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

Sandy Léger, Xavier Balguerie, Alice Goldenberg, Valérie Drouin-Garraud, Annick Cabot, Isabelle Amstutz-Montadert, Paul Young, Pascal Joly, Virginie Bodereau, Muriel Holder-Espinasse, et al.

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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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21  
22 **Keywords:** Waardenburg syndrome, Tietz syndrome, MITF, Freckles, Pigmentation  
23  
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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.<sup>1</sup> 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.<sup>2-3</sup> 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).<sup>3-4</sup>

13 A notable relationship between non-truncating mutations of the basic domain and  
14 Tietz syndrome has been described.<sup>3, 5-10</sup> 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.<sup>11</sup>  
21 The absence of total or partial gene deletion was assessed by QMF-PCR (Quantitative  
22 Multiplex Fluorescent PCR).<sup>12</sup> 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 v2.4<sup>15</sup>  
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.<sup>16</sup>

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.<sup>17</sup> 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.<sup>3</sup> 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-A<sup>18</sup> is  
22 shown in the supplementary Figure. Equivalent amino-acids that are mutated in MITF  
23 are on the side of the basic domain  $\alpha$ -helix that is localized in contact with the DNA  
24 groove, while the unbound side of the  $\alpha$ -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.<sup>9,19</sup>

11           Among the abundant mouse *Mitf* alleles, several are similar to the human  
12 mutations we identified or affect the same residue: *microphthalmia* (*Mitf*<sup>Mi</sup>) is similar to  
13 p.Arg217del, *Oak-ridge* (*Mitf*<sup>Mi-Or</sup>) to p.Arg216Lys, and *White* (*Mitf*<sup>Mi-wh</sup>) affects the  
14 Ile212 that is changed to Asn.<sup>19</sup> 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.<sup>17</sup> 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 function<sup>8</sup>. 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.<sup>17, 20-22</sup> However freckles have not usually been  
20 considered as part of the WS pigmentary disorders<sup>2</sup> so far and their occurrence might be  
21 underestimated. Chen et al. recently proposed it to be a Chinese variant of the WS  
22 phenotype<sup>17</sup> but we found it in some European patients as well. The melanocortin-1  
23 receptor gene, *MC1R*, described as the major freckle gene,<sup>23</sup> is a good candidate to  
24 influence this phenotype. It encodes a G-protein-coupled receptor that mediates the  $\alpha$ -  
25 MSH (melanocyte-stimulating hormone) effect in melanocytes, resulting in an

1 upregulation of *MITF*. *MC1R* is characterized by a remarkably polymorphic sequence.<sup>24</sup>  
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.<sup>25</sup>

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.<sup>26</sup> In cases with other *MITF* mutations, strabismus has  
11 only been reported in one family of WS2 with OA,<sup>22</sup> a condition well-known for its  
12 strabismus association, or with a polygenic deletion.<sup>27</sup> 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.<sup>19</sup>

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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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	<b>p.Lys206Gln</b>	Familial	+	+/-	-/-	+/-/Blond	-/-	-/-	<b>WS2</b>	France/Italy	2	This study	
	c.630C>G	<b>p.Asn210Lys</b>	Familial	+	+/-	+/-	-/-/Blond	+/-	-/-	<b>Tietz syndrome</b>	USA / Ireland		6	
Exon 7	c.635T>G	<b>p.Ile212Ser</b>	Familial	-	+/+	+/+	-/+Brown	+/+	-/-	<b>WS2</b>	Vietnam/Martinique	1	This study	
	c.639A>C (+ c.635-5delT)	<b>p.Glu213Asp</b>	De novo	+	+/-	-/+	+/+Red	+/-	+/-	<b>WS2</b>	Europe/South Africa	3	This study	
	c.647G>A	<b>p.Arg216Lys</b>	De novo	+	+/-	-/-	+/+Light brown	-/-	+/+	<b>WS2</b>	France	4	<sup>3</sup> + this study	
	c.649_651delAGA	<b>p.Arg217del</b>	Familial	+	+/-	+/-	-/+Red	+/-	-/-	<b>Tietz syndrome</b>	Europe		5	
	c.649_651delAGA	<b>p.Arg217del</b>	De novo	+	+/-	+/-	-/(24)/Blond	+/-	-/-	<b>Tietz syndrome</b>	? (Japanese paper)		8	
	c.649_651delAGA	<b>p.Arg217del</b>	Familial	+	+/-	?§/-	-/(1)#/Red	-/-	-/-	<b>WS2/Tietz syndrome</b>	? (US paper)		<sup>10</sup> The index case also had OA + P513R in the <i>TYRP1</i> gene	
	c.649_651delAGA	<b>p.Arg217del</b>	Familial	+	+/-	+/-	-/(3)/Brown	-/-	+/?	<b>Tietz syndrome</b>	France	5	<sup>3</sup> + this study	
	c.649_651delAGA	<b>p.Arg217del</b>	Familial	+	+/-	+/-	-/(15)/Blond	-/-	-/-	<b>Tietz syndrome</b>	Japan		9	
	c.649_651delAGA	<b>p.Arg217del</b>	De novo	+	+/-	-/-	-/(10)/Brown	+/-	-/+	<b>WS2</b>	China		<sup>17</sup> (completed)	
	c.649_651delAGA	<b>p.Arg217del</b>	De novo	+	+/-	-/-	-/(12)/Brown	+/-	-/+	<b>WS2</b>	China		<sup>17</sup> (completed)	
c.649_651delAGA	<b>p.Arg217del</b>	De novo	+	+/-	-/-	-/(12)/Brown	+/-	-/+	<b>WS2</b>	China		<sup>17</sup> (completed)		
c.650G>T	<b>p.Arg217Ile</b>	Familial	+	+/-	+/-	+/- (3)#/Blond	-/-	-/-	<b>Tietz syndrome</b>	Portugal	6	This study		
c.650G>T	<b>p.Arg217Ile</b>	De novo	+	+/-	-/-	-/+Brown	+/-	-/-	<b>WS2</b>	China		<sup>17</sup> (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**

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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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