Waardenburg Syndrome: Description of two novel mutations in the PAX3 gene, one of which incompletely penetrant

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Abstract

We describe two different novel mutations in the PAX3 gene, detected in two families with cases of Waardenburg syndrome type I (WSI). The missense mutation detected in one family involved a single substitution in exon 2 (c.142 G > T) and was present both in the affected individual and in his clinically normal father. The mutation found in the second family consisted of a deletion of 13 bases, c.764-776del(TTACCCTGACATT), in exon 5.

Key words: Waardenburg syndrome, PAX3 gene, incomplete penetrance, sensorineural hearing impairment, telecanthus.

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Introduction

The Waardenburg syndrome (WS) is characterized by the association of craniofacial dysmorphisms (synophrys, telecanthus, broad and high nasal root, and lower lacrimal dystopia), pigmentation defects (heterochromic and bright hypochromic blue irides, hypopigmented skin spots, and partial hair albinism - white forelock or early graying), and sensorineural congenital hearing impairment. WS is a genetically heterogeneous condition with a wide clinical spectrum and a very high degree of phenotypic expressivity. The two most frequent variants (WSI and WSII) out of the four described so far differ from each other by telecanthus, present only in the first variant. WSI is caused by mutations in the PAX3 gene (2q35), that encodes a DNA-binding transcription factor known to be active mainly in the embryonic neural crest; among the genes this factor controls are Met, MyoD and MITF (Tajbakhsh et al., 1997; Epstein et al., 1996). The second variant (WSII) comprises a heterogeneous group of defects affecting melanocyte proliferation or survival. Two causative genes of this variant have been identified so far, MITF and SLUG, both being transcription factors that map to chromosome 3p12 and 8q11, respectively. The MITF gene, controlled by PAX3, plays an important role in melanocyte differentiation, which probably explains the similar hearing and pigmentation defects in both WSI and WSII (Read and Newton, 1997), whereas telecanthus is probably the result of an independent action of the PAX3 protein on the development of the craniofacial bones. Telecanthus is the most important sign for the differentiation between WSI and WSII, but its penetrance rate is incomplete, making the clinical diagnosis of the variant (I or II) in an isolated affected individual without the trait troublesome. Methods based on the frequencies of signs or on craniofacial measurements can be used in such cases (Pardono et al., 2003). Molecular analysis can be very helpful in the correct differentiation between the two variants and thus enabling the proper estimation of the deafness risk for a child to be born from an affected individual, which is significantly different in variants I and II. This risk can be obtained by simply halving the estimates calculated for the penetrance values of the deafness trait in the two conditions, which were estimated in 47% for WSI and 79% for WSII.

Over 60 different PAX3 mutations have been described up to now in families with cases of WSI. It is noteworthy that generally each family presents a different alteration, and only a few new mutations seem to occur in different genealogies, as it is the case with the G insertion at position 874 of PAX3 (Ishikiriyama et al., 1989; Lin et al., 1992; Morell et al., 1992; Tassabehji et al., 1993; Baldwin et al., 1995; Wildhardt et al., 1996). The detection rate of mutations in gene PAX3 is about 80-90% for the classical form of WSI, using different screening techniques. No clear-cut correlation between genotype and phenotype in WSI has been found so far. MITF mutations have been found in a modest number (about 20%) of families fitting...
the diagnostic criteria for WSII, and in one family with the phenotype of Tietz-Smith syndrome (Smith et al., 2000).

Here we report two novel mutations in the \textit{PAX3} gene, associated with WSI in two families.

\textbf{Material and Methods}

\textbf{Case descriptions}

To classify affected individuals into each of the two variants WSI and WSII, we used discriminant functions based on metric (craniofacial) measurements and on categoric data (frequencies of the cardinal signs or symptoms described below), as detailed in Pardono \textit{et al.} (2003). The patients were examined for the presence of eight major cardinal signs of the condition (telecanthus, synophrys, iris pigmentation disorders, localized hair albinism, sensorineural deafness or hearing impairment, hypopigmented skin spots, nasal root hyperplasia, and inferior lacrimal dystopia).

The two novel mutations were found in two families. In Family 1, the isolated propositus presented sensorineural congenital hearing impairment and early graying, synophrys, telecanthus, bright hypochromic blue irides, and broad and high nasal root. His parents did not present any cardinal signs and symptoms of WSI, but surprisingly the father carried the same mutation found in the propositus. In Family 2 (Figure 1), ten different WSI patients distributed over three generations were personally examined, and it was noteworthy that they all presented most cardinal signs and symptoms of the condition (Table 1). The propositus himself, referred to us as an affected individual, was a boy who did not have the mutation; he had normally pigmented blue eyes and was deaf, but he presented no other signs suggesting the diagnosis of WSI.

\textbf{Molecular analysis}

In order to confirm the role of \textit{PAX3} as the causative gene in Family 2, we performed a microsatellite linkage analysis (ABI PRISM Linkage Mapping Set v. 2.5-MD 10 kit, Applied Biosystems) with markers close to \textit{PAX3} (D2S396, D2S126 e D2S2382). The amplified fluorescence-labeled PCR products were analyzed in a MegaBACE 1000 DNA Analysis System with the Genetic Profiler software version 1.5 (Amersham Biosciences).

Mutational screening was performed by SSCP (single-strand conformation polymorphism). PCR primers were designed as described by Baldwin \textit{et al.} (1992) and Macina \textit{et al.} (1995). The products were electrophoresed on 5% MDE® gel (3% glycerol) (Cambrex Bio Science Rockland, Inc.) at 6 W for 10-14 h at 20 °C, and visualized after silver staining (Bassam \textit{et al.}, 1991). Amplicons with altered migration patterns were sequenced using the DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences) and analyzed in a MegaBACE 1000 DNA Analysis System (Amersham Biosciences).

To investigate the pathogenic status of the missense mutation, we tested 150 normal controls (80 of predominantly Caucasian extraction, and 70 of predominantly African extraction) by PCR followed by restriction-endonuclease digestion with \textit{PstI}.

\textbf{Results and Discussion}

In the affected individual of Family 1, we detected a missense mutation in exon 2 (c.142G>T) that involved an amino acid substitution (G48C) in the paired domain of the \textit{PAX3} protein. Using PolyPhen (http://www.bork.embl-heidelberg.de/PolyPhen/) as a prediction tool, this mutation was predicted to be probably damaging. Although not presenting any of the eight cardinal signs or symptoms of WSI, the patient’s father carried this same mutation. To our knowledge, this is the first molecularly documented case of lack of penetrance in a carrier of a mutation responsible for WSI. No normal control out of 150 tested presented this mutation.

In Family 2, linkage analysis with microsatellite markers close to \textit{PAX3} confirmed the involvement of \textit{PAX3} in the etiology of the condition. Figure 1 shows the cosegregation of the haplotype D2S126/D2S396 with the \textit{PAX3} gene in all affected individuals in which molecular analysis was performed. The maximum lod-score value (3.20), cor-

\begin{table}[h]
\centering
\begin{tabular}{|l|cccccccccc|}
\hline
Clinical signs & III-5 & IV-2 & IV-13 & IV-14 & V-1 & V-2 & V-3 & V-6 & V-8 & VI-1 \\
\hline
Telecanthus & + & + & + & - & + & + & + & + & + & + \\
Synophrys & + & + & + & + & + & + & + & + & + & + \\
Iris pigmentation disorders & + & + & + & + & + & + & + & + & + & + \\
Localized hair albinism & + & + & - & - & + & + & + & + & - & - \\
Inferior lacrimal dystopia & - & + & - & - & + & + & + & + & - & + \\
\hline
\end{tabular}
\caption{Distribution of the cardinal signs and symptoms of WS in ten personally examined patients of Family 2 (see Figure 1).}
\end{table}
responding to marker D2S126 alone, was found in the family for a recombination fraction value of $\theta = 0$ (zero). The novel mutation identified in this gene consisted of a deletion of 13 base pairs [c.764-776del(TTACCCTGACATT)] in exon 5, responsible for the generation of a frameshift and of a truncated protein. In the family, this deletion was present in nine tested affected individuals (III-5, IV-2, IV-13, IV-14, V-1, V-2, V-3, V-6, and V-8) and absent in nine tested non-affected individuals (V-5, V-13, V-19, V-20, V-21, VI-1, VI-3, VI-5, and VI-8). The mutations described here, besides contributing to the WS mutation database, are important for a correct differentiation between WS variants and for the identification of carriers exhibiting few or no clinical manifestations. Previously described mutational screenings in PAX3 among WSI patients failed to detect mutations in at least 10% of the patients. The situation is even worse with regard to WSII, in which only about 20% of the patients present mutations in MITF, and very few in SLUG. If no mutation is detected in an isolated case of WS without telecanthus, the clinical data gain more importance in the differential diagnosis of the condition. As we pointed out elsewhere (Pardono et al., 2003), discriminant analysis based on the frequency of cardinal signs/symptoms or ocular measurements are efficient in correctly classifying patients affected by variants I and II of WS.

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References


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