Novel compound heterozygous pathogenic variants in ASCC1 in a Chinese patient with spinal muscular atrophy with congenital bone fractures 2 : evidence supporting a "Definitive" gene-disease relationship

Weiliang Lu
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region
ORCiD: https://orcid.org/0000-0002-8671-8413

Mingxing Liang
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Regions

Jiasun Su
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region

Jin Wang
Maternal and Child Health Hospital of guangxi Zhuang Autonomous Region

Lingxiao Li
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region

Shujie Zhang
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region

Zailong Qin
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region

Limei Huang
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region

Yingchi Lu
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region

Shang Yi
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region

Sheng Yi

Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region

BoBo Xie
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region

Haiyang Zheng
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region

Jingsi Luo
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region

Xiaoyan Gao
Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region

Yiping Shen

yiping.shen@childrens.harvard.edu*Corresponding Author*
ORCiD: https://orcid.org/0000-0001-5820-0839

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Abstract

Background: A very limited spectrum of ASCC1 pathogenic variants had been reported in five (mostly consanguineous) families with spinal muscular atrophy with congenital bone fractures 2 [OMIM #616867] since 2016.

Methods:A proband from a non-consanguineous Chinese family presented with neonatal severe hypotonia, respiratory distress, muscle weakness and atrophy, as well as congenital bone fractures was examined by exome sequencing.

Results: A compound heterozygosity of a nonsense (c.932C>G ,p.Ser311Ter) and an exon 5 deletion in ASCC1 segregating with phenotypes was detected, both variants are novel and pathogenic. Since ASCC1 is a relative new disease gene, we performed the gene curation following ClinGen SOP. The existing evidence is sufficient to support a "Definitive" level of disease-gene relationship.

Conclusion: This case report expended the mutation spectrum of ASCC1 and support the notion that this novel disease also occur in outbreed populations and this is a rare disease but may still be underdiagnosed due to its perinatal lethal outcomes.

Keywords: spinal muscular atrophy with congenital bone fractures 2; ASCC1 ; compound heterozygous; gene curation; exome sequencing

Background

ASCC1 encodes a subunit of the activating signal cointegrator 1 (ASC-1) complex. The ASC-1 complex is a transcriptional coactivator that plays an important role in gene transactivation by multiple transcription factors. Previous study showed that ASCC1 pathogenic variants were associated with Barrett esophagus and esophageal adenocarcinoma[1]. Recently, using WES, ASCC1 pathogenic variants were reported to cause bone fractures in neonates with lethal outcomes. Knierim et al identified a homozygous frameshift variant (c.157dupG, p.Glu53Glyfs*19) in ASCC1 in a patient with prenatal-onset spinal muscular atrophy (SMA), multiple congenital contractures (arthrogryposis multiplex congenita), respiratory distress, and congenital bone fractures[2]. The same variant at homozygous status were identified by Oliveira et al. in a patient with severe neonatal hypotonia, lack of spontaneous movements, microretrognathia and arthrogryposis, bilateral femoral fractures and

thin, gracile ribs [3]. Three additional families were reported by Böhm et al. with prenatal-onset muscle weakness with arthrogryposis and congenital bone fractures[4]. Two of the families were consanguineous and five affected individuals had the same homozygous variant. Only one family showed a compound heterozygous variant involving a nonsense variant (c.667C > T/p.Glu223*) and the recurrent frameshift variant. Thus, a total of ten patients from five independent families had been reported to be similarly affected. A total of three variants were uncovered so far and the recurrent frameshift variant occurred in six patients from three families. The narrow mutation spectrum of ASCC1 in patients mostly from consanguineous families limited our understanding of this severe condition. Here we report the first Chinese case and revealed two novel pathogenic variants, implicating the condition may be more widespread.

Methods

Whole exome sequencing

Genomic DNA was extracted from the peripheral blood samples. Target capture was done using an Agilent SureSelect Human All Exon kit (Agilent, Santa Clara, California, USA) according to the manufacturer's protocols, and sequencing was performed on an Illumina HiSeq2000 (Illumina). Sequence data were processed by GATK pipeline for SNV[5] and a custom pipeline for CNV detection combined with visual inspection of sequence read depth by IGV for possible exonic deletion. Variant filtering and prioritization were done using TGex (LifeMap Sciences, Alameda, CA). The variant pathogenicity was assessed according to ACMG/AMP guidelines[6].

ASCC1 targeted variant analysis

Sanger sequencing was carried out in the patient and in parental samples to confirm the nonsense variant identified by WES. The variant was described by the accession number NM_001198798.2 (ENST00000394919) for ASCC1. To confirm deletions involving exonic sequences, we designed one pair of MLPA probes for 3–8 exons. Since exon 5 is relatively large, we designed two pairs of probes for exon 5 (Table 1).

Table 1

The MLPA probe sequences

exon (Ex)	Target-speific hybridization sequences (5'--3')	GC(%)	Tm (°C)	Target sequence length (bp)	Total amplicom size (bp)
ASCC1 EX 3	C TCCATGGAGT GTGCTGATGA GCCCTGTGAT GCCTACGAGG TGGAGCAGAC CCCACAA	55 63	79.95 80.26	58	100
ASCC1 EX 4	GAGAGGGGAC ACTAGGAAGA AAATAGAAAT GGAGA CCAAA ACTTCTATTAGCATTCCTAA ACCTGGACAAGACGGGGA	43 44	74.26 80.08	78	120
ASCC1 EX 5a	CACTGG CCAGCATCGA AATGGTGTAATTTCA GCCCG AACACGGATT GATGTTCTTT TGGACACT	45 48	76.41 79.21	64	108
ASCC1 EX 5b	C CTCAATGAAG TTGAGGTTCAGGAAGGATTC CTGA GATTCC AGGAGGAAGT ACTGGCGAAG TGCTCCATG	46 54	78.03 81.22	70	112
ASCC1 EX 6	CATCTAACTA TTGGGATGTTGGTGCTTTTG AGTGAGGAAG AGATCCAGCA GACATGTGAG ATGCTACAGCAGTG	42 50	77.61 77.10	74	116
ASCC1 EX 7	GATATTTC TGGGGGTAAA CCCCTAGAAGTGGAGATGGCAG GGATAGAA TACATGAATG ATGATCCTGG CATGGTGGATGTTC	50 43	80.92 79.50	82	124
ASCC1 EX 8	GC TACAAGAATT AGTTGATCGAGTGCTGGAAC GTTTTCAGGC ATCTGGACTA ATAGTGAAAG AGTGGAATAGTGTGAAACTGCATGC	45 40	79.83 76.86	87	129

The MLPA was performed using the MLPA kit (P200-Reference-1- B1;MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer’s instructions.

Results

Clinical description

The proband was delivery at 37 weeks of gestation to a healthy mother (G2P2) from a non-consanguineous family (Fig. 1A). At 32 weeks of gestation, fetal ultrasound examination revealed an abnormal posture of upper limbs and club feet. The brain MRI indicated a mild bilateral lateral ventricle dilation with the left and right lateral ventricle posterior horn of approximately 1.26 cm and 1.12 cm in size, respectively (Fig. 1B). The delivery was uneventful with APGAR scores 5, 8, 8 at 1, 3 and 5 min, respectively. The neonate presented with pervasive edema, hypotonia, talipes equinovarus and humeral fractures (Fig. 1C-E). He had respiratory distress accompanied by pneumonia, coagulation abnormality and cryptorchidism. His ears were posteriorly rotated and low-set. He passed away at day 2 after birth. Detailed clinical data was shown in Table 2.

Table 2

Clinical features of the patient reported in this work and comparison with published cases with ASC-1-related neuromuscular diseases

	Family 1[Ref[8]]	Family 2[Ref[9]]	Family 3[Ref[10]]	Family 4[Ref[10]]	Family 5[Ref[10]]	This case
Number of patients (n)	2	2	2	1	3	1
Country	Turkey	Portugal	Tunisia	Morocco	Sri Lanka	China
Gender	Female	Male/Female	Male	Female	Male/Female	Male
Locus	ASCC1	ASCC1	ASCC1	ASCC1	ASCC1	ASCC1
Pathogenic variant	c.157dupG (p.Glu53Glyfs*19) homo	c.157dupG (p.Glu53Glyfs*19) homo	c.157dupG (p.Glu53fs19*) homo	c.157dupG (p.Glu53fs19*) c.466C > T (p.Arg156*) compound heterozygous	c.667C > T (p.Glu223*) homo	c.932C > G (p.Ser311Ter); Exon 5del compound heterozygous
Reduced/absent fetal movements	Y	Y	U	U	Y	Y
Poly/hydramnios	Y	N	U	U	U	N
Oligohydramnios	N	N	U	U	U	N
Premature delivery(< 37 wk)	Y	N	U	N	U	N
Neonatal hypotonia	Y	Y	Y	Y	Y	Y
Neonatal respiratory distress	Y	Y	Y	Y	Y	Y
Congenital bone fractures	Y	Y	Y	Y	Y	Y
Joint contractures	Y	Y	Y	Y	Y	Y
Muscle weakness and atrophy	Y	Y	Y	Y	Y	Y
Cardiomyopathy	N	N	N	N	N	N
Skin changes	U	N	U	U	U	Y
Brain imaging	Abnormal cortical gyration (MRI)	N (transfontanel ultrasonography)	NP	NP	N	Y(lateral ventricle dilatation,mild)
Skeletal muscle histology	Fiber size variation and atrophy. Type 1 fiber grouping	Fiber atrophy (limited analysis, in the context of autopsy)	Fibre size variability, oxidative rims	Fibre size variability, oxidative rims	Fibre size variability, oxidative rims, type I fibre predominance	NP
Severity	U(died between 2 weeks and 16 months of life	died within a few days of life	Deceased shortly after birth	deceased at 13 days	Deceased shortly after birth	deceased at 2 days

N, no; NP, not performed; NA, not applicable; U, unknown; Y, yes.

Mutation analysis

A heterozygous nonsense variant, c.932C > G/p.Ser311Ter in ASCC1was detected in the proband and the father. It is predicted to lead to nonsense mediated decay. (Fig. 2A). We also detected an intragenic deletion involving exon 5 in the proband(Fig. 2B). The mother was the carrier of the deletion. (Fig. 2C). Both variants had not been previously reported. Both are null variants and are pathogenic according to the ACMG/AMP guidelines[6].

Discussion

ASCC1 (OMIM: 614215), contains 10 coding exons, and encodes a protein of the ASC-1 cointegrator complex that mediates the interaction of transcription factors with the basal transcription machinery to modulate gene expression[7]. More recently, ASCC1 was suggested to be a ribonucleoprotein complex involved in transcriptional coactivation of a wide range of genes and in RNA processing[2] and loss of function mutations were detected to be associated with a type of spinal muscular atrophy (MIM: 253300)[2]. Pathogenic variants in ASCC1 downregulated genes associated with neurogenesis, neuronal migration, and pathfinding, as well as with bone development[2]. So far, a total of ten patients from five independent families had been reported to be similarly affected by this perinatal lethal condition. Three variants including one highly recurrent frameshift variant (c.157dup (p.Glu53Glyfs*19) accounted for most of the pathogenic variants. Since this severe meuromuscular disorder caused by biallelic LOF variants in ASCC1 is a relative new disease-gene relationship and only a limited number of patient/pathogenic variants had been reported, we performed gene curation following the GlinGen gene curation protocol (https://clinicalgenome.org/site/assets/files/3907/gene-disease_validity_standard_operating_procedures_version_7-1.pdf). Based on previously published cases and this Chinese family, the total clinical validity score reaches 14 (10 points from genetic evidence and 4 points from experimental evidence), thus sufficient evidence support a "Strong" gene-disease relationship. Now three years had passed since the first reported cases[2] and all affected individuals exhibited similar phenotypes and prognosis, overall, a "Definitive" level of disease-gene relationship can be concluded. We reported the second case with a compound heterozygous variant. In the previously reported ten cases, nonsense or frameshift variants are generally at the homozygous state and only one patient carried a compound heterozygous variant[4]. Our patient is the second case carrying a compound heterozygous variant in ASCC1.The report of the two affected individuals from this Chinese family extended the mutational spectrum and provided further evidence that this condition also occur in out populations.

Loss of function variants in ASCC1 are overall rare and never found to be homozygous in gnomAD (http://gnomad.broadinstitute.org/) except for the p.Ser78* nonsense variant which is located in an alternatively spliced exon and are believed to be not clinically relevant. There are 31 heterozygous LOF variants (Fig. 3) (should be at least likely pathogenic since both PVS1 and PM2 apply) and the overall pathogenic allele carrier rate is 0.0003, thus the estimated disease incidence would be in the order of 9/40,000,000. Only 3 LOF variants were

found in East Asian population, including two splice donor mutations and one frameshift mutation, the carrier AF was lower than 0.0001, thus the disease incidence is expected to be lower in Asian. The mutation spectrum also different from population to population. Pathogenic variants from general population are found to be concentrated in exons 3, 5 and 6, and it is consistent with the distribution of pathogenic variants detected in patients (Fig. 2D), indicated that these 3 exons are more clinically relevant.

The clinical presentations of all reported patients are summarized in Table 2. This condition is mainly characterized by prenatal symptoms including decreased fetal movement, hydramnios, premature delivery, multiple fetal long bone fractures, arthrogryposis multiplex congenita and stiffness and neonatal sympotoms such as severe hypotonia, absence of deep tendon reflex, muscle weakness, muscle fiber abnormalities; abnormal cerebral cortical cerebral gyrus [2–4]. Our patient presented with hypotonia, congenital multiple deformity, neonatal respiratory distress, talipes equinovarus and humeral fractures, which are all major features of this condition. Interestingly, our patient also exhibited additional features not previously reported including cyanosis, ecchymoses, pneumonia, and cryptorchidism. In addition, his prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) were significantly elevated (19.0 seconds, 76.3 seconds and 26.5 seconds, respectively), indicating coagulation abnormality. These novel features may added to our more complete understanding of this condition and can be used for differential diagnosis with other with spinal muscular atrophy related disorders.

Conclusion

We reported the first Chinese ASCC1 case with two novel pathogenic variants (the second compound heterozygous case). While the main clinical presentation of our patient is consistent with those of previously reported, novel phenotypes were also noticed. Although rare, this condition has poor prognosis and can be missed due to a neonatal lethal outcome. The current evidence support a definitive gene-disease relationship. Additional cases are expected even in non-consanguineous populations.

Abbreviations

ASCC1:activating signal cointegrator 1 complex subunit 1; LOF:loss of function; WES:Whole-exome sequencing; GATK:Genome Analysis Toolkit; SNV:single nucleotide variant; CNV:Copy number variant; IGV:Integrative Genomics Viewer; ACMG/AMP:The American College of Medical Genetics and Genomics/The Association for

Molecular Pathology; PVS1:very strong pathogenic; PM2:moderate pathogenic; AF:Allele Frequency; MLPA:multiplex ligation-dependent probe amplification.

Declarations

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Authors’contributions

WLL and MXL: study design, data analysis and manuscript writing. JW and ZLQ: study design and data analysis, LXL and JSL: collection of the clinical samples and clinical evaluation of the patients. JSS and SJZ: sequencing data analysis. SY and HYZ□bioinformatics analysis. LMH and YCL: genomic DNA extraction. SY and BBX: MLPA anlysis. XYG and YPS: study design, data analysis, clinical evaluation of the patients and manuscript revision. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets are available upon request.

Ethics approval and consent to participate

This work was approved by the ethics committee of Guangxi Maternal and Child Health Hospital. Written informed consent was obtained from the family.

Consent for publication

Consent for publication was obtained from all participants.

Competing interests

The authors declare no conflict of interest.

Author details

[a] Genetic and Metabolic Central Laboratory, Birth Defect Prevention Research Institute, Maternal and Child Health Hospital, Children's Hospital of Guangxi Zhuang Autonomous Region, Nanning, 530002 China.

[b] Department of Medical Genetics and Molecular Diagnostic Laboratory, Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai, 200127, China

[c] Division of Genetics and Genomics, Boston Children's Hospital; Department of Neurology, Harvard Medical School, Boston, Massachusetts, 02115 USA.

[d] Department of Neonatology, Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region, Nanning 530003, Guangxi, China.

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Figures

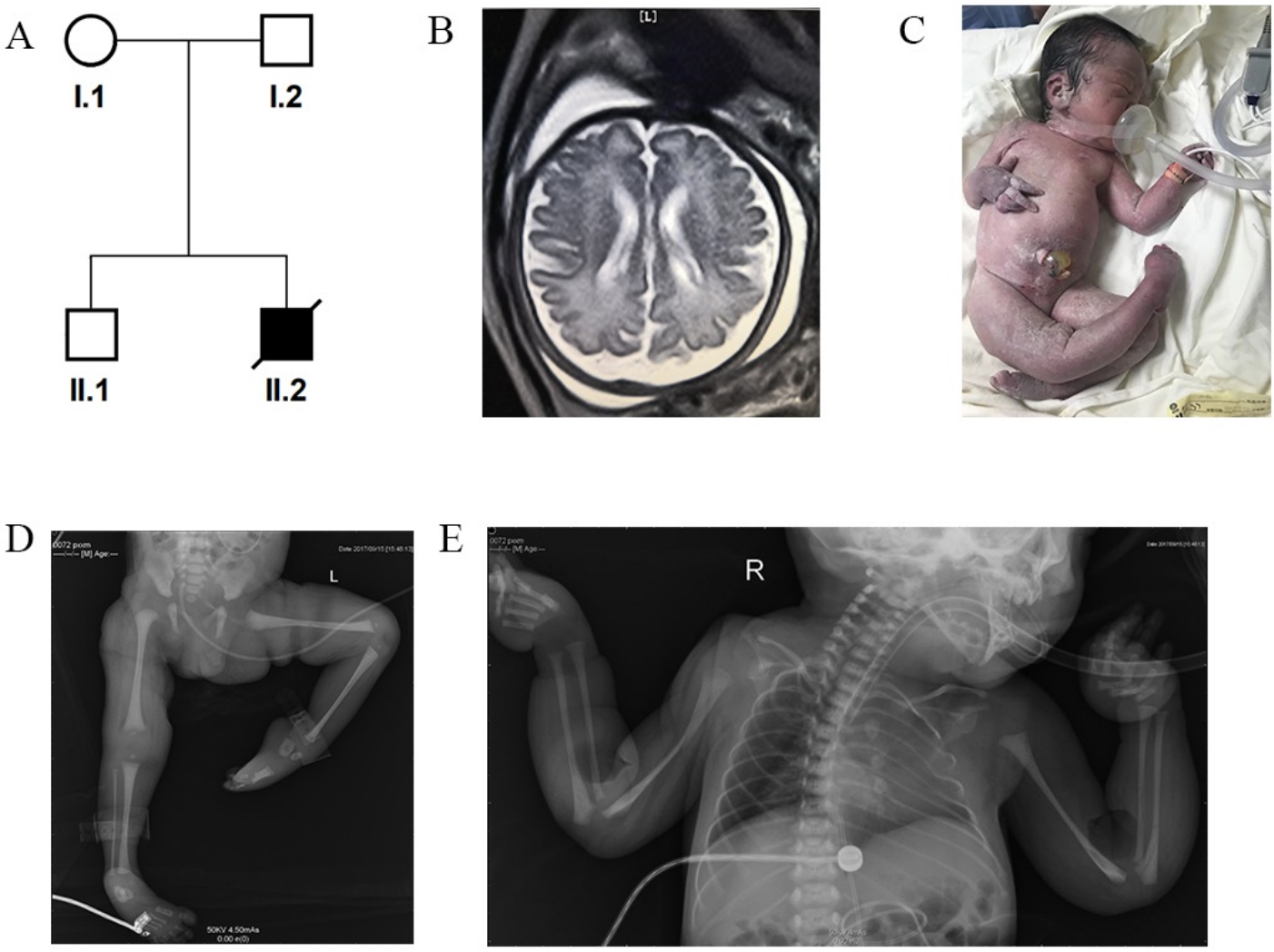

Figure 1

Family pedigree and patient's main clinical features. A: Pedigree of the family compatible with an autosomal recessive inheritance pattern. B: The MRI images of affected children II.2 (A). C: Myopathic appearance, including congenital multiple deformity, neonatal respiratory distress and talipes equinovarus. D-E: The malformation of lower limb and the bilateral congenital femoral fractures.

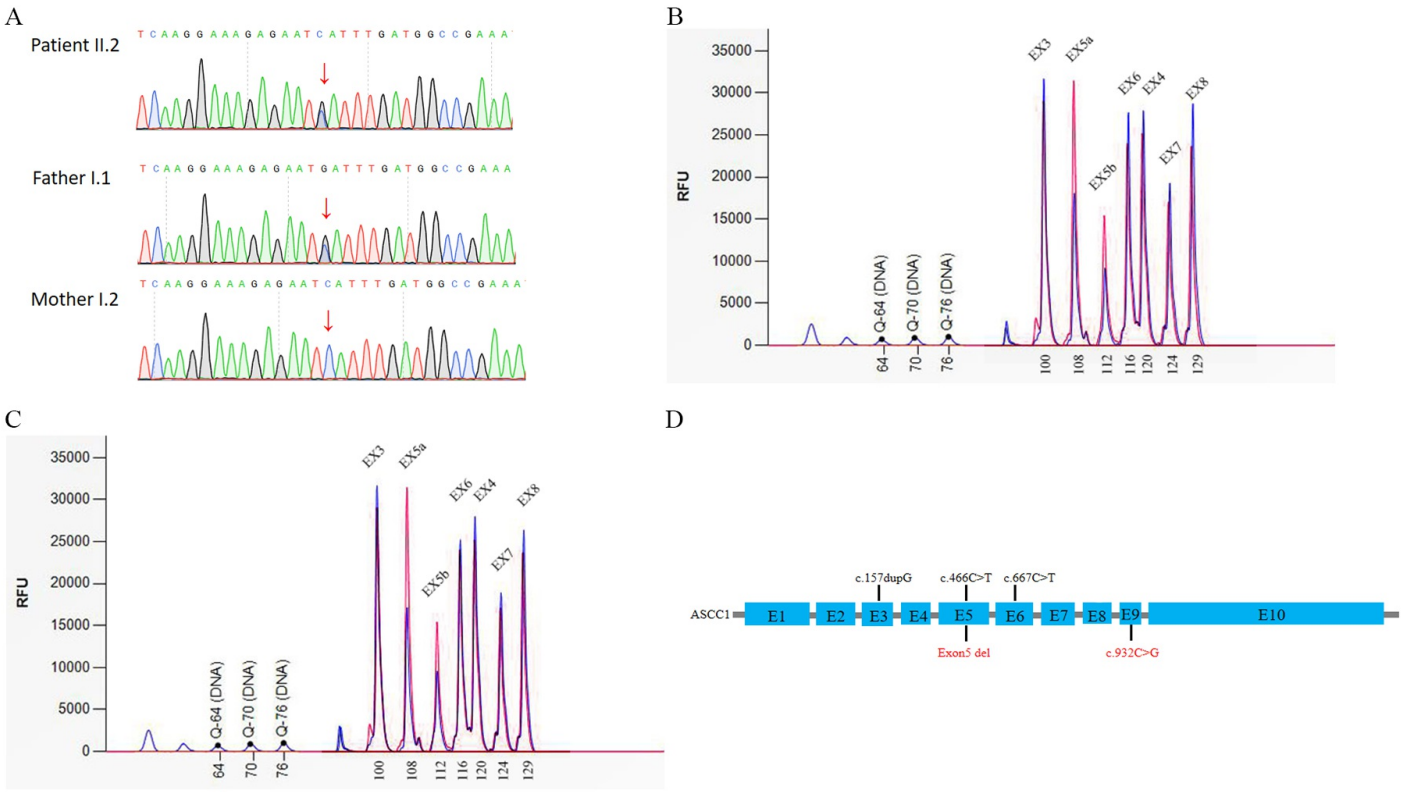

Figure 2

Identification of NM_001198799.2:c.932C>G and Exon 5 del variants in ASCC1 gene.A: Confirmation of variant by Sanger sequencing in the patient and in both parents.B: a deletion mutation in exon 5 was detected in the patient by using MLPA, and the mother was the carrier of this variant(C). The red peak represent the control that carried a complete ASCC1 gene, the blue peak represent the deletion of exon 5 in the proband and his mother; the X-axis represents the amplicoms of 3-8 exons by MLPA. D: Exon structure of the ASCC1 gene and distribution of the mutations. The published mutations are depicted in black, the new ASCC1 mutations in our patient are highlighted in red.

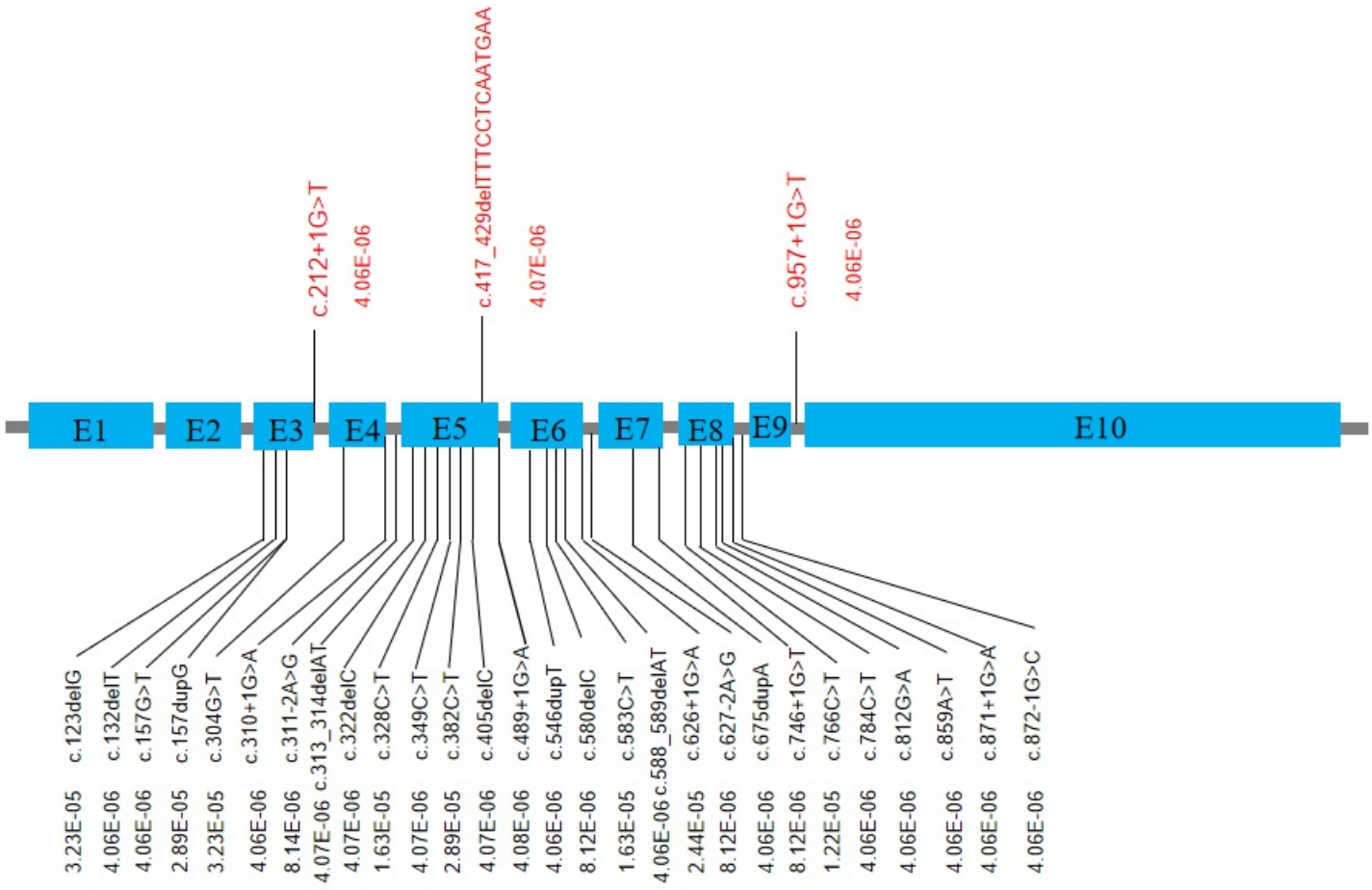

Figure 3

Schematic representation of exons and introns of ASCC1 with the heterozygous mutations identified in East Asian population(red) and in global population(black).