

A novel STS mutation and an Xp22.3 microdeletion from one Chinese family with X-linked ichthyosis

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Research article

Keywords: X-linked ichthyosis, exome sequencing, STS, mutation, deletion

Posted Date: July 15th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-23257/v1>

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Abstract

Background X-linked ichthyosis (XLI, OMIM# 308100) is a relatively common type of skin disorder, characterized by widespread, dark brown, polygonal scales and generalized dryness. Most patients (90%) are contributed to genomic deletion of the entire *STS* (steroid sulfatase) gene encoding steroid sulfatase, with the remaining cases being caused by point mutations or partial deletions. The patient carrying genomic deletion comprising the entire *STS* gene and adjacent genes often manifests neurological symptoms.

Methods Herein, we reported a large four-generation Chinese family with 5 individuals with XLI with/without mental delay. Genetic analysis was conducted by whole exome sequencing and confirmed by Sanger sequencing and quantitative Real-time PCR.

Results These patients present generalized dryness and scaling of the skin with dark scales of the skin on trunk, and limbs. Two pathogenic variants, a novel *STS* mutation (c.1532A > G, p.E511G, NM_000351) and a ~ 2 Mb genomic microdeletion at Xp22.3 (6,451,785-8,434,424) embracing entire *STS* gene were identified in this family. In addition, an individual with Xp22.3 deletion has mental delay.

Conclusion Our study expands the genetic profile of the *STS* gene in XLI patients and highlights the clinical application of exome sequencing.

Background

The ichthyoses are a heterogeneous group of dermatological conditions characterized by widespread, dark brown, polygonal scales and generalized dryness. Cutaneous manifestations are frequently present few weeks after birth, usually do not improve with age but affect a person throughout life [1]. The majority of ichthyoses is inherited and can be pinpointed to characteristic genetic mutations. X-linked recessive ichthyosis (XLI, OMIM#308100) is a relatively common type of ichthyosis with an estimated prevalence ranging from 1:1500 to 1:6000 worldwide[2]. XLI was first described in 1927 by Lundborg[3]. It may occur solely as a skin disorder or may be associated with other physical findings such as corneal opacities (up to 50% of cases), cryptorchidism (20% of cases), chondrodysplasia punctata, and nephritic syndrome [4]. X-linked ichthyosis is caused by the deletion of or mutations in the *STS* (steroid sulfatase) gene encoding steroid sulfatase. The *STS* gene is located on chromosome Xp22.3, containing 10 exons and spans about 146 kb[5]. No homologous sequences were detected on the Y chromosome[6] and non-X-inactivation was reported in this gene[7]. Most patients (85%-90%) have a complete or partial deletion of the *STS* gene, while point mutations within *STS* gene account for about 10% of cases[8]. The protein encoded by *STS* gene is a steroid sulfatase enzyme, a membrane-bounding protein present in most organs and tissues including the skin, breast, blood and brain[9]. Patients with mutations involving solely the *STS* gene may present with attention deficit hyperactivity disorder (ADHD), while those with deletions including neighboring genes such as *neuroligin 4* (*NLGN4*) may be associated with multiple neurodevelopmental disorders, including mental retardation, autism spectrum disorders (ASDs), ADHD,

and seizures[4]. In addition, a recent study showed adult males with XLI disease-causing deletions are apparently at increased risk of cardiac arrhythmias and self-reported mood problems[10].

In this study, we report a four-generation Chinese family with XLI and identified a novel hemizygote point mutation (c.1532 A > G, p.E511G, NM_000351) in one branch and a recurrent ~ 2 Mb genomic deletion (Xp22.3: 6,451,785-8,434,424) comprising the entire *STS* gene in other branch. These variants were absent in healthy family members and cosegregated with XLI in this pedigree. Our study extended the knowledge of mutation spectrum in XLI patients and highlighted the clinical application of exome sequencing.

Methods

Subjects

The XLI family in this study has 47 members across four generation and 5 patients were diagnosed with ichthyosis. The family pedigree is shown in Fig. 1A. Written informed consents were obtained from all subjects or the patient (III:6)'s guardian, and the study was approved by the institutional review board of Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science & Technology (2019011). All experiments were performed in accordance with the Declaration of Helsinki.

Whole-exome Sequencing

Whole exome sequencing was carried out by a commercial company (Chigene, Beijing, China) following standard experimental procedures. The exome was captured using IDT the xGen Exome Research Panel v1.0 Kit. The high-throughput sequencing was based on Illumina NovaSeq 6000 (Illumina, San Diego, CA, USA). The read length of the sequencing was paired-end 150 bp, and the read depth was 120×. Trio whole exome sequencing was carried out, respectively, on the proband family (III:1, II:1, and II:2) and his cousin family (III:6, II:7, and II:8).

Bioinformatics Analysis

The possible effects of the variant on the function and structure of protein, and likelihood of pathological damage were analyzed by SIFT, MutationTaster, PolyPhen2-HDIV, Provean, PolyPhen2-HVAR, M-CAP and REVEL. The sequences of steroid sulfatase protein from zebrafish, trubripes, Mmulatta, human and chimpanzee were aligned using Clustal Omega Sanger sequencing.

Segregation Analysis

Direct Sanger sequencing was carried out in III:1, II:1, II:2, III:3, III:5 to identify the potential disease-causing mutations. Real-time PCR was used to determine the copy number of *STS* gene of II:7, II:8, III:6,

III:7, III:9, III:11, III:19, and of *PNPLA4* gene of II:8, III:6, III:19. All primers were shown in Table 1.

Table 1
Sequences for primers for PCR and real-time RT-PCR analyses

Primers	Forward	Reverse
Gene or fragments	GCAGCATAATTCCGCATCACTT	CCACAGAAGGCATAAACCTACCA
STS-1Q	CCTCACATTATCTGCCAAGCAC	TTGAAGCAGGCCAATCCTACTCA
STS-5Q	CCCTTACATCACGGCTTCAATTATT	AGTAGCCCCAGACAATTGAGTG
STS-10Q	CTTCTGTTCGGGAGTTATGTCACC	CATGAAAATGATCGGGCACCT
PNPLA4	GCGGGATCGTTGGTTGCTTCT	GCTGGTGTTCGCCTTGGTCTT
CYBB	AGTCACTCTGCTCCCTTCC	CGACAGACTGGCAAGAGAATC
36B4	CAGCAAGTGGAAAGGTGTAATCC	CCATTCTATCATCACGGGTACAA

Results

Clinical information

The proband (III:1, male, 32 years old, shown in Fig. 1A) came to our genetic clinic for counseling as his sister (III:3) was pregnant. The proband complained scattered ichthyosis all over the body, which became more severe in winter with dry and cold weather (shown in Fig. 1B). Our patient has one aunt and three uncles from his mother's side, and his aunt's son (III:5) also displayed similar symptom. In addition to the classic feature of ichthyosis, the son (II:6, 37 years old) of his youngest uncle (II:7) exhibited intellectual disability and speech delay, with a history of febrile convolution (shown in Fig. 1A). Three daughters (III:7, III:9, III:11) of this uncle did not show signs of ichthyosis. Interestingly, the branch of his aunt (II:8)'s family also have another two individuals with ichthyosis and barriers to effective communication (II:11 and II:19), with normal intelligence (Fig. 1A).

Whole-exome Sequencing, Bioinformatics And Segregation Analysis

Through whole exome sequencing, after the filtering steps, a solitary heterozygous missense variant, c.1532A > G (p.E511G), in exon 10 of the *STS* gene (NM_000351) was identified in the proband III:1 based on sequences from NCBI database. Further Sanger sequencing revealed that individual III:5 was hemizygous while II:1, II:5 and III:3 were carriers (shown in Fig. 1C). This mutation is predicted to be pathogenic by bioinformatics tools and conserved (Fig. 1D) among different species, and it is not observed in other family members. The c.1532A > G mutation has not been reported previously, and is not present in any public genomic variants database, including Human Genome Mutation Database,

1000Genomes, ESP6500, ExAC Browser (exome aggregation consortium), and COSMIC (the catalog of somatic mutations in cancer), indicating that c.1532A > G in *STS* is a novel mutation that may cause ichthyosis.

As the mutation, c.1532A > G (p.E511G), was not detected in the individual II:6 with ichthyosis, intellectual disability and speech delay, a trio whole-exome sequencing was performed. After data analysis, a maternal genomic deletion of 1.98 Mb in chromosome Xp22.31 (6,451,785-8,434,424) comprising the entire *STS* gene and 6 neighboring genes (*HDHD1*, *PNPLA4*, *VX*, *VCX2*, *VCX3A*, and *VCX3B*) (Fig. 1E), and a de novo duplication of 2.59 Mb of in chromosome 11q14.3 (89,947,185 – 92,538,515) containing two protein-coding genes (*FAT3* and *CHORDC1*) were observed. Further RT-PCR was performed on mRNA from the patient III:6 and his siblings (III:7, III:9, and III:11) using primers for *STS* and one of deleted neighboring gene, *PNPLA4*. RT-PCR showed that the *STS* and *PNPLA4* gene were absent in II:6 but not in his siblings. In addition, individual III:19, who had similar ichthyoses with normal intelligence, was also tested and found to have the deletion of *STS* and *PNPLA4* gene. The individual II:11, who had same symptom as individual III:19, was not genetically tested, as blood sample was not available. All these RT-PCR results were shown in Fig. 1F and 1G. Taken together, two genetic variants, point mutation (c.1532A > G, p.E511G) and a maternal genomic deletion of 1.98 Mb in chromosome Xp22.31, were observed in the family.

Discussion

In this study, we reported two variants from one large family, one is a novel missense mutation in the proband with ichthyosis, and the other one is a maternal deletion in Xp22.31 in his cousin (the son of his uncle from his mother's side) with ichthyosis and disability. XLI is a recessive disorder and it almost exclusively affects males. Four XLI female cases have been described, three of these patients were offspring of an affected father and a carrier mother[11, 12], and one patient was adopted girl who was homozygous nonsense mutation in the *STS* gene[8].

To date, in addition intragenic large deletions, a total of 29 *STS* variants, including the variant (c.1532A > G, p.E511G) reported in this study, have been identified. As shown in Fig. 2 and Table 2, of the 29 variants, 5 are truncating, 3 are frameshift, 20 are missense, and 1 is a deletion involving one amino acid, and it seems that exon 7 and 8 are the hotspot variation region.

Table 2
Point mutations in the STS gene reported to date in the literature/ database

No.	Sequence change	Amino acid change	exon	Reference/database
1	c.184G > T	G62R	3	Clinvar
2	c.261C > G	Y87X	3	del Refugio Rivera Vega et [21] et al[21]
3	c.268C > T	R90X	3	Winge et al[22]
4	c.287G > A	W96X	4	Afzal et al[23]
5	c.323C > T	S108L	4	Diociaiuti et al[9]
6	c.452C > G	P151R	5	Diociaiuti et al[24]
7	c.494C > T	T165I	5	Liao et al[25]
8	c.529_532del4insAG	p.V177Sfs81	5	Takeichi et al[26]
9	c.1022C > T	S341L	7	Basler et al[27]
10	c.1030G > A	G344R	7	Diociaiuti1 et al[9]
11	c.1032C > T	G344X	7	Morita et al[28]
12	c.1046_1048delAAG	E349del	7	Valdes-Flores et al[29]
13	c.1049T > G	V350G	7	Oyama et al[30]
14	c.1075G > A	G359R	7	Diociaiuti et al[24]; Oyama et al[30]
15	c.1099G > A	G367R	8	Wei et al[31]
16	c.1114T > A	W372R	8	Basler et al, Alperin et al[27]
17	c.1115G > C	W372S	8	Alperin et al[5]
18	c.1138G > C	G380R	8	Oyama et al[32]
19	c.1165C > T	Q389X	8	Oyama et al[32]
20	c.1213_1214insTC	P405Lfs408*	8	Murtaza et al[33]
21	c.1256 + 1G > T	R419Sfs427*	8	Alperin et al[5]
22	c.1331A > G	H444R	9	Alperin et al[5]
23	c.1337G > A	C446Y	9	Basler et al[27]
24	c.1360C > T	R454C	9	Gonzalez-Huerta et al[34]
25	c.1361G > A	R454H	9	Valdes-Flores et al[29]
26	c.1393A > G	K465E	10	Trevisson et al[35]
27	c.1532A > G	E511G	10	This study

No.	Sequence change	Amino acid change	exon	Reference/database
28	c.1679A > C	Q560P	10	Sugawara et al[12]
29	c.1679A > G	Q560R	10	Goodwin et al[36]

XLI is characterized by scaly skin on the scalp, trunk and limbs, aggravates in winter and dry climates and alleviate in summer [13, 14], which is consistent with the clinical features of our patient and the affected individuals in his family. Previous studies demonstrated that, in addition to skin problems, XLI patients with Xp22.31 deletion usually present with contiguous gene deletion symptoms[15], including short stature(*SHOX*), Kallmann syndrome(*KAL*), X-linked chondrodysplasia punctata (*ARSE*) and X-linked ocular albinism(*OA1*). In addition, neurodevelopmental conditions, including ADHD, autism, and mental retardation, were also observed in patients with XLI[16, 17]. Recently, a study showed adult males with XLI disease-causing deletions are apparently at increased risk of cardiac arrhythmias and self-reported mood problems[10], and altered basal ganglia structure was observed in these patients[10]. In this pedigree in the present study, the patient III:6, who had ichthyosis, mental retardation, delayed speech, and history of febrile convolution, was found to harbor a Xp22.31 deletion of 1.98 Mb, and the patient has not yet showed heart rhythm problems at the age of 35 years old. Follow-up should be done by regular examination. In addition to *STS* gene, the deleted region contains 6 neighboring gene (*HDHD1*, *PNPLA4*, *VX*, *VCX2*, *VCX3A*, and *VCX3B*). The *VCX3A* gene has been reported to be necessary to maintain normal intellectual development and proposed to be responsible for mental retardation in some XLI individuals[18]. However, highly variable phenotypes were observed in individuals with *VCX3A* deletions, which also happened in our study. Based on the RT-PCR results, it is likely that two patients (II:11 and III:19) has Xp22.31 deletion, however they did not present mental retardation but with barriers to effective communication, which further showed that the cognitive behavioral syndromes vary from normal intelligence to mental retardation, even with same size of deletion. Previous report also showed that no difference in deletion size of fragment including *VCX-A* for XLI patients with and without mental retardation[18]. We also noted that the patient (II:6) harbored a *de novo* duplication of 2.59 Mb in 11q14.3 containing 12 genes, inducing two protein-coding genes (*FAT3* and *CHORDC1*), 8 noncoding genes (*DISC1FP1*, *NDUFB11P1*, *OSBPL9P2*, *OSBPL9P3*, *PGAM1P9*, *RPL7AP57*, *RPS3AP42* and *TUBB4BP4*) and microRNAs(*MIR1261* and *MIR4490*), it is unclear that whether this duplication is correlated to mental retardation in this patient. Thus, the detailed molecular mechanism underlying mental retardation in patients with XLI and Xp22.31 deletion need further investigation.

Both Xp22.31 deletion and point mutations in the *STS* gene were also correlated with ADHD, suggesting that STS deficiency has a curial role in the pathogenesis of inattentive/hyperactivity symptoms. Mouse models also support this hypothesis[19]. In addition to ADHD, other symptoms, such as short stature, epilepsy, bone density reduction, and cryptorchidism, were observed in two siblings [20]. However, our XLI patients with point mutation in the *STS* gene did not display any symptoms of ADHD or other related cognitive behavioral problems.

Conclusion

Through high throughput sequencing of a four-generation Chinese family, we identified a novel missense mutation of *STS* gene in a patient with ichthyosis and a ~ 2 Mb Xp22.3 deletion in his cousin with ichthyosis and mental retardation, expanding the mutation spectrum of XLI. Meanwhile, the study also demonstrates that the genotype-phenotype correlation of XLI is very complicated, further study is needed. Finally, our study emphasizes the importance of whole exome sequencing for genetic diagnosis in rare diseases.

Abbreviations

XLI
X-linked ichthyosis
ADHD
attention deficit hyperactivity disorder
NLGN4
neuroligin 4
ASD
autism spectrum disorder
SIFT
sorting intolerant from tolerant

Declarations

Ethics approval and consent to participate

The study was done after obtaining the ethics approval from the institutional review board of Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science & Technology(approval number 2019011), and with the patient (proband) or the guardian of one patient who has mental delay.

Consent for publication

Written informed consent for publication of clinical details and/or clinical images was obtained from all of the participants or the guardian of one patient who has mental delay.

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the grants of Wuhan Municipal Health Commission (NO.WX17Z12 from Xuelian He, WX19C19 from Yufeng Huang, WX14A06 from Peiwei Zhao); Youth Program of National Natural Science Foundation of China (NO.81700302) from Yufeng Huang; Natural Science Foundation of Hubei Province (2017CFB322) from Sukun Luo.

Authors' contributions

Study concepts: XH, YH

Study design: YH

Literature research: SL

Clinical information collection: GF, YH

Data acquisition: YH, PZ

Data analysis/interpretation: LT

Manuscript preparation: YH

Manuscript editing/ revision/review: XH

All authors have read and approved the manuscript

Acknowledgements

We thank all patients for their participation in the study.

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Figures

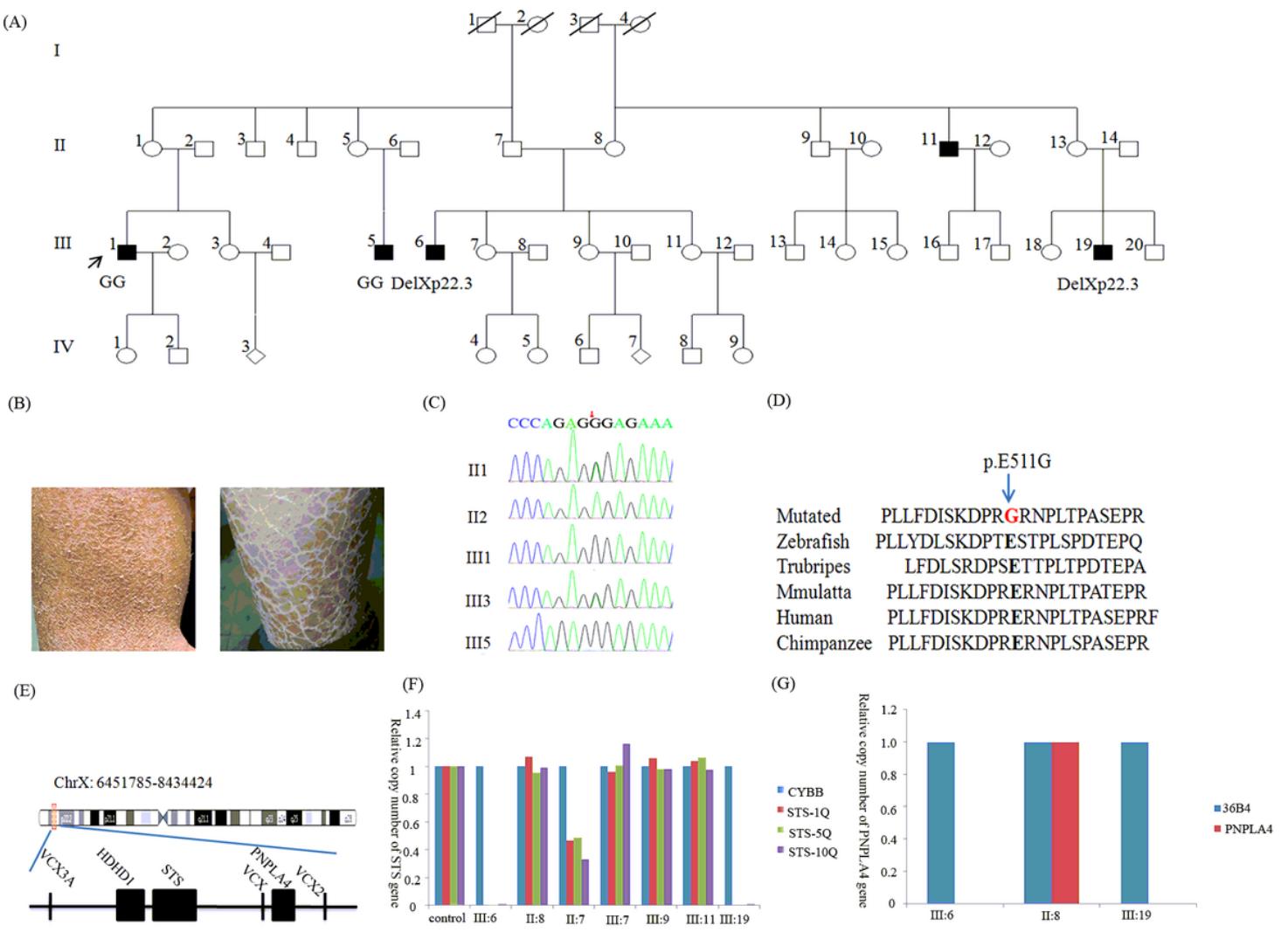


Figure 1

Identification of a novel nonsense mutation in STS gene and a microdeletion in Xq22.3 in a larger family with ichthyosis. (A) The affected members with XLI are depicted by a shaded black square (male). Generations are shown as I to IV. The proband (III:1) is indicated by an arrow. (B) Skin changes of proband (III:1) in spring (left) and in III:6 in winter (right), respectively. (C) Sanger sequencing to confirm the STS c.1532A>G mutation in the proband (III:1), affected member (III:5), and the unaffected control (II:1, II:2, III:3). The mutation is marked by an arrow. (D) Conservation analysis of p.E511G in STS across species. (E) RT-PCR to confirm the STS deletion in the affected member (III:6, III:19) and the unaffected control (II:7, II:8, III:7, III:9, III:11). RT-PCR of three exons of STS genes. (F) RT-PCR to confirm PNPLA4 another gene in Xp22.311 deletion in the affected member (III:6, III:19) and the unaffected control (II:8). RT-PCR of one exons of PNPLA4 genes. (G) Schematic representation of the Xp22.31 region ideogram and some relevant genes of the region (according to UCSC).

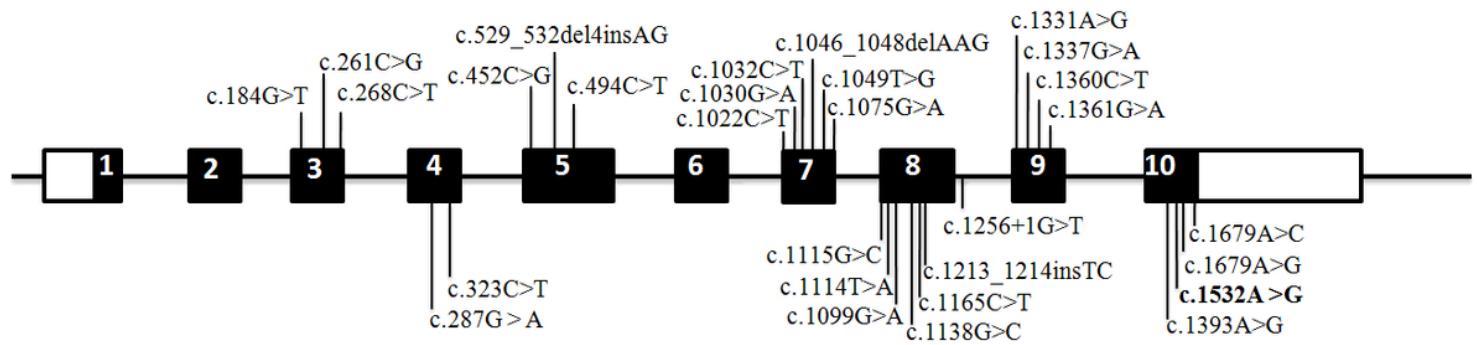


Figure 2

Identified mutations in STS gene in literature and in our study. The mutation in bold was reported in this study.