones relative to the known human *GLI3* sequence (Ruppert et al. 1990) was determined by partial sequencing (Fig. 1a).

The distal mouse cDNA clone, pMGli20, corresponds to nucleotides 3232–4734 of the human cDNA. Hybridization with genomic DNA of homozygous *Xt*/*Xt* mice and normal control mice did not show any alteration in the 3′ part of the gene (Fig. 1d).

The proximal mouse cDNA clone pMGli11 starts approximately 500 bp upstream of the published *GLI3* cDNA and reaches down to nucleotide 415. The probe hybridizes with multiple *Eco*RI and *Pst*I fragments in normal mouse DNA that are completely deleted in homozygous *Xt*/*Xt* embryos (data not shown). To further characterize the deletion boundary within the *GLI3* gene, we hybridized the human cDNA fragment pcrGliprox (nucleotides 98–570) with genomic DNA of homozygous *Xt*/*Xt* and normal control mice. This probe overlaps with a 300-bp *Bgl*II/*Not*I fragment (pMGli11BN), representing the 3′ third of pMGli11, but reaches 160 bp further downstream. Like probe pMGli11BN (Fig. 1b), pcrGliprox hybridizes with an 8-kbp *Pst*I fragment that is deleted in the DNA of *Xt*/*Xt* embryos. Furthermore, two additional *Pst*I frag-

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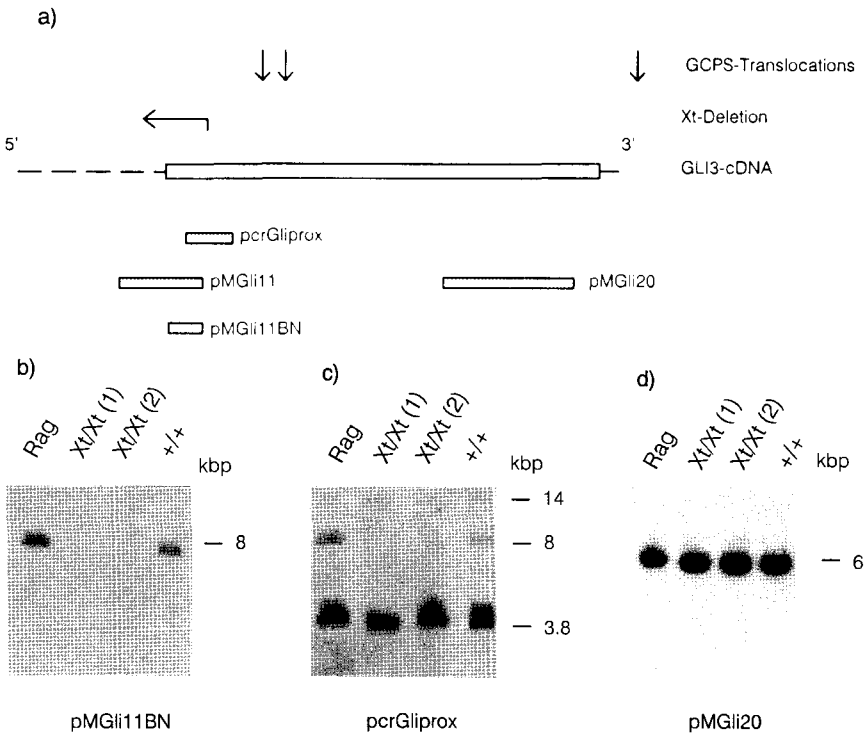

Fig. 1. The *GLI3* gene is partially deleted in *Xt* mutants. **(a)** The different GCPS translocations and the *Xt* deletion **(arrows)** are drawn in relation to the human cDNA published by Ruppert and co-workers (1991). The open reading frame of *GLI3* is indicated by an **open box;** the 5′ end, not yet determined, by a **broken line.** The positions of the cDNA fragments used in this study are marked by **shaded boxes. (b–d)** DNA from two homozygous *Xt/Xt* embryos, a normal (+/+) sib and the mouse tumor cell line Rag, was digested with *Pst*I **(b,c)** and *Eco*RI **(d)** and hybridized with different probes from the *GLI3* gene. **(b)** Probe pMGli11BN, a 300-bp *Bgl*II/*Not*I fragment of pMGli11 detects an 8-kbp *Pst*I fragment that is deleted in *Xt/Xt* embryos. This fragment overlaps with the human probe pcrGliprox. **(c)** Probe pcrGliprox reaches 160 bp further downstream and hybridizes with two additional *Pst*I fragments that are both not altered in the DNAs tested. **(d)** Hybridization of pMGli20 does not show any indication for a mutation in *Xt/Xt* mice. *Xt/Xt* breeding pairs in a (C3Hx101) F_2 background were purchased from the MRC Radiobiology Unit, Harwell, UK. High-molecular-weight DNA was prepared from embryos on day 16 of gestation. *Xt/Xt* embryos were identified macroscopically by their polydactyly and the malformation of the eye. The DNA was isolated according to the method of Kunkel and co-workers (1982), and Southern blot analysis was carried out under standard conditions (Sambrook et al. 1989). The mouse cDNA probes, pMGli11 and pMGli20, which are available upon request, were isolated from an 8.5-day fetal mouse cDNA library (Fahrner et al. 1987), with the human cDNA fragments pcrGliprox and pcrGlidist (Vortkamp et al. 1991a) as probes.

ments of 14 kbp and 3.8 kbp are detected, both of which are not deleted in the *Xt/Xt* embryos (Fig. 1c).

Thus, the breakpoint of the *Xt* deletion must be localized between nucleotides 415 and 570 of the corresponding human cDNA. The breakpoint is probably located in an intron of the *GLI3* gene, as hybridization of *Eco*RI (not shown) and *Pst*I (Fig. 1b,c)-digested *Xt*-DNA showed no rearranged fragments. At present we do not know the size of the deletion, but it seems likely that the deletion region includes 80 kbp of genomic DNA from the *add* locus (Pohl et al. 1990) that was shown to be deleted in *Xt*.

The deletion of the 5′ region of the *GLI3* cDNA in *Xt/+* mice prevents the formation of a functional protein product from one allele. Therefore, a reduction in gene dosage for *GLI3* is the likely cause for the malformations seen in both, the mouse *Xt* mutant and the human GCPS syndrome, confirming the homology of these syndromes. As the integration site of the transgene in the *add* mutant has been localized within the *Xt* deletion, it will be of great interest to determine the precise physical relationship between the genomic *GLI3* locus and the *add* integration site. *Xt* mutant mice exhibit a much more severe phenotype compared with the *add* mutants, leaving the possibility that there are additional genes in the *Xt* deletion region that may contribute to the observed phenotype.

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