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variations in Waardenburg and Tietz syndromes.**

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

4

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22 **Keywords:** Waardenburg syndrome, Tietz syndrome, MITF, Freckles, Pigmentation
23
24 **Running title:** Non-truncating mutations of the MITF basic domain
25

1 **ABSTRACT**

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

18

19

1 INTRODUCTION

2 The microphthalmia-associated transcription factor (MITF) is a basic helix-loop-
3 helix (bHLH) leucine zipper transcription factor which regulates melanocyte
4 development and the biosynthetic melanin pathway. Its gene has several alternative
5 promoters and first exons that produce differentially expressed isoforms.¹ Mutations in
6 the M (melanocytic) isoform of *MITF* are known to lead to Waardenburg syndrome type
7 2A (WS2A, MIM 193510), an autosomal dominant disorder characterized by variable
8 degrees of sensorineural hearing loss and pigmentation disorders of the skin, skin
9 appendages and irides.²⁻³ Rarely, *MITF* mutations lead to Tietz syndrome (MIM
10 103500), an allelic condition characterized by a more severe phenotype of hearing loss
11 and generalized, albinoid-like hypopigmentation of the skin and hair from birth, rather
12 than the patchy depigmentation seen in Waardenburg syndrome (WS).³⁻⁴

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

19 PATIENTS AND METHODS

20 Sequencing of the *MITF-M* isoform exons was modified from Tassabehji et al.¹¹
21 The absence of total or partial gene deletion was assessed by QMF-PCR (Quantitative
22 Multiplex Fluorescent PCR).¹² Mutations were named according to the international
23 nomenclature based on Genbank NM_000248.2 for *MITF-M* (isoform 4) cDNA. More
24 details are given in the supplementary data.
25

1 Twelve patients from six families, with new or recurrent non-truncating mutations
2 of the MITF basic domain, were enrolled in this study. None of the mutations was
3 described as a polymorphism in the relevant databases
4 (<http://www.ncbi.nlm.nih.gov/snp>, <http://browser.1000genomes.org>). When necessary to
5 confirm the de novo occurrence, six microsatellites were analysed using the linkage
6 mapping set (Applied Biosystems, Foster City, CA). Mutations were analysed using
7 several software packages including Human splicing finder v2.4¹⁵
8 (<http://www.umd.be/HSF/HSF.html>) and Polyphen-2
9 (<http://genetics.bwh.harvard.edu/pph2/>) in order to evaluate their effect. The
10 conformation files for Srebp1-A, Usf, Myc, Mad and Max were imported from the
11 protein data bank (accession codes 1AM9, 1AN4, 1HLO, 1NKP) and represented using
12 the Swiss-Pdb Viewer software.¹⁶

13

14 **RESULTS**

15 **Clinical Data**

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

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

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

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

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

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

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

24

25 **Identification of mutations**

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

14 All the non-truncating mutations of the MITF basic domain (missense
15 substitutions and in-frame deletions, here described or previously published) are
16 reported in Table 1. They all involve amino-acids highly conserved across evolution.
17 None of them is predicted to result in a truncating protein through splice alteration. All
18 are predicted as probably damaging by polyphen-2. In order to further document
19 pathogenicity, we looked at their localisation in tertiary structure. The three-dimensional
20 (3D) structure of MITF has not been determined but several other bHLH factors have
21 been studied in their bound-to-DNA conformation. An example using SREBP1-A¹⁸ is
22 shown in the supplementary Figure. Equivalent amino-acids that are mutated in MITF
23 are on the side of the basic domain α -helix that is localized in contact with the DNA
24 groove, while the unbound side of the α -helix appears devoid of mutations.

25

1 **DISCUSSION**

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

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

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

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

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

1 According to the diagnostic criteria for WS proposed by the Waardenburg
2 Consortium, all the patients could be diagnosed as having WS. Indeed, Tietz syndrome
3 is characterized as a variant with a “more severe” phenotype: association of congenital
4 profound sensorineural hearing loss and uniform dilution of pigmentation (skin, eyes
5 and hair). The observation that melanocyte density is normal in the hypopigmented
6 areas suggests that the migration of melanocytes progenitors occurs normally and argues
7 for an abnormality of melanocyte function⁸. However, both mechanisms may coexist, as
8 generalized hypopigmentation and WS-type depigmented patches are sometimes
9 observed in the same patients (Figure 1c). However, the difference between diffuse
10 hypopigmentation and normal fair skin may be unclear in some cases, and distinction
11 between Tietz and WS is sometimes difficult. Diffuse hypopigmentation could be
12 considered as another variable phenotypic feature of WS, being associated with some,
13 but not all, MITF basic domain mutations. Of note, the patients who independently carry
14 recurrent mutations (p.Arg217del or p.Arg217Ile) do not all show the same phenotype,
15 with only some being classified as Tietz syndrome.

16 We observed a striking frequency of freckles (60%), mainly in Asian populations
17 (66%). They were not observed within the depigmented patches, possibly because of a
18 complete absence of melanocytes. In the literature, we found only three cases of freckles
19 in patients with other MITF mutations.^{17, 20-22} However freckles have not usually been
20 considered as part of the WS pigmentary disorders² so far and their occurrence might be
21 underestimated. Chen et al. recently proposed it to be a Chinese variant of the WS
22 phenotype¹⁷ but we found it in some European patients as well. The melanocortin-1
23 receptor gene, *MC1R*, described as the major freckle gene,²³ is a good candidate to
24 influence this phenotype. It encodes a G-protein-coupled receptor that mediates the α -
25 MSH (melanocyte-stimulating hormone) effect in melanocytes, resulting in an

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

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

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

1

2 **CONFLICT OF INTEREST**

3 The authors declare no conflicts of interest.

4

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10

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24

25

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

Exon	cDNA*	ARN/protéine	Inheritance	Hear CHL	Pigmentary disorders				Other F/CALM	Vision S/A	Phenotype	Origin	Family	Reference
					Eye BI/HI	Skin GH/PH	Hair WF/PG+/HC							
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 <i>TYRP1</i> 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).

1 **LEGENDS TO FIGURES**

2

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

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