Post-lingual non-syndromic hearing loss phenotype: a novel homozygous missense mutation in MITF

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Abstract

Background Hearing loss (HL) represents the most common congenital sensory impairment with an incidence of 1-5 per 1000 live births. Non-syndromic hearing loss (NSHL) is an isolated finding that is not part of any other disorder accounting for 70% of all genetic hearing loss cases. Methods In the current study, we report a multifactorial genetic mode of inheritance in a NSHL consanguineous family using exome sequencing technology. We evaluated the possible effects of the single nucleotide variants (SNVs) detected in our patients using in silico methods. Results Two bi-allelic SNVs were detected in the affected patients; a MYO15A (. p.V485A) variant, and a novel MITF (p.P338L) variant. Along with these homozygous mutations, we detected two heterozygous variants in well described hearing loss genes (MYO7A and MYH14). The novel p. Pro338Leu missense mutation on the MITF protein was predicted to change the protein structure and function. Conclusion The novel MITF variant is the first bi-allelic SNV in this gene to be associated with an autosomal recessive non-syndromic HL case with a post-lingual onset. Our findings highlight the importance of whole exome sequencing for a comprehensive assessment of the genetic heterogeneity of HL.

Background

With a prevalence of 1 to 5 per 1000 births, hearing loss represents the most common congenital sensory impairment. Congenital HL could be either due to hereditary and non-hereditary genetic factors, or to certain complications during pregnancy and childbirth[1]. Most of the cases (around 60%) are attributed to genetic causes with more than 150 identified genes that are associated with either syndromic or non-syndromic form of this disease [2,3]. Non-syndromic hearing loss (NSHL) accounts for 70% of genetic hearing loss cases which are not usually associated with other signs and symptoms. a NSHL can be either inherited in an autosomal recessive manner (75–80%), autosomal dominant manner(20–25%) or as X linked or mitochondrial inheritance in rare cases (1–2%)[4]. To date, over 115 genes have been linked to non-syndromic hearing loss with *GJB2, SLC26A4, MYO15A, OTOF,* and *CDH23* being considered the most commonly identified genes were some of which have been associated with both recessive and dominant form of the disease [6]. On the other hand, some other HL cases were either attributed to digenic interactions or to certain genetic-environmental factors [5].

With the advent of next-generation sequencing (NGS), genetic mapping within large, clinically well-characterized families with NSHL provides a powerful approach for mapping critical chromosomal intervals which when mutated could be responsible for this phenotype. In the Middle East, the high rate of consanguineous marriages favors the incidence of autosomal recessive diseases such in that of NSHL [7]. Unfortunately, despite this high prevalence, the needed genetic linkage studies using NGS technologies are still not very well established[8].

In this study, we report a multifactorial genetic mode of inheritance in an NSHL consanguineous family using exome sequencing analysis, and we propose for the first time the involvement of a novel *MITF* variant in non-syndromic hearing loss disease with post-lingual onset.

Methods

Subjects

Two young siblings presented to the Department of Otolaryngology - Head and Neck Surgery at American University of Beirut (AUB) with a complaint of late-onset HL. These patients, along with their consanguineous family, were included in the ongoing study of the genetic basis of HL in Lebanon. Family members received a complete otolaryngologic examination, in addition to pure tone audiometry testing. They were also referred to Ophthalmology, Cardiology and Nephrology for identification of possible other congenital abnormalities and ruling out syndromic HL. A follow-up examination was done for one available affected patient (II.5) and her parents after 4 years from the first visit. The study was approved by the Institutional Review Board (IRB) at the American University of Beirut (protocol number:OTO.MB1.02).

Exome Sequencing

Blood samples were collected from the family members and DNA extraction was performed using the QIAamp Blood Midi Kit (Qiagen Sciences, Inc., Germantown, MD), using the manufacturer's instructions. DNA quantification was also performed through the NanoDrop (Thermo Fisher Scientific, Inc., Waltham, MA) at the molecular core facility at AUB. One microgram of coded DNA samples from both parents and the two patients were shipped to Macrogen (South Korea), where exome sequencing was performed using the V5 SureSelect Target Enrichment Capture system from Agilent on a HiSeq 4000 platform from Illumina.

Data Analysis

Primary analysis was done at Macrogen. Generated FASTQ files were mapped to the reference genome using the SureCall software from Agilent technologies. The Illimuna Variant Studio was used for annotation and variant calls. To assess the pathogenicity of possible candidates, we used SIFT (http://sift. jcvi.org/), PolyPhen2 (http://genetics.bwh.harvard.edu/ pph2/),MutationTaster (http://www.mutationtaster.org/), and GERP++ (http://mendel.stanford.edu/ SidowLab/downloads/gerp/) scores to predict deleterious variants. To predict the effect of the detected mutations on the protein structure and stability, we used DUET software (http://biosig.unimelb.edu.au/duet/stability)

Results

Clinical manifestation

The family consists of consanguineous parents with two sisters diagnosed with post-lingual hearing impairment and four unaffected brothers (Fig 1). HL was noted in the two sisters (II.5/II.6) at the age of six and twelve, respectively. Physical examination did not demonstrate any dysmorphic features suggestive of a syndromic disease. Both patients were not reported to have any pigmentary changes in hair, eyes, or skin. No visual complaints including night blindness, visual field loss and decrease in central vision were detected.. Audiogram analysis of this family revealed that the two siblings had a bilateral HL. Puretone audiometry for patients revealed approximately similar pattern of a “cookie-bite audiogram” with mild HL in the low frequencies, sloping to borderline severe in mid frequencies, and rising to moderate in high frequencies (Fig 2). Word discrimination score was excellent for both patients at the time of referral.

A follow-up audiogram for the patient (II.5) indicated a stable hearing after 4 years from the initial diagnosis. In addition no features of any syndromic disease,which are usually initiated after puberty, were detected.

Mutational analysis

Exome sequencing of the four family members achieved approximately (95%) mean exome coverage, at coverage of (8X). From a total number of around 58000 variants, we only analyzed those that occur in the coding regions of the genes. We filtered variants via a list of 155 genes used for clinical diagnosis of HL while only including missense, frameshift, splice and stop gained alterations and a minor allele frequency (MAF) of <0.01 (S1 Table). Possible causative variants for each patient were summarized in (S1 and S2 Tables). The strong candidate variants that might underlie the mild to moderate NSHL in the two patients were those detected in *MITF, MYO15A, MYO7A,* and *MYH14* genes (Fig 1)[9].

Two bi-allelic single nucleotide variants (SNVs) were detected in the two patients; a previously described *MYO15A* (NM_016239.3:c.1454T>C) mutation and a novel one *MITF* one (NM_198159.2:c.1013C>T) resulting in the missense mutations p.V485A and p.P338L respectively (Supplementary Table 2). On the top of the variants that were detected amongst the known HL genes were a mono-allelic variant in *MYO7A* (NM_000260.3:c.5563C>T) resulting in the nonsense mutation p.Q1855* inherited from the mother. In addition, a heterozygous variant in *MYH14* (NM_001145809.1:c.1150G>T) inherited from the father, results in a heterozygous missense mutation p.G384C. (S2 and Figure 1).

Finally, a search for unbiased bi-allelic mutations in the family did not yield additional variants with a MAF <1% except for TRPV2 (rs756373391). The latter is a close member of the *TRPV4* gene that is implicated in some cases of HL (S4 and S4 Tables).

In silico prediction and modulation for the novel MITF variant

Using in silico method, we evaluated the possible effect of the novel p. Pro338Leu on the structure of MITF protein. This mutation changed a highly conserved Pro338residue in the α-helix of the bHLH motif into Leucine (Fig 3). The amino acid substitution in MITF p. Pro237Leu is predicted to be probably damaging by Polyphen2 (score 1; range 0–1 with 0 = benign and 1 = probably damaging). SIFT predicts that the substitution is tolerated (score 0.92; a score ≤0.05 predicts the change to be damaging and >0.05 predicts it to be tolerated). However, mutation taster predicts the substitution to be disease causing with a probability of 1 (0–1) (Table 1). Molecular modeling predicts that substitution of proline for leucine can destabilize the protein (NMA Based Predictions ΔΔG ENCoM: 0.207 kcal/mol). Therefore, it is expected that this missense mutation changes the structure of the protein, hence, affects the protein function.

Discussion

Consanguinity is a double-edged sword as it facilitates novel gene discovery for diseases and at the same time it challenges genetic testing as the likelihood of a high impact single genetic cause is decreased [3]. Interestingly, in this study we showed a multifactorial inheritance form of NSHL with the involvement of two independent homozygous alterations in well-known HL genes. To best our knowledge, this is the first study to report a novel *MITF* variant to be involved in a NSHL with autosomal recessive mode of inheritance and a post-lingual onset.

MYO15A and *MITF* homozygous alterations: the dilemma of predictive tools?

MYO15A encodes for the XVA myosin protein which plays a vital role in elongation and development of stereocilia and actin filaments. More than forty mutations in *MYO15* have been reported in the motor domain of the protein with generally autosomal recessive hearing loss impairment characterized by a profound phenotype at all frequencies [10]. The detected homozygous mutation p.V485A in *MYO15A* in this report was previously associated with a HL phenotype in an Iranian family[3]. Mutations in the N-terminal domain are thought to be associated with a milder form of HL as it affects only one of the two major isoforms of the gene[11]. The p.V485A mutation is located within the N-terminal domain, but our indexed patients suffer from a mild to severe phenotype. In addition, two healthy individuals from the Gnomad Exome database harbor this variant, thus arguing against a major role for this mutation in the affected individuals. Accordingly, we postulate that other players might be linked- in collaboration or independently from MYO15A- to the underlying phenotype.

As such we looked at the second shared bi-allelic mutation between the two sisters which is a novel p.P338L missense mutation in the *MITF* gene. Thisgene encodes for the melanocyte-specific promoter of microphthalmia-associated bHLH transcription factor. A total of >40 *MITF* mutations have been proven to be disease-causing in patients with either the Waardenburg's syndrome type 2)WS2) (OMIM#193510) or the Tietz syndrome (OMIM #103500) (Chen et al., 2016). Both syndromes are autosomal dominant and

are characterized by overlapping phenotypes that encompass HL and pigmentary abnormalities with variable penetrance To our knowledge, only 2 homozygous *MITF* cases were detected in WS2 and WS4 [12,13]. In the present study, the detected homozygous p.P338L missense mutation was neither reported in the dbSNP database, nor in the Gnomad Exome/Genome database. It was also absent from more than 300 Lebanese exomes. The heterozygous frequency of this variant is less than 0.00001 in these databases as it is only present in 3 individuals. Being localized in the bHLH DNA-binding domain along with the predictive deleterious effect of this variant, we hypothesize that this mutation is disease causing (Table–1). Structure-function assays are compulsory to assess the effect of this mutation on the ability of *MITF* to heterodimerize, bind DNA, and/or translocate to the nucleus.

Widening the *MITF* disease spectrum through exome sequencing

Previously, patients who presented with HL as the only phenotypic feature were thought to have NSHL and thus only mutations in genes associated with this type of HL were investigated. But it is now well known that in some syndromes special tests are required to detect secondary features or that the penetrance of the secondary features is either incomplete or age dependent. An example on this is Usher syndrome which presents as NSHL case early in life since the onset of the secondary symptom (retinitis pigmentosa) does not appear until puberty. This might cause false clinical categorization of some patients with SHL who can benefit from appropriate implementation of visual rehabilitation at early stages [5]. As such, it is very critical to categorize genes and variants that are involved in both forms of HL and those that are unique to each type. Another example is a heterozygous variant in *MITF* (p.R110X) that was usually associated only with SHL but was recently detected in an NSHL case that presented without pigmentary changes in hair, eyes, or skin of the patient, which are usually features of WS2[15]. Combining these facts with our results, we propose expanding the implications of *MITF* variants from syndromic to non-syndromic HL with an autosomal recessive mode of inheritance.

Additionally, it is widely known that most autosomal dominant loci cause post-lingual hearing impairment (example: *MYO7A* and *MYH14)* while autosomal recessive HL with delayed childhood onset is a rare clinical finding [9]. Herein, we are the first to propose *MITF* and *MYO15A* variants as autosomal recessive loci causing stable post-lingual hearing impairment rather than progressive pre-lingual one.

Multifactorial inheritance

Although most cases of genetic deafness result from mutations in a single gene, an emerging number of examples are being documented in which recessive mutations at two loci are being involved. An example on this is the digenic interaction that underlies the cause of deafness in individuals by carrying a single mutation at the *GJB2* locus along with a deletion which involves the functionally related *GJB6* gene[17]. Thus, we propose another case of digenic nature of inheritance mainly through the involvement of both

MITF and *MYO15A* variants coupled with two detected heterozygous variants in *MYO7A* and *MYH14* genes. Different compound heterozygous or homozygous mutations related to *MYO7A* have been reported in variety of autosomal recessive usher syndromes families[18]. However, mutations in *MYH14* gene are associated with autosomal dominant hearing impairment[19]. Thus, we speculate that the detected *MYH14* and *MYO7A* mutations are not the direct cause of the HL in our patients since their parents presented as healthy carriers for each variant. Further functional studies are needed to assess the independent and combined effect of these mutations on the development of hearing loss.

Conclusion

The present study describes a rare form of hereditary non-syndromic autosomal recessive post-lingual sensorineural hearing loss that is associated with polygenic inheritance mode of bi- and mono- allelic variants. In this study, we unraveled the association of a novel *MITF* variant in NSHL which suggests its inclusion in any diagnostic targeted exome screening for both forms of HL. Moreover, our study reveals the importance of clinical exome sequencing in comprehensively addressing the genetic heterogeneity of HL and in detecting novel variants associated with NSHL. Yet, our findings challenge the idea of single genetic disease causing variant suggesting an aggregate of risk loci causing NSHL after exceeding a certain threshold.

Abbreviations

HL: Hearing loss; NSHL: Non-syndromic hearing loss;Single Nucleotide Variants: SNVs; NGS: Next Generation Sequencing; Waardenburg’s Syndrome: WS

Declarations

Acknowledgment

We thank the patients and the family for their contribution to this research.

Author ‘s Contributions

AK and SBK: did all the experiments, and participate in the analysis and writing up of the manuscript. RB,GD,SAR, and AK did the clinical assessment and recruitment of the patients and their family members. MB and GN designed the study, secured the funding, analyzed the data, and wrote up the manuscript. All authors read and approved the final manuscript.

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Availability of Data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon a reasonable request.

Ethics approval and consent to participate

The entire procedure was approved by the Institutional Review Board (IRB) at the American University of Beirut (protocol number:OTO.MB1.02) and carried out with written informed consent of the patients.

Consent for publication

All patients, parents and legal guardians signed an informed consent for data publication.

Competing Interests

The authors declare that they have no competing interests

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Table 1

Table 1. Pathogenicity scores of detected variants assessed by SIFT, PolyPhen2, Mutation Taster, and GERP++ software.

Allelic Variant	Zygosity	Amino acid change	SIFT	Polyphen-2	MutationTaster	GERP++
MITF NM_198159.2 c.1013C>T	Homozygous	P338L	Tolerated Score:0.92	Probably damaging Score:1	Disease causing Score:1	Conserved Score:5.06
MYO15A NM_016239 c.1454T>C	Homozygous	V485A	Damaging Score:0	Probably damaging Score:0.9	Disease causing Score:0.9	Conserved Score:5.1
MYH14 NM_001145809 c.1150G>T	Heterozygous	G384C	Damaging Score: 0	Probably damaging Score:1	Disease causing Score:1	Conserved Score:3.42
MYO7A NM_000260.3 c.5835 C>T	Heterozygous	Q1855*	NA	NA	Disease causing Score:1	NA

Figures

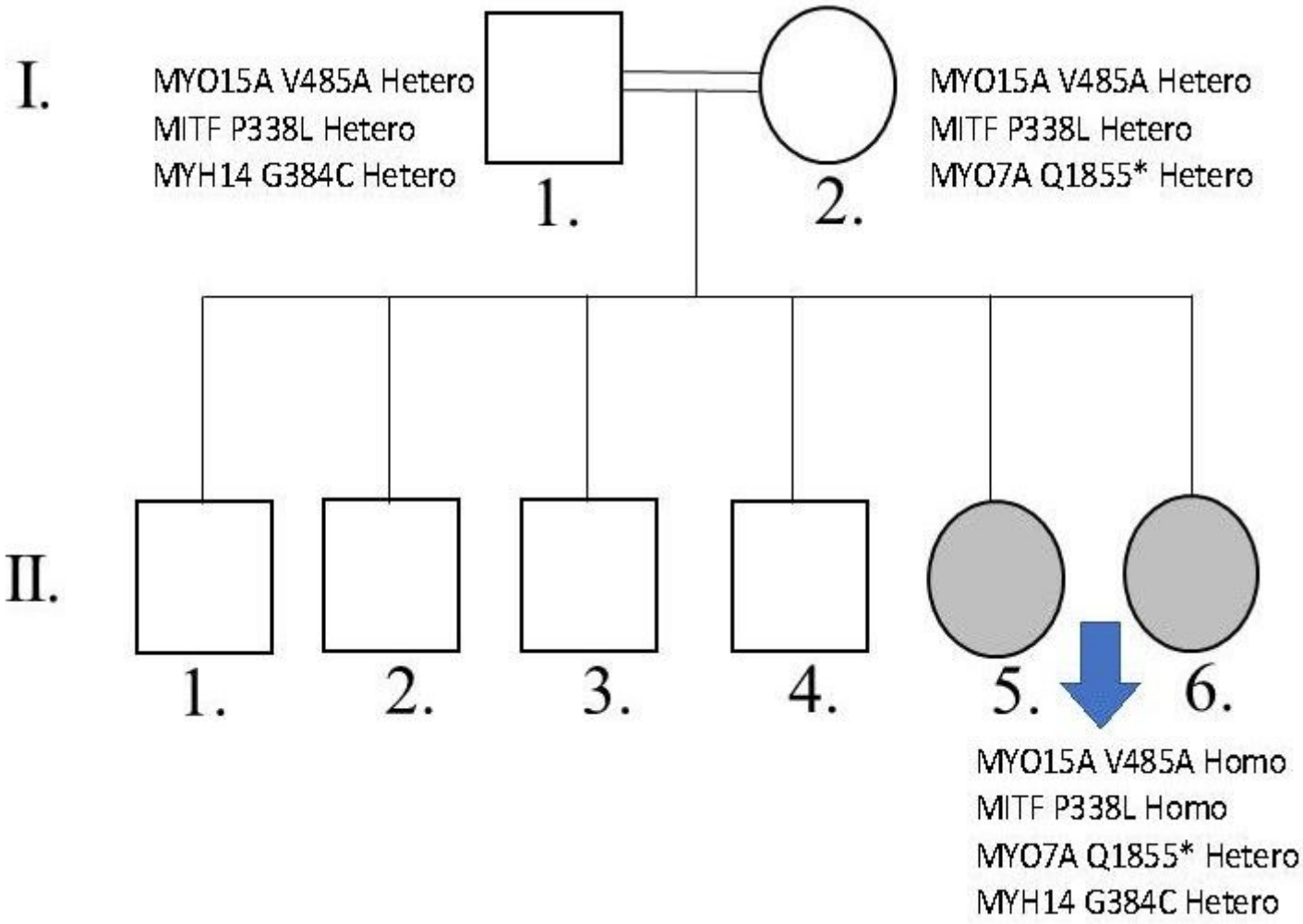
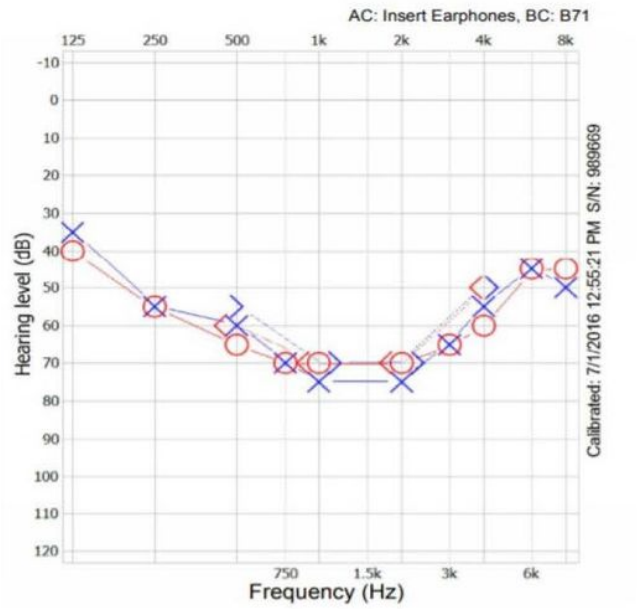
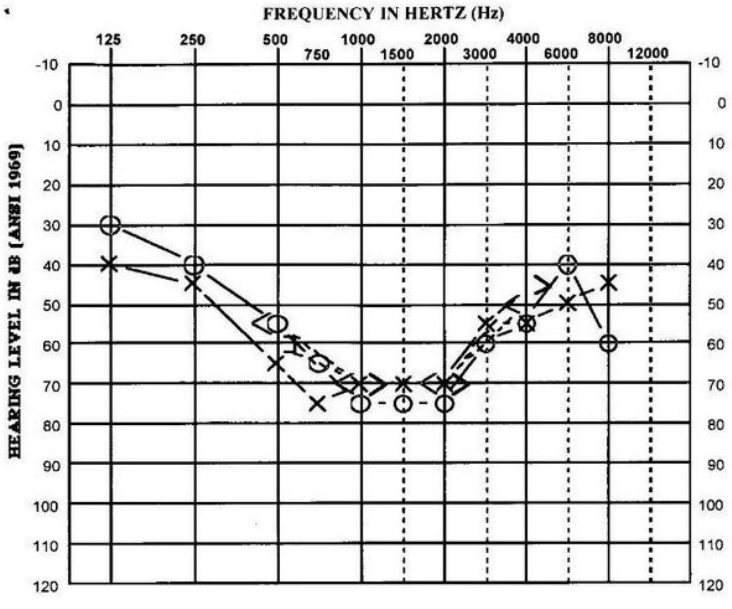


Figure 1

Family's phenotype and genotype. The pedigree of the enrolled family, with affected individuals marked in grey. Possible causative variants of the affected sisters and those of the parents are listed.



II.5



II.6

Figure 2

Audiograms of the affected probands. The audiograms show mild to severe progressive hearing loss in both ears for both affected individuals (II.5) and (II.6). The audiograms were taken at the time of diagnosis.

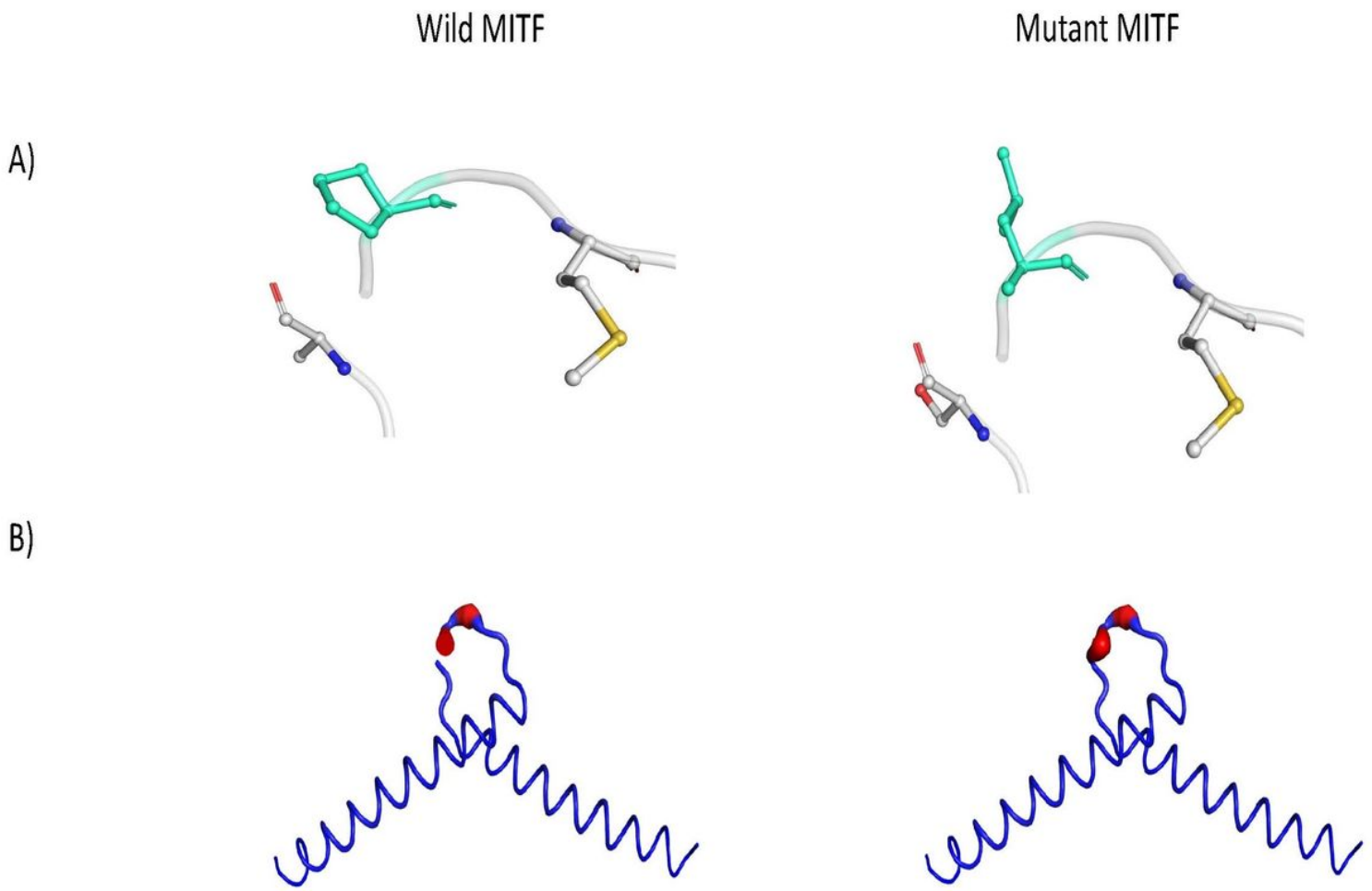


Figure 3

Protein structure prediction of the novel MITF variant (p.P338L). In silico modeling (A) the effect of the MITF mutation using the DUET software shows a general destabilization of the structure (B). Wild-type and mutant residues are colored in light-green and are also represented as sticks alongside with the surrounding residues which are involved on any type of interactions (A). The magnitude of the fluctuation is represented by thin to thick tube colored blue (low), white (moderate) and red (high) (B).

Supplementary Files

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