**Supplementary Material**

**Table S1: Detected mutations in *TSC1* (exons 3 to 23) and *TSC2* (exons 1 to 41) genes (nonsense, missense, Synonymous, intron and duplication mutations)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Patients** | **Gene** | **cDNA Variant** | **Protein Variant** | **Variant Type** | **1000 Genomes** | **ClinVar** | **Pathogenicity** |
| (Patient.1) | *TSC1* | c-7C>T | N/A | Intronic Variant | rs62621221 | RCV000210810.1 | Benign |
| c.2626-5\_2626-4dup | N/A | Duplication | [rs5901000](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs5901000) | [RCV000586176.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000586176.1) | Benign |
| *TSC2* | c.4375C>T | p(Arg1459\*) | Nonsense | rs45517340 | RCV000190004.2 | Pathogenic |
|  (Patient 2) | *TSC1* | c.1142-33A>G | N/A | Intronic Variant | [rs6597586](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs6597586) | [RCV000254427.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000254427.1) | Benign |
| c.1439-37C>T | N/A | Intronic Variant | [rs10901220](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs10901220) | [RCV000042031.2](https://www.ncbi.nlm.nih.gov/clinvar/RCV000042031.2) | Benign |
| c.2625+68G>A | N/A | Intronic Variant | [rs1076160](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs1076160) | [RCV000829852.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000829852.1) | Benign |
| *TSC2* | c.226-101C>T | N/A | Intronic Variant | [rs144408387](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs144408387) | Not Reported | Not provided |
| c.482-3C>T | N/A | Intronic Variant | [rs1800720](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs1800720) | [RCV000576589.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000576589.1) | Benign |
| c.482-68C>G | N/A | Intronic Variant | rs2516734 | [RCV000043225.2](https://www.ncbi.nlm.nih.gov/clinvar/RCV000043225.2) | Not provided |
| c.976-100C>G | N/A | Intronic Variant | [rs2074968](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs2074968) | [RCV000834380.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000834380.1) | Benign |
| c.1600-39C>T | N/A | Intronic Variant | [rs45477195](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs45477195) | [CV000254340.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000254340.1) | Benign |
| c.5161-10A>C | N/A | Intronic Variant | [rs1800718](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs1800718) | [RCV000589236.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000589236.1) | Benign |
| c.5202T>C | *p.(Asp1734=)* | Synonymous variant | [rs1748](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs1748) | [RCV000162957.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000162957.1) | Benign |
| c.5260-49C>T | N/A | Intronic Variant | [rs13332221](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs13332221) | [RCV000042680.2](https://www.ncbi.nlm.nih.gov/clinvar/RCV000042680.2) | Benign |
| c.5260-25C>G | N/A | Intronic Variant | [rs13332222](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs13332222) | [RCV000576507.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000576507.1) | Benign |
|  (Patient 3) | *TSC1* | c.1142-33A>G | N/A | Intronic Variant | [rs6597586](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs6597586) | [RCV000254427.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000254427.1) | Benign |
| c.1439-37C>T | N/A | Intronic Variant | [rs10901220](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs10901220) | [RCV000042031.2](https://www.ncbi.nlm.nih.gov/clinvar/RCV000042031.2) | Benign |
| c.2625+68G>A | N/A | Intronic Variant | rs1076160 | [RCV000829852.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000829852.1) | Benign |
| c.2626-5\_2626-4dup | N/A | Duplication | [rs5901000](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs5901000) | [RCV000586176.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000586176.1) | Benign |
| *TSC2* | c.482-3C>T | N/A | Intronic Variant | [rs1800720](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs1800720) | [RCV000576589.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000576589.1) | Benign |
| c.482-68C>T | N/A | Intronic Variant | [rs2516734](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs2516734) | [RCV000043225.2](https://www.ncbi.nlm.nih.gov/clinvar/RCV000043225.2) | Not provided |
| c.1070C>T | p.(Ala357Val) | Missense | [rs150195368](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs150195368) | [RCV000230505.5](https://www.ncbi.nlm.nih.gov/clinvar/RCV000230505.5) | VUS/ benign\*1 |
| c.976-16C>T | N/A | Intronic Variant | [rs773016098](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs773016098) | Not Reported | Not provided |
| c.976-100C>T | N/A | Intronic Variant | Not provided | Not Reported | Not provided |
| c.1513C>T | p.(Arg505\*) | Nonsense | rs45517179 | [RCV000517734.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000517734.1) | Pathogenic |
| c.4569+10G>A | N/A | Intronic Variant | Not provided | Not Reported | Not provided |
| Patient 4  | *TSC1* | c.2503-59C>T | N/A | Intronic Variant | rs930922255 | Not Reported | Benign |
| c.2626-5\_2626-4dup | N/A | Duplication | [rs5901000](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs5901000) | [RCV000586176.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000586176.1) | Benign |
| *TSC2* | C.4375C>T | p(Arg1459\*) | Nonsense | rs45517340 | RCV000201120.2 | Pathogenic |
| 16265C>G | N/A | Intronic Variant | rs2074968 | Not Reported | Benign |
|  (Patiet5) | *TSC1* | c.2625+68G>A | N/A | Intronic Variant | [rs1076160](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs1076160) | [RCV000829852.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000829852.1) | Benign |
| c.2626-5\_2626-4dup | N/A | Duplication | [rs5901000](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs5901000) | [RCV000586176.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000586176.1) | Benign |
| *TSC2* | c.4569+10G>A | N/A | Intronic Variant | Not provided | Not Reported | Not provided |
| c.618C>A | p.(Cys206\*) | Nonsense | [rs45498496](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs45498496) | [RCV000043149.2](https://www.ncbi.nlm.nih.gov/clinvar/RCV000043149.2) | Not provided |
| c.5331T>C | p.(Pro1777=) | Synonymous variant | [rs370820622](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs370820622) | [RCV000574389.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000574389.1) | Likely benign |
| c.465C>T | p.(Tyr155=) | Synonymous variant | [rs45444196](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs45444196) | [RCV000569174.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000569174.1) | Likely benign |
| c.1119+23G>A | N/A | Intronic Variant | [rs180840428](https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=rs180840428) | [RCV000055550.1](https://www.ncbi.nlm.nih.gov/clinvar/RCV000055550.1) | Not provided |
| c.1119+64A>G | N/A | Intronic Variant | Not provided | Not Reported | Not provided |
| c.1717-55T>C | N/A | Intronic Variant | [rs7187438](https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/?chr=16&from=2120402&to=2120402&gts=rs7187438&mk=2120402:2120402%7Crs7187438) | RCV000055373.1 | Not provided |

\*1 conflicting information about classification as VUS and as benign ( Richards et al., 2015; Nykamp et al., 2017).

**Table S2 : *TSC1* Primers :**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Exon** | **Forward 5΄- 3΄** | **Reverse 5΄- 3΄** | **Annealing temperature** | **Product size** |
| **3** | GCATTAGTTTGTCTTGCAGG | TATCAGCAGGATTCTAGTGG | 61°C | 349bp |
| **4** | CTGGATTTTGTGACAGGAAGC | GCTCAGGACAAGTTGCACAG | 65°C | 278bp |
| **5** | AGACAAGACTTGAGAGATTGG | AGCTTCCTTGCTTTAAGTTGC | 62°C | 334bp |
| **6** | TCAGTGTTTAGAGCCTCTTC | ATTCCCTCATCTGTCTGGTG | 62°C | 376bp |
| **7** | GGAGTGAGGTGGGAATATAC | AGTGGCGAGGAAGAAAACTG | 63°C | 402bp |
| **8** | GTTCTCAAGGTTCCATCTGTG | TCCCAAATATACCTCAACAGG | 63°C | 295bp |
| **9** | GCTTGTTCTTTGCTATCAGAG | TAGGAACTGAACTAAGTCTTAC | 61°C | 391bp |
| **10** | CTCTGAAGTGTTACTTTGTCC | GATCCTTAAAAGTGACTCCTG | 62°C | 428bp |
| **11** | CATGGATATAAACCTCGTGGA | CACTTTACACATTCTGAAAGC | 61°C | 336bp |
| **12** | TCAGAATGTGTAAAGTGAACC | TCAAACCCATTGCATTTTAGG | 60°C | 412bp |
| **13** | ATTTGAGAATCACTGCACTCG | CCCAGAATTTCCTTGTTTCCA | 62°C | 275bp |
| **14** | TAAGCAGTTTTACACTTACAGG | GTGAGCAATGGCACAAAATCC | 63°C | 256bp |
| **15A** | TCTCCTCTCTCTGAGTGACA | GGGAGTAAAGGCTTGCTTTG | 63°C | 335bp |
| **15B** | ACTCGGCAGCCTCCAGTTC | CTTTTGCTTTCTTTAACAGCTC | 63°C | 339bp |
| **15C** | CGACGAGAGTGGGCTTTGG | CTGCCTCAAGGTGGGAGTG | 67°C | 336bp |
| **16** | GCCATTGCAGATTTTGACCAC | CTGTTTCCCAGAGGGCACC | 65°C | 255bp |
| **17** | AAGCTAACAACACATGGGAAG | CCAAAGGAGTGGGAAGGAC | 63.5°C | 370bp |
| **18** | TATTCAGATGAAATGTTCGCAG | AACAAGTCAAGGACACCCAG | 62°C | 362bp |
| **19** | AGAAAGTAGAGCCGTTGAGC | CCATGACACAGACACTCAAG | 63°C | 333bp |
| **20** | AACATTGAAATAGTGTGCTCC | CATCAAGCCATTCTCTATGC | 61°C | 317bp |
| **21** | ACTAAAATGCCTTCTCAGTCC | GAAAGATACAGACCAGCCAG | 62°C | 369bp |
| **22** | TCATCAGGAAAGGCTTTTGTG | CCTGTCTAGTCAGCAGTAAC | 62°C | 335bp |
| **23A** | AGTGAGTGTCAGTGTAATTCC | CTTCTCCCATAGTCGTCTCC | 63.5°C | 317bp |
| **23B** | AGCAGCAGCGAGCTTTCTAC | ACACCCAAGTCTTTGCCCAG | 65°C | 260bp |
| **23C** | TTTCGTAATAAGAGCGAGAGC | TTTGAAACGTGCATTCACACC | 62°C | 282bp |

**Table S3 : *TSC2* Primers :**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Exon** | **Forward 5΄- 3΄** | **Reverse 5΄- 3΄** | **Annealing temperature** | **Product size** |
| **1** | GTCCCCATTCCTGTTTCGTT | TCTCTGCATTTCCCTCTAGC | 63°C | 242bp |
| **2** | TGGAAAATGCAGTGGGAGTC | GCAAACCAGATCATCGGCAG | 63°C | 296bp |
| **3** | GTGGGGCTCTCAGTCACAAG | TAGACCCTGTGCCCAGCAAC | 67°C | 350bp |
| **4** | CTGCACTTCAGGGACTTCTTG | CATCCAGGTTACACTTGATCG | 64°C | 282bp |
| **5** | GACTGATGATGGGGTTTCTG | ACTGCGGAGCTGAACTTAGG | 63°C | 294bp |
| **6** | ACGTCATAGAGTGACTAGACC | CTGAGGCTCAGAGAGACCGA | 66°C | 269bp |
| **7** | TGAAGGAGGTGGGAAGGAAG | AGACAACCATTCATGGGAGAC | 64°C | 299bp |
| **8** | GTCTTTGGGAGGAGATGGTG | GTGTGTGCTTGGCAAGGGAC | 65°C | 295bp |
| **9** | CCTCGGCAACCTCACACATC | TTCCTGCCAGCTCACTGCAC | 67°C | 243bp |
| **10** | ATAAACGTGTGGTGGGCACTG | ATCTTCTGAGTGGGCCGAGC | 67°C | 337bp |
| **11** | GGCTGAGGGTGTCTCCATGC | TTCTGAGGCTCAGAAAGCTGC | 67°C | 330bp |
| **12** | CTGGGCTCTGACAGCAAACC | CTCATCGGGCATCCTGAGAC | 67°C | 298bp |
| **13** | CTCTGTGCCCTGTGTGCCTG | CACGGAGAAATAGCCCTGAGC | 68°C | 257bp |
| **14** | GGTCGGTTCCTGAGGAATTGG | GAGCTCTGGCACGCTAGCAC | 68°C | 286bp |
| **15** | AGGTGTTTGTGGTAGAAAGTG | GGAACAGACTCCAACACAAC | 63°C | 287bp |
| **16** | GTGTTTTGAAGCACGCACTCTA | GGAACCACACCTGCCGAGAC | 66°C | 316bp |
| **17** | GTGTCTGTGTTGGGATGTGG | CCCTCAGACATGCAAACAGC | 65°C | 343bp |
| **18** | GCGCTGTTTGCATGTCTGAGG | AGGCCAGACAGGGAGGGAG | 68°C | 350bp |
| **19** | CTGAGAACAGGGCTCCATAG | CAGAGGCTCGCAGTCTTTTG | 65°C | 316bp |
| **20** | CACTCCCACCACTCCGAAAG | CATCAAGCTCGCCTGTCCTG | 67°C | 338bp |
| **21** | TTCAGGGACTTGCTAAGCCTC | GCACAGGTTACACAGCCCGA | 67°C | 366bp |
| **22** | CGTGGCCTTCTCTCCTCTG | CCTTGGGGACAAAGGCTGC | 67°C | 300bp |
| **23** | CTTTGTCCCCAAGGCCTGAG | CAAAGCAAGCCCTGGCCTAC | 67°C | 257bp |
| **24** | CCTGCAGCTTGTCCCTGG | CTCACTGACTTGGAGGCAC | 65°C | 286bp |
| **25** | CTCTCCATCCTGACCCTGTG | CAGACGATGAGGTCATGCAAG | 66°C | 345bp |
| **26** | CTGTTGGGGTCTTTCCGAGC | ACAGGGAGCGTGAAACCCAG | 67°C | 359bp |
| **27** | CTGGGTTTCACGCTCCCTGT | TTAGTCCCCAGGCTGGTACG | 67°C | 277bp |
| **28** | TGACTGCACCTTCCTTTCCTC | ACACTGGGACCAGCAGCAAC | 66°C | 308bp |
| **29** | TCTGGCTGCCTGTGGCACTA | AAGAGGGCCAAGTCTGCAATC | 66°C | 415bp |
| **30** | TGAGGATTGTGGGAGGGAGC | GCAGAGAGGAGCCACATTGC | 67°C | 408bp |
| **31** | CAGCTTGTAGCTAGCACTGG | ACAGCACCTATGAGGCAGAG | 65°C | 282bp |
| **32** | TGTCCCTCTGGTCAGGAGAAG | AGAGCCCTGCCTCCCCTAAG | 68°C | 285bp |
| **33A** | CTGGGATGGAGGACAGATAG | TGACCGGGCCTTAACCTCAG | 67°C | 351bp |
| **33B** | CCTCTGTGGACCTCTCCTTC | TACTCTCTTGCCCCTGCGTG | 67°C | 317bp |
| **33C** | GTTAAGGCCCGGTCACAGTC | CAAGTGAGGCACCAGGTTCC | 67°C | 319bp |
| **34** | CGGTGGAGTGGGAGATGG | GAGCCCTCTGCAAGCCTG | 66°C | 280bp |
| **35** | GGAGCTCAGGCTTGCAGAGG | ACTTCATGCTGTAGGGGACC | 66°C | 345bp |
| **36** | CTGCTGGAATGGATGGTCTTG | GTAGCAGGACTGGATGGGAG | 66°C | 394bp |
| **37** | CTGGAGTAATCAGGAGGTGC | CGAAGCTCAGGGAGGAGG | 66°C | 318bp |
| **38** | TGCCAGAGGGGAAAGTTCAG | GGGAGACTTGGTGGTCACG | 66°C | 317bp |
| **39** | TGCAGTGCAGGAAAGGTAGG | GTGAGCTCACTATCAGGTGG  | 65°C | 281bp |
| **40** | TGGCCAAGATCGTGTCTGAC | GACAGGCAATACCGTCCAAG | 65°C | 583bp |
| **41** | TCACAGGTGCATCATAGCCG | GACAGGCAATACCGTCCAAG | 65°C | 425bp |

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Forward

Reverse



**Figure S1 :** A full four-color chromatogram showing the results of the sequencing run of Patient 1 (forward and reverse). Sequence analysis of *TSC2* : exon 34, resulting in pathogenic mutations: c.4375C>T mutation.



Forward

Reverse



**Figure S2 :** A full four-color chromatogram showing the results of the sequencing run of Patient 4 (forward and reverse). Sequence analysis of *TSC2* : exon 15, resulting in pathogenic mutations: c.1513C>T mutation.