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Novel and recurrent non-truncating mutations of the MITF basic domain: genotypic and phenotypic variations in Waardenburg and Tietz syndromes

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ABSTRACT

The microphthalmia-associated transcription factor (MITF) is a basic helix-loop-helix leucine zipper transcription factor which regulates melanocyte development and the biosynthetic melanin pathway. A notable relationship has been described between non-truncating mutations of its basic domain and Tietz syndrome, which is characterized by albinoid-like hypopigmentation of the skin and hair, rather than the patchy depigmentation seen in Waardenburg syndrome, and severe hearing loss. Twelve patients with new or recurrent non-truncating mutations of the MITF basic domain from six families were enrolled in this study. We observed a wide range of phenotypes and some unexpected features. The patients all had blue irides and pigmentation abnormalities that ranged from diffuse hypopigmentation to Waardenburg-like patches. In addition, they showed congenital complete hearing loss, diffuse hypopigmentation of the skin, freckling and ocular abnormalities, more frequently than patients with MITF mutations outside the basic domain. In conclusion, the non-truncating mutations of the basic domain do not always lead to Tietz syndrome but rather to a large range of phenotypes. Sun-exposed freckles are interestingly observed more frequently in Asian populations. This variability argues for the possible interaction with modifier loci.

INTRODUCTION

The microphthalmia-associated transcription factor (MITF) is a basic helix-loop-helix (bHLH) leucine zipper transcription factor which regulates melanocyte development and the biosynthetic melanin pathway. Its gene has several alternative promoters and first exons that produce differentially expressed isoforms.[1] Mutations in the M (melanocytic) isoform of *MITF* are known to lead to Waardenburg syndrome type 2A (WS2A, MIM 193510), an autosomal dominant disorder characterized by variable degrees of sensorineural hearing loss and pigmentation disorders of the skin, skin appendages and irides.[2-3] Rarely, *MITF* mutations lead to Tietz syndrome (MIM 103500), an allelic condition characterized by a more severe phenotype of hearing loss and generalized, albinoid-like hypopigmentation of the skin and hair from birth, rather than the patchy depigmentation seen in Waardenburg syndrome (WS).[3-4]

A notable relationship between non-truncating mutations of the basic domain and Tietz syndrome has been described.[3, 5-10] The basic domain of bHLH transcription factors is the DNA binding domain, necessary to recognize and bind their transcriptional targets. In contrast to previous reports, we identify new families with such MITF mutations associated with phenotypic features ranging from from Tietz to Waardenburg syndrome, and the literature was reviewed to assess the genotype-phenotype correlation.

PATIENTS AND METHODS

Sequencing of the *MITF-M* isoform exons was modified from Tassabehji et al.[11] The absence of total or partial gene deletion was assessed by QMF-PCR (Quantitative Multiplex Fluorescent PCR).[12] Mutations were named according to the international nomenclature based on Genbank NM_000248.2 for *MITF-M* (isoform 4) cDNA. More details are given in the supplementary data.

Twelve patients from six families, with new or recurrent non-truncating mutations of the MITF basic domain, were enrolled in this study. None of the mutations was described as a polymorphism in the relevant databases (http://www.ncbi.nlm.nih.gov/snp, http://browser.1000genomes.org). When necessary to confirm the de novo occurrence, six microsatellites were analysed using the linkage mapping set (Applied Biosystems, Foster City, CA). Mutations were analysed using several software packages including Human splicing finder v2.4[15] (http://www.umd.be/HSF/HSF.html) and Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) in order to evaluate their effect. The conformation files for Srebp1-A, Usf, Myc, Mad and Max were imported from the protein data bank (accession codes 1AM9, 1AN4, 1HLO, 1NKP) and represented using the Swiss-Pdb Viewer software.[16]

RESULTS

Clinical Data

Family 1: six members of a French family of Vietnamese and Martinique origins were affected in three generations (Figure 1a). The proband (III.1) was a 9-year-old boy who was referred for premature greying affecting hair, eyebrows and eyelashes. In contrast with the familial dark skin pigmentation, he had generalized hypopigmentation of the skin as well as patchy depigmented macules, freckles in sun-exposed regions, lentigines and cafe-au-lait macules (Figure 1b,c,d). He had blue irides and global hypopigmentation on fundoscopic examination. W index=0.87. The auditory function was normal. A description of the whole family is presented in the supplementary data.

Family 2: a 36-year-old French woman had congenital profound sensorineural hearing loss, a white forelock, blue irides but no skin pigmentation disorder. There was

a familial history of congenital deafness in her parents and siblings. Her father had premature greying, and both her mother and brother had a white forelock with blue irides. Her son had isolated hearing loss.

Family 3: a 33-year-old South African woman of European descent had congenital profound sensorineural hearing loss (90-120dB), a white forelock preceding premature greying of hair, eyelashes and eyebrows, hypopigmented macules and freckles in the pigmented areas. She had blue irides, right exotropia and myopia. W index=1.77. Fundus examination revealed marked hypopigmentation and visual evoked potentials were normal. Her parents and two sisters had normal phenotypes, although an history of greying at about 30 years of age was reported in the father's family.

Family 4: a 21-year-old French woman had congenital profound sensorineural hearing loss, a white forelock preceding greying at the age of 16 years, and fair skin but no skin pigmentation disorders. She had blue irides, hyperopia and left esotropia complicated by amblyopia. Her parents and two sisters had normal phenotypes.

Family 5: a 3-year-old French girl had profound sensorineural hearing loss, generalized hypopigmentation, bright blue irides and albinoid hypopigmentation on fundoscopic examination. Her developmental milestones were delayed and she had axial hypotonia. She had strabismus and a suspected amblyopia of the left eye. Her mother had a similar phenotype consistent with Tietz syndrome. Her father had an isolated acquired hearing loss.

Family 6: a 3-year-old Portuguese girl had congenital sensorineural hearing loss, generalized hypopigmentation, blue irides and a white forelock. Her father had a similar phenotype with greying at the age of 20 years.

Identification of mutations

Three novel mutations were characterized. A nucleotide substitution, c.635T>G, that predicts a missense variation at the protein level (p.Ile212Ser) was found in all affected members of family 1. A c.616A>C (p.Lys206Gln) mutation was found in the proband of family 2 (parents not tested). In family 3, two variations were located on the same allele: c.635-5delT and c.639A>C (p.Glu213Asp). The intronic variation (c.635-5delT) was inherited from the unaffected father and was not predicted to result in splice alteration by *in silico* analysis, while the missense (p.Glu213Asp) mutation occurred de novo and is thought to be responsible for the disease. The proband of family 6 carried a previously reported c.650G>T (p.Arg217Ile) mutation.[17] Parental samples were not available for testing. We briefly reported the mutations found in families 4 (c.647G>A, p.Arg216Lys, de novo) and 5 (c.649_651delAGA, p.Arg217del, in the mother and daughter) in a recent review without a clinical description.[3] All mutations were identified in the heterozygous state.

All the non-truncating mutations of the MITF basic domain (missense substitutions and in-frame deletions, here described or previously published) are reported in Table 1. They all involve amino-acids highly conserved across evolution. None of them is predicted to result in a truncating protein through splice alteration. All are predicted as probably damaging by polyphen-2. In order to further document pathogenicity, we looked at their localisation in tertiary structure. The three-dimensional (3D) structure of MITF has not been determined but several other bHLH factors have been studied in their bound-to-DNA conformation. An example using SREBP1-A[18] is shown in the supplementary Figure. Equivalent amino-acids that are mutated in MITF are on the side of the basic domain α-helix that is localized in contact with the DNA groove, while the unbound side of the α-helix appears devoid of mutations.

DISCUSSION

We report the clinical features and genotypes of six unrelated families segregating missense mutations or in-frame deletions located in the MITF basic domain. Three of these mutations have not been previously reported.

Our report brings to fifteen the number of cases with mutations specifically affecting this domain. The p.Arg217del mutation is peculiar in that it is the only in-frame deletion and it represents half of the cases. It has been found in at least two ethnic groups and often occurs de novo. Its recurrence might be partly due to the presence of a short nucleotide triplet repeat. Functional tests have suggested that this mutation, or its mouse homolog, may act as a dominant negative allele.[9, 19]

Among the abundant mouse *Mitf* alleles, several are similar to the human mutations we identified or affect the same residue: *microphthalmia* ($Mitf^{Mi}$) is similar to p.Arg217del, *Oak-ridge* ($Mitf^{Mi\text{-}Or}$) to p.Arg216Lys, and *White* ($Mitf^{Mi\text{-}wh}$) affects the Ile212 that is changed to Asn.[19] Due to the difference of transmission between mouse and human and to the influence of the background strain in mouse, it is difficult to speculate about the phenotypic correlations between species.

Table 1 regroups the clinical features observed in all fifteen families. The data published initially have been completed here when the first description was brief.[17] Our study reveals a great variability of clinical features, and not exclusively Tietz syndrome as previously hypothesized.

Patient 1 differs from the other cases by the absence of congenital hearing loss. Deafness has a high frequency in our study, affecting 14 out of the 15 families. Pigmentary disorders are always present including blue irides or partial heterochromia, patchy to diffuse skin hypopigmentation, light blond hair from birth or a white forelock, premature greying, freckles, lentigines and cafe-au-lait macules (Table 1).

According to the diagnostic criteria for WS proposed by the Waardenburg Consortium, all the patients could be diagnosed as having WS. Indeed, Tietz syndrome is characterized as a variant with a "more severe" phenotype: association of congenital profound sensorineural hearing loss and uniform dilution of pigmentation (skin, eyes and hair). The observation that melanocyte density is normal in the hypopigmented areas suggests that the migration of melanocytes progenitors occurs normally and argues for an abnormality of melanocyte function[8]. However, both mechanisms may coexist, as generalized hypopigmentation and WS-type depigmented patches are sometimes observed in the same patients (Figure 1c). However, the difference between diffuse hypopigmentation and normal fair skin may be unclear in some cases, and distinction between Tietz and WS is sometimes difficult. Diffuse hypopigmentation could be considered as another variable phenotypic feature of WS, being associated with some, but not all, MITF basic domain mutations. Of note, the patients who independently carry recurrent mutations (p.Arg217del or p.Arg217Ile) do not all show the same phenotype, with only some being classified as Tietz syndrome.

We observed a striking frequency of freckles (60%), mainly in Asian populations (66%). They were not observed within the depigmented patches, possibly because of a complete absence of melanocytes. In the literature, we found only three cases of freckles in patients with other MITF mutations.[17, 20-22] However freckles have not usually been considered as part of the WS pigmentary disorders[2] so far and their occurrence might be underestimated. Chen et al. recently proposed it to be a Chinese variant of the WS phenotype[17] but we found it in some European patients as well. The melanocortin-1 receptor gene, *MC1R*, described as the major freckle gene,[23] is a good candidate to influence this phenotype. It encodes a G-protein-coupled receptor that mediates the α-MSH (melanocyte-stimulating hormone) effect in melanocytes, resulting in an

upregulation of *MITF*. *MC1R* is characterized by a remarkably polymorphic sequence.[24] Some missense changes result in lower eumelanin induction that favors a eumelanin to pheomelanin shift, and explains the association found between the presence of *MC1R* variant alleles and the occurrence of red hair, fair skin and sun sensitivity.[25]

Among features not classically described in WS, we also found frequent eye and vision problems including strabismus in 3 cases and amblyopia in 4 or 5. These problems are not commonly reported to be associated with WS, but Delleman et al. reported that 5 out of 26 WS patients had convergent strabismus (with or without amblyopia), including one with WS2, leading to a 19% occurrence that is notably higher than in the general population.[26] In cases with other MITF mutations, strabismus has only been reported in one family of WS2 with OA,[22] a condition well-known for its strabismus association, or with a polygenic deletion.[27] In our study the high rate (40%) of ocular abnormalities leads to the possibility that they could be more frequently or specifically associated with MITF basic domain mutations. In mouse *Mitf* mutants, eye abnormalities range from severe microphthalmia to late retinal degeneration that were not described in human.[19]

In conclusion, this study highlights the existence of unexpected features and a wide range of phenotypes associated with non-truncating mutations of the MITF basic domain. Congenital complete hearing loss, ocular abnormalities, freckles and diffuse hypopigmentation of skin are more frequent than in patients with MITF truncating mutations or missense mutations located elsewhere in the protein. The large range of phenotype observed and the variability argues for the possible interaction with modifier loci. Freckles are interestingly observed more frequently in Asian populations, which also suggests the impact of genetic modifiers in the development of sun-exposed freckles.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Table 1 Phenotypic features associated with non-truncating mutations of the MITF basic domain.

					Pigmentary disorders								
Exon	**cDNA***	**ARN/protéine**	**Inheritance**	**Hear CHL**	**Eye BI/HI**	**Skin GH/PH**	**Hair WF/PG†/HC**	**Other F/CALM**	**Vision S/A**	**Phenotype**	**Origin**	**Family**	Reference
Exon 6	c.616A>C	**p.Lys206Gln**	Familial	+	+/-	-/-	+/-/Blond	-/-	-/-	**WS2**	France/Italy	2	This study
	c.630C>G	**p.Asn210Lys**	Familial	+	+/-	+/-	-/-/Blond	+/-	-/-	**Tietz syndrome**	USA / Ireland		[6]
Exon 7	c.635T>G	**p.Ile212Ser**	Familial	-	+/+	+/+	-/+/Brown	+/+	-/-	**WS2**	Vietnam/Martinique	1	This study
	c.639A>C (+ c.635-5delT)	**p.Glu213Asp**	De novo	+	+/-	-/+	+/+/Red	+/-	+/-	**WS2**	Europe/South Africa	3	This study
	c.647G>A	**p.Arg216Lys**	De novo	+	+/-	-/-	+/+/Light brown	-/-	+/+	**WS2**	France	4	[3] + this study
	c.649_651delAGA	**p.Arg217del**	Familial	+	+/-	+/-	-/+/Red	+/-	-/-	**Tietz syndrome**	Europe		[5]
	c.649_651delAGA	**p.Arg217del**	De novo	+	+/-	+/-	-/-(24)/Blond	+/-	-/-	**Tietz syndrome**	? (Japanese paper)		[8]
	c.649_651delAGA	**p.Arg217del**	Familial	+	+/-	**?§/-**	-/-(1)#/Red	-/-	-/-	**WS2/Tietz syndrome**	? (US paper)		[10] The index case also had OA + P513R in the *TYRP1* gene
	c.649_651delAGA	**p.Arg217del**	Familial	+	+/-	+/-	-/-(3)/Brown	-/-	+/+**?**	**Tietz syndrome**	France	5	[3] + this study
	c.649_651delAGA	**p.Arg217del**	Familial	+	+/-	+/-	-/-(15)/Blond	-/-	-/-	**Tietz syndrome**	Japan		[9]
	c.649_651delAGA	**p.Arg217del**	De novo	+	+/-	-/-	-/-(10)/Brown	+/-	-/+	**WS2**	China		[17] (completed)
	c.649_651delAGA	**p.Arg217del**	De novo	+	+/-	-/-	-/-(12)/Brown	+/-	-/+	**WS2**	China		[17] (completed)
	c.649_651delAGA	**p.Arg217del**	De novo	+	+/-	-/-	-/-(12)/Brown	+/-	-/+	**WS2**	China		[17] (completed)
	c.650G>T	**p.Arg217Ile**	Familial	+	+/-	+/-	+/-(3)#/Blond	-/-	-/-	**Tietz syndrome**	Portugal	6	This study
	c.650G>T	**p.Arg217Ile**	De novo	+	+/-	-/-	-/+/Brown	+/-	-/-	**WS2**	China		[17] (completed)

CHL: congenital hearing loss, BI/HI: blue irides/heterochromia irides, GH/PH: generalized/patchy hypopigmentation, WF/PG/HC white forelock/premature greying/hair color, F/CALM : freckles/cafe-au-lait macules, S/A: strabismus/amblyopia, WS2: Waardenburg syndrome type 2, OA: ocular albinism.

*cDNA nucleotide numbering with +1 as the A of the initiation codon in the reference sequence NM_000248.2 corresponding to the M(melanocytic)-isoform of MITF.

†: when propositus did not show PG, age at the last consultation is indicated between brackets if < 20 years for Caucasian or < 25 years for Asian.

§: reported as a “fair complexion”.

#: premature greying in other family member(s).

LEGENDS TO FIGURES

Figure 1 Family 1. (a) Pedigree. (b) Photographs of the proband III.1 at the age of 11 years, showing generalized hypopigmentation (in contrast with familial dark skin), premature greying affecting hair, eyelashes and eyebrows, blue irides, freckles, with (c) depigmented patches and (d) cafe-au-lait macules. Color figure can be seen in the online issue.