Research Article

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

Eliete Pardono, Juliana F. Mazzeu, Karina Lezirovitz, Maria Teresa B.M. Auricchio, Paula Iughetti, Rafaella M.P. Nascimento, Regina C. Mingroni-Netto and Paulo A. Otto

Centro de Estudos do Genoma Humano, Departamento de Genética e Biologia Evolutiva,
Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil.

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.

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

Send correspondence to Paulo A. Otto. Centro de Estudos do Genoma Humano, Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, Caixa Postal 11461, 05422-970 São Paulo, SP, Brazil. E-mail: otto@usp.br.

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 *PAX3* gene, associated with WSI in two families.

Material and Methods

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 *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.

Molecular analysis

In order to confirm the role of *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 *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 *et al.* (1992) and Macina *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 *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 *PstI*.

Results and Discussion

In the affected individual of Family 1, we detected a missense mutation in exon 2 (c.142 G > T) that involved an amino acid substitution (G48C) in the paired domain of the 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 *PAX3* confirmed the involvement of *PAX3* in the etiology of the condition. Figure 1 shows the cosegregation of the haplotype D2S126/D2S396 with the *PAX3* gene in all affected individuals in which molecular analysis was performed. The maximum lod-score value (3.20), cor-

Table 1 - Distribution of the cardinal signs and symptoms of WS in ten personally examined patients of Family 2 (see Figure 1).

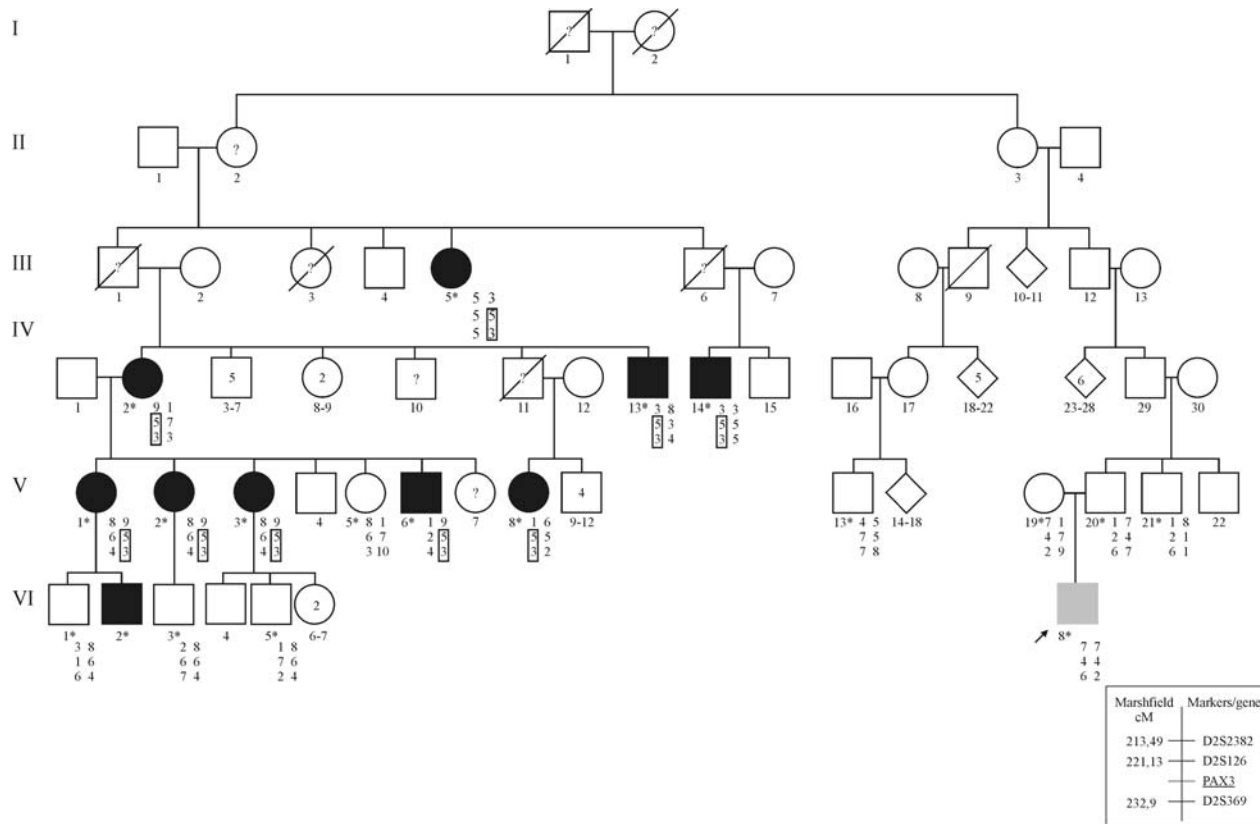| Clinical signs	Individuals									
	III-5	IV-2	IV-13	IV-14	V-1	V-2	V-3	V-6	V-8	VI-1
Telecanthus	+	+	+	-	+	+	+	+	+	+
Synophrys	+	+	+	+	+	+	+	+	+	+
Iris pigmentation disorders	+	+	+	+	+	+	+	+	+	+
Localized hair albinism	+	+	+	-	+	+	+	+	+	-
Deafness	+	-	+	+	+	+	-	-	-	?
Hypopigmented skin spots	-	-	-	-	+	+	-	+	-	-
Nasal root hyperplasia	-	+	+	-	+	+	-	-	+	+
Inferior lacrimal dystopia	-	+	-	-	+	+	+	+	-	+

Figure 1 - Pedigree of Family 2. The boxed haplotype defined by markers D2S126 and D2S396 segregates with the WSI trait in the clinically evaluated family members. The proband, indicated in gray, is a deaf individual not affected by WSI.

responding to marker D2S126 alone, was found in the family for a recombination fraction value of θ = 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.

Acknowledgments

References

Baldwin CT, Hoth CF, Amos JA, da-Silva EO and Milunsky A (1992) An exonic mutation in the *Hup2* paired domain gene causes Waardenburg's syndrome. Nature 355:637-638.

Baldwin CT, Hoth CF, Macina RA and Milunsky A (1995) Mutation in *PAX3* that cause Waardenburg syndrome type I: Ten new mutations and review of the literature. Am J Med Genet 58:115-122.

Bassam BJ, Caetano-Anolles and Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:80-3.

Epstein JA, Shapiro DN, Cheng JLPY and Mass RL (1996) *Pax3* modulates expression of the c-Met receptor during limb muscle development. Proc Natl Acad Sci USA 93:4213-4218.

Ishikiriyama S, Tonoki H and Shibuya Y (1989) Waardenburg syndrome type I in a child with de novo inversion (2)(q35q37.3). Am J Med Genet 33:505-507.

Lin SP, Petty EM, Gibson LH, Inserra J, Seashore MR and Yang-Feng TL (1992) Smallest terminal deletion of the long arm of chromosome 2 in a mildly affected boy. Am J Med Genet 44:500-502.

Macina RA, Barr FG, Galili N and Riethman HC (1995) Genomic organization of the human *PAX3* gene: DNA sequence analysis of the region disrupted in alveolar rhabdomyosarcoma. Genomics 26:1-8.

Morell R, Friedman TB, Moeljopawiro S, Hartono N, Soewito and Asher JH (1992) A frameshift mutation in the *HuP2* paired domain of the probable human homolog of murine *Pax-3* is responsible for Waardenburg syndrome type 1 in an Indonesian family. Hum Molec Genet 1:243-247.

Pardono E, van Bever Y, van den Ende J, Havrenne PC, Iughetti P, Maestrelli SRP, Costa Filho O, Richieri-Costa A, Frota-Pessoa O and Otto PA (2003) Waardenburg syndrome: Clinical differentiation between types I and II. Am J Med Genet 117A:223-235.

Read AP and Newton VE (1997) Waardenburg syndrome. J Med Genet 34:656-665.

Smith SD, Kelley PM, Kenyon JB and Hoover D (2000) Tietz syndrome (hypopigmentation/deafness) caused by mutation of *MITF*. J Med Genet 37:446-448.

Tajbakhsh S, Rocancourt D, Cossu G and Buckingham M (1997) Redefining the genetic hierarchies controlling skeletal myogenesis: *Pax3* and *Myf-5* act upstream of *MyoD*. Cell 89:127-138.

Tassabehji M, Read AP, Newton VE, Patton M, Gruss P, Harris R and Strachan T (1993) Mutation in the *PAX3* gene causing Waardenburg syndrome type 1 and type 2. Nature Genet 3:26-30.

Wildhardt G, Winterpacht A, Hibert K, Menger H and Zabel B (1996) Two different *PAX3* gene mutations causing Waardenburg syndrome type I. Molec Cell Probes 10:229-231.

Associate Editor: Francisco Mauro Salzano