Naxos disease in an Arab family is not caused by the Pk21 57del2 mutation

Evidence for exclusion of the plakoglobin gene

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ABSTRACT

Objective: Naxos disease is a rare hereditary disorder characterized by palmoplantar keratoderma, woolly hair and cardiomyopathy. This study aims to determine whether Naxos disease in a Saudi Arab family is caused by the Pk2157del2 mutation that was identified in Greek families from Naxos Island where the disease had originally been described.

Methods: This study was undertaken at King Fahad Hospital of the University, Al-Khobar, and the Medical University of Hannover, in the spring of 2003. Naxos disease has been encountered in a 2-year-old girl and her 30-year-old aunt of a Saudi Arab family. Deoxyribonucleic acid samples of this family were analyzed by polymerase chain-reaction (PCR) amplification of the respective region of the plakoglobin gene, and direct nucleotide sequencing of the PCR-products. Segregation analysis was performed employing the newly detected IVS11+22G/A polymorphism.

Results: Molecular genetic analysis of the DNA sample of the child diagnosed with Naxos disease showed absence of the Pk2157del2 mutation. In addition, the segregation analysis revealed heterozygosity for IVS11+22G/A in the affected girl.

Conclusion: Absence of the Pk2157del2 frameshift in the affected child proved that Naxos disease in this Saudi Arab family is not caused by the same mutation that was identified in the Greek families. Furthermore, heterozygosity for the IVS11+22G/A polymorphism provided evidence for exclusion of the plakoglobin gene in this consanguineous family.

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Naxos disease is a rare hereditary disorder characterized by palmoplantar keratoderma, woolly hair and cardiomyopathy. Palmoplantar keratodermas are a diverse group of skin diseases characterized by hyperkeratosis of the palmoplantar epidermis. The majority of the palmoplantar keratodermas have been attributed to mutations in structural proteins such as keratins, desmosomal proteins, and connexins.[1] The cardiac manifestations appear during the teenage years and are severe and progressive and may end with arrhythmia and sudden death. In 1983, Crosti et al[2] reported on a family from Italy with a typical palmoplantar keratoderma and woolly hair but without cardiac abnormalities. The designation Naxos disease was first used in a report on 4 families from the Greek Island Naxos, whereby 7 out of 9 affected persons showed symptoms of heart disease including

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cardiomegaly, electrocardiogram (ECG) abnormalities, episodes of ventricular tachycardia, and sudden death of one patient.[3] The cardiac abnormalities were confirmed in later reports.[4,5] Research in recent years has shown extensive genetic heterogeneity in Naxos disease from 2 aspects:

First, although the mode of inheritance of Naxos disease has been shown as autosomal recessive in the majority of cases, autosomal dominant transmission of the disease has also been reported. In 1998 Coonar et al,[6] mapped the gene for Naxos disease in the Greek families to chromosome 17q21.2. The involved gene encodes for plakoglobin, a key protein component of desmosomes adherence junction, and is important for the tight adhesion of many cell types, including those in the heart and skin. The mutation, Pk2157del2, in the plakoglobin gene was subsequently identified as responsible for the disease in those families.[7] This type of Naxos disease is believed to be transmitted in an autosomal recessive mode of inheritance. In 1998 Carvajal-Huerta[8] reported on patients from Ecuador with a similar autosomal recessive type of the disease with combined palmoplantar keratoderma with woolly hair and dilated cardiomyopathy. In this Ecuadorian family, the recessive mutation 7901delG in the desmoplakin gene (mapped to chromosome 6p23-24) causes a generalized striate keratoderma particularly affecting the palmoplantar epidermis, woolly hair and a dilated left ventricular cardiomyopathy.[9] On the other hand, autosomal dominant association of woolly hair, and cardiac abnormalities have been reported in an Italian family affected with Naxos disease.[10]

Second, heterogeneity of Naxos disease with regard to the disease-causing locus. On the basis of the role of both desmoplakin and plakoglobin in cell adhesion, several genes coding for components of the desmosomes, could be considered as candidate genes for Naxos disease. Such genes are those coding for type I keratins on chromosomes 17q21.2 and type II on chromosomes 12p13, desmoyokin on 11q13.1, the desmocollins/desmogleins cluster on 18q12.1, plakophilin-1 on 1q32, plakophilin-2 on 12p13, and plakophilin-4 on 2q23-q31. In fact, all of those candidate genes, in addition to the above mentioned 2 genes; plakoglobin and desmoplakin, now known to be involved with Naxos disease, have recently been excluded by Djabali et al[11] as candidate genes for the disease in 2 Palestinian Arab families originating from villages near Jerusalem.

Recently, 2 Saudi Arab patients have been diagnosed with Naxos disease, and it seemed interesting to focus on the genetics of this rare disease reported for the first time in this community.[12]

Methods. In the spring of 2003, Naxos disease has been detected in 2 persons of a Saudi Arab family. This study was undertaken at King Fahad Hospital of the University, Al-Khobar, and the Medical University of Hannover. The first case is a 2-year-old girl who presented with diffuse, thick, yellow, scaly lesions in the palms and soles, and woolly curly slow-growing scalp hair. The second case was a 30-year-old aunt of the child (from the mother‘s side) who presented with similar palmoplantar lesions and woolly hair. Both patients were free of any signs of cardiac disease. Upon careful dermatological evaluation, the diagnosis of 2 cases as Naxos disease was reached.[12]

All individuals involved in this investigation consented to participate in the study. However, a blood sample of the affected aunt was not available for the molecular investigations. Detection of the already known allele for Naxos disease - the Pk2157del2 mutation in the plakoglobin gene[7] - by direct nucleotide sequencing, seemed most appropriate. Genomic DNAs of 7 individuals of this family (parents and 5 siblings including the 2-year-old patient) were extracted from lymphocytes according to the standard procedure. The respective region of the gene was amplified by polymerase chain-reaction (PCR) using the primer pair Pk3F (5‘-CTGTTCCGCATCTCCGAGGA-3‘) and Pk3 (5´-CACTACTTCCATCTGCTAGGA-3‘) and the conditions of the PCR were those as described elsewhere.[7] The PCR products were purified using a Pharmacia kit (GFXTM PCR, Amersham Pharmacia Biotech, Piscataway, New Jersey). The sequencing reaction was performed using a Big Dye DNA sequencing kit (PE Applied Bio-systems, Foster City, CA, USA) according to standard procedures. The sequencing products were purified using Centri Sep Columns (Princeton Separations) and analyzed in an ABI Prism 310-Genetic Analyzer (Perkin-Elmer, Foster City, CA, USA).

Results. The sequencing results showed absence of the Pk2157del2 mutation in all of the 7 samples tested (**Figure 1a**). A G/A polymorphism (IVS11+22G/A) was detected at nucleotide +22 of intron 11 of the plakoglobin gene (**Figure 1b**). This polymorphism involves nucleotide 2853 of the plakoglobin sequence (GenBank accession number AF306723) and was not listed in the Single Nucleotide Polymorphism database of the The National Center for Biotechnology Information. Heterozygosity for IVS11+22G/A was detected in the 2 parents as well as in 3 siblings including the child with Naxos disease (**Figure 1b)**. One sibling was homozygous for the A allele, and another sibling presented with homozygosity for the G allele. The IVS11+22G/A polymorphism has also been detected in the heterozygous state in 3 out of

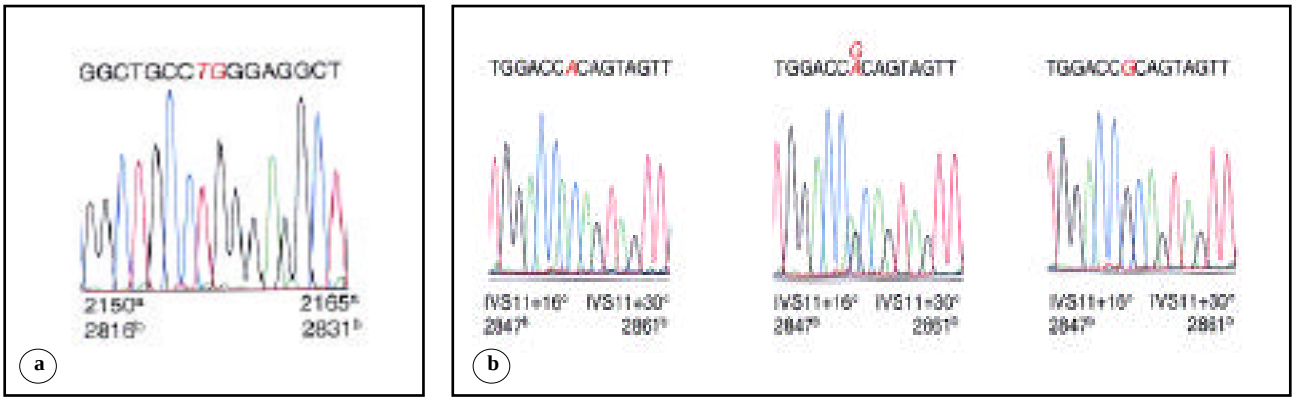

Figure 1 - Sequencing of parts of **a)** Exon 11 - Sequencing of DNA from the affected child revealed absence of the Pk2157del2 mutation. This mutation involves the deletion of nucleotides T and G (marked in red and Italics) at positions 2157 and 2158 (numbering according to reference 7). The patient is homozygous for the wildtype sequence, which is shown from nucleotides 2150 to 2165 (a), corresponding to nucleotides 2816 to 2831 (numbering according to GenBank accession number AF306723). **b)** Intron 11 - detection of a G/A polymorphism at nucleotide +22 of intron 11 of the plakoglobin gene (IVS11+22G/A, nucleotide 2853 according to (b). Refers to the nucleotide position in intron 11 (IVS11) of the plakoglobin gene. Left: Homozygosity for A; middle: Heterozygosity for G and A; right: homozygosity for G (nucleotides marked in red and italics).

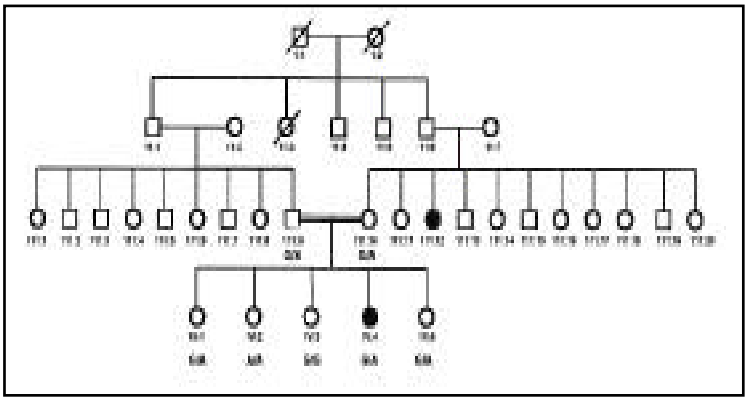

Figure 2 - Pedigree of the consanguineous Saudi Arab family affected with Naxos disease and segregation of the IVS11+22G/A polymorphism, showing that the affected child (IV:4), the parents (III:9 and III:10) and 2 siblings (IV:1 and IV:5) are heterozygous. Affected patients designated in bold symbols.

10 normal controls not related to this family. The segregation of the polymorphism in the affected family is shown in **Figure 2**.

Discussion. We focussed our work on the investigation on the plakoglobin gene, since this was the gene where a recessive mutation was found in those Greek families from Naxos, in which the disease was described originally.[7] The absence of the Pk2175del2 mutation in the affected child proved that Naxos disease in this Arab family is not caused by the same recessive plakoglobin mutation. In addition, the segregation analysis performed employing the newly detected IVS11+22G/A polymorphism (**Figure 2**) clearly revealed that the affected child was heterozygous, which strongly argues against the presence of a recessive disease causing mutation in the plakoglobin gene in this Saudi Arab family.

So far, the mode of inheritance and the gene involved in this family remains undetermined. One possibility may be that the disease is caused by a recessive mutation in the desmoplakin gene, as demonstrated in 3 Ecuadorian families,[9] or in any other of the candidate genes mentioned in the introduction. Autosomal recessive inheritance is assumed due to consanguinity in this family. Alternatively, it is possible that the disease segregates in an autosomal dominant manner with reduced penetrance, a pattern of inheritance which was reported previously to be present in an Italian family with Naxos disease.[10] Interestingly, the plakoglobin gene, the desmoplakin gene, as well as 7 other potential candidate genes for Naxos disease, have been excluded in another Arab community, such as Palestinian Arab families.[11] From their extensive work Djabali et al[11] postulated the existence of a novel causative gene underlying Naxos disease. The recessive mutations in both the plakoglobin as well as the desmoplakin genes are

known to be associated with dilated cardiomyopathy. In this Saudi family, both affected patients presently do not show any cardiac complications, which usually are known to appear during the teenage years. In the work by Crosti et al[2] from Italy, they similarly described presentation of palmoplantar keratoderma with woolly hair, but without cardiac abnormalities.

In conclusion, further molecular genetic studies are recommended in order to determine exactly the mode of inheritance and the locus involved with Naxos disease in this Saudi Arab family.

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