Phenotypic Similarities and Differences in Patients with a p.Met112Ile Mutation in *SOX10*

Veronique Pingault,[1,2,3*] Laurence Pierre-Louis,[4] Asma Chaoui,[2,3] Alain Verloes,[5] Elisabeth Sarrazin,[6] Goran Brandberg,[7] Nadege Bondurand,[2,3] Peter Uldall,[8] Sylvie Manouvrier-Hanu[9,10]

[1]Hôpital Henri Mondor, Laboratoire de Biochimie et Génétique, F-94000, Créteil, France

[2] INSERM, U955, Equipe 11, F-94000, Créteil, France ;

[3] Université Paris-Est, UMR_S955, UPEC, F-94000, Créteil, France

[4] Maison de la Femme de la Mère et de l'Enfant (MFME), Service de réanimation, de soins intensifs néonatals et de néonatologie, CHU Martinique, F-97200 Fort-de-France

[5] Department of Genetics, APHP-Robert Debre University Hospital, Paris VII-Denis Diderot University and INSERM U676, Paris, France

[6] Centre de référence caribéen des maladies rares neuromusculaires et neurologiques adultes et enfant, CERCA, Hôpital Pierre Zobda Quitman, CHU Martinique, F-97200 Fort-de-France

[7] Pediatric and Adolescent Clinic, County Hospital, Falun , Sweden

[8] Pediatric and Adolescent Clinic, The Juliane Marie Centre, Rigshospitalet, and University Hospital, Copenhagen, Denmark

[9] Service de Génétique Clinique, Centre Hospitalo-Universitaire de Lille, 59037 Lille, France

[10] Faculté de Médecine, Université Lille 2, 59045 Lille, France

Running title: SOX10 mutations and phenotypic variability

***Correspondence to:** Veronique Pingault, IMRB (Inserm U955), Equipe 11, Hopital Henri Mondor, 94010 Creteil Cedex, France; Phone 33-1-49-81-28-96; Fax 33-1-48-99-33-45; email: veronique.pingault@inserm.fr

ABSTRACT

Waardenburg syndrome (WS) is characterized by an association of pigmentation abnormalities and sensorineural hearing loss. Four types, defined on clinical grounds, have been delineated, but this phenotypic classification correlates imperfectly with known molecular anomalies. *SOX10* mutations have been found in patients with type II and type IV WS (i.e., with Hirschsprung disease), more complex syndromes, and partial forms of the disease. The phenotype induced by *SOX10* mutations is highly variable and, except for the neurological forms of the disease, no genotype-phenotype correlation has been characterized to date. There is no mutation hotspot in *SOX10* and most cases are sporadic, making it particularly difficult to correlate the phenotypic and genetic variability.

This study reports on three independent families with *SOX10* mutations predicted to result in the same missense mutation at the protein level (p.Met112Ile), offering a rare opportunity to improve our understanding of the mechanisms underlying phenotypic variability. The pigmentation defects of these patients are very similar, and the neurological symptoms showed a somewhat similar evolution over time, indicating a potential partial genotype-phenotype correlation. However, variability in gastrointestinal symptoms suggests that other genetic factors contribute to the expression of these phenotypes. No correlation between the rs2435357 polymorphism of *RET* and the expression of Hirschsprung disease was found. In addition, one of the patients has esophageal achalasia, which has rarely been described in WS.

Key words: SOX10 protein, Waardenburg syndrome, Hirschsprung disease, Pigmentation disorders, c-ret, Proto-Oncogene Protein, Phenotype, Genotype

INTRODUCTION

Waardenburg syndrome (WS) is characterized by an association of pigmentation abnormalities, including depigmented patches of skin and hair, early graying, vivid blue eyes or heterochromia of the irises, and sensorineural hearing loss. Four types, defined clinically, have been described; however, this phenotypic classification correlates imperfectly with known molecular anomalies. Six genes are associated with WS, with very different frequencies; *PAX3*, *MITF*, *EDN3*, *EDNRB*, *SNAI2*, and *SOX10* (for a review, see [Pingault et al., 2010]).

SOX10 is one of 20 proteins belonging to the SOX family of transcription factors. The name 'SOX', a contraction of 'SRY box', refers to their homology to the testis determining factor. These proteins are involved in cell fate determination and cell lineage development [Kuhlbrodt et al., 1998], with SOX10 specifically contributing to the early development of the neural crest (from which melanocytes, the enteric nervous system, and part of the peripheral nervous system originate) and to oligodendrocyte differentiation. Accordingly, *SOX10* mutations can result in type II WS (WS2 (MIM# 611584); depigmentation and deafness) [Bondurand et al., 2007], type IV WS (WS4 (MIM# 613266); depigmentation, deafness, and Hirschsprung disease (HD, absence of enteric ganglia in the distal part of the intestine, MIM# 142623)) [Pingault et al., 1998], PCWH (peripheral demyelinating neuropathy, central dysmyelinating leucodystrophy, WS, HD (MIM# 609136)) [Inoue et al., 2004], a more complex syndrome also involving the central and/or peripheral nervous system, and Kallmann syndrome (hypogonadism and anosmia (MIM# 308700)) through a defect affecting the neural crest-derived olfactory ensheathing cells [Pingault et al., 2013]. The influence of modifier genes has been hypothesized to explain this high degree of phenotypic variability.

SOX10 mutations are usually private; that is, almost all mutations found in families are novel. Moreover, most cases are sporadic. This situation makes it considerably more difficult to decipher the origin of the phenotypic variability among patients. Here, we describe three

independent families all carrying mutations predicted to result in the same missense change at the protein level, offering a rare opportunity to improve our understanding of the mechanisms underlying phenotypic variability.

CLINICAL REPORTS

Patient 1 (Denmark):

This male patient was born from healthy parents, at term, and with normal growth parameters (birth weight 3450 g (0SD), height and OFD were reported as normal without precision). He was hypotonic and had bilateral cryptorchidism. Hormonal evaluation was normal. Hearing problems were suspected at neonatal screening and further examination revealed profound sensorineural deafness with absent auditory evoked potentials. A temporal bone CT scan was normal. Cochlear implantation was performed at the age of 1 year. Bilateral nystagmus, observed when the patient was 1 month old, disappeared spontaneously 2 months later. When first examined at 3 months of age, he had bright blue irides, normal fundi oculi, a white forelock, a depigmented spot on the forehead, large areas of depigmentation extending from the knee to the heel on the left side, and a sock-like depigmentation on the right foot. Cafe-au-lait spots were also present on the lower extremities. Although meconium emission has not been retarded, severe constipation had begun soon after birth, leading to a diagnosis of HD and the patient underwent surgery at 5 months of age. Aganglionosis extended towards the right angle of the colon (long-segment form). Furthermore, neurological examinations during infancy were abnormal. At 5 months of age, hypotonia was associated with some spasticity and abnormal movements. Motor milestones were delayed (sitting age 10 months, walking age 18 months). At 2 years of age, he was still hypotonic and had minor muscular weakness; however, his fine motor abilities were not delayed and his tendon reflexes were normal. Speech was moderately delayed (30 words), which was to be expected when taking into account his hearing problems.

Nevertheless, at the age of 6 years, the patient has no cognitive impairments and is able to start school without assistance. Speech is normalized. Growth parameters are normal (height 122 cm: +1.5SD; weight 21 kg: +0.5SD) and he has normal genitalia.

Patient 2 (Martinique):

This male patient is the first child of young, unrelated, and healthy parents. He was born at 40 weeks of gestation, with severe intra-uterine growth retardation (IUGR): birth weight 1830 g (−3.9 SD), length 44 cm (−3 SD), and OFC 33 cm (−2 SD). Meconium emission was normal. When examined at 3 months of age, he had constipation, axial hypotonia, bilateral nystagmus and strabismus, bright blue irises, a white forelock, depigmented spots on the medial forehead, the philtrum, and both hands and feet (Fig. 1A-E). At 9 months, examination revealed developmental delay (the baby was unable to sit) with axial hypotonia and limb spasticity. His head circumference was 46.5 cm (+1.7 SD). The parents noted premature graying of the hair and eyebrows, beginning at 8 months of age. Nystagmus was associated with severe visual impairment. Profound bilateral sensorineural deafness was diagnosed and a temporal bone CT scan showed no other anomaly than slight bilateral dilation of the vestibules and lateral semicircular canals. Simple dietary management relieved constipation. Genitalia were normal male. Later, the neurological development improved and the patient walked at 18 months of age. Unilateral cochlear implantation was performed when he was 20 months of age. At 4 years, his motor and comprehension abilities are described as normal, even if he is still unable to speak. Height is 116 cm (+4SD), weight 16.5 kg (+0.5SD) and OFC 53 cm (+1,5SD).

Family 3 (France):

Patient 3 is the second child of healthy parents. Her brother is also healthy and she was born at term. At birth weight was 3000 g (-0.5 SD) and length 50 cm (0 SD). According to her parents OFC was “normal”, however the patient was unable to precise it. Profound bilateral sensorineural deafness was diagnosed at the age of 6 months, and she was taught to use sign

language. Early motor development was slightly delayed (she walked at 2 years of age), but subsequent development was normal, and she attended mainstream school. When examined at age 46 years, her height was 158 cm (-1SD) and her weight 60 kg (+1SD), She had blue irides, bilateral iris hypoplasia, and albinoid fundi. Neurological examination was normal. She had white-blond hair and eyebrows, and reported premature graying of her hair and eyebrows at 16 years of age (Fig. 1F,G). Furthermore, she had depigmented spots mimicking a left glove and a right sock (Figure 1H). In adulthood, she developed some freckles on the face. She did not complain of constipation. She had numerous caries. She declined carrying out a CT Scan. Her husband had type I WS (dystopia canthorum, deafness, and white forelock) and he carried a heterozygous *PAX3* mutation (c.936C>A, p.Tyr312X). They had two daughters; the first one is patient 4 and the other was not affected by WS.

Patient 4:

This girl was born at term (39 SA) with normal growth parameters (birth weight 3120 g (0SD), length 49 cm (0SD), and OFD 34,5 cm (0SD)). She was not hypotonic, and meconium emission was not delayed. Profound bilateral sensorineural deafness was diagnosed soon after birth. When examined at the age of 3 months, she had bright blue irises and nystagmus. Ophthalmologic examination revealed bilateral albinoid fundi. Electroretinogram and visual evoked potentials were normal. Neurological examination was normal, but pigmentation anomalies were observed (white forelock and eyebrows, median depigmented spot on the forehead, and a depigmented left hand mimicking a glove) (Fig. 1I–K). Genitalia were normal female. Later, neurological development was slightly delayed (walking at 2 years of age). She used sign language and her hair and eyelashes became white at around 12 years of age. She noted some balance disorders. She was slightly constipated, but had no HD. At 13 years, dysphagia and retro-esophageal pain led to a diagnosis of esophageal dyskinesia / achalasia. She was treated with NIFEDIPINE®; however, the treatment was discontinued, as it caused dizziness. When last examined at 15 years, she attended a mainstream school for deaf

children. Her height was 152 cm (-1,7SD), her weight was 50 kg (-0,2SD. Genitalia were normal and puberty had begun but she had not started menstruation. The hormonal assessment highlighted a pituitary insufficiency in GH and gonadotropic axes. CT Scan revealed a normal pituitary gland and left olfactory bulb but the absence of the right olfactory bulb.

Patients clinical features are summarized in Table I.

MOLECULAR ANALYSIS AND RESULTS

SOX10 mutations were found in all four patients using standard Sanger sequencing. Mutations were named according to international nomenclature, with +1 corresponding to the A of the ATG translation initiation codon in the cDNA reference sequence: GenBank NM_006941.3 for *SOX10*, and NM_181459.2 for *PAX3*. Patients 1 and 2 both carry a *de novo* heterozygous c.336G>A substitution in *SOX10* that is predicted to result in a missense mutation, p.Met112Ile, at the protein level. Patient 3 and her daughter, patient 4, both carry a *SOX10* heterozygous c.336G>C substitution that is also predicted to result in a p.Met112Ile missense mutation. The *PAX3* mutation found in patient 3's husband was not transmitted to his daughters. The *SOX10* mutations are not predicted to alter splicing and were previously reported (without a clinical description) in a paper addressing their functional consequences *in vitro* [Chaoui et al., 2011].

In a search for genetic modifiers of the intestinal phenotype in these patients, we genotyped the rs2435357 polymorphism of *RET* in genomic DNA from all patients and available family members using the following primers: 5'CATGATCTGAGTCACTGCCT, 3'CTGGCCAGGATGATGGGCGC. PCR amplicons (after 35 cycles at an annealing temperature of 60°C) were sequenced by standard protocols on a 16-capillary ABI Prism sequencer using an internal primer (TGCTGAGTGCATGGGGACAG). Patients 1, 2, and 3 are homozygous for the C allele, while patient 4 and her sister are heterozygous for the T allele, which was inherited from their father. The molecular results are presented in Figure 2.

DISCUSSION

Approximately 100 *SOX10* point mutations and deletions have been published to date (http://grenada.lumc.nl/LOVD2/WS/home.php?select_db=SOX10). Each is specifically linked to a family, with the notable exception of p.Arg313X, which has been found four times (resulting from three different nucleotide variations, a situation very similar to the p.Met112Ile mutation described here) and results in PCWH [Inoue et al., 2004; Touraine et al., 2000]. Independently of the presence or absence of neurological features, *SOX10* mutations represent about half of WS4 and 15% of WS2 patients [Bondurand et al., 2007]. All features show incomplete penetrance and variable expressivity. *SOX10* mutations have also been reported in patients with atypical WS4 with intestinal pseudo-obstruction [Pingault et al., 2002], isolated HD [Sanchez-Mejias et al., 2010], and Kallmann syndrome with deafness [Pingault et al., 2013], highlighting the high level of phenotypic variability associated with changes at this locus. To date, no obvious genotype-phenotype correlations have been reported, other than neurological defects associated with some of the truncating mutations that appear to escape nonsense-mediated RNA decay (NMD) [Inoue et al., 2004].

Initially, most *SOX10* mutations (nonsense, splice, frameshift, or deletions were predicted to result in a truncated or absent protein. However, we and others recently found that missense changes in the *SOX10* HMG domain (its DNA binding domain) also account for a significant proportion of mutations [Barnett et al., 2009; Bondurand et al., 1999; Chaoui et al., 2011; Morin et al., 2008]. As they are missense changes, these mutations are not recognized by the NMD pathway and have been associated with all forms of WS (WS2, WS4, PCWH, PCW (PCWH without HD), and Kallmann syndrome with deafness) [Chaoui et al., 2011; Pingault et al., 2013]. We previously analyzed the functional consequences of these missense mutations to investigate potential genotype-phenotype correlations, taking advantage of the fact that the mutated transcripts avoid NMD-mediated RNA degradation. We

found no obvious relationship between their functional effects *in vitro* and the phenotypic features present in the patients [Chaoui et al., 2011]. However, in this study, a p.Met112Ile mutation was identified in three independent families, providing a rare opportunity to study the phenotypic similarities and differences between the patients.

The four patients carrying this mutation have very similar pigmentation defects: bright blue irises, a white forelock, a white spot on the forehead, and large depigmented areas on the extremities that mimic gloves and/or socks. Although the latter aspect is described in WS, it is uncommon. Premature graying of hair and eyebrows has not yet been observed in patient 1, who is very young, but was apparent in the other three patients. The four patients all had profound bilateral sensorineural hearing loss. Transient nystagmus was present in early childhood in three patients; neonatally in patients 1 and 2, and during the first weeks of life for patient 4. This information was not available for patient 3. Furthermore the four patients had transient motor delay (more severe in patient 2), which recovered during early childhood, without obvious intellectual disability. These similarities in three independent families, especially the pigmentation pattern, suggest a genotype-phenotype correlation.

Conversely, the phenotypes of patients differ in other ways: patient 2 had severe IUGR of unknown cause, however, further survey showed normal postnatal growth, so that a placental cause is likely. In regards to Kallmann syndrome features, patient 4 showed an absence of the right olfactory bulb and gonadotropic hormonal insufficiency, but these aspects could not be fully evaluated in the other patients. More specifically, the gastrointestinal features of the patients are also discordant: a single patient (patient 1) has HD, whereas the others suffer from slight constipation (patient 4), transient constipation in early infancy (patient 2), or has normal transit in adulthood (patient 3).

This clinical variability in patients carrying the same (or similar) mutations suggests that the genetic environment modifies the phenotypic expression of the intestinal phenotype. An obvious candidate genetic modifier is rs2435357, a common *RET* intronic polymorphism

located in an enhancer. The T allele (frequency 0.25 in Europe) alters a functional *SOX10* binding site and results in a lower level of expression of *RET in vitro*. It is statistically associated with HD susceptibility [Emison et al., 2010; Emison et al., 2005]. rs2435357 genotyping showed that the only patient with HD does not carry the T allele, arguing against an association between the presence of the T allele and HD in patients with the p.Met112Ile mutation. This observation does not allow any generalization from a statistical point of view; however, it is complementary of a previous study showing an absence of correlation between the rs2435357 genotype and HD in 24 WS4 patients carrying various *SOX10* mutations [de Pontual et al., 2007].

In addition to the intestinal involvement, the diagnosis of esophageal achalasia in patient 4 is remarkable. Esophageal achalasia is related to other disorders of gastrointestinal motility, especially HD, even at the molecular level [Gockel et al., 2012], but it has, to our knowledge not been described with WS. Achalasia is considered a disease of delayed onset disease, and is caused by the degeneration of a subpopulation of enteric neurons in the esophagus, whereas HD is a developmental disease associated with congenital absence of intrinsic ganglion cells in the myenteric and submucosal plexuses of the distal bowel. The association of achalasia and WS may be coincidental, but may also point to an underdiagnosed complication with low penetrance.

Apart from the impact of NMD in the neurological phenotype of patients carrying *SOX10* point mutations [Inoue et al., 2004], no obvious genotype-phenotype correlations have been described to date. Our observation of the same mutation at the protein level in four patients from three families provides a rare opportunity to move toward precise interpretation, and allows the following conclusions: 1) the very similar pigmentation defects (especially the glove / sock pattern affecting the extremities) and the somewhat similar neurological evolution (hypotonia in early childhood without intellectual disability in later development) suggest a partial genotype-phenotype correlation; and 2) variable gastrointestinal defects

suggest that other genetic factors contribute to the expression of this phenotype. Finally, we would like to highlight the unusual association with esophageal achalasia, appearing in one patient at 13 years of age. Given the difficulty of diagnosing achalasia, we recommend that this should be considered as a possible manifestation in WS patients, particularly in case of dysphagia. Further observations are required to determine if achalasia is a rare feature of WS, or coincidental.

ACKNOWLEDGMENTS

We thank the patients and their families for their contribution to the study. We acknowledge Dr Monique Elmaleh (Hopital Robert Debré, Paris) for her radiological expertise. This work was supported by the Institut de la Santé et de la Recherche Médicale (INSERM), the Assistance Publique des Hôpitaux de Paris (AP-HP), and the Agence Nationale de la Recherche (ANR-JCJC-2010). A.C. is a recipient of a fellowship from the Fondation pour la Recherche Médicale (FRM). The authors declare no conflict of interest.

REFERENCES

Barnett CP, Mendoza-Londono R, Blaser S, Gillis J, Dupuis L, Levin AV, Chiang PW, Spector E, Reardon W. 2009. Aplasia of cochlear nerves and olfactory bulbs in association with SOX10 mutation. Am J Med Genet A 149A:431-436.

Bondurand N, Dastot-Le Moal F, Stanchina L, Collot N, Baral V, Marlin S, Attie-Bitach T, Giurgea I, Skopinski L, Reardon W, Toutain A, Sarda P, Echaieb A, Lackmy-Port-Lis M, Touraine R, Amiel J, Goossens M, Pingault V. 2007. Deletions at the SOX10 gene locus cause Waardenburg syndrome types 2 and 4. Am J Hum Genet 81:1169-1185.

Bondurand N, Kuhlbrodt K, Pingault V, Enderich J, Sajus M, Tommerup N, Warburg M, Hennekam RC, Read AP, Wegner M, Goossens M. 1999. A molecular analysis of the yemenite deaf-blind hypopigmentation syndrome: SOX10 dysfunction causes different neurocristopathies. Hum Mol Genet 8:1785-1789.

Chaoui A, Watanabe Y, Touraine R, Baral V, Goossens M, Pingault V, Bondurand N. 2011. Identification and functional analysis of SOX10 missense mutations in different subtypes of Waardenburg syndrome. Hum Mutat 32:1436-1449.

de Pontual L, Pelet A, Clement-Ziza M, Trochet D, Antonarakis SE, Attie-Bitach T, Beales PL, Blouin JL, Dastot-Le Moal F, Dollfus H, Goossens M, Katsanis N, Touraine R, Feingold J, Munnich A, Lyonnet S, Amiel J. 2007. Epistatic interactions with a common hypomorphic RET allele in syndromic Hirschsprung disease. Hum Mutat 28:790-796.

Emison ES, Garcia-Barcelo M, Grice EA, Lantieri F, Amiel J, Burzynski G, Fernandez RM, Hao L, Kashuk C, West K, Miao X, Tam PK, Griseri P, Ceccherini I, Pelet A, Jannot AS, de Pontual L, Henrion-Caude A, Lyonnet S, Verheij JB, Hofstra RM, Antinolo G, Borrego S, McCallion AS, Chakravarti A. 2010. Differential contributions of rare and common, coding and noncoding Ret mutations to multifactorial Hirschsprung disease liability. Am J Hum Genet 87:60-74.

Emison ES, McCallion AS, Kashuk CS, Bush RT, Grice E, Lin S, Portnoy ME, Cutler DJ, Green ED, Chakravarti A. 2005. A common sex-dependent mutation in a RET enhancer underlies Hirschsprung disease risk. Nature 434:857-863.

Gockel HR, Gockel I, Schimanski CC, Schier F, Schumacher J, Nothen MM, Lang H, Muller M, Eckardt AJ, Eckardt VF. 2012. Etiopathological aspects of achalasia: lessons learned with Hirschsprung's disease. Dis Esophagus 25:566-572.

Inoue K, Khajavi M, Ohyama T, Hirabayashi S, Wilson J, Reggin JD, Mancias P, Butler IJ, Wilkinson MF, Wegner M, Lupski JR. 2004. Molecular mechanism for distinct neurological phenotypes conveyed by allelic truncating mutations. Nat Genet 36:361-369.

Kuhlbrodt K, Herbarth B, Sock E, Hermans-Borgmeyer I, Wegner M. 1998. Sox10, a novel transcriptional modulator in glial cells. J Neurosci 18:237-250.

Morin M, Vinuela A, Rivera T, Villamar M, Moreno-Pelayo MA, Moreno F, del Castillo I. 2008. A de novo missense mutation in the gene encoding the SOX10 transcription factor in a Spanish sporadic case of Waardenburg syndrome type IV. Am J Med Genet A 146A:1032-1037.

Pingault V, Bodereau V, Baral V, Marcos S, Watanabe Y, Chaoui A, Fouveaut C, Leroy C, Verier-Mine O, Francannet C, Dupin-Deguine D, Archambeaud F, Kurtz FJ, Young J, Bertherat J, Marlin S, Goossens M, Hardelin JP, Dode C, Bondurand N. 2013. Loss-of-function mutations in SOX10 cause Kallmann syndrome with deafness. Am J Hum Genet 92:707-724.

Pingault V, Bondurand N, Kuhlbrodt K, Goerich DE, Prehu MO, Puliti A, Herbarth B, Hermans-Borgmeyer I, Legius E, Matthijs G, Amiel J, Lyonnet S, Ceccherini I, Romeo G, Smith JC, Read AP, Wegner M, Goossens M. 1998. SOX10 mutations in patients with Waardenburg-Hirschsprung disease. Nat Genet 18:171-173.

Pingault V, Ente D, Dastot-Le Moal F, Goossens M, Marlin S, Bondurand N. 2010. Review and update of mutations causing Waardenburg syndrome. Hum Mutat 31:391-406.

Pingault V, Girard M, Bondurand N, Dorkins H, Van Maldergem L, Mowat D, Shimotake T, Verma I, Baumann C, Goossens M. 2002. SOX10 mutations in chronic intestinal pseudo-obstruction suggest a complex physiopathological mechanism. Hum Genet 111:198-206.

Sanchez-Mejias A, Watanabe Y, Fernandez MR, Lopez-Alonso M, Antinolo G, Bondurand N, Borrego S. 2010. Involvement of SOX10 in the pathogenesis of Hirschsprung disease: report of a truncating mutation in an isolated patient. J Mol Med 88:507-514.

Touraine RL, Attie-Bitach T, Manceau E, Korsch E, Sarda P, Pingault V, Encha-Razavi F, Pelet A, Auge J, Nivelon-Chevallier A, Holschneider AM, Munnes M, Doerfler W, Goossens M, Munnich A, Vekemans M, Lyonnet S. 2000. Neurological phenotype in Waardenburg syndrome type 4 correlates with novel SOX10 truncating mutations and expression in developing brain. Am J Hum Genet 66:1496-1503.

FIGURE LEGENDS

FIG. 1. Clinical features. A-D: Patient 2 at age 9 months. Note the bright blue irises (A), the white forelock and the depigmented spot on the medial forehead and philtrum (B), the depigmented extremities (C and D). E-G: Patient 3. Note the blue eyes and premature graying of the hair (E, at 28 years of age; F, at 45 years of age), and the depigmented spot mimicking a glove on the left upper limb (G). H-J: Patient 4. Note the bright blue irises, the white forelock and the slight depigmented spot on the medial forehead in early infancy (H, at 1 years of age), premature graying of the hair and the depigmented spot mimicking a glove on the left upper limb (I and J, at 15 years of age).

FIG 2. Summary of molecular and familial data. A, Patient 1; B, Patient 2; and C, Patients 3 and 4. The presence or absence of Hirschsprung disease is indicated under each patient. N, normal.