

Syndromic Ectrodactyly with Severe Limb, Ectodermal, Urogenital, and Palatal Defects Maps to Chromosome 19

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Summary

Congenital limb malformations rank behind only congenital heart disease as the most common birth defects observed in infants. Finding genes that cause defects in human limb patterning should be straightforward but has been limited, in part, by the bewildering spectrum of phenotypes, which are difficult to separate into etiologically distinct disorders. One approach to the identification of relevant genes is to take advantage of unique extended kindreds in which a defect in limb patterning is segregating. Recently, a large Dutch family with ectrodactyly, ectodermal dysplasia, cleft palate, and urogenital defects (EEC) was described by Maas et al. We have studied this kindred and localized a gene causing EEC to a locus on chromosome 19, in a region defined by D19S894 and D19S416. A second extended kindred with EEC does not map to this locus, indicating that EEC is a genetically heterogeneous disorder. Growth and patterning of the limbs, teeth, hair, and genitourinary system are mediated in part by epithelial-mesenchyme inductive interactions. The identification of both the gene causing EEC and its mutation may further elucidate the general signals mediating inductive mechanisms.

Introduction

The vertebrate limb is a paradigmatic structure for developmental biologists studying growth, patterning, and axis determination. During the past decade, a number of the pivotal genetic elements controlling these processes have become better understood (Tickle 1995). Remarkably, many of the genes and pathways mediating

limb development in quite distantly related model organisms (e.g., *Drosophila*) appear to be conserved in humans (Serrano and O'Farrell 1997; Shubin et al. 1997). Yet translation of these results into a more lucid understanding of limb patterning defects in humans has been slow. Of the several hundred human malformation syndromes characterized by defects in limb patterning, only a handful have been mapped to specific chromosomal segments, and considerably fewer have been characterized at the molecular level.

Ectrodactyly (i.e., split-hand/split-foot malformation [SHFM]) is a human limb malformation characterized by defects of the central elements of the autopod (i.e., the hands and feet; Temtamy and McKusick 1978). However, the spectrum of patterning defects observed in SHFM ranges from cutaneous syndactyly of two digits to absence of the entire autopod. The prevalence of SHFM is between 1/10,000 and 1/90,000; X-linked and autosomal dominant forms have been described (Faiyazul-Haque et al. 1993; Nunes et al. 1995; Crackower et al. 1996). Furthermore, autosomal dominant SHFM syndromes are genetically heterogeneous (Nunes et al. 1995).

SHFM can be observed as an isolated finding or in combination with other anomalies, such as tibial aplasia, craniofacial defects, and genitourinary abnormalities (e.g., syndromic ectrodactyly; Scherer et al. 1994). The prototypical example of an ectrodactyly syndrome accompanied by multiple organ defects is ectrodactyly with ectodermal dysplasia and cleft-lip/cleft-palate syndrome (EEC; OMIM 129900; <http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispim?129900>).

EEC is an uncommon autosomal dominant disorder characterized by SHFMs; urogenital defects; anomalies of skin, hair, teeth, and nails; nasolacrimal abnormalities; and cleft palate with or without cleft lip (Buss et al. 1995). Hypohydrosis is not a prominent feature of EEC. Intra- and interfamilial expression is highly variable, and penetrance is incomplete (Preus and Fraser 1973).

Previous investigations have established a causative relationship between cytogenetic abnormalities of chromosome 7 and syndromic ectrodactyly, including EEC

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(Scherer et al. 1994). Yet this conclusion is based on analysis of only one family, segregating a balanced chromosome 7 translocation, and two sporadic cases. Moreover, none of the affected individuals were reported to have any of the canonical defects of EEC (e.g., no lacrimal-duct, genitourinary, or nail anomalies were reported), and two individuals exhibited features uncommon in EEC (i.e., bilateral accessory nipples and mental retardation). Consequently, association of EEC with a locus on chromosome 7 should be interpreted with caution. To our knowledge, no locus for a dominantly inherited syndromic ectrodactyly disorder has been identified previously.

In 1996, Maas et al. described a Dutch EEC kindred with 14 affected individuals in three generations (fig. 1, family A). All affected individuals in family A have limb and ectodermal defects (fig. 2), and 10 individuals suffer from urogenital anomalies, most notably an atrophic bladder epithelium that causes pain with micturition. We evaluated a second family (fig. 1, family B) with six individuals in three generations affected with EEC. All affected individuals in family B have limb, ectodermal, and genitourinary abnormalities. We report the mapping of a locus causing EEC to the pericentromeric region of chromosome 19.

Subjects and Methods

Clinical Evaluation

All studies were performed with the approval of the Institutional Review Board of the University of Utah, the Academic Medical Center in Amsterdam, and the General Counsel of the Shriners Hospitals for Children. Individuals in family A were examined by R.C.M.H., those in family B by M.B. All affected individuals in families A and B have limb and ectodermal defects, including sparse scalp hair and eyebrows, dystrophic nails, hypodontia, and lacrimal-duct abnormalities. All but three individuals in family A have urogenital abnormalities. Affected individuals in family B have urethral stenosis or pain with micturition. Two individuals in family A had a cleft palate and/or cleft lip. One individual in family B had anal stenosis. A detailed description of the clinical features of family A is available in the report by Maas et al. (1996).

Ascertainment of Genotype

Fifteen milliliters of blood was obtained from each affected individual and at least first-degree relatives. Genomic DNA was prepared from lymphocytes and cell lines derived from Epstein-Barr virus-transformed lymphocytes, as described elsewhere (Bell et al. 1981; Anderson and Gusella 1984). Primers were end-labeled by

use of γ - ^{32}P -ATP and polynucleotide kinase. One picomole of end-labeled primer was added to the PCR mix. Genomic DNA sequences were amplified in 1 \times buffer (10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM MgCl_2) with 25 ng of template genomic DNA, 50 μM dNTPs, 5% dimethyl sulfoxide, 20 pmol of each primer, and 1 U of *Taq* DNA polymerase in a total reaction volume of 25 μl . Samples were cycled 30 times in a Perkin-Elmer 9600 PCR machine using an initial annealing temperature of 62°C. The annealing temperature was decreased 4°C on the sixth cycle. Twenty microliters of 50% formamide-loading dye was added to each reaction after PCR. Samples were denatured for 3 min at 94°C and then were electrophoresed through 5% denaturing polyacrylamide gels. Bands were visualized by autoradiography. All experiments with markers used to construct haplotypes in the EEC critical region were confirmed by repetition.

Linkage Analysis

Two-point analysis was performed by use of MLINK of the LINKAGE package (Lathrop et al. 1984). Disease penetrance was set at .95, without a sex difference. Normal and disease allele frequencies were set at .9999 and .0001, respectively. Genotypes for all individuals typed in the reported families are available on request.

Results

Initially, we tested and excluded linkage to markers surrounding previously identified SHFM loci on chromosomes 7q21.3-q22.1 and 10q24-q25 (data not shown). Next, we began a genomewide search using short tandem repeats (STRs) chosen on the basis of their PIC and location sufficient to exclude linkage within ~30 cM (Dib et al. 1996). After screening ~10% of the genome, a pairwise maximum LOD score (Z_{max}) of 4.06 was obtained, with the completely informative marker D19S49 at a recombination fraction (θ) of .00. Twenty additional STRs spanning ~40 cM around D19S226 were tested for linkage (table 1, family A), and haplotypes were constructed on the basis of the marker-order estimates of the regional linkage map of chromosome 19 (http://www~bio.llnl.gov/bbrp/genome/html/chrom_map.html).

Examination of haplotypes demonstrates recombination events between the EEC locus and D19S894 in individuals V-13 and V-17 (fig. 1, family A) and between the EEC locus and D19S416 in individual V-13. These recombinants narrow the critical region containing the EEC locus to an ~33-cM interval between D19S216 and D19S416. These markers span the cytogenetic region from 19p13.1 to 19q13.1. Linkage between D19S566 and EEC in family B (fig. 1) was excluded ($Z_{\text{max}} = -5.12$

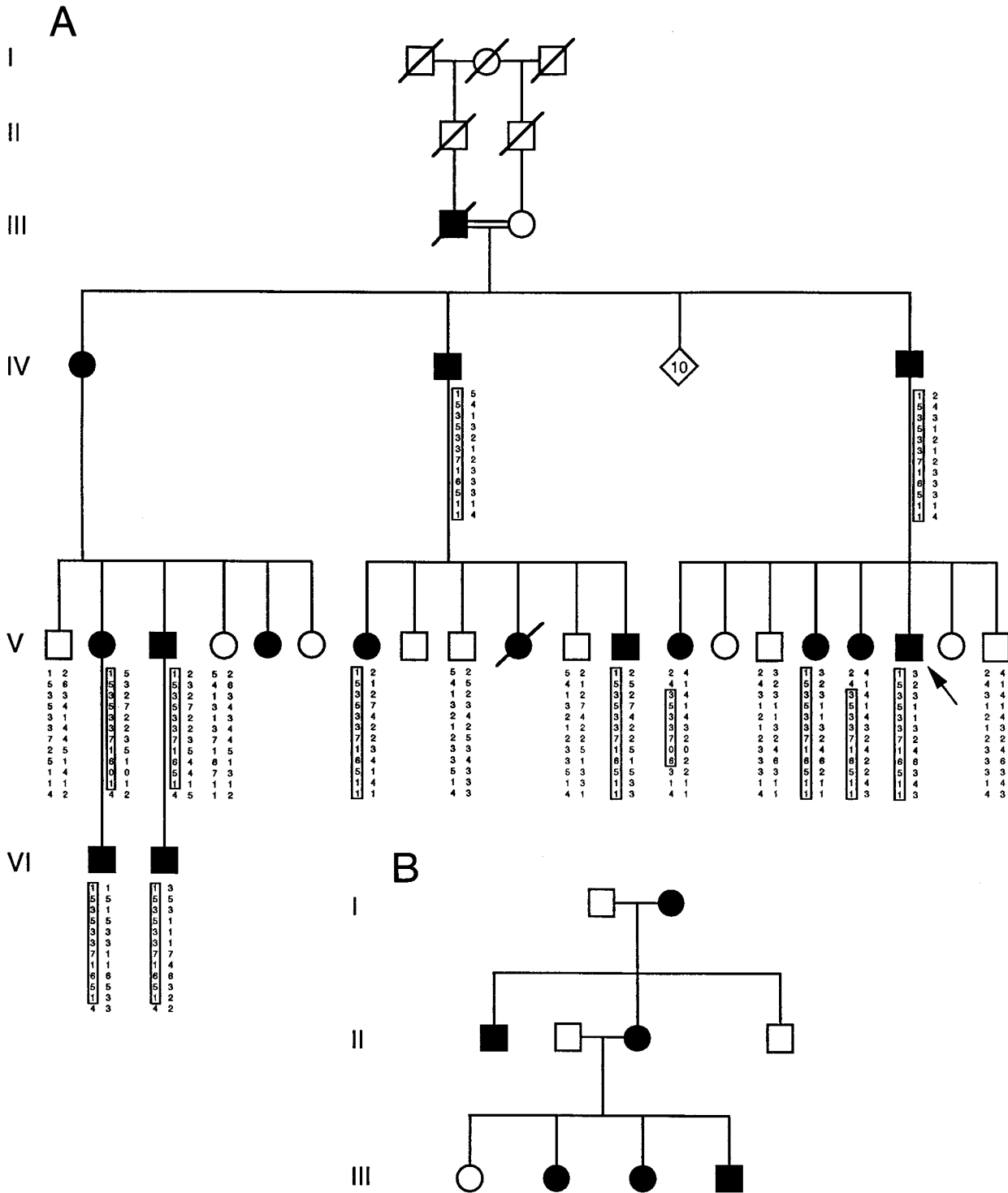


Figure 1 Pedigrees of families A and B with EEC syndrome. Affected individuals are denoted by a blackened symbol, and unaffected individuals are denoted by an unblackened symbol. In family A, the haplotype segregating with EEC is boxed, and genotypes for the microsatellite markers D19S209, D19S894, D19S216, D19S922, D19S840, D19S226, D19S410, D19S566, D19S931, D19S416, D19S223, and D19S412 are shown.

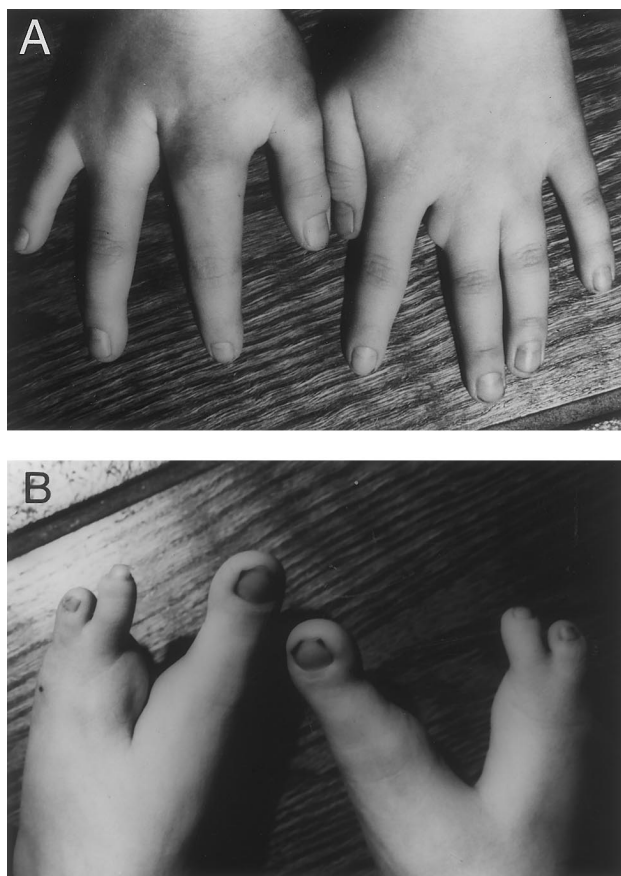


Figure 2 Split-hand/split-foot anomalies of individual V-12 in family A. A, Proximal fusion of digits 3 and 4 of the left hand, with a partial duplication of the distal 3d metacarpal, and absence of digit 3 of the right hand. B, Absence of digits 2 and 3 of the right and left feet, producing a median cleft in each foot.

at $\theta = .00$; table 1), indicating that EEC is a genetically heterogeneous disorder. Additionally, EEC was not linked to markers on chromosomes 7q21.3-q22.1 and 10q24-q25 in family B (data not shown).

Discussion

We believe that the two phenotypes of EEC in family A—SHFM and ectodermal dysplasia—are caused by a single pleiotropic gene. However, the cosegregation of two closely linked genes cannot be excluded. Cytogenetic analysis of the proband demonstrated no detectable chromosomal abnormalities. Although >165 transcripts have been mapped to the EEC critical region, a BLAST search of dbEST, for transcripts that are highly homologous to genes expressed in the developing limb bud and that map to the SHFM1 critical region (e.g., *Dlx5* and *DSS*), was negative. There are conflicting reports indicating an association between cleft lip with or without cleft palate and a locus on chromosome 19q, suggesting

Table 1

Pairwise LOD Scores between Chromosome 19 Markers and Ectrodactyly Families A and B

Locus	LOD SCORE AT $\theta =$						
	0	.01	.05	.10	.20	.30	.40
Family A:							
D19S209	-4.06	-.92	.42	.86	.96	.70	.28
D19S894	-3.64	-.50	.84	1.26	1.32	.98	.45
D19S216	1.57	1.60	1.62	1.53	1.21	.78	.32
D19S922	3.35	3.35	3.26	3.03	2.39	1.60	.70
D19S840	3.38	3.32	3.04	2.70	2.00	1.25	.47
D19S226	3.52	3.45	3.18	2.84	2.13	1.39	.59
D19S410	3.44	3.37	3.10	2.77	2.07	1.33	.55
D19S566	3.01	3.01	2.94	2.73	2.15	1.42	.60
D19S49	4.06	3.99	3.70	3.32	2.52	1.65	.71
D19S416	-1.86	1.90	2.34	2.29	1.84	1.21	.49
D19S412	-6.99	-1.99	-.30	.36	.74	.63	.30
Family B:							
D19S922	-4.84	-3.46	-1.94	-1.25	-.63	-.33	-.16
D19S410	-4.84	-4.75	-3.17	-2.14	-1.13	-.57	-.22
D19S566	-5.12	-4.01	-2.45	-1.70	-.93	-.49	-.20

that EEC could be an allelic variant (Stein et al. 1995; Wyszynski et al. 1997). However, the families studied have nonsyndromic cleft lip/cleft palate, and no exceptional candidate gene has been proposed. Nevertheless, one very reasonable candidate gene within the EEC critical interval is *TGF- β 1*, located at 19q13.1.

TGF- β 1 and related molecules in the *TGF- β* superfamily are involved in diverse biological processes, including growth and differentiation. Epithelially synthesized *TGF- β 1* is localized predominantly to adjacent mesenchyme, which is consistent with its role as a paracrine regulator of morphogenetic interactions (Millan et al. 1991). In chick and mouse, *TGF- β s* are expressed in the developing limb prechondrogenic condensations and are potent chondrogenic stimuli for limb mesoderm in vitro (Roark and Greer 1994). Surgical manipulation of limb ectoderm results in both a rapid increase in *TGF- β 1* transcripts in the adjacent mesoderm and the formation of interdigital extra digits (Martin et al. 1993). Moreover, implantation of beads soaked in *TGF- β 1* into the 3d interdigit of a chick consistently produces truncation of digits 3 and 4, interdigital chondrogenesis, and a phenotype strikingly similar to SHFM (Ganan et al. 1996). *TGF- β 1* partly regulates murine epidermal keratinocyte function by controlling the transcription of keratin genes. It is expressed in the whisker follicle, tooth bud, and embryonic palatal tissue (Millan et al. 1991). Furthermore, *TGF- β 1* may be the mesenchymal signal that partly controls epithelial branching in the kidney and glandular organs. Thus, the *TGF- β 1* signaling pathway is involved in the development of each tissue affected in individuals with EEC.

To date, exons 4–7 of *TGF- β 1* have been screened for mutations, by SSCP analysis, but no polymorphisms that

segregate with the affected individuals have been found. Sequencing of the exons of *TGF- β 1*, as well as of control elements, is currently under way.

Considerable overlap exists between EEC and a variety of syndromes characterized by defects in limb patterning, including ectrodactyly-cleft palate (OMIM 129830; <http://www3.ncbi.nlm.nih.gov/omimhttp://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/disp-mim?129830>), lacrimo-auriculo-dento-digital syndrome (OMIM 149730; <http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/disp-mim?149730>), and acro-dermato-ungual-lacrimal-tooth (OMIM 103285; <http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/disp-mim?103285>). To date, the molecular basis of these disorders remains unknown. A kindred with SHFM accompanied by mammary-gland, ectodermal, and palatal defects has been reported but does not map to chromosomes 7 or 12 (Hamel et al. 1996). We suggest that these disorders may be allelic to the EEC locus on chromosome 19.

Ulnar-mammary syndrome (UMS; OMIM 181450; <http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/disp-mim?181450>) is a limb-malformation syndrome characterized by altered limb, apocrine, dental, and urogenital development (Bamshad et al. 1996). The same ectodermal-derived structures develop abnormally in individuals with EEC. Moreover, both an individual with UMS and SHFM (Franceschini et al. 1992) and a family with features overlapping EEC and UMS have been reported (Hamel et al. 1996). Mutations in *TBX3*, a member of the T-box family of transcription factors, have been characterized recently in families with UMS (Bamshad et al. 1997). Consequently, we hypothesize that the product encoded by the EEC locus participates in some of the same developmental pathways as do T-box genes, notably *TBX3*.

Growth and patterning of the limbs, teeth, hair, and genitourinary system are mediated in part by epithelial-mesenchyme inductive interactions that stimulate and sustain branching networks. This signaling pathway appears to be disturbed in individuals with EEC caused by mutations in a gene on chromosome 19. Consequently, characterization of this gene and its mutation may further elucidate the general signals mediating induction and branching.

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