Continuum of phenotypes in HMSN-P and CMT patients with *TFG* mutation

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Abstract

Charcot-Marie-Tooth (CMT) is a common neuropathy, and hereditary motor and sensory neuropathy with proximal predominance (HMSN-P) is a recently described rare neuromuscular disease. Whereas many genes have been implicated for CMT, *TFG* is the only known HMSN-P causing gene. Within the framework of diagnostic criteria, clinical variation is evident among CMT and also HMSN-P diagnosed individuals. Mutations that cause p.(Pro285Leu) and p.(Gly269Val) in TFG were earlier reported as cause of HMSN-P in two Iranian pedigrees. Here, we report identification of p.(Gly269Val) in TFG as cause of CMT in a large Iranian pedigree. The clinical features of patients of the three pedigrees are presented and critically compared. Similarities between the two HMSN-P diagnosed pedigrees with different *TFG* mutations, and differences between the two differentially diagnosed pedigrees with the same p.(Gly269Val) mutation were evident. The clinical features of the HMSN-P pedigree with the p.(Pro285Leu) and the CMT pedigree with the p.(Gly269Val) mutation were clearly congruent with the respective diagnoses, whereas the features of the HMSN-P diagnosed pedigree with the p.(Gly269Val) were intermediate between the other two pedigrees. It is therefore suggested that the clinical features of the three Iranian pedigrees with *TFG* mutations and diagnosed with HMSN-P or CMT represent a continuum.

Keywords: CMT, HMSN-P, p.(Gly269Val), *TFG***,** tropomyosin-receptor kinase fused gene

Introduction

Charcot-Marie-Tooth (CMT) disease constitutes a heterogeneous group of inherited peripheral neuropathies (Hoebeke et al., 2018; Mathis et al., 2015) that occurs worldwide. It is the most common inherited neuropathy with an estimated prevalence of one in a few thousand in most populations (Gonzaga-Jauregui et al., 2015). The clinical features of adult-onset CMT are highly variable but commonly include symmetric slowly progressive distal muscle weakness and atrophy that first affect the lower limbs, foot deformities, slight or moderate distal sensory impairment, and depressed tendon reflexes (Lupski et al., 2010; Marttila et al., 2017; Mathis et al., 2015). The disease can become apparent anytime from early childhood through late adulthood, but onset is usually in adolescence or early adulthood. CMT is commonly classified as CMT type 1 (CMT1; demyelinating) or CMT type 2 (CMT2; axonal) on the basis of median motor nerve conduction velocity (NCV) (Brennan, Bai, & Shy, 2015) . More than 80 genes have been implicated in the etiology of CMT and related disorders, and these account for disease status in the majority of CMT1 patients and in a smaller fraction of CMT2 patients (Hoebeke et al., 2018; Marttila et al., 2017; Mathis et al., 2015). Mutations in *PMP22* that encodes the peripheral myelin protein cause demyelinating CMT and are the most common genetic cause of CMT (Gonzaga-Jauregui et al., 2015).

Hereditary motor and sensory neuropathy with proximal predominance (HMSN-P) is the name given to a recently described rare neuromuscular disease. Its original description emphasized proximal dominant muscle weakness and atrophy and also included mild sensory dysfunction, fasciculations, reduced deep tendon reflexes, and axonal degeneration in the peripheral nerves (Takashima et al., 1997). In 2012 and 2013, it was shown that *TFG* that encodes the tropomyosin-receptor kinase (TRK-) fused protein is the cause of HMSN-P. The TFG protein is localized at endoplasmic reticulum (ER) exit sites in various tissues including muscle (Witte et al., 2011). Various studies have shown that the protein functions in protein transport and secretory processes (Hanna et al., 2017; Johnson et al., 2015; McCaughey et al., 2016; Witte et al., 2011). TFG depletion affects associations between the ER and the ER-Golgi intermediate compartment (ERGIC) membranes and COPII-coated carrier mediated transport (Johnson et al., 2015). In addition to HMSN-P, some mutations in *TFG* cause hereditary spastic paraplegia (HSP) (Beetz et al., 2013; Catania et al., 2018; Elsayed et al., 2016; Harlalka et al., 2016; Tariq & Naz, 2017), suggesting that vesicular transport is also important in the etiology of this disorder. Inheritance pattern of HSP caused by *TFG* mutations is autosomal recessive. Most recently, studies in human stem cell derived neurospheres that expressed a mutated form of TFG that is known to cause hereditary spastic paraplegia revealed decreased self-association of axons (Slosarek et al., 2018). Reduced levels of L1CAM at

cell surfaces was shown in these cells, consistent with the proposal that the defect in axon bundling may be due to impaired trafficking of this adhesion molecule. Additionally, TFG affects intracellular protein homeostasis and ER stress by its effects on the ubiquitin-proteasome system (UPS) (Slosarek et al., 2018; Yagi, Ito, & Suzuki, 2014). In recent years, mutations in several genes that affect ER structure and function have been associated with various neurodegenerative diseases, suggesting that the ER has critical roles in neural functions (Fowler, Byrne, & O'Sullivan, 2016; Roussel et al., 2013).

Except for two recently described families from Iran, all reported HMSN-P patients had Far East, usually Japanese, ancestry (Campellone, 2013; Elison Sarapura-Castro, 2018; Ishiura et al., 2012; Lee et al., 2013; Miura et al., 2008; Patroclo, Lino, Marchiori, Brotto, & Hirata, 2009; Takahashi et al., 2007; Takashima et al., 1997; Takashima et al., 1999). Clinical descriptions in the more recently diagnosed patients from Iran emphasized variability in presentations. For example, presence of prominent effects on sensory nerves, comparable involvement of proximal and distal muscles, cranial nerve involvement, and rapid progression were observed in some patients (Alavi et al., 2015; Khani, Shamshiri, Alavi, Nafissi, & Elahi, 2016). Pattern of disease inheritance in all families identified was autosomal dominant. A c.854C>T mutation in *TFG* that causes p.(Pro285Leu) was identified in all HMSN-P families of the Far East and in one of the Iranian families. A different mutation in *TFG* (c.806G>T) that causes p.(Gly269Val) was identified in the second Iranian HMSN-P pedigree (Khani et al., 2016). Interestingly, the same p.(Gly269Val) causing mutation had recently been reported as cause of CMT2 in a Taiwanese pedigree (Tsai et al., 2014). Considering the overlaps in CMT and HMSN-P clinical features that were partly described above and the variability in presentations of both diseases, we suggested that diagnosis of different diseases in the Taiwanese and Iranian patients may be inappropriate (Khani et al., 2016). In fact, in a recent proposal for classification of Charcot-Marie-Tooth diseases, HMSN-P was classified as a form of autosomal dominant axonal type CMT (Mathis et al., 2015). Identification of mutations in *TFG* as cause of CMT in some patients and as cause of HMSN-P in other patients is consistent with close association between the two diseases that is suggested by this classification.

Here, we report finding a p.(Gly269Val) causing mutation in *TFG* in patients of a new Iranian pedigree. The presentations of the patients in this pedigree clearly justify diagnosis of CMT2. We present subjective, clinical, biochemical, electrodiagnostic (EDX), and muscle magnetic resonance imaging (MRI) data on patients of this pedigree. We compare features of the three identified Iranian pedigrees with mutations in *TFG* which have been identified and discuss the variability in presentations.

Subjects and Methods

This research was performed in accordance with the Declaration of Helsinki and with approval of the ethics board of the University of Tehran.

The CMT-100 pedigree studied here includes at least 26 affected individuals distributed in four generations (Fig. 1A). The proband (CMT-IV25) and subsequently four additional family members (CMT-III3, -IV10, -IV19, and -IV29) were definitively diagnosed with CMT2 by neurologist SN who was very familiar with the clinical features of patients of the earlier Iranian HMSN-P pedigrees (HMSN-159 and HMSN-160) (Alavi et al., 2015; Khani et al., 2016). CMT2 diagnosis was confirmed by neurologist HS who was also familiar with presentations of the Iranian HMSN-P pedigrees. The clinical features of the patients are described below. The proband of CMT-100 and several affected and non-affected family members were referred to us for genetic analysis. Inheritance was autosomal dominant. The health status of generation I individuals is unknown.

To confirm or rule out existence of a mutation in one of the known CMT causing genes in CMT-100 patients and possibly identify a candidate disease causing variation in a novel gene, the DNA of the proband CMT-IV25 was exome sequenced on an Illumina HiSeq 4000 system (Illumina, CA, USA). Sequence alignment was performed against human reference genome GRCh37/hg19, and variant callings were done by using ENSEMBL Variant Effect Predictor (https://asia.ensembl.org/Tools/VEP) and wANNOVAR (http://wannovar.wglab.org/). Subsequently, a file of heterozygous variations was prepared by removing SNPs with a MAF of > 0.01 in the dbSNP database (http://www.ncbi.nlm.nih.gov/), the Trans-Omics for Precision Medicine Program (https://www.nhlbiwgs.org/), the 1000 Genomes database (www.1000genomes.org), the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), the Exome Aggregation Consortium database (http://exac.broadinstitute.org/), the Genome Aggregation Database (http://genomad. broadinstitute.org/), the Greater Middle East Variome Project (http://igm.ucsd.edu/gme/), ENSEMBL (https://asia.ensembl.org/index.html), The HEX database (https://www.alzforum.org/exomes/hex), or Iranome database (http://iranome.com/) , or observed in in-house exome data belonging to approximately 50 unrelated Iranians affected with non-neurologic diseases. Variations in this file that did not affect amino acid change or splicing were also filtered out. Variations that remained were scrutinized to identify those within any of 74 genes previously reported to cause or confer susceptibility to CMT disease (Supplementary Table 1). The genes considered were those reported in the Inherited Peripheral Neuropathies Mutation Database (http://www.molgen.ua.ac.be/CMTMutations/), in the Neuromuscular Disease Center site

(https://neuromuscular.wustl.edu/time/hmsn.html), and/or in any of four publications that discuss CMT causing genes (Bird, 1993; Gonzaga-Jauregui et al., 2015; Mathis et al., 2015; Tazir, Hamadouche, Nouioua, Mathis, & Vallat, 2014). Candidate disease causing variations identified in any of these genes were screened for segregation with disease status in CMT-100 by direct sequencing. The identified mutation had been earlier screened in 300 Iranian control individuals by an allele specific PCR protocol (Khani et al., 2016).

Electrodiagnostic studies **(**EDX) including nerve conduction studies (NCS) and needle electromyography (EMG) were done in upper and lower extremities, truncal regions, and cranial regions according to standard procedures (Dantec Keypoint G4, Natus, CA, USA). Lower extremity (calf, thigh and pelvic regions) magnetic resonance imaging (MRI) was performed using a 1.5-T system (MAGNETOM Avanto 1.5 Tesla, Siemens, Germany). T1-and T2-weighted spin echo protocols were performed.

Results

The clinical features of five patients of CMT-100 who were examined are presented in Table 1. These features were remarkably similar in the patients, except that the ambulatory state of the patient with longest disease duration (CMT-III3) was worse. Twenty one additional individuals designated as affected in the pedigree were reported by family members to have presentations similar to the five patients who were critically examined (Fig. 1A). Age at onset in the patients was in their 3rd decade of life. Motor deficit was the major complaint at the early stages of the disease and sensory problems including mild paresthesia, dysesthesia and numbness were not troublesome even when atrophy and deformity occurred due to progressive motor deficit. The patients first noticed distal motor deficit in lower limbs, especially plantar flexion weakness. Foot deformity and distal upper limb weakness and atrophy were reported to gradually ensue during the following 5-10 years. None of CMT-100 patients complained about symptoms pertaining to cranial or trunk regions or about fasciculations.

Examination showed decreased muscle force which was significantly more prominent in distal regions and in the lower extremities. Atrophy and deformity in calf muscles and in intrinsic hand and foot muscles were obvious in distal regions. Mild distal sensory impairment was evidenced by decreased vibration sense and decreased light touch and pain sense. Deep tendon reflexes were decreased in both upper and lower extremities. Superficial abdominal reflex was normal. Cranial examination was normal and autonomic or respiratory abnormality was not detected. Electrodiagnostic study was performed on III3, IV10, IV25 and IV29 with disease durations of, respectively, 32, 12, 13, and 6 years. Results of nerve conduction studies were similar in all

the patients. They showed decreased motor and sensory action potential amplitude, with almost normal conduction velocities in the patients. Electromyography showed neurogenic motor unit potential (MUP) pattern in conjunction with spontaneous fibrillation potentials in the extremities which evidence chronic denervation and reinnervation. Complex repetitive discharges and myokymia were also seen during electromyography of patients IV25 and IV29. Fasciculation potential was not detected in any of the patients. No electrodiagnostic evidence of significant asymmetry was detected.

Muscle MRI was performed for patients IV10, IV25, IV19 and III3, whose disease durations were, respectively, 12, 13, 21, and 32 years, in order to assess the proportional effect of neuropathy in proximal and distal regions (Fig. 2). Involvement of distal muscles was much more prominent than proximal muscles in all the patients. Abnormal signal change suggestive of edema and fat deposition was most severely seen in calf muscles. The abnormal signal change in the thigh region, particularly in the medial compartment of thigh that is innervated by the obturator nerve, was much less. More proximal regions (the pelvis) were spared except in patient III3 who was at late stage disease progression. Fat replacement and atrophy were symmetric in all examined patients except for distal muscle regions of patient IV10 in which significant asymmetry was detected.

The filtering protocol used on the exome sequence data of patient CMT-IV25 identified candidate disease causing variations in two CMT associated genes. C.1204G>A that causes p.(Val402Ile) and c.806G>T that causes p.(Gly269Val) were found, respectively, in *SH3TC2* and *TFG*. *SH3TC2* encodes the SH3 domain and tetratricopeptide repeat domain 2 protein which functions in the endocytic recycling pathway (Roberts et al., 2010). Mutations in *SH3TC2* are cause of Charcot–Marie–Tooth type 4C disease (CMT4C), which is an autosomal recessive form of demyelinating Charcot-Marie-Tooth disease (Azzedine et al., 2006; Gooding et al., 2005; Senderek et al., 2003). In one CMT4C family, heterozygous carriers of a single mutated allele (p.(Tyr169His)) presented with a subclinical axonopathy phenotype detectable only by electrophysiological studies (Lupski et al., 2013). The p.(Val402Ile) causing variation in *SH3TC2* was not considered the major cause of disease in pedigree CMT-100, most importantly because of lack of segregation with disease status. Four of seven affected individuals screened did not carry the mutation, and unaffected individual CMT-IV26 did carry the mutation (Table 2). The unaffected status of CMT-IV26 was confirmed by EMG testing. Four (CMT-III3, CMT-IV10, CMT-IV25, and CMT-IV29) of the affected individuals screened were among the five patients who had been examined by physicians. Disease severity was similar in CMT-IV10 and CMT-IV25 whose present age (43 and 40 years) and disease duration (12 and 13 years) were close. CMT-IV10 was homozygous

for the *SH3TC2* wild type allele, whereas CMT-IV25 was a heterozygous carrier of the variant allele. This further supports the proposal that the p.(Val402Ile) causing variation in *SH3TC2* did not significantly affect disease status. Severity of disease in CMT-IV29, another carrier of the variant allele, was also similar to CMT-IV10 and CMT-IV25. Maximum severity in CMT-III3 (homozygous for the wild type allele) is best attributed to long duration of disease. The c.806G>T mutation found in the *TFG* gene completely segregated with disease status among 26 members of the CMT-100 pedigree who were available for screening; it was present in 18 CMT affected individuals and absent in eight unaffected individuals (Fig. 1A and 1B). Furthermore, as reported in the introduction section, the same p.(Gly269Val) causing mutation was earlier reported as cause of CMT in one family and as cause of HMSN-P in another family (Khani et al., 2016; Tsai et al., 2014). Based on six intragenic single nucleotide changes or deletions, the mutated *TFG* allele in the Iranian HMSN-P and CMT-100 families are identical by descent (Table 3). Both families originate from the same province in Iran.

Discussion

We have identified p.(Gly269Val) causing mutation in *TFG* as cause of CMT2 in patients of Iranian pedigree CMT-100. Definitive CMT diagnosis is justified as the patients present with distal muscle weakness, foot deformities, mild distal sensory impairment, and depressed tendon reflexes. Axonal CMT (CMT2) diagnosis was based on results of electrophysiologic and neuropathologic studies. Previously, we had found the same p.(Gly269Val) causing mutation in *TFG* in an Iranian HMSN-P diagnosed pedigree and a p.(Pro285Leu) causing mutation in *TFG* in another Iranian HMSN-P pedigree (Alavi et al., 2015; Khani et al., 2016). Table 4 presents a summary of distinguishing presentations of HMSN-P and CMT2 diagnosed patients of the three Iranian pedigrees with p.(Gly269Val) or p.(Pro285Leu) causing mutations in *TFG*. The comparison emphasizes similarities between the patients of the two HMSN-P diagnosed pedigrees with different *TFG* mutations, and differences between the two pedigrees with the same p.(Gly269Val) mutation. Asymmetric manifestations and presence of prominent fasciculation and cramps that are typical features of neuronopathies were among features observed only in the HMSN-P pedigrees. These presentations have prompted some authors to propose that *TFG* related diseases may be considered within the category of motor neuron diseases (Tsai et al., 2014). Cranial nerve involvement, another characteristic feature of neuronopathies, was seen only in the HMSN-P pedigree with the p.(Pro285Leu) mutation. There was no feature that was shared between the HMSN-P pedigree with the p.(Pro285Leu) mutation and the CMT-100 pedigree with the p.(Gly269Val) mutation, but absent in the HMSN-

P pedigree with the p.(Gly269Val) mutation. It is reasonable to conclude that the clinical features of the HMSN-P pedigree with the p.(Gly269Val) mutation (HMSN-160) are intermediate between those of the HMSN-P pedigree with the p. (Pro285Leu) mutation and the CMT2 pedigree with the p.(Gly269Val) mutation (CMT-100). As patients of the three Iranian pedigrees were examined by the same neurologists (SN and HS), clinical bias is unlikely to have contributed to differential evaluations or diagnosis. Available clinical data on the patients of the CMT2 Taiwanese pedigree with the p.(Gly269Val) mutation suggest that their presentations were similar to those of the Iranian CMT-100 pedigree. Symmetry was reported and spontaneous muscle activity or truncal and cranial involvement were not reported (Tsai et al., 2014).

The presence of different clinical features among patients with the same p.(Gly269Val) mutation in *TFG* begs an explanation. Differences in genetic background and environmental factors are obvious potential contributing causes. Variable presentations associated with mutations in the same gene and even identical mutations are not limited to mutations in *TFG* and are being increasingly noted (Armstrong, 2012; Lesage et al., 2013; Lindquist et al., 2013; Nicolas et al., 2018). Stochastic events during growth and development or variations in other genes may contribute to differences in presentations even for Mendelain diseases (Badano & Katsanis, 2002; Dipple & McCabe, 2000; Gonzaga-Jauregui et al., 2015; JBS, 1941; W, 1939). Interestingly, a CMT diagnosis among patients who harbor the p.(Pro285Leu) mutation has not been reported, even though many more patients and families with this mutation have been described (Fujisaki et al., 2018). It may be that despite the fact that p.Gly269 and p.Pro285 are positioned within the same proline and glutamine-rich domain of the TFG protein, the range of potential consequences of changes in p.Gly269 may be wider than that of p.Pro285. In fact, it has been shown that the immediate functional consequences of the two mutations are not the same (Tsai et al., 2014; Yagi et al., 2014).

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethical Standards

All participants, after being informed of the nature of the research, consented to participate. This research was performed in accordance with the Declaration of Helsinki and with approval of the ethics board of the University of Tehran.

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436 **Figure legends**

437 **Figure 1- Iranian CMT-100 pedigree with p.(Gly269Val) mutation in *TFG*.** A- *TFG* genotypes of
438 individuals tested are presented. Present age of individuals is provided when known. Filled circles and squares,
439 CMT2 affected; unfilled circles and squares, asymptomatic at time of examination. Among the affected
440 individuals, only those designated with * were clinically examined, and the others were reported to be affected
441 by family members. M, mutated *TFG* allele; N, wild-type *TFG* allele. B- DNA sequence chromatograms
442 showing the heterozygous c.806G>T mutation in *TFG*, and the wild type sequence.

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444

445 **Figure 2 - Axial view of T1 weighted muscle MRI of lower extremities of CMT-100 patients**. Years after
446 onset of symptoms in IV-10, IV-25, IV-19, and III-3 are, respectively, 12, 13, 21, and 32 years. More prominent
447 involvement of distal regions is evident, especially at early stages of disease. There is relatively more
448 involvement of anterolateral compartments in the thigh region, and relatively more involvement of posterior calf
449 region. Asymmetry is detected only in distal regions of patient IV10.

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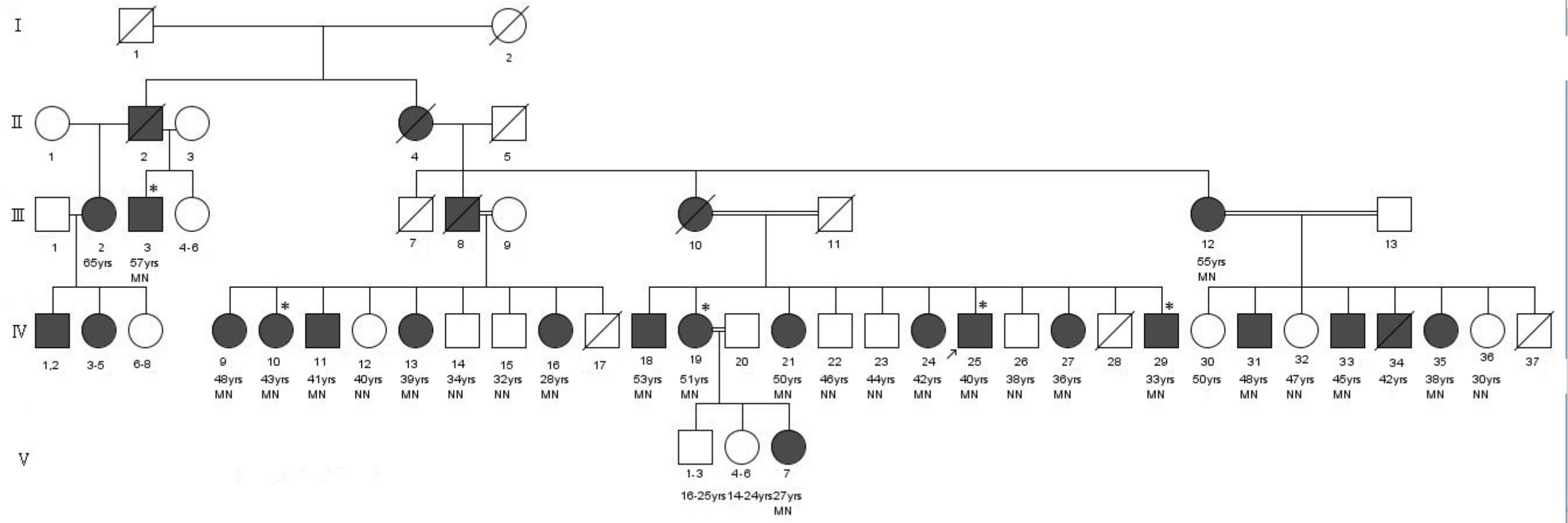
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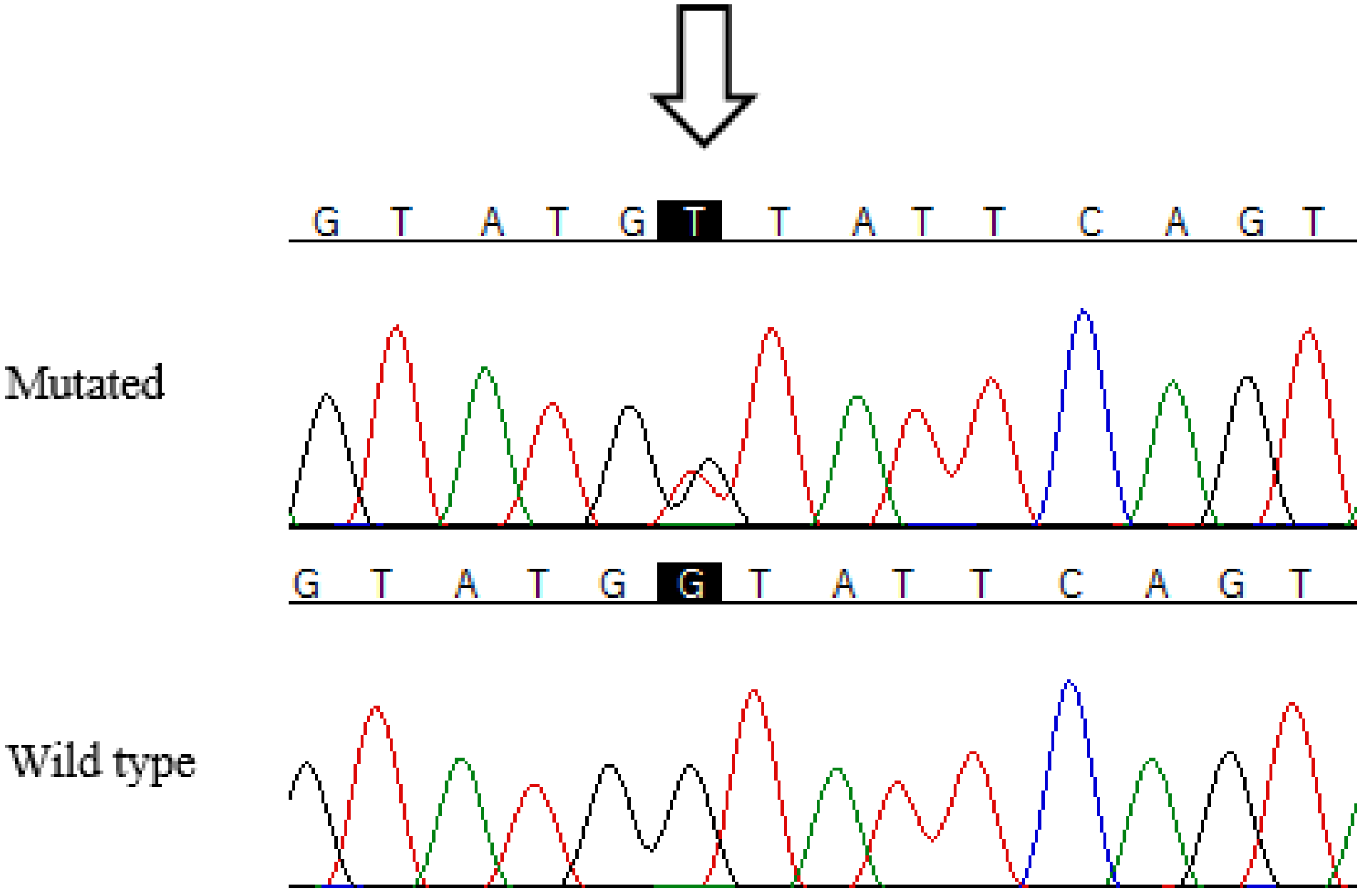
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