Evidence for Extensive Locus Heterogeneity in Naxos Disease

Karima Djabali[*], Amalia Martínez-Mir[*], Liran Horev, Laurent Klapholz, Benjamin Glaser[†], Angela M Christiano[*] and Abraham Zlotogorski

Department of Dermatology, Hadassah Medical Center, Jerusalem, Israel

[*]Departments of Dermatology and Genetics & Development, Columbia University, New York, New York, U.S.A.

[†]Departments of Endocrinology, Hadassah Medical Center, Jerusalem, Israel

Correspondence: Dr Abraham Zlotogorski, Department of Dermatology, Hadassah Medical Center, P.O.B. 12000, Jerusalem, Israel, 91120. Email: zloto@cc.huji.ac.il

To the Editor:

Naxos disease is a rare autosomal recessive disease that consists of an associated triad of woolly hair, thickened palms and soles (keratoderma), and heart involvement. The hair phenotype is unique, characterized by congenital woolly, curly, rough, and light colored scalp hair and sparse eyebrows. The nonepidermolytic keratoderma appears during the first years of life and involves mainly pressure areas in the palms and soles. The heart manifestations appear during the teenage years and are severe and progressive and may end with arrhythmia and premature sudden death. The disease was originally described in individuals from the Greek Island Naxos by Protonotarios *et al* in 1986 and byBarker *et al*in 1998. In 1998, Carvajal-Huerta reported patients from Guayaquil, Ecuador with a similar autosomal recessive triad, with combined epidermolytic palmoplantar keratoderma with woolly hair and dilated cardiomyopathy, and described the skin manifestations. Another family with the autosomal dominant association of nonepidermolytic palmoplantar keratoderma, woolly hair, and dilated right ventricle was reported by Tosti *et al* (1994) in an Italian family. Recently, Coonar *et al* (1998) mapped the gene for the Greek families (Naxos disease) to 17q21, and a mutation in the plakoglobin gene was identified as responsible for the disease in the Greek families (McKoy *et al*, 2000). In the Ecuadorian family, a mutation in the desmoplakin gene was found to be responsible for the disease (Norgett *et al*, 2000). In this study, we report the clinical findings in two new Arab families with Naxos disease originating from villages near Jerusalem. Importantly, we have excluded both plakoglobin and desmoplakin as the candidate genes in these families. Furthermore, we have analyzed several other regions harboring candidate genes of interest, and found no evidence for linkage.

The pedigree structures of the two families are shown in **Figure 1(a, b)**. The history and clinical examination of patients from families A and B included the congenital appearance of woolly, curly, rough, light colored scalp hair with sparse eyebrows, axillary, and pubic hair (**Figure 1c**). Skin involvement included palmoplantar keratoderma (**Figure 1d, e**) starting around age 3, as well as

follicular keratosis on extensor arms, shins, back, and cheeks, lichenoid papules mainly on the lower shins and psoriasiform keratosis. A plantar skin biopsy taken from affected individuals in these families showed two types of keratoderma, specifically, epidermolytic in family A and nonepidermolytic in family B. Furthermore, hair plucked from the scalp of members of the two families revealed in affected members plenty of trauma-related hair shaft abnormalities, including longitudinal and oblique fractures, tapered hairs, trichorrhexis nodosa like lesions, pseudomonilethrix, twisted and corkscrew-like hair, without pili torti. Hairs were of different diameters and some of them were curly. Electrocardiogram and echocardiogram were performed, following the history of a sudden death at age 18 in one affected family member (from family A) and complaints of dizziness, syncope, and chest pain in other members. The electrocardiogram demonstrated tachycardia in the young members and different types of arrhythmia, including ventricular premature beats, couplets, triplets, and nonsustained VT in older members. In two members (family A, V-1, V-6) the echocardiogram showed right ventricular dysplasia with right ventricular dilatation and decreased function of the right and left ventricles. These clinical observations provided the final diagnosis of arrhytmogenic right ventricular cardiomyopathy (ARVC).

Since all the affected members descended from consanguineous couples, we hypothesized that the impaired gene must have arisen from the same ancestral mutation in both alleles. Thus, heterozygosity for the candidate gene/region was considered sufficient for exclusion of genetic linkage. Although both families originated from the same geographic region in Israel, suggesting they might be related, we elected to evaluate each candidate locus allowing for genetic heterogeneity. First, we screened for mutations in the human desmoplakin (NM_004415) and plakoglobin (AJ249711) genes (Franke *et al,* 1989; Virata *et al*, 1992). Sequence analysis of each exon and splice junctions of the desmoplakin and plakoglobin genes (Whittock *et al*, 1999,2000) from affected individuals revealed no mutation in both genes compared with unrelated unaffected individuals. Furthermore, we identified several heterozygous sequence changes in the introns of affected individuals, supporting the exclusion of these genes (data not shown).

Second, on the basis of the role of both desmoplakin and plakoglobin in cell adhesion, several other genes coding for components of the desmosomes or proteins involved in different aspects of cell adhesion were considered as candidate genes for the disease in our families. These included the genes coding for type I and type II keratins on chromosomes 17 and 12, respectively (Romano *et al*, 1988;Yoon *et al*, 1994;Aberle *et al*, 1995;Coonar *et al*, 1998), desmoyokin on 11q13.1 (Courseaux *et al*, 1996), and the desmocollin/ desmoglein cluster on 18q12.1 (Arnemann *et al*, 1991). We also analyzed markers for plakophilin 1 at 1q32 (Cowley *et al*, 1997), plakophilin 2 at

12p13 (Bonne *et al*, 1998), and plakophilin 4 (or p0071) at 2q23-q31 (Hatzfeld and Nachtsheim, 1996; Bonne *et al*, 1998). As the gene structures were not available for each of these genes, we performed cosegregation and homozygosity studies using microsatellite markers covering these loci (**Table I**). As a result, none of the regions analyzed showed cosegregation with the disease trait. Moreover, heterozygosity in the affected members could be used to exclude the regions on chromosomes 12, 17, and 18, harboring the keratins and desmocollin/desmoglein gene clusters, respectively, as well as for the chromosomal regions containing plakophilin 1, 2, and 4 as specified in **Table I**.

These findings emphasize the hetergeneous genetic basis of Naxos disease, which appears to segregate in a dominant manner as well as the autosomal recessive forms (Tosti *et al*, 1994). Our future work will be directed toward the evaluation of additional desmosomal proteins, as well as conducting a whole genome scan to find regions homozygous by descent, and identify a novel causative gene(s) underlying Naxos disease.

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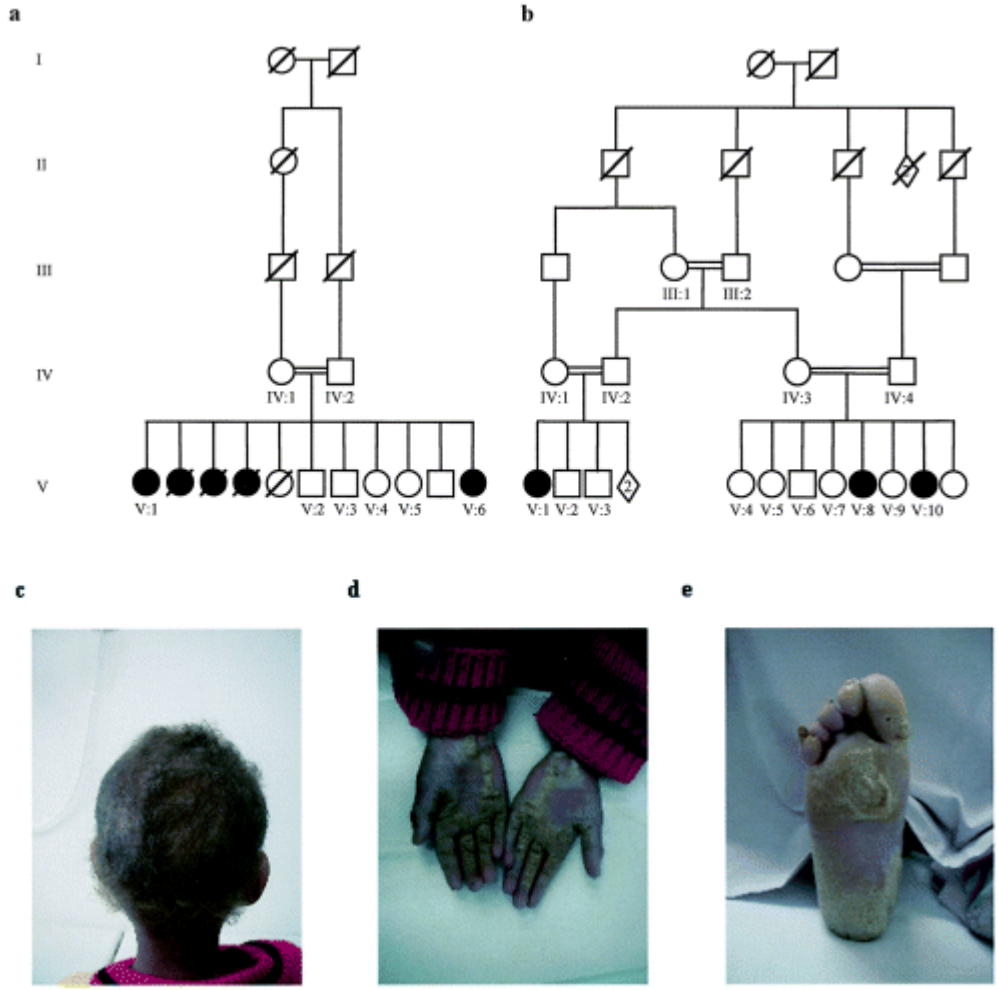
Figures

Figure I



Pedrigrees and clinical presentation of Naxos families A(a) and B(b). (a, b) Pedigrees of the two Naxos disease families from Israel. Filled circles and squares represent affected females and males, respectively. A diagonal line through a symbol indicates a deceased individual. Double lines are indicative of consanguinity. (c–e) Clinical presentation of the Naxos disease phenotype. Note in (c) the woolly and light colored, sparse hair typical of affected patients. (d, e) The presence of marked palmoplantar hyperkeratosis is also a hallmark of this phenotype.

Tables

Table I. Candidate genes excluded from linkage to Naxos disease

Gene/loci	Chromosome location	Markers[a]	Distance (cM)
Plakophilin 1	1q32-q44	D1S2622	214.08
		D1S477	215.17
		D1S2686	216.46
Plakophilin 4	2q23-q31	D2S2241	156.92
		D2S321	157.55
		D2S284	161.81

Gene/loci	Chromosome location	Markers[a]	Distance (cM)
Desmoplakin	6p24.3	D6S477	9.18
		D6S470	18.22
		D6S2434	25.08
Desmoyokin	11q13.1	D11S1765	61.78
		D11S1883	65.05
Type II keratin	12q13.13	D12S1661	63.89
		D12S390	67.63
		D12S398	68.16
Plakophilin 2	12p13	D12S1053	51.99
		GATA63D01	53.64
		GATA123B12	55.50
Type I keratin	17q21.2	D17S800	62.01
		D17S1789	63.09
Plakoglobin	17q21.1	D17S800	62.01
		D17S1789	63.09
Desmocollins/desmogleins	18q12.1	D18S819	52.86
		D18S847	56.71
		GATA173A03	62.29

[a] PCR primers for the microsatellite markers were designed according to the sequences on the Genome Database and the Cooperative Human Linkage Center (http://www.gdb.org; http://www.lpg.nci.nih.gov/CHLC)