Original Article

The homozygous R504C mutation in *MTO1* gene is responsible for ONCE syndrome

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Conflicts of interest

The authors declare that they have no conflict of interest or financial relationship to disclose.

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Abstract

We report clinical and biochemical finding from three unrelated patients presenting ONCE (Optic Neuropathy, Cardiomyopathy and Encephalopathy with lactic acidosis and combined OXPHOS deficiency) syndrome. Whole-exome sequencing (WES) of the three patients and the healthy sister of one of them were used to identify the carry gene. Clinical and biochemical findings were used to filter variants, and molecular, *in silico* and genetic studies were performed to characterize the candidate variants. Mitochondrial DNA (mtDNA) defects involving mutations, deletions or depletion were discarded, whereas WES uncovered a double homozygous mutation in the *MTO1* gene (NM_001123226:c.1510C>T, p.R504C, and c.1669G>A, p.V557M) in two of the patients and the homozygous mutation p.R504C in the other. Therefore, our data confirm p.R504C as pathogenic mutation responsible of ONCE syndrome, and p.V557M as a rare polymorphism variant.

Key words

mitochondria; mtDNA; WES; *MTO1*; hypertrophic cardiomyopathy; encephalopathy; optic atrophy

Introduction

Mitochondrial disorders are a group of syndromes associated with severe dysfunction of oxidative phosphorylation (OXPHOS). Cardiomyocytes are one of the major targets of OXPHOS defects due to their extremely high demand of energy, and hence, infantile hypertrophic cardiomyopathy is a usual clinical feature displayed in many mitochondrial disorders. On the other hand, defects in genes involved in the mitochondrial translation machinery are increasingly recognized as a cause of OXPHOS diseases. Recently, patients exhibiting hypertrophic cardiomyopathy and lactic acidosis as a consequence of a combined respiratory chain deficiency have been found to carry mutations in *MTO1* (MIM#614667) (1, 2) and *GTPBP3* (MIM#608536) genes (3). In humans, the *MTO1* gene encodes an evolutionarily conserved protein which works together with the protein encoded by the *GTPBP3* gene to catalyze the biosynthesis of 5-taurinomethyl (τm^5U_{34}) of the wobble uridine base in mt-$tRNA^{Leu(UUR)}$, mt-$tRNA^{Trp}$, mt-$tRNA^{Gln}$, mt-$tRNA^{Glu}$, and mt-$tRNA^{Lys}$ (4). This modification is important to the accuracy and efficiency of mtDNA translation (5).

In the present study, we defined a new clinical subgroup of combined OXPHOS deficiency 10 (MIM#614702; COXPD10) with optic neuropathy and encephalopathy, that we termed ONCE syndrome (Optic Neuropathy, Cardiomyopathy, and Encephalopathy with lactic acidosis and combined OXPHOS deficiency). Through WES analysis of three unrelated patients, we identified the homozygous p.R504C variant in the *MTO1* gene, thus clarifying its contribution to ONCE syndrome.

Material and methods

Written informed consent was obtained from the patients' parents, and the Ethic Committee of the respective institutions approved the studies.

Whole-exome sequencing (WES) was performed on genomic DNA obtained from patient blood following standard protocol described before (6). Nuclear variants and indels were prioritized according to the following criteria: (i) variants that were rare in healthy individuals (allele frequency below 0.01) or new (not described within public databases); (ii) variants predicted to modify protein function (nonsense, splice site, coding indel, or missense variants); (iii) variants consistent with a recessive model of pathogenesis (including homozygous variants or two heterozygous variants present in the same gene). Additional indications to prioritize the candidate genes were obtained by using predictive software scoring the likelihood for pathogenicity SIFT, Polyphen-2, MutPred and Mutation Taster. Sanger sequencing of patients, healthy sister and their parents was performed for candidate gene mutations to confirm segregation.

Results

Cases report

Patient 1 (Pt1) is the second child of non-consanguineous, healthy parents from western Spain. His older sister is healthy. The patient was born at 42 weeks of gestation of an uneventful pregnancy and delivery. During infancy he presented with failure to thrive, developmental delay with axial hypotonia, slight ataxia of the trunk and episodes of bronchitis. At 22 months old he was found to have a hypertrophic non obstructive cardiomyopathy that was treated with verapamil. At 2 years old his developmental delay was more prominent in cognitive areas than motor milestones. Basal laboratory test results were normal except for hyperlactacidemia. Visual-evoked potentials and brain MRI (magnetic resonance images) were normal. A muscle biopsy showed a significant reduction of complex I and IV activities of mitochondrial respiratory chain (MRC) whereas histomorphology was normal. Mitochondrial DNA (mtDNA) studies showed absence of deletions and depletion, and the whole mtDNA sequencing didn't show any pathological variant (Caucasian haplogroup U5a1b). He started a treatment with coenzyme Q10 (CoQ_{10}), L-ascorbic acid (vitamin C), riboflavin (vitamin B_2) and L-carnitine. Clinical examination at the age of 3 showed little face expressivity and tendency to open his mouth and drooling. He had scapula alata and equinovarus feet, his weight was 12.6 kg (-1.6 SD), his length 90 cm (-2 SD) and his cranial circumference 51.1 cm (median). He was able to walk alone and go up and down stairs although clumsily; however, the language was delayed. Liver and kidney function test were normal. Serum creatine kinase (CK) was slightly elevated (193 IU/L, normal < 130 IU/L), with occasional levels of 367 and 404 IU/L. Lactic acid was mildly elevated (3.7 mM, normal < 2.30 mM), with a lactate/pyruvate ratio of 16 (normal < 20). Other metabolic investigations showed increased blood levels of alanine (568 μM, normal 170-430 μM). Abdominal ultrasonography showed bilateral cortical kidney hyperechogenicity, which persisted along evolution. Hearing studies were normal. Holter record was normal but heart ultrasonography showed hypertrophy of the septum and posterior wall of left ventricle with slight tricuspid insufficiency. At 6 years old he started with epileptic seizures, initially with complex partial seizures, with repetitive crisis for 2 or 3 seconds

during hours, sometimes secondary generalized. Then he was treated with lamotrigine and clonazepam. At age of 7, he presented inconstant bilateral ptosis and mental deterioration. EEG (electroencephalography) showed generalized polyspikes-waves discharges and photosensitivity. He also presented optic atrophy with reduction in visual acuity (10% of normal), that interfered in his learning abilities, especially in writing and reading. Brain MRI spectroscopy showed no abnormalities. At 11 years old, he presented moderate intellectual disability (IQ of 54), slight ataxia, dysmetria and some difficulties to stand up on one foot and stand up from the floor. A new brain MR spectroscopy didn't show abnormalities. Laboratory examinations in cerebrospinal fluid (CSF) showed a slight decreased of 5-methyltetrahydrofolate (5-MTHF 29 nmol/l, normal 35-124 nmol/l) and a slight increased of homovanillic acid (579 nM, normal 156-410 nM), while the plasma folic acid was normal. In addition to previous therapy, he started treatment with folinic acid with subsequent normalization of biochemical parameters and a slight improvement of his behavior and scholar performance but without major changes in her cognitive status.

Currently, at 16 years old, the clinical conditions remain stable. Ophthalmological examination revealed several disturbance in distance visual acuity without correction in both eyes (20/200), orthophoria in primary position, but divergent exotropia (-2º) with dominant right eye. Fundoscopy revealed papillary pallor indicating that he had bilateral papillary atrophy more prominent on right eye (Fig. 1a). Optical Coherence Tomography (OCT) showed significant thinning of macular ganglion cell layer (Fig. 1b), and thinning of macular and peripapillary retinal nerve fiber layer (RNLF) in both eyes (Fig. 1c).

Patient 2 (Pt2) is the only child of non-consanguineous parents from southeastern Spain. Her maternal grandmother is diabetic and a paternal half-brother is healthy. Pt2 was born of an uneventful pregnancy and forceps delivery. The neonatal period and early development were normal. In the scholar period she had learning difficulties and developmental delay. At 8 years old, she presented intellectual disability, clumsiness and slight fatigability. Laboratory determinations showed elevated plasma alanine and hyperlactacidemia (lactic acid levels between 8.9 and 5.7 mM, normal 0.50-2.30

mM), while lactic acid in CSF was normal. EEG was normal and brain MRI showed arachnoid cyst on the pontocerebellar right side. At 12 years old, she weighed 34 kg (-1.2 SD) and her length was 138 cm (-2.1 SD), and presented dysmetria and scoliosis. She showed bradypsychia, clumsiness and fatigability, and was unable to read and write or made simple maths calculus properly, but not showed clear ataxia, muscle weakness or pyramidal affectation. Electromyogram (EMG) showed a myopathic pattern. Cardiologic ultrasonography and heart MRI showed hypertrophic concentric non obstructive cardiomyopathy, mostly in septum and left ventricle. Systolic and diastolic functions were normal. Muscle histochemistry showed numerous ragged-red fibers (RRF), and MRC analysis evidenced a combined deficiency of complexes I, III and IV. The mtDNA molecular analysis showed neither deletions nor depletion, and whole mtDNA sequencing revealed known polymorphisms corresponding to the Caucasian haplogroup H1c without the presence of pathological variants. Ophthalmologic studies and blood testing for liver, kidney and thyroid function were normal, as well as coagulation studies and CK levels. However, she had metabolic acidosis (HCO_3^- 17 mEq/l, normal >24 mEq/l), with elevated plasma lactic acid level (6.4 mM, normal < 2.30 mM), lactate/pyruvate ratio (36, normal <20), alanine (998 μM, normal 170-430 μM) and homocysteine (23 μM, normal 4.0-12.3 μM). By contrast, the level of vitamin B_{12} was low (143 pg/ml, normal 222-753 pg/ml) while folic acid was normal (3.33 ng/ml, normal 2.61-13.6 ng/ml). Studies of CSF showed normal cytochemistry, lactic acid, aminoacids, neurotransmitter and 5-MTHF levels. She was treated by L-carnitine, CoQ_{10}, vitamin C, vitamin B_2, folic acid (vitamin B_9), thiamine (vitamin B_1), pyridoxine (vitamin B_6), and cobalamin (vitamin B_{12}). Later, the levels of vitamin B_{12} and homocysteine were normalized, but hyperlactacidemia persisted (ranging 2.4-6.8 mM). Her clinical condition also improved (she weighted 43.5 Kg (-0.9 SD) and her length was 149 cm (-1.6 SD) and cardiomyopathy was controlled (maximal left ventricle wall was 18mm, posterior and lateral, and right ventricle was less affected and systolic function was normal). She received also scholar support.

Currently, at 19 years old, she presents effort dyspnoea, frequent palpebral eye tics that worsen when she is nervous, and she is able to read and count from 1 to 20. Cardiac evaluation showed

functional limitation to exercise with early tachycardia, and cardiac ultrasonography showed hypertrophic concentric left ventricle (12 mm), while systolic and diastolic function were normal. In ophthalmological examination, she showed a distance visual acuity without correction of 20/40 and 20/30 in right and left eye respectively, and orthophoria in primary position. The fundoscopy exam showed papillary pallor in both eyes indicating the she had bilateral partial papillary atrophy. OCT showed thinning of macular ganglion cell layer and thinning of macular and peripapillary RNLF in left eye, while it was unable to measure in the right eye because palpebral tics and poor collaboration.

Patient 3 (Pt3) is the only child of healthy non-consanguineous parents from Germany. She was born at term after regular pregnancy and delivery. During the first days of life, she presented with metabolic acidosis followed by complete resolution. She could sit at 6 months and walk at 16 months, but language was slightly delayed with first words at 2 years. She attended regular school till the age of 14 years despite some learning difficulties; since 14 years she needed scholar support. A muscle biopsy performed when she was 6 months old showed respiratory chain complexes I and IV deficiency whereas histology was normal. During childhood she suffered fatigability and clumsiness without any other symptom. At 11 years, after a syncope, heart ultrasonography showed hypertrophic concentric non-obstructive cardiomyopathy, mostly affecting septum and left ventricle. Systolic and diastolic functions were normal. A treatment by beta blockers agents was started. At 14 years, she started to present short lasting focal epileptic seizures characterized by myoclonic arms movements followed by loss of contact and tonic head deviation. Seizures were initially refractory to antiepileptic treatment (levetiracetam, 2000 mg/day) with persistence of daily episodes.

Currently, at 16 years of age, seizures have a frequency of one per month under levetiracetam (2000 mg/day) and clobazam (20 mg/day). Neurological exam is normal. EEG showed a slow background activity with theta/delta waves and right frontotemporal spikes. She presents fatigability and clumsiness. She is treated by L-carnitine (2.5 g/day), idebenone (135 mg/day), nadolol (60 mg/day), levetiracetam (2000 mg/day) and clobazam (20 mg/day).

Brain MRI showed a bilateral signal hyperintensity involving dentate nuclei and a pre-central cortex. Ophthalmological exam detected a slight bilateral optic atrophy with normal right and 8/10 left visual acuity. Fundoscopy revealed temporal papillary pallor predominating on left eye, and OCT showed thinning of peripapillary RNLF in both eyes. Laboratory examinations showed hyperlactacidemia (lactate 7.8 mM, normal 1.0-1.5 mM), and elevated plasma alanine (822 μM, normal 221-481 μM).

Genetic analysis

The aforementioned analytic pipeline of WES data, enabling us to prioritize variants in genes that were rare and were predicted to be deleterious. We confirmed the presence of two homozygous missense mutations in Pt1 and Pt2: p.R504C (NM_001123226:c.1510C>T) and p.V557M (c.1669G>A,) in the *MTO1* (Mitochondrial tRNA translation optimization 1) gene. The mutations segregate with the disease in both families through a recessive inheritance pattern. The parents of both families are heterozygous for the mutations and the healthy sister of the family 1 is wild type (Fig. 2a). In order to discard a common ancestor between these two families, we studied the rare variants (allele frequency below 0.01 or new) identified by WES in chromosome 6. Of around 500 variants, only 5 were present in both patients: two homozygous in *MTO1* gene and three heterozygous in different genes. These data make it unlikely that both families have a recent common ancestor. In Pt3 was only detected the homozygous p.R504C variation in *MTO1* gene. The mother is heterozygous for this variant but DNA from the father is not available (Fig. 2a). A heterozygous *MTO1* deletion affecting the other allele could be excluded by NGS. The p.R504C mutation was ascribed as "likely pathogenic" by using well established *in-silico* tools of pathogenesis prediction (SIFT, PolyPhen2, MutPrep and Mutation Taster) (Table 1). It is located in a conserved region of *MTO1* gene (Fig. 2b) and the aminoacid residue is conserved from *Homo sapiens* to *Saccharomyces cerevisiae* (Fig. 2c). However, the p.V557M variation was ascribed by *in silico* prediction as "polymorphism", and the aminoacid is not conserved.

Discussion

We describe three adolescent patients from three unrelated families (Table 2) presenting with defects of MRC activities, encephalopathy (intellectual disability), hypertrophic cardiomyopathy diagnosed during later infancy or childhood, lactic acidosis, and optic nerve atrophy developed in adolescence, suggesting a mitochondrial disease (Table 2). In addition, two of them had epilepsy. However, molecular mtDNA analysis in the patients ruled out depletion, rearrangements or known or putative pathogenic mutations. Using WES, we identified two homozygous mutations in the nuclear-encoded gene *MTO1* (mitochondrial tRNA translation optimization 1) (Table 1) that are located inside of GidAad3 domain of the protein (Fig. 2b). GidA is the eubacterial ortholog of MTO1, and the GidA associated domain 3 (GidAad3) has been suggested to be implicated in binding of FAD (Flavin Adenine Dinucleotide) and the D-stem of tRNA (7), and to be responsible for the interaction with GTPBP3 protein (8). The first missense mutation (R504C), founded in the three patients, changes a conserved arginine to cysteine (Fig. 2), and it is not described in any genomic database (2016-5): 1000G, EVS and ExAC Browser. The R504C mutation is described as "likely pathogenic" by *in-silico* analysis software (SIFT, PolyPhen2, MutPrep and Mutation Taster) (Table 1), and the aminoacid is evolutionarily conserved among species (Fig. 2c). Furthermore, we identified the R504 of *Homo sapiens MTO1* as equivalent to R434 of *Aquifex aeolicus* GidA, which is located in FAD binding site (N1 atom of R434 binds to the flavin N3 and O4 atoms by hydrogen bond) (7). The second variant (V577M), that was only founded in Pt1 and Pt2, is described in genomic databases (rs139608228) as very rare variant (allele frequency of 0.001504) and as "polymorphism" by *in-silico* analysis (Table 1). Furthermore, the aminoacid V577 is not conserved among species (Fig. 2c).

The mutation R504C has been recently reported (9) in two siblings who presented a multisystemic disorder associating optic atrophy, cardiomyopathy, cognitive disability and epileptic seizures. These patients also carried a homoplasmic mutation (m.593T>G) in the mitochondrial $tRNA^{Phe}$ that could act synergistically to worsen the disease (9). The phenotype of these two siblings is almost identical to the shown by our three patients (cardiomyopathy, lactic acidosis, encephalopathy,

defects of MRC activities, optic atrophy and epilepsy), but the mtDNA sequence in Pt1 and Pt2 excluded the presence of any mtDNA pathological mutation. Therefore, it is unlikely that the mutation in the mitochondrial $tRNA^{Phe}$ (that is not present in our patients) or the variant V557M in *MTO1* gene (that is not present in the two siblings and Pt3) may contribute to the core clinical phenotype. As the two siblings (9) and our three patients developed optic nerve atrophy during adolescence or adulthood, this phenotype could considered as characteristic of this mutation (R504C), in contrast to epilepsy, since only one of the two siblings and Pt1 and Pt3 have epilepsy. Accordingly, the core phenotype associated to R504C mutation in *MTO1* gene is cardiomyopathy, lactic acidosis, encephalopathy with optic neuropathy and combined OXPHOS deficiency, being secondary phenotype epilepsy. Based on the association of the clinical features and biochemical abnormalities of these five patients, we suggest that they form a new clinical subgroup of combined OXPHOS deficiency 10 (MIM#614702; COXPD10, also "cardiomyopathy, infantile hypertrophic mitochondrial, and lactic acidosis"), with optic neuropathy and encephalopathy, or ONCE syndrome (acronym for Optic Neuropathy, Cardiomyopathy, and Encephalopathy with lactic acidosis and combined OXPHOS deficiency). And the R504C mutation in *MTO1* gene is the responsible for this syndrome.

So far, only 11 mutations, 9 missense and 2 frameshift, in the *MTO1* gene have been described in 16 patients (Table 3). Cardiomyopathy were diagnosed in 14 patients, from which 7 died in neonatal or infancy period and 7 patients have survived until adolescence or adulthood. The phenotype and severity of the disease are partially correlated with the onset and clinical presentation. Thus, patients with neonatal-onset deceased during the first year of live, while patients with onset in infancy were able to survive to the adolescence and adulthood. Others phenotypes such as intellectual disability, epileptic seizures and optic nerve atrophy seen to be associated with long term evolution.

The patients Pt1 and Pt2 had other metabolic deficiencies that have not been reported before in patients with *MTO1* gene mutations. Thus, Pt1 had cerebral folate deficiency without abnormalities in brain MRI, unlike it has been demonstrated in other mitochondrial syndromes (10). Supplementation with folinic acid normalized the biochemical parameters in CSF, as described in cerebral folate

deficiency syndrome (11), but without major changes in her cognitive status. On the other hand, Pt2 showed hyperhomocysteinemia due to vitamin B_{12} deficiency, which were normalized after vitamin B_{12} treatment. Cerebral folate deficiency or Vitamin B_{12} chronic deficiency could cause several neurological problems and mental deterioration that could contribute to worsen the phenotype. Thus, CSF folate levels and vitamin B_{12} levels should be investigated in patients with *MTO1* mutations in order to define the phenotypic spectrum.

Web resources

The URLs for the data presented herein are as follows:

OMIM, http://www.omim.org/
SIFT, http://sift.jcvi.org/
Polyphen-2, http://genetics.bwh.harvard.edu/pph2/
MutPred, http://mutpred.mutdb.org/
Mutation Taster, http://www.mutationtaster.org/
1000G, *http://www.1000genomes.org/*
EVS, http://evs.gs.washington.edu/EVS/
ExAC Browser, http://exac.broadinstitute.org

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Figure legend

Fig. 1. Ophthalmological finding using 3D-OCT (Topcon model 2000). **(a)** The temporal papillary pallor in both eyes of Pt1 is shown. **(b)** Macular ganglion cell complex analysis by OCT scan in both eyes of Pt1 is shown. All retinal fovea sublayer (retinal nerve fiber layer (RNFL), ganglion cell layer (GCL)) showed significant thinning. The average thickness is compared to the normative data and displayed according to color. **(c)** The thinning of peripapillary RNFL in both eyes of Pt1 is shown.

Fig. 2. Genetic and functional analysis. **(a)** Family pedigrees showing segregation of R504C (C/T) and V557M (G/A) mutations in *MTO1*. **(b)** Schematic representation of human MTO1 showing the position of R504C (red) and V557M (green) mutations, and the GidAad3 domain. **(c)** Multiple sequence alignment of MTO1 protein region surrounding the R504 (red) and V557 (green) in various species is also shown.

Table 1
WES candidate genes

Candidate genes after filtering and prioritization

Chr	Position[a]	SNP	Ref	Status	Gene	Codon	Change	MAF[b]
6	74191892	New	C	Hom[c]	***MTO1***[d]	CGC=>TGC	R504C	
6	74192255	rs139608228	G	Hom	***MTO1***	GTG=>ATG	V557M	0.0015

"In silico" analysis

Gene	Change	SIFT	PolyPhen-2	Mutation Taster	MutPred
MTO1[c]	R504C	Damaging	Probably damaging	Disease causing	Disease-associated
MTO1	V557M	Tolerated	Benign	Polymorphism	Neutral

[a]GRCh37.p13; [b]MAF: Minor Allele Frequency from ExAC browser (2016-5-1); [c]Hom: Homozygous
[d]RefSeq NM_001123226.1

Table 2
Patients with mutations in *MTO1* gene

Characteristics	Pt1	Pt2	Pt3
Sex	Male	Female	Female
Age	16y	19y	16y
Onset	Infancy	Childhood	Childhood
Consanguinity	No	No	No
Cardiomyopathy	22m	12y	11y
Optic Neuropathy	7y	19y	16y
Encephalopathy	2y	8y	2y
Lactic acidosis	yes	yes	yes
OXPHOS deficiency	2y (I+IV)	12y (I+III+IV)	14y (I+IV)
Epilepsy	6y	No	14y
EEG	6y: a	8y: n	16y: a
Brain MRI	2y: n, 11y: n	8y: a[1]	16y: a[2]
Folic acid deficiency	11y	No	No
Vitamin B_{12} deficiency	No	12y	No
EMG	nd	12y: mp	nd
Genetic study	R504C[3]+V557M[3]	R504C[3]+V557M[3]	R504C[3]

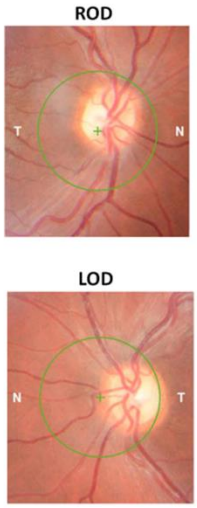
y: year; m: month; nd: not determined; n: normal; a: altered; mp: myopathic pattern
EEG: electroencephalography; MRI: magnetic resonance images; EMG: electromyography
[1]Arachnoid cyst on the pontocerebellar right side
[2]Bilateral hyperintensity involving dentate nuclei and pre-central cortex
[3]Homozygous

Table 3
Selected *MTO1*[a] Pathogenic Variants

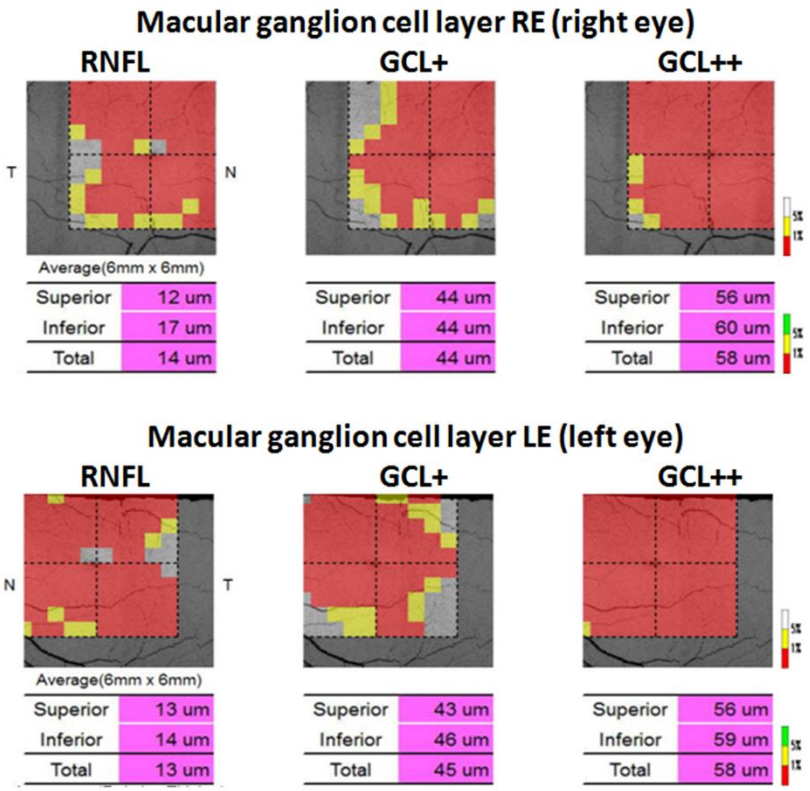
Amino Acid Change	Mutation type	Reference
p.R660Lfs*8	Frameshift (NMD[b])	Ghezzi *et al* [2012]
p.A468T	Missense	
p.G59A	Missense	Vasta *et al* [2012]
p.T308A	Missense	
p.R517H	Missense	Baruffini *et al* [2013]
p.T411I	Missense	
p.G211Dfs*3	Frameshift (NMD)	Taylor *et al* [2014]
p.V41G	Missense	
p.H256R	Missense	
p.I408F	Missense	Tischner *et al* [2015]
p.R504C	Missense	Charif *et al* [2015]

[a]RefSeq NM_001123226.1
[b]NMD, nonsense-mediated mRNA decay

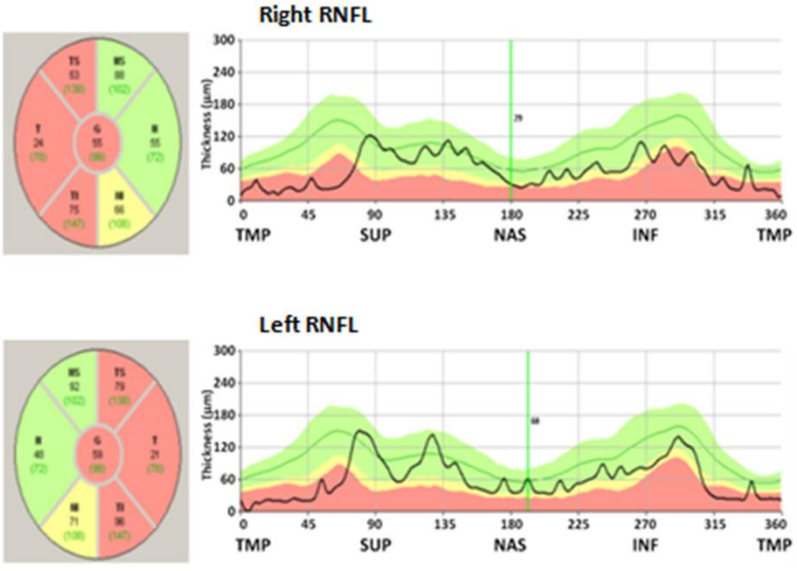
(a)


ROD
T
N
LOD
N
T

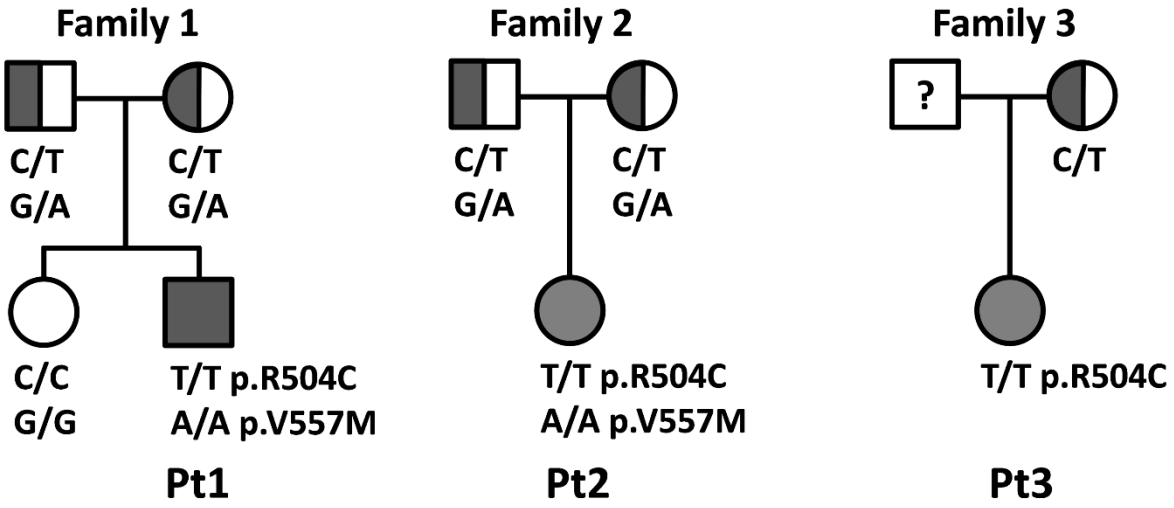
(b)


Macular ganglion cell layer RE (right eye)
RNFL
GCL+
GCL++
T
N
Average(6mm x 6mm)
Superior 12 um
Inferior 17 um
Total 14 um
Superior 44 um
Inferior 44 um
Total 44 um
Superior 56 um
Inferior 60 um
Total 58 um
Macular ganglion cell layer LE (left eye)
RNFL
GCL+
GCL++
N
T
Average(6mm x 6mm)
Superior 13 um
Inferior 14 um
Total 13 um
Superior 43 um
Inferior 46 um
Total 45 um
Superior 56 um
Inferior 59 um
Total 58 um

(c)


Right RNFL
Thickness (µm)
300
240
180
120
60
0
0 45 90 135 180 225 270 315 360
TMP SUP NAS INF TMP
Left RNFL
Thickness (µm)
300
240
180
120
60
0
0 45 90 135 180 225 270 315 360
TMP SUP NAS INF TMP

(a)



(b)

(c)

H.sapiens	LTTLGTSEPY**R**MFTSRVEFRL	MKSSLEEGIS**V**LKSIEFLSSK
P.troglodytes	LTTLGTSEPY**R**MFTSRVEFRL	MKSSLEEGIS**V**LKSIEFLSSK
C.lupus	LTTLGTNEPY**R**MFTSRAEFRL	MKSSLEEGIS**V**LKSIEFSSSK
B.taurus	LTTLGTNEPY**R**MFTSRAEFRL	MKSSLEEGIS**M**LKSIEFLSTK
M.musculus	LTTLGTSEPY**R**MFTSRVEFRL	MKSSLEEGIS**V**LKSIKFSSSK
R.norvegicus	LTTLGTSEPY**R**MFTSRVEFRL	MKSSLEEGIS**V**LKSIKFSSSK
G.gallus	LTTLGTTEPY**R**MFTSRVEFRM	MRAALEDGIA**T**LKSLQFSISK
D.rerio	LVCHGVTEPY**R**MFTSRAEFRT	VQASLKQALS**T**LQSIHLSAAR
D.melanogaster	LTSLGTNEPY**R**MFTSRAEFRL	TEARLQSAIE**S**LRRLRKHTHY
C.elegans	LTSLGTNEPY**R**MLTSRAEFRL	TKGELNNLTQ**R**TEEMKMSMVK
S.cerevisiae	LINNGVIEPY**R**MFTSRSEFRI	DKHLYDETIR**A**LQNFKLSSQK