Case Report

Clinical and Genetic Analysis of A Father-Son Duo with Monomelic Amyotrophy: Case Report

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Abstract

Monomelic Amyotrophy (MMA) is a rare neurological disorder restricted to one upper limb, predominantly affecting young males with an unknown aetiopathogenesis. We report a familial case of father-son duo affected by MMA. Whole exome sequencing identified genetic variations in *SLIT1, RYR3* and *ARPP21* involved in axon guidance, calcium homeostasis and regulation of calmodulin signaling respectively. This is the first attempt to define genetic modifiers associated with MMA from India and advocates to extend genetic screening to a larger cohort. Deciphering the functional consequences of variations in these genes will be crucial for unravelling the pathogenesis of MMA.

Keywords: ARPP21, familial monomelic amyotrophy, RYR3, SLIT1, whole exome sequencing

Introduction

Monomelic amyotrophy (MMA), also known as Hirayama disease, is characterized by atrophy of muscles in distal extremities followed by spontaneous arrest over a few years, predominantly affecting muscles innervated by seventh and eighth cervical segments.[1,2] There is no involvement of lower limbs and bulbar muscles, and deep tendon reflexes are sluggish or absent. The disorder usually develops in the second or early third decade with male preponderance[3,4] with a few exceptions of late onset of the disease.[5,6] Majority of MMA cases are sporadic, but rare familial occurrences have also been reported.[3,6-12] Usually progression occurs for 2 to 5 years followed by a stationary course. Though the disease is not fatal, early onset severely affects the progressive years of adolescence and young adulthood. Due to rarity of this disease, very few studies have described genetic association with MMA.[6,13] Here, we report a familial MMA case of affected father and son sharing deleterious genetic variations in SLIT1, RYR3 and ARPP21.

Methods

Patient enrolment

Two patients with MMA from one family from North India were diagnosed and clinically evaluated at Sir Ganga Ram Hospital, New Delhi, India. Informed written consent was taken from patients before withdrawal of blood samples. Control samples (n = 40) with the same ethnic background were also included.

Clinical workup comprised of i) detailed neurological examination; ii) motor and sensory nerve conduction in upper and lower limbs; iii) electromyography (EMG) of the affected upper limb, contralateral upper limb and both lower limbs; iv) magnetic resonance imaging (MRI) with flexion and extension of neck with gadolinium enhancement; and v) routine biochemical tests, ganglioside antibodies.

Whole-exome sequencing analysis

Whole-exome sequencing (WES) was done using Twist Comprehensive Exome Panel (Twist Biosciences) and Illumina NextSeq 550 Platform. Potential damaging variants were identified by filtering based on: 1) minor allele frequency below 1% in 1000 Genome and Exome aggregation consortium (ExAC); 2) non-synonymous, frame-shift deletion, insertion and stop gain variants with a quality score of ≥ 25 and a Combined Annotation Dependent Depletion (CADD) phred score of ≥ 20; 3) variants expected to be deleterious in PolyPhen, SIFT and Mutation Taster.

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Furthermore, pathogenicity was assigned in accordance with recommendations from the American College of Medical Genetics and Genomics (ACMG). Potential damaging variants were validated by Sanger sequencing [Supp Figure 1].

Gene tolerance assessment

Identified genes were assessed for intolerance using pLI, missense z-conservation (http://exac.broadinstitute.org/) and Residual Variation Intolerance Score (RVIS).[14-16] Genes with pLI scores ≥0.9, deviation of the observed number of missense variants from the expected number with z-scores greater than zero and bottom 25th percentile computed by RVIS indicated intolerance to genetic variations.

Results

Clinical description of patients

Case 1: A 55-year-old male presented on 15 July 2015 to Sir Ganga Ram Hospital, New Delhi with a complaint of weakness of the left upper limb of 45 years duration. Diagnosis of MMA was considered in the year 1972 at the age of 12 years with complaints of progressive weakness and twitching of muscles of the left upper limb of 2 years duration. He had come for revaluation. On neurological examination, there was atrophy and weakness of the thenar, hypothenar, interossei, forearm flexor and extensor group of muscles of both upper limbs with the changes more marked on the left upper limb [Figure 1-1A, 1B]. Nerve conduction studies showed asymmetrical large fibre motor axonal neuropathy in both upper limbs with no evidence of conduction block. Sensory conduction studies were normal. Electromyography (EMG) did not show evidence of active denervation in the first dorsal interosseous, biceps and brachioradialis muscles of both upper limbs; however, chronic denervation with reinnervation in these muscles was present. Thus, C5 to T1 innervated muscles in both upper limbs were affected. Lower limbs did not show any abnormality.

The MRI of the cervical spinal cord showed straightening of cervical spine, asymmetric cord atrophy, left more than right, from C3 to C7 level and most prominent at C5 to C6 levels, and intramedullary linear high signal intensity from C3 to C7, [Figure 1-C]. 'Snake-eye appearance' was seen corresponding to ventral horn cell region at C6–C7 level in axial T2-weighted image [Figure 1 and 1D]. On dynamic cervical spine MRI, anterior shifting of the posterior cervical dura on flexion and epidural flow voids were not observed. Biochemical tests were normal, and ganglioside antibodies were not present.

Case 2: This 28-year-old male, son of case 1, was seen on 15 July 2015 with complaints of atrophy and weakness of right hand and forearm of 7 years duration. For the initial 3 years, there was a progression of symptoms followed by a stationary phase. He noted fasciculations in the right forearm and a feeling of coldness and excessive sweating of the right hand. There was no involvement of either the left upper limb or both lower limbs. On neurological examination, atrophy and weakness of thenar, hypothenar, interossei and muscles of medial compartment and extensor muscles of

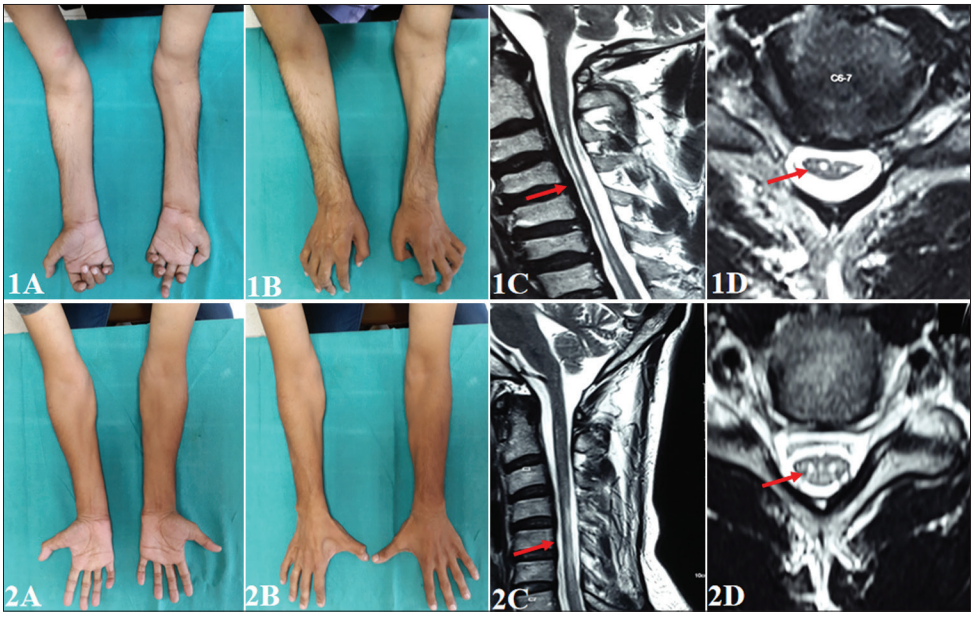

Figure 1: Case 1. 1A and 1B: Atrophy of thenar, hypothenar, interossei, forearm flexor and extensor group of muscles of both upper limbs, more marked changes on the left upper limb. MRI of cervical spine. 1C: Asymmetric cord atrophy from C3 to C7 level, left more than right, and most prominent at C5 and C6 levels. Intramedullary linear high signal intensity from C3 to C7 levels. 1D: 'Snake-eye appearance' corresponding to ventral horn cell region at C6-C7 levels. Case 2. 2A and 2B: Atrophy of thenar, hypothenar, interossei and muscles of the medial compartment and extensors of the forearm with sparing of brachioradialis of the right upper limb. MRI of the cervical spine. 2C: Straightening of cervical spine, focal cord atrophy at C5–C6 level on the right side and intramedullary hyperintensity from C4 to C7 level. 2D: 'Snake-eye appearance' corresponding to ventral horn cell region at C6

forearm with sparing of brachioradialis of right upper limb were observed [Figure 1-2A, 2B]. Fasciculations were seen in forearm muscles. Tendon reflexes were sluggish in the right upper limb and normal in other limbs and plantars were flexors. Nerve conduction study showed large fibre motor axonal neuropathy in the right upper limb with no evidence of conduction block. Sensory conduction studies were normal. EMG showed active denervation (fibrillations and positive sharp waves) in the right first dorsal interosseous. Chronic denervation with reinnervation changes were noted in the triceps and first dorsal interosseous muscles of the right upper limb. Mild chronic reinnervation was also observed in the left first dorsal interosseous muscle. MRI of the cervical spinal cord showed straightening of cervical spine, focal cord atrophy at C5–C6 level on the right side and intramedullary hyperintensity from C4 to C7 level [Figure 1 and 2C] and 'snake-eye appearance' corresponding to ventral horn cell region at C6 level in axial T2- weighted image [Figure 1 and 2D]. On dynamic cervical spine MRI in flexion, forward displacement of posterior dural wall, epidural flow voids or enhancing epidural mass were not present. Biochemical tests were normal, and ganglioside antibodies were not present.

Genetic analysis of father–son duo

Exome sequencing of the father–son duo combinedly identified a total of 49,116 variations. Variant filtering prioritized 1,917 variations based on the genotype quality score of ≥25 and minor allele frequency of ≤0.01. After filtering out synonymous variations, 1,397 variations (non-synonymous, frameshift deletion and insertion) were assessed for deleteriousness by CADD (≥20), SIFT, PolyPhen and MutationTaster scores which narrowed down to 173 variations, with 41 shared rare and damaging variations [Supplemetary Table] between father and son. Interestingly, among these shared variations, two-point variations in SLIT1 and RYR3 qualified as likely pathogenic whereas variations in 28 other genes were classified as variants of uncertain significance (VUS) (https://franklin.genoox.com/clinical-db/home) [Table 1]. The likely pathogenic variations included a novel non-synonymous heterozygous variation (c. 2276T > A; p.Ile759Asn) in exon 21 of SLIT1 gene

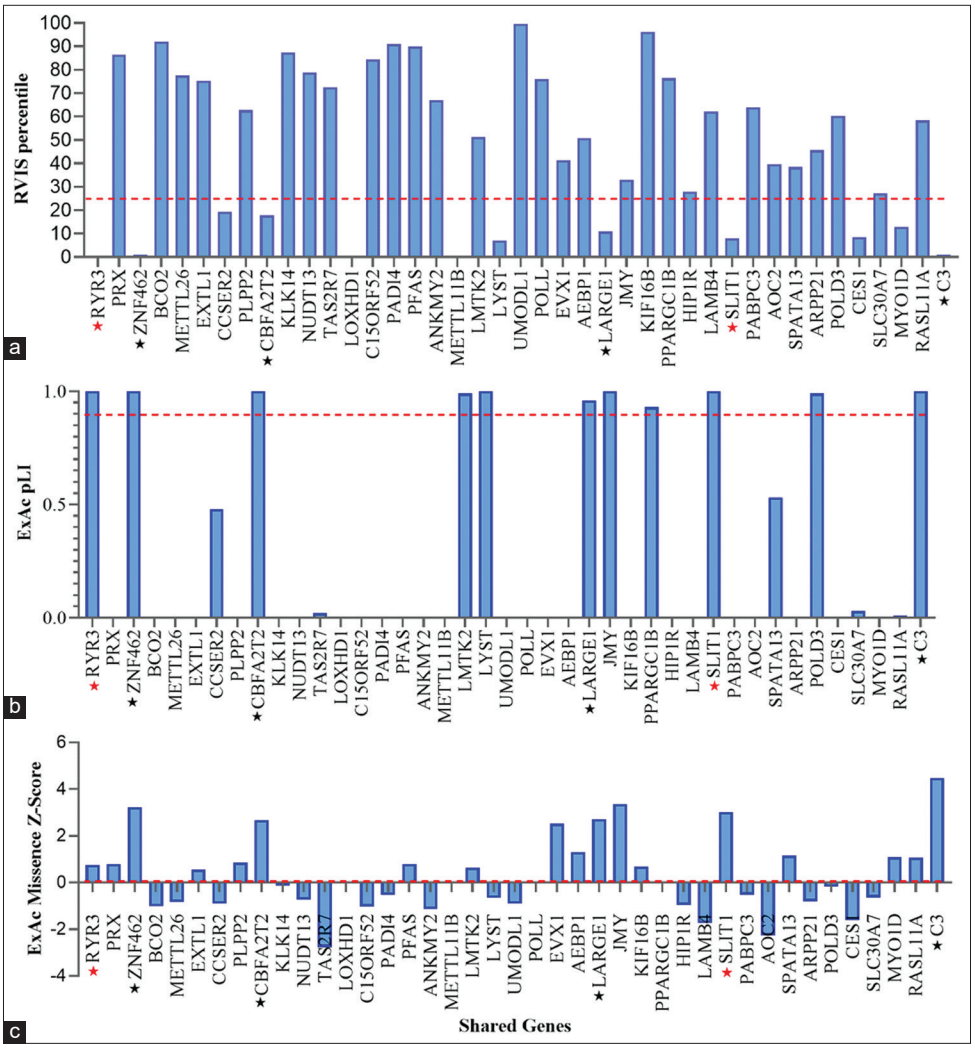

Figure 2: Mutation intolerance scores computed by a) RVIS (Bottom 25th percentile), b) pLI (≥0.9) and c) missense z-score (>0). The recommended cutoff beyond which variations are expected to be deleterious is indicated by the red dotted line. Genes intolerant to these scores are indicated by red and black asterisks

Table 1: Deleterious variants identified in father–son duo

Gene	Putative biological function	Variant	1000 G and ExAC Frequency	dbSNP ID	SIFT SCORE (<0.05 is pathogenic)	POLYPHEN SCORE (>0.85 is pathogenic)	MUTATION TASTER SCORE (value near to 1 is more deleterious)	ACMG Classification
SLIT1 (Slit guidance ligand 1)	Molecular guidance cue in cellular migration, interacts with roundabout homologue receptors.	c.T2276A p.I759N	-	--	D (0)	D (0.935)	D (1)	Likely Pathogenic
RYR3 (Ryanodine receptor 3)	Calcium channel that mediates the release of Ca2+ from sarcoplasmic reticulum into the cytoplasm in muscle and thereby plays a role in triggering muscle contraction.	c.G4505T p.R1502L	0.0012 0.0022	rs545597409	D (0.001)	D (0.999)	D (1)	Likely Pathogenic
ARPP21 (cAMP-regulated phosphoprotein 21)	Regulator of calmodulin signalling	c.G2163T p.Q721H	--	--	D (0.017)	D (0.998)	D (0.986)	VUS

and a non-synonymous heterozygous variation (c. 4505G > T; p.Arg1502Leu) in exon 34 of RYR3 gene. These two genes appeared to be intolerant based on loss-of-function as computed by RVIS, pLI and missense z-scores (Figure 2, marked with a red asterisk). These variations in SLIT1 and RYR3 were not present in healthy controls. In addition, LARGE1, C3, ZNF462 and CBFA2T2 with VUS were also intolerant (Figure 2, marked with a black asterisk). A novel VUS was identified in ARPP21, a gene recently shown to be associated with amyotrophic lateral sclerosis (ALS) [Supplementary Table]. However, it appears to be tolerant based on the constraint metrics [Figure 2]. Previously reported variations in KIAA1377 and C5orf42 genes were not found in the affected patients.[13]

Discussion

Familial cases of MMA are extremely rare. Two prior reports have documented a 54-year-old lady with Brachial Monomelic Amyotrophy (BMMA) of the right upper limb and her son with BMMA of the left upper limb; two siblings with classical manifestations of MMA of the upper limb.[7,8] Reports of a 53-year-old man and his 18-year-old son suffering from benign focal muscular atrophy of upper extremities from Germany and two siblings with proximal muscle weakness restricted to one arm from Turkey also document the familial cases.[10,11] Only one study has reported genetic association with familial MMA where a heteroplasmic mutation, the 7472insC in the $tRNA^{Ser\ (UCN)}$ gene was observed in an Italian patient with adult-onset MMA and his maternal relatives.[6] In this case report, we have identified deleterious variations in SLIT1, RYR3 and ARPP21 in the affected father–son duo. The novel mutation identified in this familial MMA case lies in the N-terminal LRR (LRRNT4) domain of SLIT1 which is highly conserved and flanks Leucine-rich repeat (LRR) region. The LRR motifs in various proteins play an important role in the myelination of axons, axon guidance, synapse formation and stabilization of neuronal circuits. SLIT1 expression is specific to the brain and nervous system.[17] The SLIT/ROBO signalling pathway is crucial for muscle-cell formation, neuronal axon guidance and migration in the nervous system.[18,19] Further, SLIT guidance molecules from floor plates regulate positioning of cranial motor neurons and direct their axons out to muscle targets.[20-22] Dysfunction of Slit/Robo leads to ectopic migration of motor neurons which causes cell death or loss of motor neuron features.[23] RYR3 is a ryanodine receptor, which releases calcium from the endoplasmic reticulum in neurons. Mutations of RYR3 may disrupt intracellular calcium homeostasis, impairing neuronal function.[24] Thus, it is plausible that mutations in SLIT1 and RYR3 may contribute to pathogenesis of MMA and hence need to be evaluated in a larger subset of patients. It is important to mention that mutations in SLIT1 have been reported in patients affected with neuroblastoma, acquired aplastic anaemia, supernormal coronary arteries and attention-deficit hyperactivity disorder,[25-28] and mutations in RYR3 have been implicated in nemaline myopathy, Alzheimer's disease, gender dysphoria and autism spectrum disorders.[24,29-31] Another identified deleterious variant worth mentioning is ARPP21, which has been shown to be associated with ALS in Europe and the United Kingdom,[32-34] but did not appear to be a risk factor in patients from Australia and mainland China[35,36] and hence may be investigated in MMA as well as ALS patients from different ethnic groups. Further studies are required to elucidate the functional impact of SLIT1, RYR3 and ARPP21 in MMA.

Abbreviations

MMA: Monomelic Amyotrophy; SLIT1: Slit guidance ligand 1; RYR3: Ryanodine receptor 3; ARPP21: cAMP-regulated phosphoprotein 21; RVIS: Residual Variation Intolerance

Score; ACMG: American College of Medical Genetics and Genomics; ALS: Amyotrophic lateral sclerosis; MRI: Magnetic Resonance Imaging.

Availability of data and materials

Genomic dataset generated during the current study is available in the ClinVar repository (https://www.ncbi.nlm.nih.gov/clinvar/) (accession number SCV003920657.1).

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Conflicts of interest

There are no conflicts of interest.

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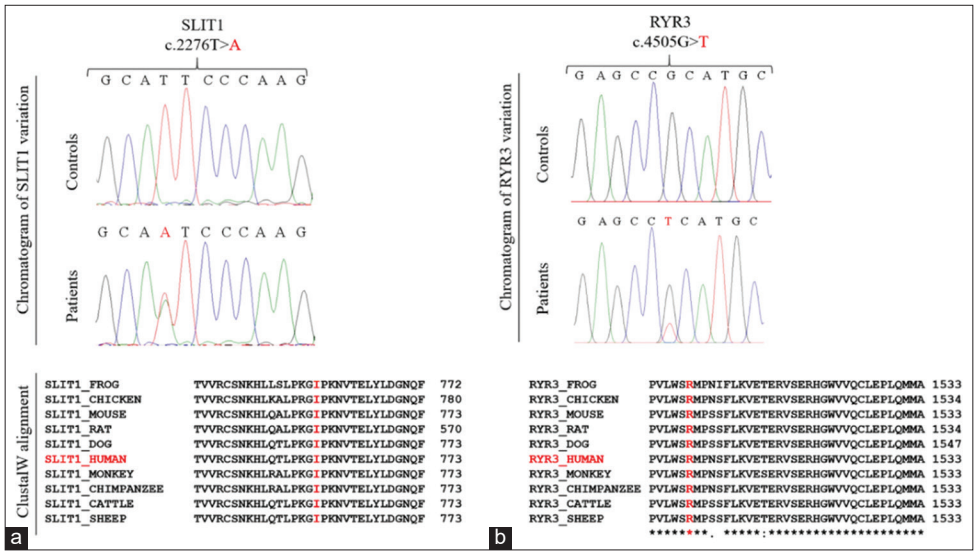

Supp Figure 1: Validation of wild type and mutant a) SLIT1 and b) RYR3 by Sanger sequencing in controls and patients. Sequence alignment showing conservation of mutated amino acid sequence among various species

Supplementary Table: 41 shared rare and damaging variations between father and son.

Gene	RefSeq	Variant	Chromosome and Exon No.	Allele Frequency (1000G)	Allele Frequency (ExAc)	dbSNP ID	SIFT SCORE	POLYPHEN SCORE	MUTATION TASTER SCORE	ACMG classification
SLIT1	NM_003061	c.T2276A:p.I759N	Chr 10 Exon 21	.	.	.	0	0.935	1	Likely Pathogenic
RYR3	NM_001036	c.G4505T:R1502L	Chr 15 Exon 34	0.0012	0.0022	rs545597409	0.001	0.999	1	Likely Pathogenic
EXTL1	NM_004455	c.A1477C:p.I493L	Chr 1 Exon 8	0.0014	0.0014	rs569774724	0.008	0.966	0.533	VUS
CCSER2	NM_001284242	c.C65T:p.P22L	Chr 10 Exon 2	0.0082	0.0039	rs368729802	0.028	0.649	1	VUS
PLPP2	NM_003712	c.C253T:p.R85C	Chr 19 Exon 3	0.0032	0.0055	rs61745392	0.01	0.998	1	VUS
KLK14	NM_001311182	c.A656G:p.D219G	Chr 19 Exon 7	0.005	0.0052	rs201296079	0	1	0.995	NA
NUDT13	NM_001283014	c.T71C:p.V24A	Chr 10 Exon 2	0.0016	0.0038	rs143864075	0.006	0.997	0.989	VUS
LOXHD1	NM_001308013	c.A1265G:p.Y422C	Chr 18 Exon 12	0.0028	0.0052	rs201536647	0	1	1	Benign
PFAS	NM_012393	c.C1754G:p.P585R	Chr 17 Exon 15	0.0012	0.0006	rs144294972	0.008	1	1	VUS
ANKMY2	NM_020319	c.C112T:p.R38C	Chr 7 Exon 2	0.0036	0.0031	rs199676294	0.019	0.992	0.989	Benign
LYST	NM_000081	c.G8050A:p.E2684K	Chr 1 Exon 30	0.0002	0.0003	rs185605358	0.031	0.931	0.999	VUS
EVX1	NM_001304519	c.C554T:p.A185V	Chr 7 Exon 3	0.0006	0.0012	rs541750417	0.008	0.745	0.986	VUS
AEBP1	NM_001129	c.G2234A:p.R745Q	Chr 7 Exon 18	0.001	0.0003	rs138705367	0.018	0.993	0.707	Likely Benign
KIF16B	NM_001199865	c.G3175A:p.A1059T	Chr 20 Exon 19	0.0008	0.001	rs117138500	0.005	0.726	0.794	VUS
MYO1D	NM_001303279	c.C1751A:p.P584Q	Chr 17 Exon 15	.	.	.	0	1	1	VUS (Pathogenic moderate)
PPARGC1B	NM_001172698	c.C2164T:p.R722C	Chr 5 Exon 7	0.0036	0.0013	rs146710258	0	1	0.997	VUS
LAMB4	NM_001318046	c.C1843A:p.P615T	Chr 7 Exon 15	0.0002	0.0003	rs201909531	0.002	0.972	1	VUS
PRX	NM_181882	c.C493T:p.R165C	Chr 19 Exon 7	0.0014	0.0014	rs555499679	0.045	1	0.996	Benign
ZNF462	NM_001347997	c.C2428G:p.H810D	Chr 9 Exon 3	0.0002	0.0002	rs564684817	0.002	0.599	0.688	VUS
POLD3	NM_006591	c.G207T:p.Q69H	Chr 11 Exon 3	.	.	.	0.014	0.996	0.686	VUS
BCO2	NM_001256398	c.G1166A:p.G389D	Chr 11 Exon 8	0.0006	0.0026	rs139612323	0.002	0.997	1	VUS (Pathogenic moderate)
METTL26	NM_001040160	c.C244T:p.P82S	Chr 16 Exon 2	0.0006	0.0031	rs530101742	0.016	0.975	0.991	VUS
ARPP21	NM_001267617	c.G2163T:p.Q721H	Chr 3 Exon 19	.	.	.	0.017	0.998	0.986	VUS
CES1	NM_001025194	c.A611G:p.N204S	Chr 16 Exon 5	.	.	.	0	0.999	1	VUS
CBFA2T2	NM_001032999	c.A86G:p.K29R	Chr 20 Exon 2	0.0006	0.0004	rs562416059	0.002	0.997	1	VUS
TAS2R7	NM_023919	c.T359A:p.L120H	Chr 12 Exon 1	0.0034	0.0016	rs202246571	0	1	0.508	VUS
C15orf52	NM_207380	c.C724T:p.R242W	Chr 15 Exon 6	0.0004	0.0003	rs367889605	0	1	0.98	NA
PADI4	NM_012387	c.A926G:p.Y309C	Chr 1 Exon 8	0.0052	0.0084	rs33981382	0.001	0.997	0.929	Benign
LMTK2	NM_014916	c.G178A:p.V60M	Chr 7 Exon 2	0.0008	0.0002	rs562493853	0.015	0.771	0.999	VUS
METTL11B	NM_001136107	c.G387T:p.R129S	Chr 1 Exon 30	0.0034	0.003	rs535550660	0	1	1	Likely Benign
PABPC3	NM_030979	c.C440T:p.T147I	Chr 13 Exon 1	.	.	rs78432860	0	0.978	0.999	VUS

Contd...

Supplementary Table: Contd...

Gene	RefSeq	Variant	Chromosome and Exon No.	Allele Frequency (1000G)	Allele Frequency (ExAc)	dbSNP ID	SIFT SCORE	POLYPHEN SCORE	MUTATION TASTER SCORE	ACMG classification
AOC2	NM_001158	c.C1896A:p.F632L	Chr 17 Exon 3	.	.	.	0.002	0.997	1	VUS
UMODL1	NM_001004416	c.T1653G:p.C551W	Chr 21 Exon 10	0.0018	0.0006	rs540886339	0	1	1	VUS
POLL	NM_001174084	c.C169T:p.R57W	Chr 10 Exon 3	0.0004	0.0012	rs61757734	0	1	1	VUS
SPATA13	NM_001286793	c.C391A:p.R131S	Chr 13 Exon 4	.	.	.	0.045	0.985	1	VUS
LARGE1	NM_133642	c.G1994A:p.R665H	Chr 22 Exon 14	0.0024	0.005	rs1046166	0.001	1	1	Benign
JMY	NM_152405	c.C134G:p.T45S	Chr 5 Exon 1	0.0002	0.0005	rs528007143	0	0.999	1	VUS
SLC30A7	NM_001144884	c.A482C:p.H161P	Chr 1 Exon 5	.	.	.	0.007	0.999	1	VUS
RASL11A	NM_206827	c.G5C:p.R2P	Chr 13 Exon 1	.	.	.	0.018	0.799	0.99	VUS
C3	NM_000064	c.C4534G:p.R1512G	Chr 19 Exon 37	.	.	.	0.022	0.999	0.793	VUS
HIP1R	NM_001303097	c.C455T:p.A152V	Chr 12 Exon 6	0.0002	0.0004	rs151322438	0.005	0.907	1	NA