1. **The procedures of recombinant plasmid construction**
2. Recombinant plasmid containing the ALPL-coding sequences (designated as ALPL-mRNA)

The ALPL-coding sequences with attached restriction enzyme sites (recognition sequences of XhoI at the 5′ end and recognition sequences of EcoRI at the 3′ end) were provided in **Table S 1** and synthesized by Fuzhou Sunya Biotechnology Co.,Ltd. Both the synthesized products and the pEGFP-N1 vector were digested by XhoI/EcoRI and then recombined using Exnase II.

1. Recombinant plasmid containing the ALPL-coding sequences with c.269A>G (designated as c.269A>G)

Firstly, with ALPL-mRNA serving as an amplification template, primer 1 and primer 3 were utilized to obtain amplified product 1, while primer 2 and primer 4 were utilized to obtain amplified product 2. Then, taking amplified product 1 and amplified product 2 as amplification templates, primer 3 and primer 4were utilized to obtain the ALPL-coding sequences with c.269A>G. Both the ALPL-coding sequences with c.269A>G and pEGFP-N1 vector were digested by XhoI/EcoRI and then recombined using Exnase II.

1. Recombinant plasmid containing the ALPL-coding sequences with c.787T>C (designated as c.787T>C)

Firstly, with ALPL-mRNA serving as an amplification template, primer 5 and primer 8 were utilized to obtain amplified product 1, primer 6 and primer 7 were utilized to obtain amplified product 2. Then, taking amplified product 1 and amplified product 2 as amplification templates, primer 5 and primer 6 were utilized to obtain the ALPL-coding sequences with c.787T>C. Both the ALPL-coding sequences with c.787T>C and pEGFP-N1 vector were digested by XhoI/EcoRI and then recombined using Exnase II.

1. Recombinant plasmid containing the ALPL gene-exon3, intron3, exon4, intron4, exon5 (designated as ALPL-WT)

Firstly, with the genomic DNA of the proband’s grandmother (a normal individual) serving as an amplification template, the ALPL gene-exon3, intron3, exon4, intron4, exon5 with attached restriction enzyme sites (recognition sequences of BamHI at the 5′ end and recognition sequences of XhoI at the 3′ end, and the full length of this sequence was provided in **Table S 1**, were obtained using primer 9 and primer 10. Both the amplified product and pMini-CopGFP vector were digested by BamHI/XhoI and then recombined using Exnase II.

1. Recombinant plasmid containing the ALPL gene-exon3, intron3, exon4, intron4, exon5 with c.182-9C>T (designated as ALPL-MT)

Firstly, with ALPL-WT serving as an amplification template, primer 11 and primer 12 were utilized to obtain the ALPL gene-exon3, intron3, exon4, intron4, exon5 with c.182-9 C>T. Both the amplified product and pMini-CopGFP vector were digested by BamHI/XhoI and then recombined using Exnase II.

1. Recombinant plasmid containing the ALPL-coding sequences with c.182\_199del (designated as c.182\_199del)

Since minigene analysis results indicated that c.182-9C>T affected the *ALPL* gene pre-mRNA splicing process and ultimately led to c.182\_199del (The deletion protein sequence of TNSALP was provided in **Table S 2**), a recombinant plasmid containing the ALPL-coding sequences with c.182\_199del also needed to be constructed. First, with ALPL-mRNA serving as an amplification template, primer 13 and primer 15 were utilized to obtain amplified product 1, primer 14 and primer 16were utilized to obtain amplified product 2. Then, taking amplified product 1 and amplified product 2 as amplification templates, primer 13 and primer 16 were utilized to obtain the ALPL-coding sequences with c.182\_199del. Both the ALPL-coding sequences with c.182\_199del and pEGFP-N1 vector were digested by XhoI/EcoRI and then recombined using Exnase II.

**The sequences of the amplification primers and sequencing primers were shown in the Table S 3 and Table S 4.**

**Table S 1 Inserted sequences of the recombinant plasmids**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Recombinant plasmid** | **Vector** | **Restriction enzyme** | **Inserted sequences** | **Sequence length** |
| ALPL-mRNA | pEGFP-N1 | XhoI-EcoRI | **ctcgag\***ATGATTTCACCATTCTTAGTACTGGCCATTGGCACCTGCCTTACTAACTCCTTAGTGCCAGAGAAAGAGAAAGACCCCAAGTACTGGCGAGACCAAGCGCAAGAGACACTGAAATATGCCCTGGAGCTTCAGAAGCTCAACACCAACGTGGCTAAGAATGTCATCATGTTCCTGGGAGATGGGATGGGTGTCTCCACAGTGACGGCTGCCCGCATCCTCAAGGGTCAGCTCCACCACAACCCTGGGGAGGAGACCAGGCTGGAGATGGACAAGTTCCCCTTCGTGGCCCTCTCCAAGACGTACAACACCAATGCCCAGGTCCCTGACAGTGCCGGCACCGCCACCGCCTACCTGTGTGGGGTGAAGGCCAATGAGGGCACCGTGGGGGTAAGCGCAGCCACTGAGCGTTCCCGGTGCAACACCACCCAGGGGAACGAGGTCACCTCCATCCTGCGCTGGGCCAAGGACGCTGGGAAATCTGTGGGCATTGTGACCACCACGAGAGTGAACCATGCCACCCCCAGCGCCGCCTACGCCCACTCGGCTGACCGGGACTGGTACTCAGACAACGAGATGCCCCCTGAGGCCTTGAGCCAGGGCTGTAAGGACATCGCCTACCAGCTCATGCATAACATCAGGGACATTGACGTGATCATGGGGGGTGGCCGGAAATACATGTACCCCAAGAATAAAACTGATGTGGAGTATGAGAGTGACGAGAAAGCCAGGGGCACGAGGCTGGACGGCCTGGACCTCGTTGACACCTGGAAGAGCTTCAAACCGAGATACAAGCACTCCCACTTCATCTGGAACCGCACGGAACTCCTGACCCTTGACCCCCACAATGTGGACTACCTATTGGGTCTCTTCGAGCCAGGGGACATGCAGTACGAGCTGAACAGGAACAACGTGACGGACCCGTCACTCTCCGAGATGGTGGTGGTGGCCATCCAGATCCTGCGGAAGAACCCCAAAGGCTTCTTCTTGCTGGTGGAAGGAGGCAGAATTGACCACGGGCACCATGAAGGAAAAGCCAAGCAGGCCCTGCATGAGGCGGTGGAGATGGACCGGGCCATCGGGCAGGCAGGCAGCTTGACCTCCTCGGAAGACACTCTGACCGTGGTCACTGCGGACCATTCCCACGTCTTCACATTTGGTGGATACACCCCCCGTGGCAACTCTATCTTTGGTCTGGCCCCCATGCTGAGTGACACAGACAAGAAGCCCTTCACTGCCATCCTGTATGGCAATGGGCCTGGCTACAAGGTGGTGGGCGGTGAACGAGAGAATGTCTCCATGGTGGACTATGCTCACAACAACTACCAGGCGCAGTCTGCTGTGCCCCTGCGCCACGAGACCCACGGCGGGGAGGACGTGGCCGTCTTCTCCAAGGGCCCCATGGCGCACCTGCTGCACGGCGTCCACGAGCAGAACTACGTCCCCCACGTGATGGCGTATGCAGCCTGCATCGGGGCCAACCTCGGCCACTGTGCTCCTGCCAGCTCGGCAGGCAGCCTTGCTGCAGGCCCCCTGCTGCTCGCGCTGGCCCTCTACCCCCTGAGCGTCCTGTTC**c#gaattc\*** | 1585bp |
| ALPL-WT | pMini-CopGFP | BamHI-XhoI | **ggatcc\***AGAAAGAGAAAGACCCCAAGTACTGGCGAGACCAAGCGCAAGAGACACTGAAATATGCCCTGGAGCTTCAGAAGCTCAACACCAACGTGGCTAAGAATGTCATCATGTTCCTGGGAGATGGTGAGGCCCAGGGGCCTGTGGGAGGGGTGGAACAGGACACCTAGCTAGGAGCCCCGGGAGCCAGGCTGAGTTGAAGGGGGCTGTGTTGAAGGGGCTAGGGCTCTGGAGGAAGGGTGTTTAAAAGGATGAGGGGCCAGGCTGTGGATTCAAGAGGCCTGCTCTGCCAGACAGCCCCCTGAGGATCTGGGGGTGAAGGGAGAGAGGGGCTGCTCACTGACATTTACAGAGCCATGCCCAGTGCCAACTGCCCGAGCCTGCCTTGGTACCGAACTAGAGAGCTTCTGGGTACCCAAGCAGGCTGATTGGAGAGGCAGGAGCACGAGAGACTGAGGCCCCCACTCCCCACTGCAGGGATGGGTGTCTCCACAGTGACGGCTGCCCGCATCCTCAAGGGTCAGCTCCACCACAACCCTGGGGAGGAGACCAGGCTGGAGATGGACAAGTTCCCCTTCGTGGCCCTCTCCAAGGTGAGCCCCATCCCCAAGCCCAGTTCAGGTCTGTATATCCAGTATCCAGGTCGAGCATCTGAACATGACAGCAGCCAGAGGTCCCCTGACCCCCTGAGCCCCCTCCATGCCCAAGCCCACTCCCCACCTGGAGCAGCCACTGCCCTGACTTCTGACACCATAGCATCACTGTACCTGCTTGCGGGTGTGTGTTTAGAGAGAGGGTCTTGCTCTGTTGCCCAGGCTGCAGTGCAGTGGTGCAGTCATAGCTCACTGCAGCCTCCAACTCCTGGGCTCAAGCCATCCTCCCACGTTAGCCTCCCGAGTAGCTGGGACTACAGGTGTGTACCACCACATATGGATAATTAGAGACCCCCCCAAGGTCTCTAATTCTTGGGCTTAAGCGATCCTACAGCCTTGGCCTCCCAGTCTTGGGATTACAGGCGTGAGCCACCATGCACGGCCTATATATGCCTTTTTTTTTTTTTTTTTTTTGAGACAGTCTTACTCTGTCTTCCAGGCTGGAGTACAGTAGCATGACATCAGCTCACTGCAACCTCTGCCTCCCGAGTTCAAGTGATTCTTCTGCCTCAGCCTCCCAAGTAGCTGGGATTACAGGCATGCGCCACCATGCCCAGCTAATTTTTTTATTTTTAGTAGAGACAAGGTTTCACCCTGTTGGCCAGGCTGGTCTCGAACTCCTGACCCCAGGTGATCTGCCCACCTTGGCCTCCCAAAGTGCTAGGATTACAGGCATGAGCCACCGTGCCTGGCCTGTATACACTTTTGCATGAAGTTCCAGTTCATTCATTCTTTCCCGAGGCAGTTCGTGAAGTTAATTAGACTATAGGCATACCTCAGCTATACTTCGGGTTTGGTTCCAGGCCACTGTAATAAAGCAAATATTGCAATGAAGCAAGTCACACATTTTTTGGTTTCCCAGTGCATATAAAAGTTATGTTTGCACCCTACAGTAGTCTTTTAAGTGTGGGATAGCATTATGTCTAAAAAAATGTATATACCTTAATTTAAAAATATGCTATTGCTAAAAAACGCTAATGAAGTGAGCTCATGTTTTTGGAAAAAATGATGCTGATAGACTTGCTTGACACAGGGTTGCCACAGATCTTCAATTTGTAACAAACACAGTGTCTGTGGAGTACACTAAAATGAGGTGTGCCTGTACTGACCTAGGTTGTTCAAGGCAGTGTGCACTGCCGGGTAGCTATTGCTATTGGTGGTGGTGATAGTGGTGTTATGTAATAACCTCCAGACCCTGGGTCAGGCCCCAGGCTCACTTCTGGGGGAGTAGTGGGGACTATGGTGCCTTCTGTGCCTTCAAGGTGCTCTCAGGCTGGTGGGGAAGGGAGACAGGCAAATAGTTTTGGGGTCTCTGCTAAACATCCGGATGTAGGGCAACCCTGTGATGGTGGCCGCAGGGCAGGTGGCAGAGAAGTCAGCGCTGGTCTTGGCCCCACCCTTCCCTTCCCCAGTCCCCTTCCCCCTCACTTTCACTACCCTATCCCCAGACCCCCCACTCCTTCAAGAAGGCCCCTCCCTCTCCCTTCTCCAGGACTCCCCTGCTCCCCAACCCCTTGCTGGCTCCTGCAGCCCCAGGCCCGGAGAGAGGCCTCCGCTGACCCCACCTGAGCCCCAATCTTCCGCCGGACACTTCTCTGTACTTTGGAGAGCTAAGGTCCTGCCCCAGTGCTGCCAGGGTGCAGGGAATGATGGCAGGAAGCAGGCAGCTAGGTAGTCCTGTGGCTCTGGGGGGCTTCAGTGGGCAGTGGGCCTGGTCAAGGCTATGGGGTCCTCTCTGGTCCCTCACGCCCCAGTCCCCATGGTGTGAGTGTAGGCGGGGTGGGTGCCACTGGGGCGAAGGCCTGGCCATCTCCTGACCCTCCTCTCCCACCTGCAGACGTACAACACCAATGCCCAGGTCCCTGACAGTGCCGGCACCGCCACCGCCTACCTGTGTGGGGTGAAGGCCAATGAGGGCACCGTGGGGGTAAGCGCAGCCACTGAGCGTTCCCGGTGCAACACCACCCAGGGGAACGAGGTCACCTCCATCCTGCGCTGGGCCAAGGACGCTG**ctcgag\*** | 2673bp |

**ctcgag\***: Recognition sequences of XhoI; **gaattc\***: Recognition sequences of of EcoRI; **ggatcc\***: Recognition sequences of of BamHI; **c#**: Inserted base pairs to avoid frameshift that affects GFP expression.

**Table S 2 Abnormal splicing caused by c.182-9C>T on protein expression of TNSALP**

|  |  |  |
| --- | --- | --- |
|  | **Amino acid sequence of TNSALP** | **Sequence length** |
| Wild type | MISPFLVLAIGTCLTNSLVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGD**GMGVST\***VTAARILKGQLHHNPGEETRLEMDKFPFVALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIAYQLMHNIRDIDVIMGGGRKYMYPKNKTDVEYESDEKARGTRLDGLDLVDTWKSFKPRYKHSHFIWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSEDTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNGPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPMAHLLHGVHEQNYVPHVMAYAACIGANLGHCAPASSAGSLAAGPLLLALALYPLSVLF\* | 524aa |
| c.182-9C>T | MISPFLVLAIGTCLTNSLVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDVTAARILKGQLHHNPGEETRLEMDKFPFVALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIAYQLMHNIRDIDVIMGGGRKYMYPKNKTDVEYESDEKARGTRLDGLDLVDTWKSFKPRYKHSHFIWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQILRKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAIGQAGSLTSSEDTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMLSDTDKKPFTAILYGNGPGYKVVGGERENVSMVDYAHNNYQAQSAVPLRHETHGGEDVAVFSKGPMAHLLHGVHEQNYVPHVMAYAACIGANLGHCAPASSAGSLAAGPLLLALALYPLSVLF\* | 518aa |

**GMGVST\***: Missing amino acids caused by c.182-9C>T

1. **Amplification primers used in the constructionof recombinant plasmids**

**Table S 3 The sequences of amplification primers**

|  |  |  |
| --- | --- | --- |
| **Recombinant plasmid** | **Number** | **Primer sequences (5′-3′)** |
| c.269A>G | 1 | ACGAAGGGGAACTTGCCCATCTCCAGCCTGGTCTCCTCCCCAGGG |
| 2 | ACCAGGCTGGAGATGGGCAAGTTCCCCTTCGTGGCCCTCTCCAAG |
| 3 | GCTAGCGCTACCGGACTCAGATCTCGAGATGATTTCACCATTCTTAGTACTGGCCATTGGCACCTGCCTTACTAACTCCTTAGTGCCAGA |
| 4 | GCCCGCGGTACCGTCGACTGCAGAATTCGGAACAGGACGCTCAGGGGGTAGAGGGCCAGCGCGAGCAGCAGGGGGCCTGCAGCAAGGCTG |
| c.787T>C | 5 | GATCCGCTAGCGCTACCGGACTCAGATCTCGAGATGATTTCACCATTCTTAGTACTGG |
| 6 | CCCGGGCCCGCGGTACCGTCGACTGCAGAATTCGGAACAGGACGCTCAGGGGGTAGAG |
| 7 | AGCTTCAAACCGAGACACAAGCACTCCCACTTCATCTGGAA |
| 8 | CCAGATGAAGTGGGAGTGCTTGTGTCTCGGTTTGAAGCTCTTCCAGGTG |
| ALPL-WT | 9 | AAGCTTGGTACCGAGCTCGGATCCAGAAAGAGAAAGACCCCAAGTACTGGCGAG |
| 10 | TTAAACGGGCCCTCTAGACTCGAGCAGCGTCCTTGGCCCAGCGCAGGATGGA |
| ALPL-MT | 11 | ACTCCTCACTGCAGGGATGGGTGTCTCCACAG |
| 12 | TCCCTGCAGTGAGGAGTGGGGGCCTCAGTCTC |
| c.182\_199del | 13 | CCGTCAGATCCGCTAGCGCTACCGGACTCAGATCTCGAGATGATTTCACCATTCTTAGTACTGGCCATTG |
| 14 | GTGGCTAAGAATGTCATCATGTTCCTGGGAGATGTGACGGCTGCCCGCATCCTCAAGGGTCAGCTC |
| 15 | GAGCTGACCCTTGAGGATGCGGGCAGCCGTCACATCTCCCAGGAACATGATGACATTCTTAGCCAC |
| 16 | GGATCCCGGGCCCGCGGTACCGTCGACTGCAGAATTCGGAACAGGACGCTCAGGGGGTAGAGGGCCAGCGC |

1. **Sequencing primers used in the construction of recombinant plasmids**

**Table S 4 The sequences of sequencing primers**

|  |  |  |
| --- | --- | --- |
| **Recombinant plasmid** | **Number** | **Primer sequences (5′-3′)** |
| ALPL-mRNA  c.269A>G  c.787T>C  c.182\_199del | 17 | TGGGAGGTCTATATAAGCAGAG |
| 18 | CGTCGCCGTCCAGCTCGACCAG |
| 19 | AAATCTGTGGGCATTGTGAC |
| ALPL-WT  ALPL-MT | 20 | GATATACACTGTTTGAGATGAGGA |
| 21 | TGGAACCAAACCCGAAGTAT |
| 22 | GGCTAACTAGAGAACCCACTGCTTA |
| 23 | GTCCTTGGCCCAGCGCAGGAT |