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# Homozygous R627W mutations in *POLG* cause mitochondrial DNA depletion leading to encephalopathy, seizures and stroke-like episodes

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**Abstract**

Mutations in the mitochondrial DNA maintenance gene *POLG* (DNA Polymerase Gamma, Catalytic Subunit), encoding mitochondrial DNA polymerase gamma (pol γ), are associated with an extremely broad phenotypic spectrum. We identified homozygous *POLG* c.1879C>T; p.R627W mutations in two siblings from a consanguineous South Asian family following targeted resequencing of 75 nuclear-encoded mitochondrial genes. Both patients presented with encephalopathy, seizures and stroke-like episodes, and mitochondrial DNA depletion was confirmed in the proband's muscle tissue. Subsequent Sanger sequencing of *POLG* in a further 275 unrelated probands with genetically unconfirmed mitochondrial disease revealed a third unrelated proband with a similar phenotype harboring homozygous c.1879C>T; p.R627W mutations and a fourth patient, with a milder clinical disorder, harboring compound heterozygous *POLG* c.1879C>T; p.R627W and c.2341G>A; p.A781T mutations. Given endogamous practices in the Indian subcontinent, homozygous *POLG* c.1879C>T; p.R627W mutations should be excluded in South Asian patients presenting with encephalopathy, seizures and stroke-like episodes.

**Keywords:** Mitochondrial disease, *POLG*, Encephalopathy, Seizures, Status Epilepticus, Stroke-like episode, mtDNA.

## 1. INTRODUCTION

Mitochondrial disorders are complex genetic diseases, caused by mutations in either mitochondrial DNA (mtDNA) or nuclear encoded genes, that exhibit remarkable phenotypic heterogeneity with variable age of onset (Chinnery 1993). More than 99% of mitochondrial proteins required for maintaining the structure, function and stability of mtDNA molecules are encoded by nuclear genes (Boengler et al., 2011). Mitochondrial DNA polymerase gamma (pol γ) is a nuclear-encoded protein found in mitochondria and is essential for maintaining the integrity of the mitochondrial genome during replication and repair. The holoenzyme of human pol γ comprises a catalytic subunit of 140 kDa (encoded by *POLG*) and a homodimeric accessory subunit (encoded by *POLG2*). Mutations in *POLG* are a major cause of mitochondrial disease that result in the depletion and/or accumulation of multiple deletions of mtDNA (Chan and Copeland 2009).

In the present study, we report four South Asian patients from three unrelated families with multisystem mitochondrial disease harboring the known pathogenic *POLG* c.1879C>T; R627W variant in homozygous and compound heterozygous states.

## 2. PATIENTS AND METHODS

The study was approved by the Institutional Ethical Committee (IEC) of the CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India, and the Nizam's Institute of Medical Sciences (NIMS), Hyderabad, India. Informed written consent was obtained from all human subjects, prior to collection of blood and tissue samples.

### 2.1 Clinical cases

Patient 1 (P1) and Patient 2 (P2) (Fig. 1A, Family 1) were siblings, born to consanguineous parents, and exhibited similar clinical presentations and courses (Table 1). The proband (P1), a 6 year old boy, presented with sudden onset headache, vomiting, encephalopathy, myoclonus, and dysarthria, and subsequently developed generalized tonic-clonic seizures. T2-weighted brain magnetic resonance imaging (MRI) showed right sided occipital lobe, and bilateral thalamic and cerebellar, hyperintensities. Electrocardiogram (ECG), echocardiogram, abdominal ultrasonography, and nerve conduction studies were normal. Muscle tissue from P1 demonstrated no histopathological evidence of mitochondrial dysfunction, including ragged red, ragged blue or cytochrome *c* oxidase (COX) deficient muscle fibers. Quantitative PCR confirmed depletion of muscle mtDNA molecules in P1 compared with controls, but no mtDNA deletions on long range PCR.

Targeted resequencing of 75 disease-causing mitochondrial nuclear genes was performed using an Illumina NGS platform (100X coverage) using the genomic DNA extracted from the blood of P1. The sequences obtained were aligned to the GRCh37/hg19 human reference genome using BWA (Li and

Durbin 2010; Meyer et al., 2013) and analyzed with Picard and the GATK-Lite toolkit (McKenna et al., 2010; Li et al., 2009). Annotation of the variants was undertaken against the Ensembl release 75 gene model and clinically relevant mutations annotated using published pathogenic variants and a number of databases, including ClinVar, OMIM, GWAS, HGMD and SwissVar (https://www.ncbi.nlm.nih.gov/clinvar/, https://www.omim.org/, http://www.gwascentral.org/, https://www.biobase-international.com/product/hgmd, http://swissvar.expasy.org/). Filtering for non-synonymous and splice site variants within the panel of 75 nuclear genes associated with mitochondrial diseases revealed homozygous *POLG* c.1879C>T; p.R627W mutations, which were confirmed using Sanger sequencing (Fig. 1D). Homozygous *POLG* c.1879C>T; p.R627W mutations were detected in the proband's affected sister, while the mutation was present in the heterozygous state in unaffected family members (Fig. 1A).

To further investigate the prevalence of the *POLG* c.1879; p.R627W mutation in South Asian patients, we screened a further 275 unrelated probands with clinical and/or biochemical evidence of mitochondrial disease without a molecular diagnosis and identified two additional patients (P3 and P4, see results) with the *POLG* p.R627W mutation in homozygous and compound heterozygous states.

### 2.2 Molecular genetic studies

DNA was extracted from blood and/or tissue by a standard phenol-chloroform method (Thangaraj et al., 2002) with minor modifications. MtDNA was amplified using 24 primers to generate overlapping amplicons. These were purified and directionally sequenced using BigDye terminator cycle sequencing kit and ABI3730 XL Genetic Analyzer (Rieder et al., 1998). Long range PCR was undertaken to confirm the presence of large-scale rearrangements of mtDNA (Longley et al., 2006) and real-time quantitative PCR with TaqMan probes was performed to evaluate mtDNA copy number (Strauss et al., 2015), with minor modifications. To evaluate the potential functional impact of identified missense mutations, we utilized a variety of pathogenicity prediction programs, including: SIF; MutationTaster; PolyPhen-2; and Align-GVGD.

## 3. RESULTS

The major clinical and laboratory findings reported in patients harboring the *POLG* c.1879; p.R627W mutations are summarized in Table 1. Pedigrees and *POLG* genotype data are provided in Figures 1 and 2.

### 3.1 Additional probands harboring *POLG* c.1879; p.R627W mutation

**Patient 3** (Fig. 1B, Family 2, P3) presented aged 18 years, following normal early development, with headache, associated with visual disturbance and vomiting, and generalized seizures. She

subsequently developed convulsive status epilepticus. There was a past medical history of migraine. She was the product of non-consanguineous parentage and had an older sister who diedat 9 months following a febrile illness. Brain MRI during the acute phase of her illness revealed hyperintense lesions in the thalamus and basal ganglia bilaterally (Fig. 1C-I and II), and in the right temporal and occipital lobes (Fig. 1C-III). Apparent diffusion coefficient mapping confirmed low cortical signal intensity and gyral swelling affecting the right temporal and occipital lobes(Fig. 1C-IV) and diffusion-weighted MR imaging confirmed restricted diffusion abnormalities in the cerebellar hemispheres (Fig. 1C-V). Serum biochemistry, including creatine kinase and lactate levels, inflammatory and autoimmune markers were normal. ECG, echocardiography, nerve conduction studies, and CSF examination, including cell count, culture, biochemistry and cytology, were normal. Electroencephalogram revealed diffuse generalized theta range slowing. On waking, the patient exhibited a homonymous hemianopia, consistent with occipital lobe infarcts, and cerebellar ataxia. Unfortunately, she continued to experience refractory seizures, despite multiple anticonvulsant therapy, and died aged 18years. Homozygous *POLG* c.1879C>T; p.R627W mutations were confirmed in P3 using Sanger sequencing of genomic DNA extracted from blood.

**Patient 4** (Fig. 2A, Family 3, P4) was a 41-year-old male born to non-consanguineous parents. He presented with ptosis, progressive external ophthalmoplegia (PEO), proximal myopathy, and sensorineural hearing loss aged 27 years. ECG and echocardiogram were normal. Nerve conduction studies showed sensorimotor neuropathy. T2-weighted MRI revealed bilateral hyperintensities in the occipital lobes. Muscle histopathology showed ragged blue fibers (Fig. 2B-I), COX deficient fibers (Fig. 2B-II) and long-range PCR of muscle tissue confirmed multiple mtDNA deletions (Fig. 2B-III), with no evidence of mtDNA depletion on quantitative PCR. Sanger sequencing identified a single *POLG* c.1879C>T; p.R627W mutation. However, given that the mutation has not been reported to cause disease in the heterozygous state, the entire *POLG* coding region was sequenced (for primers see Paramasivam et al. 2016). This confirmed an additional pathogenic variant: c.2341G>A; p.A781T (Fig. 2D), in the polymerase domain. Segregation studies confirmed heterozygosity of both mutations among unaffected family members (Fig. 2A, Fig. 2C and Fig. 2D).

## 4. DISCUSSION

*POLG* encodes the catalytic subunit of mitochondrial pol γ, the only known polymerase for mtDNA replication and repair (Chan and Copeland 2009; Tang et al. 2012). Mutations in *POLG* cause multiple deletions and/or depletion of mtDNA in post-mitotic tissues, such as skeletal muscle, brain and liver. Since the first published disease-causing *POLG* mutation (Van Goethem et al., 2001), more than 200 pathogenic variants have been identified (http://tools.niehs.nih.gov/polg/) that exhibit both recessive and dominant inheritance patterns. These mutations are associated with an extremely broad clinical spectrum, including autosomal dominant progressive external ophthalmoplegia (adPEO),

Alpers-Huttenlocher syndrome (AHS), childhood myocerebrohepatopathy spectrum (MCHS), myoclonic epilepsymyopathy sensory ataxia (MEMSA), ataxia neuropathy spectrum (ANS), progressive external ophthalmoplegia (PEO) with or without sensory ataxic neuropathy and dysarthria (SANDO) (Stumpf et al., 2013), mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) (Tang et al. 2012), distal myopathy (Pitceathly et al., 2013), Charcot–Marie–Tooth disease, and idiopathic parkinsonism (Chan and Copeland 2009).

We report homozygous *POLG* c.1879C>T; p.R627W mutations in three patients, from two unrelated families, causing mitochondrial encephalopathy, seizures and stroke-like episodes. Despite a lack of consanguinity, P3 harbored homozygous *POLG* c.1879C>T; p.R627W mutations. This highlights the importance of recessive and population-specific genetic diseases in South Asia as a consequence of endogamous practices (Nakatsuka et al., 2017). A fourth unrelated patient (P4, Family 3), with a milder clinical phenotype comprising PEO, ptosis, hearing loss, proximal myopathy, and neuropathy, was confirmed to harbor compound heterozygous *POLG* mutations: c.1879C>T; p.R627W and c.2341G>A; p.A781T.

The *POLG* c.1879C>T; p.R627W mutation is located in the linker region, while the c.2341G>A; p.A781T mutation resides within the polymerase domain of mitochondrial pol γ. The c.2341G>A; p.A781T variant has not previously been linked with mitochondrial disease. However, several lines of evidence support its pathogenic effects. First, it is located in a highly conserved region of the protein. Second, segregation of the mutations between affected and unaffected individuals was confirmed. Finally, the mutation was absent from 310 ethnically matched control samples and has a South Asian minor allele frequency of 0.00052 (gnomAD).

Homozygous *POLG* c.1879C>T; p.R627W mutations have not previously been reported. However, three compound heterozygotes are present in the Human DNA Polymerase Gamma Mutation Database (https://tools.niehs.nih.gov/polg/) along side: 1) A467T, in a patient with sensory ataxic neuropathy, dysarthria, ophthalmoparesis, cardiomyopathy, and hearing loss (Van Goethem et al., 2003; Horvath et al., 2006); 2) T914P, in a patient with Alpers syndrome (Ashley et al., 2008); and 3) W748S, in patient with epilepsy and encephalitis (Nolte et al., 2013). In our own cohort of 2,400 South Indian controls we detected a carrier rate of 0.04% (1/2,400) for the c.1879C>T; p.R627W mutation (0.000064, gnomAD).

In conclusion, we report that homozygous *POLG* c.1879C>T; p.R627W mutations cause mtDNA depletion leading to mitochondrial encephalopathy, seizures and stroke-like episodes in consanguineous and non-consanguineous South Asian patients. Compound heterozygosity of the c.1879C>T; p.R627W mutation with the pathogenic *POLG* c.2341G>A; p.A781Tvariant is associated

with multiple mtDNA deletions that results in a milder clinical phenotype comprising autosomal recessive PEO, ptosis, hearing loss, proximal myopathy, and neuropathy.

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**COMPETING INTERESTS**

The authors declare no conflicts of interest.

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neuropathy in compound heterozygote patients with progressive external ophthalmoplegia. Neuromuscul. Disord. 13, 133–142.

**FIGURE LEGENDS**

**Fig. 1.** (A) Family 1 and (B) Family 2 pedigrees showing segregation of *POLG* c.1879C>T; p.R627W mutations among affected and unaffected family members. (C) Brain MRI of Patient 3 (P3, Family 2) during acute phase of patient's illness. (C-I) T2-weighted imaging shows bilateral hyperintense lesions in thalamus (arrow). (C-II) High FLAIR signal is present in the basal ganglia bilaterally (arrow). (C-III) diffusion-weighted imaging reveals hyperintense region in the right occipital lobe (arrow). (C-IV) apparent diffusion coefficient mapping shows low signal and gyral swelling in right temporal and occipital lobe cortices (arrow). (C-V) diffusion-weighted imaging demonstrates restricted cortical diffusion in both cerebellar hemispheres (arrow). (D) Sequencing chromatograms of *POLG* for Patient 1 (P1, Family 1, homozygous c.1879C>T; p.R627W mutations), the father of P1 (heterozygous carrier of c.1879C>T; p.R627W mutation) and a control.

**Figure 2:** (A) Family 3 pedigree showing the segregation of the *POLG* c.1879C>T; p.R627W and c.2341 G>A; p.A781T mutations among affected and unaffected family members. (B) Histochemical analysis of muscle tissue and long range PCR of muscle mitochondrial DNA from Patient 4 (P4, Family 3). (B-I) succinate dehydrogenase stain shows ragged blue fibers (asterisks). (B-II) cytochrome *c* oxidase (COX) stain shows COX deficient fibers (asterisks). (B-III) long-range PCR (9.9kb amplifications) shows multiple mtDNA deletions; lane 1 = 1kb ladder; lane 2 = control muscle; lane 3 = P3 showing multiple mtDNA deletions. (C and D) Sequencing chromatograms of *POLG* for P4 (compound heterozygous mutations c.1879C>T; p.R627W and c.2341G>A; p.A781T), the father of P4 (heterozygous carrier of c.2341G>A; p.A781T mutation) and the mother of P4 (heterozygous carrier of c.1879C>T; p.R627W mutation).

**Table 1. Clinicopathological and molecular characteristics of homozygous and compound heterozygous *POLG* c.1879C>T; p.R627W mutations**

| er | Age at symptom onset (years) | Current age / age at death (years) | Clinical features | Brain MRI | Skeletal muscle histochemistry | Multiple mtDNA deletions | mtDNA depletion | *POLG* mutations | Predicted effect on protein |
|---|---|---|---|---|---|---|---|---|---|
| | 6 | 13D | Migraine, seizures, ictal visual loss. | T2: hyperintensities right occipital lobe, bilateral hyperintensities thalamus and cerebellum. | Normal | - | + | c.[1879C>T]; [1879C>T] | p.[R627W]; [R627W] |
| | 8 | 10 | Migraine, seizures, ictal visual loss, ataxia, myoclonus, dysarthria | NA | ND | ND | ND | c.[1879C>T]; [1879C>T] | p.[R627W]; [R627W] |
| | 15 | 18D | Migraine, seizures, ataxia. | T2: hyperintensities right temporal and occipital lobe, bilateral hyperintensities thalamus, BG<br>ADC: low cortical signal intensity and gyral swelling right temporal and occipital lobes.<br>DWI: restricted diffusion abnormalities in cerebellar hemispheres | ND | ND | ND | c.[1879C>T]; [1879C>T] | p.[R627W]; [R627W] |
| | 27 | 41 | Muscle weakness, ptosis, PEO, hearing loss, sensorimotor neuropathy. | T2: bilateral hyperintensities in occipital lobes. | RRF, COX-ve | + | - | c.[1879C>T]; [2341G>A] | p.[R627W]; [A781T] |
| | 20 | 39 | Sensory ataxic neuropathy, PEO, dysarthria. | T2: bilateral hyperintensities in thalamus | Normal | + | ND | c.[1879C>T]; [1399G>A] | p.[R627W]; [A467T] |
| | 32 | 41D | PEO, cardiomyopathy, hearing loss, | ND | RRF, COX-ve | + | ND | c.[1879C>T]; [1399G>A] | p.[R627W]; [A467T] |
| | Birth | U | Epilepsy, hepatopathy, choreoathetosis,ataxia, nystagmus) | ND | ND | ND | + | c.[1879C>T]; [2740A>C] | p.[R627W]; [T914P] |
| | U | 16 | Epilepsy, encephalitis. | T2: bilateral multifocal supratentorial cortical lesions, white matter disease with periventricular involvement, bilateral cerebellar lesions. | COX-ve | ND | ND | c.[1879C>T]; [2243G >C] | p.[R627W]; [W748S] |

Abbreviations:BG, basal ganglia; COX-ve, cytochrome *c* oxidase negative fibers; D, age of death; F, female; M, male; NA, not available; mtDNA, mitochondrial DNA; ND, not done; PEO, progressive external ophthalmoplegia; RRF, ragged red fibers; U, unknown; +, present; -, absent.

**Highlights**

- The *POLG* c.1879C>T; p.R627W mutation is linked with disease in the compound heterozygous state.
- Homozygous c.1879C>T; p.R627W mutations were identified in three unrelated South Asian probands.
- Depletion of mitochondrial DNA was detected in muscle tissue.
- The clinical phenotype comprised mitochondrial encephalopathy, seizures and stroke-like episodes.

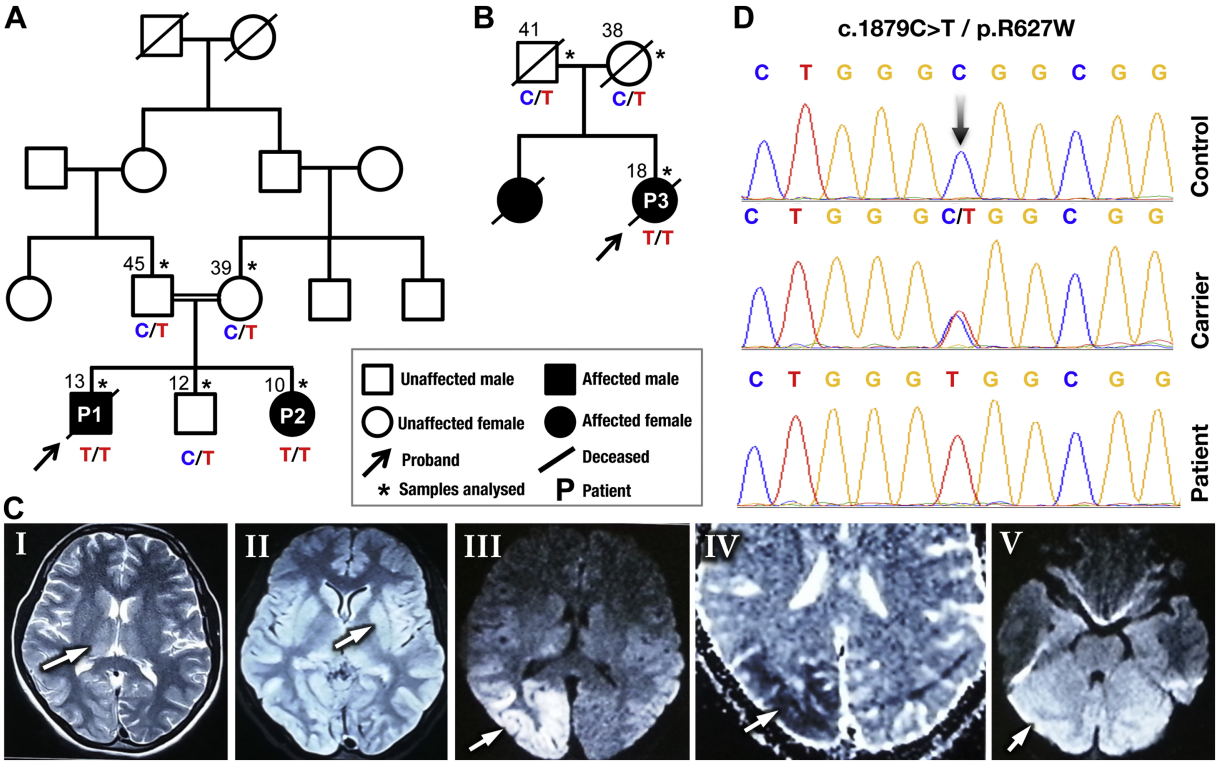
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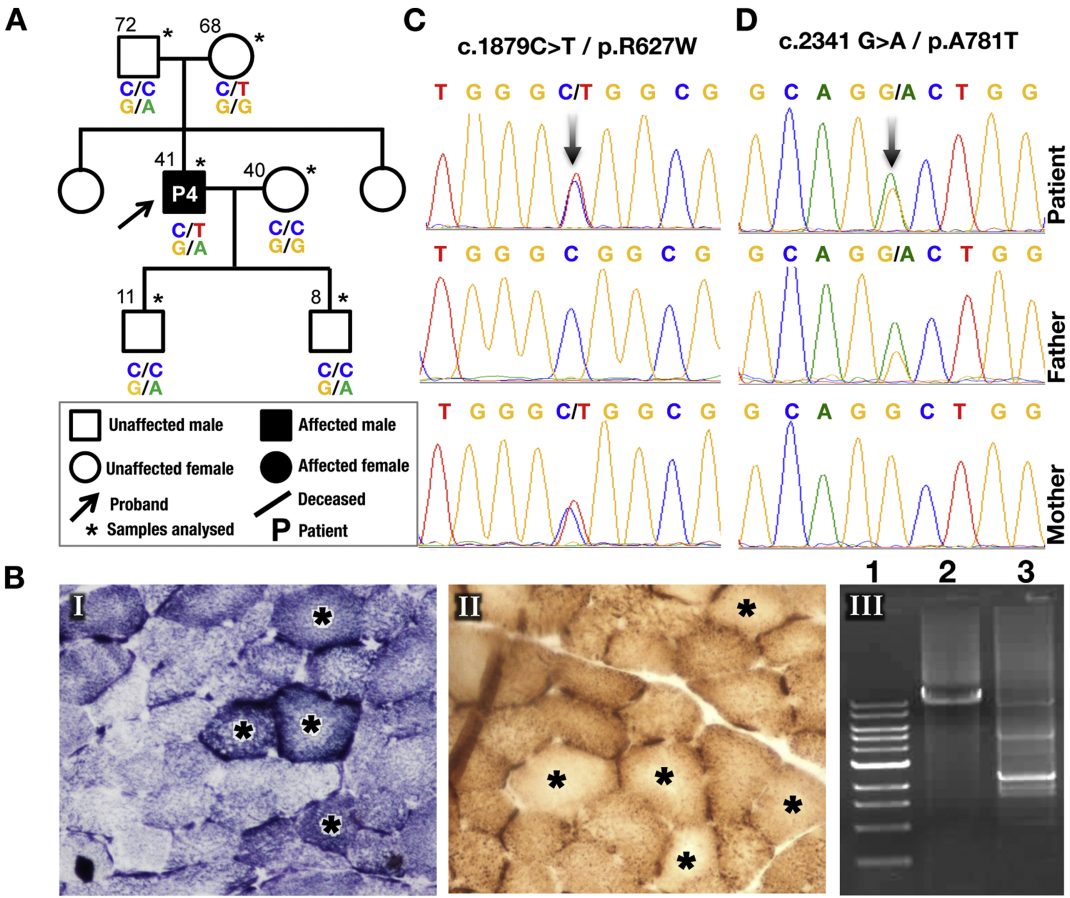
Figure 1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T G G G C/T G G C G
T G G G C G G C G
T G G G C/T G G C G
D
c.2341 G>A / p.A781T
G C A G G/A C T G G
G C A G G/A C T G G
G C A G G C T G G
Patient
Father
Mother
B
I
II
III
1
2
3

Figure 2