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Linkage Disequilibrium Mapping of the Gene for Margarita Island Ectodermal Dysplasia (*ED4*) to 11q23

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Summary

Margarita Island ectodermal dysplasia (ED4) is an autosomal recessive disorder characterized by unusual facies, dental anomalies, hypotrichosis, palmoplantar hyperkeratosis and onychodysplasia, syndactyly, and cleft lip/cleft palate. We have used an affected-only DNA-pooling strategy to carry out linkage disequilibrium mapping of the *ED4* gene to 11q23. Haplotype analysis of four complex Margarita Island ED4 families localized the *ED4* gene to an ~1–2-Mb interval spanned by just two YACs.

Introduction

Ectodermal dysplasias (EDs) are a heterogeneous group of developmental dysplasias that can involve a wide array of ectodermal structures, but particularly the skin and sweat glands, hair, nails, and teeth. More than 175 clinically and genetically distinct forms of ED have been cataloged (Freire-Maia and Pinheiro 1984, 1987; Gorlin et al. 1970; OMIM 1998). However, only a small number of ED genes have thus far been mapped or cloned.

Margarita Island ED (MIM 225060) is a rare autosomal recessive disorder occurring among the indigenous population of Isla de Margarita, a group of three islands in the south-central Caribbean, discovered by Columbus in 1498 and one of the earliest Spanish colonies in the New World. The clinical features of Margarita Island ED include unusual (100%), triangular (70%) facies, with anteverted pinnae (75%) and malar hypoplasia (70%) (Bustos et al. 1991) (fig. 1). Dental anomalies are universal (100%), and they include hypodontia, anodontia, microdontia, and other abnormalities. Hair is sparse, short, and brittle (100%), and eyebrows and lower eyelashes are scant (100%); hypotrichosis typically progresses to virtually complete alopecia by adulthood. Hand anomalies include palmoplantar hyperkeratosis (100%), onychodysplasia (60%), and extensive cutaneous syndactyly (65%). Affected individuals exhibit abnormal philtrum, cleft lip, or cleft lip/cleft palate (65%), and the philtrum is strikingly broad and flat even in many heterozygotes. Bustos et al. (1991) initially reported 27 affected individuals in seven apparently unrelated kindreds on the islands and speculated that the disorder may have arisen via a founder effect, around the time of Spanish settlement in 1500, followed by both occult and overt inbreeding. Until ~50 years ago, the indigenous population of Isla de Margarita numbered ~50,000. Thus, a rough estimate of frequency of this disorder among the indigenous population of the islands is ~1/2,000.

Here, we describe linkage disequilibrium mapping of the gene for Margarita Island ED, using an affected-only DNA-pooling strategy. Consanguineous families and inbred populations can be used to localize genes for rare recessive disorders on the basis of excess homozygosity for specific linked marker alleles due to identity by descent, an idea initially elaborated by Smith (1953) and further developed by Lander and Botstein (1987). Because ancestrally related mutant alleles in an inbred population may be separated by a great many meioses, inference of so-called "historical recombinants" by haplotype analysis of closely distributed polymorphisms can provide remarkable genetic resolution of regions of shared homozygosity. Pooling of DNA samples from affected individuals can greatly enhance genotyping efficiency, as only those markers that exhibit excess allele sharing in the affected-only DNA pool need be followed up by genotype analysis of individual DNA samples and of flanking markers (Carmi et al. 1995; Wildenberg et al. 1995; Nystuen et al. 1996; Scott et al. 1996; Sheffield et al. 1996). We used this approach to carry out an initial genome scan for the Margarita Island ED locus (*ED4*)

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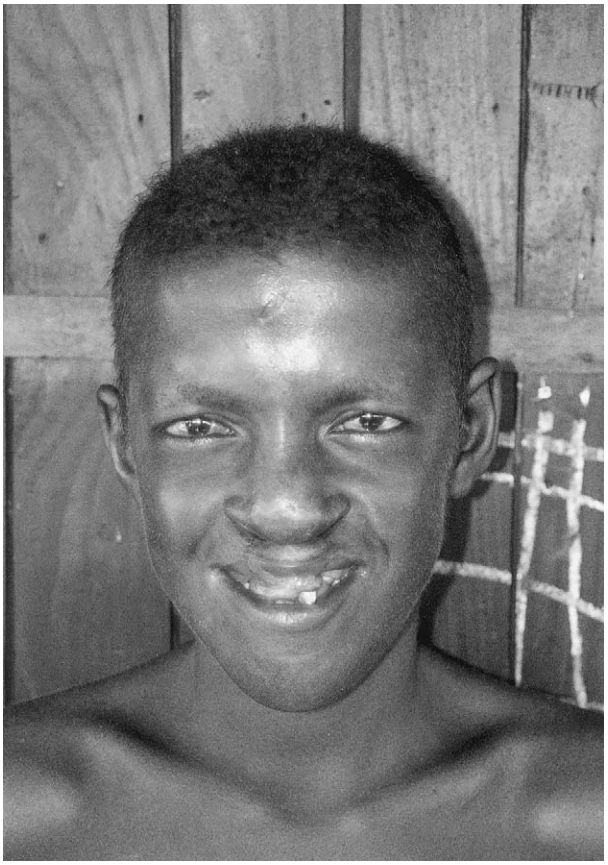

Figure 1 Typical patient with Margarita Island ED

with ~10-cM resolution and obtained results that suggest linkage of *ED4* to markers from distal chromosome segment 11q. Subsequent testing of additional nearby markers and haplotype analysis of four Margarita Island ED families allowed us to localize *ED4* to an ~1–2-Mb interval on 11q23 that is spanned by only two YACs.

Subjects and Methods

Pedigrees and Genotyping

We obtained blood samples with informed consent from four apparently unrelated, complex, indigenous, Margarita Island ED kindreds (fig. 2), including 13 affected individuals, all of whom were examined clinically. DNA was prepared by standard methods, and equimolar amounts of DNA from 11 of the affected patients, 11 obligate heterozygotes, and 10 unrelated normal individuals were pooled to prepare affected, heterozygote, and unaffected DNA pools, respectively. We then subjected DNA aliquots from each pool to PCR-based genotyping, using [^{32}P]-radiolabeled primers for polymorphic microsatellite markers from the Research Genetics Screening Set 8. Marker allele patterns were inspected visually, and markers that demonstrated an apparent shift toward homogeneity due to allele sharing in the affected pool, in particular, were chosen for further investigation by individual analysis of members of the five ED kindreds. Additional nearby microsatellite markers were selected first from genetic maps in the Marshfield Medical Foundation database and subsequently from the Whitehead Institute for Biomedical Research/MIT Center for Genome Research STS-Based Map of the Human Genome. Corresponding primer pairs were purchased from Research Genetics and used to analyze the four kindreds.

STS-Content Analysis of a YAC Contig

Preliminary information about YACs containing the relevant 11q23 markers was obtained from the Whitehead Institute for Biomedical Research/MIT Center for Genome Research STS-Based Map of the Human Genome and Généthon Human Genome Research Centre databases. The corresponding YACs were purchased from Research Genetics. YAC DNA was prepared and a detailed STS-content map of the region was established by PCR analysis of all of the relevant microsatellite markers in all YACs.

Results

Mapping of the Gene for ED4 to Chromosome 11q23

We performed a genomewide search for linkage of *ED4,* using the Research Genetics Screening Set 8, a panel of 387 polymorphic microsatellite markers distributed at ~10-cM intervals. As shown in figure 3, several markers from distal chromosome 11q—in particular, D11S1998, located in 11q23—exhibited an apparent shift toward allelic homogeneity in the affected pool and excess representation of the same allele in the heterozygote pool, relative to unrelated normals, suggesting possible linkage of these markers to *ED4*. These and additional markers from this region, selected from the Marshfield Medical Foundation genetic database, were analyzed in individual members of the four Margarita Island ED kindreds and demonstrated excess homozygosity and/or allele sharing among affected individuals, supporting linkage of *ED4* to these markers in 11q23. Furthermore, analysis of the marker alleles in the four kindreds permitted us to define an apparent ancestral marker haplotype, with presumed historical recombinants delimiting the *ED4* genetic interval to the ~10-cM region between D11S1356 and D11S4464.

D11S1356 is located on the Whitehead Institute physical map, within YAC contig WC11.10. D11S4464 is not in the Whitehead physical map, but Marshfield Med-

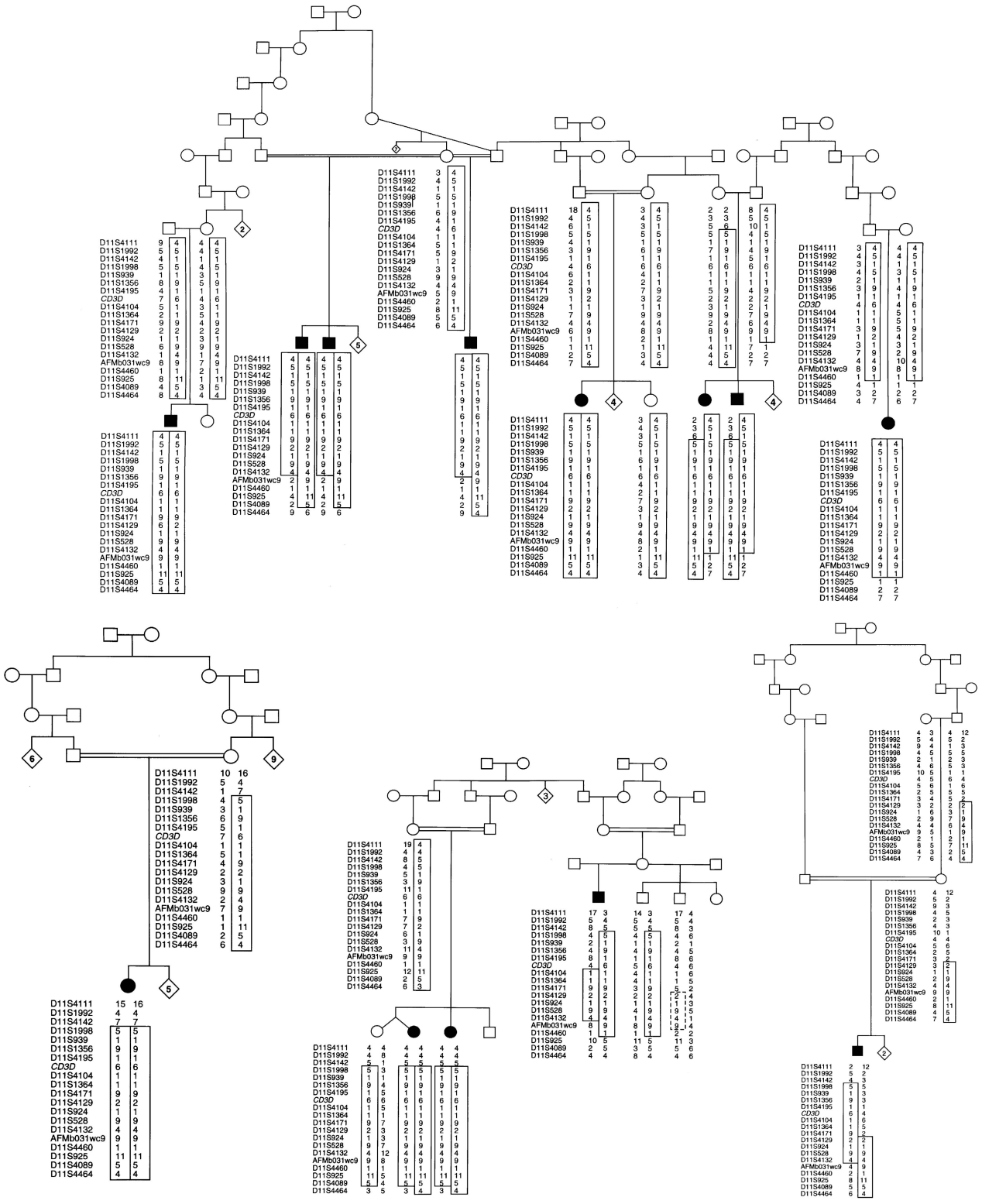

Figure 2 Pedigrees and genotypes of four Margarita Island ED kindreds. Boxes denote haplotypes or portions of haplotypes inferred as being ancestrally related to Margarita Island ED; dotted box denotes possible mutant partial haplotype in an individual whose carrier status is indeterminate. Alleles are as described in the Genome Database when given in that resource. Marker order is our best reconciliation of the Marshfield sex-averaged chromosome 11 linkage map, the Whitehead YAC contig STS-content physical map, and our own STS-content mapping of YACs from the *ED4* region of chromosome 11.

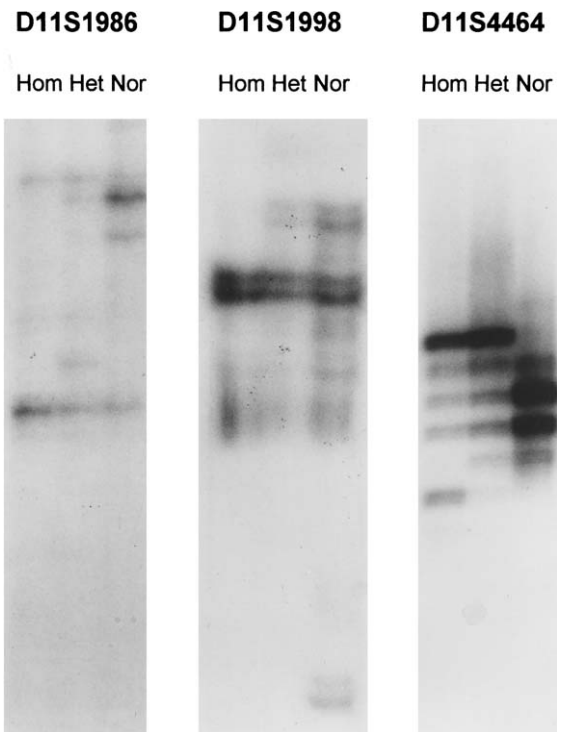

Figure 3 Allele sharing in pooled Margarita Island ED patient DNAs for markers in distal chromosome 11q. Patterns are shown for three adjacent markers from Research Genetics screening set 8: D11S1986, D11S1998, and D11S4464. Hom = homozygote pool, Het = heterozygote pool, and Nor = normal pool.

ical Foundation genetic mapping data indicate that D11S4464 is most likely in the vicinity of the distal end of YAC contig WC11.10. To refine localization of *ED4,* we therefore performed further mapping, using five markers derived from the Whitehead STS-content map of YAC contig WC11.10, eight markers derived from this region of the Marshfield genetic map, and seven more markers found on both maps. As shown in figure 2, four markers in the *ED4* region—D11S4129, D11S924, D11S528, and D11S4132—were found to be homozygous in all 13 affected patients, with D11S924 yielding a LOD score conservatively estimated at ~13.9 (the frequency of allele 1 = 0.278 among 18 unrelated normal alleles in this population). An additional marker in the region, D11S1299, yielded anomalous band sizes and so was excluded from the analysis. Genotype analysis of available family members allowed us to identify an apparent ancestral haplotype associated with Margarita Island ED, with historical recombinants defining D11S4171 as the proximal flanking marker. Distally, both AFMB031wc9 and D11S4460 were recombinant with *ED4* in families 2 and 3 (fig. 2), but their order could not be determined from available genetic map data.

Analysis of a YAC Contig across the ED4 Region

To resolve the order of markers across the *ED4* region, we constructed a physical map of the region based on STS-content of YACs. We first obtained six YACs that had been scored as positive for some of these markers in the Généthon database or in the Whitehead STS-content map of YAC contig WC11.10. We then retested these markers in these YACs, as well as additional markers that appeared to be in the region, on the basis of genetic mapping, to construct an STS-content map of the YACs. As shown in figure 4, we found that these YACs defined a contig across the *ED4* region. In particular, the contig defined by YACs 912f3 and 901a11 spanned the entire *ED4* region, including flanking markers D11S4171 and AFMb031wc9, respectively, and the four nonrecombinant markers within the *ED4* genetic interval: D11S4129, D11S924, D11S528, and D11S4132.

Discussion

Margarita Island ED was described by Bustos et al. (1991) among the indigenous population of Margarita Island in the Caribbean Sea. Affected individuals exhibit unusual triangular facies, dental anomalies, progressive hypotrichosis, palmoplantar hyperkeratosis, onychodysplasia, cutaneous syndactyly, and abnormal philtrum or cleft lip/cleft palate (fig. 1). In fact, patients with similar phenotypes had been described by others (Zlotogora et al. 1987; Ogur and Yuksel 1988; Zlotogora and Ogur 1988; Rodini and Ricieri-Costa 1990) and tentatively grouped together as the "Zlotogora-Ogur syndrome" (MIM 225000) (Rodini and Ricieri-Costa 1990). Although the Margarita Island ED patients manifest some phenotypic differences from these others, the overall similarities are striking, and these disorders may well be allelic (Zlotogora 1994), a hypothesis that could now be tested by genetic linkage analysis in these families.

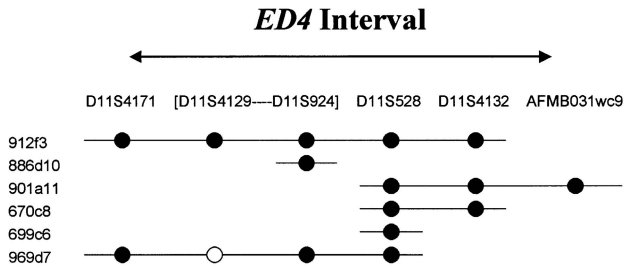

Figure 4 STS-content map of YACs in the *ED4* region. Blackened circles denote STSs present in individual YACs; unblackened circle denotes STS absent from YAC 969d7. Brackets indicate that the relative order of D11S4129 and D11S924 cannot be determined from these data.

The Science Gene Map of the Human Genome lists 13 genes and 24 anonymous expressed sequence tags located between D11S1341 and D11S925, markers that closely flank the *ED4* interval. However, none of these are obvious candidates for *ED4* on the basis of their homologies, expression, or function. Nevertheless, even in advance of cloning of the gene, our mapping of the Margarita Island ED locus would enable prenatal diagnosis of the disorder in this high-risk population, by means of genetic linkage analysis.

The localization of *ED4* to 11q23 suggests a possible mouse homologue to human Margarita Island ED. The murine *rough fur* (*ruf*) locus is located on mouse chromosome 9 at position 23.0, within a block of conserved synteny to human 11q23. The *ruf* locus, which is represented by a single allele, gives rise to recessive rough fur and hyperkeratosis, features very reminiscent of Margarita Island ED. Two known genes have been mapped to mouse chromosome 9 position 23.0, and both encode transcription factors. One of these, *Yb1a,* is probably a murine-specific pseudogene that has no direct counterpart in the human. But the other, *Pou2f3,* is homologous to human *POU2AF1,* which maps to 11q23.1, not far from the *ED4* interval. Although the expression and functional characteristics of *POU2AF1* make it an unlikely candidate gene for Margarita Island ED, analysis of *ruf* mice may well facilitate identification of the human *ED4* locus.

Finally, it is tempting to speculate about a possible relationship between the *ED4* locus and cleft lip/cleft palate. Midline facial clefts have been reported in patients with various ectodermal dysplasia syndromes (Gorlin et al. 1970; Freire-Maia and Pinheiro 1984, 1987; OMIM 1998), and abnormal philtrum, cleft lip, or cleft lip/cleft palate occurs in ~65% of individuals with Margarita Island ED. Interestingly, the philtrum is strikingly broad and flat in many Margarita Island ED obligate heterozygotes, who would be expected to number perhaps 1/22 among the indigenous population of the island. The incidence of nonsyndromic cleft lip/cleft palate is quite high on Margarita Island, ~5.4/1,000 (T. Bustos, unpublished data). Although this high incidence might result from environmental factors, such as lack of dietary folate, it is also possible that heterozygosity for Margarita Island ED might constitute a significant genetic risk factor for nonsyndromic cleft lip/cleft palate on the island.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Généthon Human Genome Research Centre, http://www.genethon.fr/genethon_en.html (for information about YACs containing the relevant 11q23 markers)

Genome Database, http://www.gdb.org (for allele descriptions in fig. 2)

Marshfield Medical Foundation, http://www.marshfield.org/genetics (for microsatellite markers used)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for Margarita Island ED [MIM 225060])

Science Gene Map of the Human Genome, http://www.ncbi.nlm.nih.gov/cgi-bin/SCIENCE96/chr?11 (for genes and anonymous expressed sequence tags located between D11S1341 and D11S925)

Whitehead Institute for Biomedical Research/MIT Center for Genome Research, STS-Based Map of the Human Genome, http://www.genome.wi.mit.edu/cgi-bin/contig/phys_map (for microsatellite markers and YACs used)

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