ORIGINAL ARTICLE

A Novel GDAP1 Mutation P78L Responsible for CMT4A Disease in Three Moroccan Families

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ABSTRACT: ***Background:*** The gene encoding the ganglioside-induced-differentiation-associated protein 1 (GDAP1) has been associated with both axonal and demyelinating neuropathy. Up to date, 25 mutations in the GDAP1 gene have been reported in patients from different origins. ***Methods:*** Three Moroccan families with early onset ARCMT1 and autosomal recessive inheritance were genotyped to test linkage to 8q21.3 and their GDAP1 gene coding exons screened for mutations. ***Results:*** A novel C233T transversion at codon 78 (P78L) was detected in 6 patients from 3 unrelated families. The mutation was found to be homozygous in two families and compound heterozygous in association with the already reported S194X mutation in one family. The P78L mutation was associated with a common haplotype suggesting a Moroccan founder mutation. The patients had symptoms within the two first years of life and developed common phenotype of CMT4A with evident hoarse-voice in two cases with the longer disease duration. ***Conclusion:*** P78L mutation was associated with a common haplotype suggesting a common ancestor.

RÉSUMÉ: Une nouvelle mutation P78L du gène GDAP1 responsable de la maladie CMT4A dans trois familles marocaines. ***Contexte :*** Le gène qui code pour la protéine GDAP1 (ganglioside-induced-differentiation-associated protein 1) a été associé à une neuropathie axonale démyélinisante. Jusqu'à maintenant, 25 mutations du gène GDAP1 ont été rapportées chez des patients de différentes origines ethniques. ***Méthodes :*** Trois familles marocaines, atteintes d'une ARCMT1 à début précoce et dont l'hérédité était autosomique récessive, ont été génotypées pour déterminer s'il existait une liaison à la région 8q21.3 et on a recherché des mutations dans les exons codants du gène GDAP1. ***Résultats :*** Une nouvelle transversion C233T au codon 78 (P78L) a été décelée chez 6 patients appartenant à 3 familles non apparentées. Il s'agissait d'une mutation à l'état homozygote dans deux familles et à l'état hétérozygote composé en association à une mutation S194X dans l'autre famille. Cette mutation a déjà été rapportée dans une famille. La mutation P78L est associée à un haplotype commun, ce qui laisse croire qu'il s'agit d'une mutation fondatrice marocaine. Les patients présentaient des symptômes au cours des deux premières années de vie et par la suite le phénotype habituel de la CMT4A. Une voix rauque a été observée chez deux patients atteints depuis longtemps. ***Conclusion :*** La mutation P78L est associée à un haplotype commun, ce qui porte à croire qu'elle provient d'un ancêtre commun.

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Charcot-Marie-Tooth (CMT) disease, a heterogeneous group of disorders affecting the peripheral nervous system, is traditionally classified into two types, demyelinating (CMT1) and axonal (CMT2) forms according to electrophysiological criteria. Charcot-Marie-Tooth (CMT1) is characterized by reduced motor nerve conduction velocities and Charcot-Marie-Tooth (CMT2) by normal or slightly reduced motor nerve conduction velocities.[1] Each type can be inherited as an autosomal dominant, autosomal recessive or X-linked trait. The autosomal recessive subgroup of CMT (ARCMT) is relatively more frequent in North Africa due to the high rate of consanguinity in theses countries. To date 11 loci and 7 genes have been reported for ARCMT and many of them have been reported in North African families. The first locus for ARCMT1

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was mapped in chromosome 8q13-21 in a consanguineous Tunisian family displaying a severe AR demyelinating neuropathy designated as CMT4A.[2] Recently, mutations in ganglioside-induced differentiation-associated protein-1 gene (GDAP1) were shown to be responsible for the CMT4A form.[3] Six other genes responsible for demyelinating ARCMT have also been identified, *ERG2*,[4] *MTMR2*,[5] *NDRG1*,[6] *PRX*,[7] *MTMR13*,[8] and *KIAA1985*.[9] An additional locus has been reported for HMSN Russe on chromosome 10q23.[10]

For the ARCMT2, three loci have been reported to date and in two of them the disease-causing gene has been discovered. The first locus (ARCMT2A) was mapped in a large inbred Moroccan family on chromosome 1q21.2,[11] and a R298C mutation in lamin A/C gene was identified to be responsible for the disease in Algerian families linked to the same locus.[12] A second locus (ARCMT2B) was assigned to chromosome 19q13.3 in a Costa Rican family with moderate phenotype[13] and the responsible gene has not yet been identified. The third locus was mapped in a Tunisian family on chromosome 8q21.3 which overlaps with CMT4A locus[14] suggesting that these two forms could be allelic. Mutations in GDAP1 gene were shown to be responsible for AR axonal CMT[15-17] as well as intermediate type ARCMT.[18]

We report here three families of Moroccan origin presenting a CMT4A phenotype, linked to 8q21.3 and associated with a novel P78L mutation in exon 2 of GDAP1 gene.

Patients and Methods

A set of 27 families of Moroccan Origin with CMT1 diagnosis and a possible autosomal recessive inheritance were examined in the Department of Clinical Neurophysiology of Rabat, Morocco from 1997 to 2004. Three of them, FEN, AME and LAH families, were selected on the basis of an early onset in the first decade and a severe clinical phenotype. Families FEN and AME are consanguineous and originated from a small village in the centre of Morocco (village of Berkin, Guerssif). Whereas family LAH is non-consanguineous but a parent, LAH-01, originated also from this location.

The six patients and five at risk relatives belonging to these three families were examined for the presence of motor and sensory loss, areflexia, foot deformities, scoliosis and other associated signs such as nerve hypertrophy, tremor, ataxia, pyramidal signs, cranial nerve involvement and dementia. Disease severity was evaluated in terms of ability to walk and run and to use hands in daily tasks. Electrophysiological examination was performed in all individuals as described previously.[19]

Blood samples from patients and healthy individuals were obtained after informed consent was given and genomic DNA was extracted using standard procedures. Since patients presented clinical signs characteristic of GDAP1 mutation, genotyping was performed directly with D8S530, D8S286, D8S1705 and D8S1757 fluorescent microsatellite markers to test linkage to 8q21.3 locus using an automated capillary DNA sequencer ABI 310. Data were collected and analysed using the ABI GENESCAN (version 2.1) and GENOTYPER (version 2.0) software (Applied Biosystems, Foster City, CA).

The six coding exons of GADP1 gene were screened for mutation using primers previously reported.[14] Both strands were sequenced with BigdyeTM dRhodamine Terminator Reaction Kit (version 1.1) according to the manufacturer's instructions using an automated DNA sequencer 310. The collected chromatogram data were analyzed with SeqScape software version 2.0 (all from Applied Biosystems, Foster City, CA).

Sequencing of GDAP1 gene was done in all FEN, AME and LAH family members and all index cases of the 24 remaining ARCMT1 families.

Results

The CMT4A markers were found to be homozygous in patients from families FEN and AME who shared the same haplotypes (Figure 1). Sequencing of the six codon exons of GDAP1 gene revealed a novel homozygous C to T transition at nucleotide 233 of exon 2 (C233T) resulting in a missense mutation of Pro to Leu at codon 78 (P78L) in patients FEN-19, FEN-21, FEN-25 and AME-09. The unaffected parents FEN-06, FEN-17 and AME-07 are heterozygous for this mutation.

Patients LAH-05 and LAH-06 were not homozygous for CMT4A markers used here but had the same genotype. Since their father LAH-1 originated from the same village as the parents of families FEN and AME, sequencing of exon 2 showed the P78L mutation at heterozygous state in these two patients and their father (Figure 1). Screening of the other GDAP1 exons identified the already reported S194X mutation at heterozygous state in the two patients and their mother. We did not find the P78L mutation in 198 ethnically matched control chromosomes. Comparison of the human GDAP1 protein sequence with those in the peptide sequence data base indicates that P78, the substituted amino acid in the six patients studied, is highly conserved among diverse group of species and is located in a highly conserved sequence region of the protein (Figure 2).

The chromosomes bearing the P78L mutation shared a common haplotype between D8S530 and P78L mutation in the three families studied, but different in the two distal markers in family LAH (Figure 1). This difference suggests that a recombination event occurred between GDAP1 gene and D8S1705 marker.

No mutation was found in the coding exons of GDAP1 gene in the index cases of all the 24 remaining ARCMT1 families.

In the six patients belonging to the three unrelated families sharing the novel mutation P78L, clinical symptoms (Table 1) were similar to the majority of patients with GDAP1 gene mutations reported up to date. For all patients, age at onset was early in childhood under two years. One patient (LAH-06) had hypotonia at birth, and delay of walking was observed in three patients (AME-09, LAH-05 and LAH-06). The patients had a predominately distal motor deficit and atrophy of both upper and lower limbs. Atrophy and weakness of proximal muscles in all limbs were also noticed in patients FEN-21 and AME-09 whose examination age was 19 and 15 years respectively. All patients had four limbs areflexia and foot deformities. Only the three patients of the family FEN-21 displayed a distal sensory impairment involving particularly proprioception in the lower limbs. A hoarse voice was evident in patients FEN-21 and AME-09 with the longer disease durations (17.5 and 14 years respectively).

We noted intra- and inter-familial variability in term of the functional disability since patient FEN-19 became wheelchair

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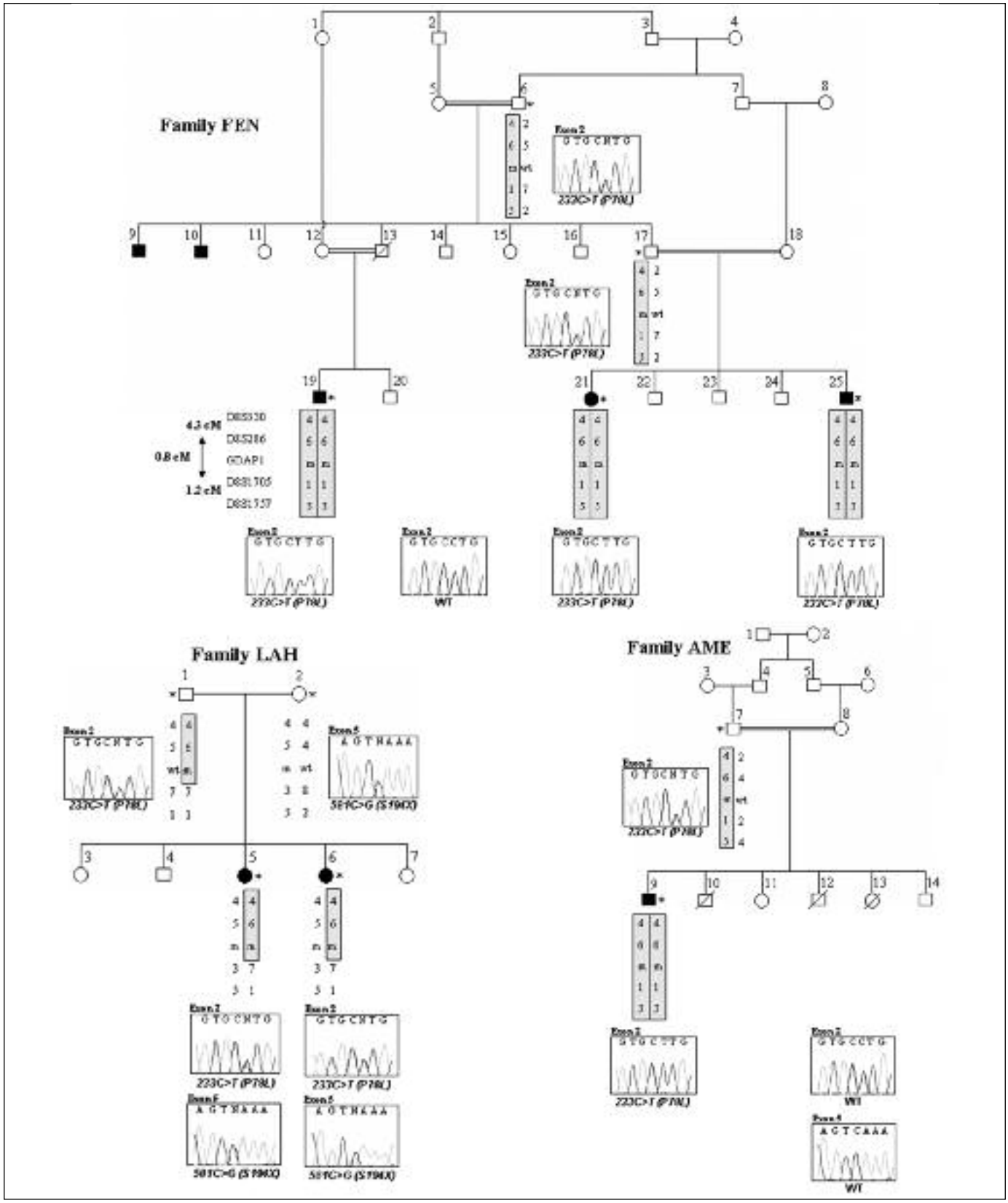

Figure 1: *Pedigrees of three Moroccan families with ARCMT1 and electrophoregrams showing the C233T sequence variation in part of exon 2 of the GDAP1 gene, resulting in the P78L missense mutation. Black and white symbols correspond to affected and asymptomatic members respectively. Members who were included in the study are indicated with a star.*

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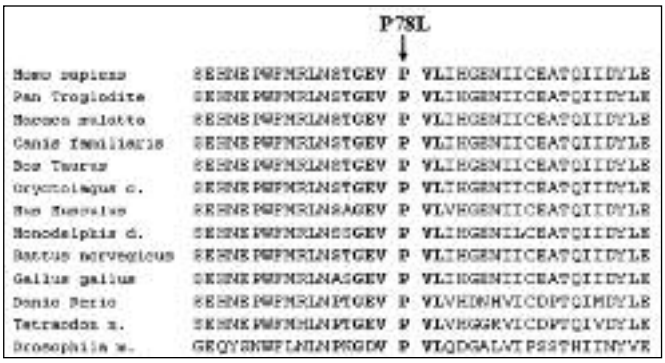

Figure 2: *Clustal W alignment of the GDAP1 orthologs of the region surrounding the P78L missense mutation.*

dependent at 6 years of age, her cousin FEN-21 at 15 years of age, whereas patient AME-09 walked with a cane at age of 15 years. The disease severity was relatively moderate in the two patients belonging to the family LAH with the compound heterozygous mutations P78L and S194X but with the shorter disease durations.

Electrophysiological findings are showed in Table 2. The motor nerve conduction velocity (MNCV) of median nerves could be measured in four patients, three of which (FEN-19, FEN-25 and LAH-05) had reduced values bellow 25m/s with reduced compound muscle action potential (CMAP) amplitudes. Only one patient LAH-06, with compound heterozygous mutation, had slightly reduced MNCVs. The peroneal nerve motor response could not be recorded in all patients because of important muscle atrophy in the legs. Sensory nerves responses were abnormal in all cases either abolished or with reduced amplitudes and sensory nerve conduction velocity (SNCV). The needle electromyography showed neurogenic pattern in distal muscles in all patients and also in proximal muscles in patient FEN-21. According to these electrophysiological data the neuropathy seems to be mixed with both demyelination and axonal damage.

Discussion

We report a novel pathogenic mutation P78L in the GDAP1 gene segregating in three unrelated Moroccan families with CMT4A disease. This mutation was found to be homozygous in four patients belonging to two families and compound heterozygous in association with the already reported S194X mutation in two patients from the third family. Heterozygous carriers have been shown to be healthy in clinical and electrophysiological examinations.

The pathogenic effect of the P78L mutation could be supported by the fact that the mutation was not observed in 198 ethnically matched chromosomes, was found in homozygous state and compound heterozygous in association with a known pathogenic stop mutation S194X, and P78 is highly conserved among diverse group of species.

Haplotype analysis in these three unrelated families from a restricted geographical area of Morocco showed that the P78L mutation was associated with a common ancestor with a possible origin in the centre of Morocco.

Among the AR forms, CMT4A is the most frequent.[20] To date, 25 CMT-associated GDAP1 mutations (OMIM 606598)

Table 1: Clinical findings in patients with P78L mutation in the GDAP1 gene

Case N°	FEN-19	FEN-21	FEN-25	AME-09	LAH-05	LAH-06
Age at onset (years)	2	1.5	2	< 1	< 2	< 1
Age at examination (years)	7	19	8	15	6	4
Disease duration (years)	5	17.5	6	14	4	3
Age of walking (months)	< 18	< 18	< 18	24	24	24
Hypotonia at birth	no	no	no	no	no	yes
Distal motor deficit	UL + LL	UL + LL	UL + LL	UL + LL	UL + LL	UL + LL
Proximal motor deficit	LL	UL + LL	no	UL + LL	no	no
Distal motor atrophy	UL + LL	UL + LL	UL + LL	UL + LL	UL + LL	UL + LL
Proximal motor atrophy	no	UL + LL	no	UL + LL	no	no
Sensory loss						
Pain and touch	no	LL	no	no	no	no
Proprioception	LL	LL	LL	no	no	no
Areflexia	UL + LL	UL + LL	UL + LL	UL + LL	UL + LL	LL
Foot deformities	severe	moderate	severe	moderate	moderate	moderate
Scoliosis	no	yes	no	no	no	no
Hoarse voice	no	yes	no	yes	no	no
Functional disability						
upper limbs	clawfingers	clawfingers	clawfingers	clawfingers	clawfingers	moderate
lower limbs	wheelchair bound	wheelchair bound	walk with a cane	walk with a cane	walk with aid	walk unaided

Table 2: Electrophysiological findings in patients with P78L mutation in the GDAP1 gene

Case N°	FEN-19	FEN-21	FEN-25	AME-09	LAH-05	LAH-06
Median nerve						
DML (ms)	16.8	NE	6	NE	5.2	3.7
MNCV (m/s)	13.2	NE	24.5	NE	22	45.6
CMAP amplitude (mV)	0.3	NE	0.4	NE	0.2	1.8
SNCV (mV)	NE	NE	ND	32	31	29
SNAP amplitude (µV)	NE	NE	ND	0.8	2.3	2.5
Ulnar nerve						
DML (ms)	NE	NE	7.5	NE	3.2	2.6
MNCV (m/s)	NE	NE	15	NE	31.6	45
CMAP amplitude (mV)	NE	NE	0.2	NE	0.4	0.8
SNCV (mV)	NE	NE	ND	27	ND	ND
SNAP amplitude (µV)	NE	NE	ND	0.1	ND	4.2
Peroneal nerve						
DML (ms)	NE	NE	NE	NE	NE	NE
MNCV (mV)	NE	NE	NE	NE	NE	NE
CMAP amplitude (mV)	NE	NE	NE	NE	NE	NE
Sural nerve						
SNCV (mV)	NE	NE	NE	NE	NE	NE
SNAP amplitude (µV)	NE	NE	NE	NE	NE	NE

DML: distal motor latency; MNCV: motor nerve conduction velocity; CMAP: compound muscle action potential, SNCV: sensory nerve conduction velocity; SNAP: sensory nerve action potential. NE: Not elicitable; ND: Not done.

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have been described, causing both axonal and demyelinating forms of CMT probably by disturbing communication between the degenerating axons and the ensheathing Schwann cells.

Pedrola et al[21] demonstrated that GDAP1 protein is predominately expressed in axons rather than in myelin and it is localized in the mitochondrial membrane of the cell, suggesting that CMT4A is primarily an axonal neuropathy caused by abnormalities in mitochondrial function. However, Niemann et al[22] demonstrated in the peripheral nervous system that GDAP1 is expressed by myelinating Schwann cells as well as motor and sensory neurons, suggesting that the disease may primarily affect Schwann cells, neurons or both.

GDAP1 encodes a protein with two transmembrane domains and a region that contains a glutathione S-transferase, and thus was initially classified as a novel family of the GST proteins due to phylogenetic and structural analysis. However, it has been demonstrated that GDAP1 does not express GST activity and could be a member of another family of GSTs.[21,23]

GDAP1 is an integral membrane protein of the outer mitochondria with the NH2 terminus and the GST domains exposed to the extracellular cytoplasm and the COOH terminal transmembrane domain that contains the mitochondrial-targeting information, since it was demonstrated that mutations leading to GST-C truncations lost mitochondrial localization whereas GDAP1 protein carrying missense mutations remained correctly targeted to mitochondria.[21-22]

The present discovered C233T mutation, the first mutation described in the GDAP1 exon 2, is localized in the GST-N domain of GDAP1 protein, and thus could not affect the protein localization in mitochondria. How the P78L mutant causes neuropathy in the studied families remains unexplained.

In a subset of 40 ARCMT families (27 ARCMT1 and 13 ARCMT2) of Moroccan ancestry, we observed only two mutations in the coding regions of GDAP1 gene, the S194X mutation in 11 patients belonging to 6 families with axonal neuropathy,[24] and the P78L mutation in 6 patients belonging to 3 families with a mixed neuropathy (the present study). Therefore GDAP1 mutations can currently be considered as the most frequent cause of ARCMT in Morocco with 22.5%.

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