

## **Appendix 1.**

**Table S1. Sequence variants identified in COL2A1.**

| <b>Exon Location</b> | <b>Variants Identified</b> | <b>Position (NM_001844.4)</b> | <b>Function</b>      | <b>Amino Acid Change</b> |
|----------------------|----------------------------|-------------------------------|----------------------|--------------------------|
| Exon 2               | *previously reported       | c.258C>A                      | Coding Nonsynonymous | Cys86X                   |
| Exon 7               | rs3737548                  | c.504C>A                      | Coding Synonymous    | -                        |
| Intron 11            | rs10875716                 | c.762+15G>A                   | Intronic             | -                        |
| Intron 11            | rs3216168                  | c.762+42delG                  | Intronic             | -                        |
| Intron 12            | Novel                      | c.816+215C>T                  | Intronic             | -                        |
| Intron 21            | rs10875714                 | c.1366-11C>T                  | Intronic             | -                        |
| Intron 23            | rs3829734                  | c.1528-62C>T                  | Intronic             | -                        |
| Intron 31            | rs11168338                 | c.2050-49G>T                  | Intronic             | -                        |
| Intron 33            | rs11168337                 | c.2193+101G>T                 | Intronic             | -                        |
| Intron 33            | rs3829736                  | c.2194-111G>A                 | Intronic             | -                        |
| Intron 33            | rs3829737                  | c.2194-55T>C                  | Intronic             | -                        |
| Intron 33            | rs3842680                  | c.2194-52insG                 | Intronic             | -                        |
| Intron 34            | rs2276456                  | c.2302-32T>C                  | Intronic             | -                        |
| Intron 34            | rs2276457                  | c.2302-22G>A                  | Intronic             | -                        |
| Exon 36              | rs1635553                  | c.2400T>C                     | Coding Synonymous    | -                        |
| Intron 37            | rs2276458                  | c.2464-75A>C                  | Intronic             | -                        |
| Exon 53              | rs2070739                  | c.4213G>A                     | Coding Nonsynonymous | Gly1405Ser               |
| Intron 53            | rs1635560                  | c.4317+43C>T                  | Intronic             | -                        |

\* Donoso LA, Edwards AO, Frost AT, Ritter R, Ahmad NN, Vrabec T, Rogers J, Meyer D. Identification of a stop codon mutation in exon 2 of the collagen 2A1 gene in a large stickler syndrome family. American journal of ophthalmology 2002; 134(5):720-7.

**Table S2. Primers for VCAN Sequencing**

| Exon         | Forward Primer         | Reverse Primer            | Product size (base pairs) |
|--------------|------------------------|---------------------------|---------------------------|
| Exon-1       | GTGAATGAACCCTCCTC      | TTTCTTAACTTCGCCTTAC       | 517                       |
| Exon-2       | ACCCTTATTACATAACAATGC  | ATCAATCTTTTATTCCAGTG      | 270                       |
| Exon-3       | CAAACACTATTATAAAGGCTGC | CAAACAACAGTTACTTCTGAG     | 562                       |
| Exons-4/5    | CTAAGTTGATACATTGCTCC   | ACAAGCACGAAGACTTG         | 569                       |
| Exon-6.1     | AAAGTATTACATGCTCCTCC   | TGTGGTTTTAACCATATCAC      | 578                       |
| Exon-6.2     | CATTGATGTTCAACTAGCC    | AGTAAACCTAACTAAAGATACTCTG | 581                       |
| Exon-7.1     | TCCCTACAAAATACTCAGG    | ATTTTCTTTTCAGTTTTGG       | 657                       |
| Exon-7.2     | CCTTTGGTAACATCTATGG    | GTATCTGTTCTCATCTCTGG      | 605                       |
| Exon-7.3     | AGAATTGTTTCCTTATTCTG   | AATAAGAGTAAATTCATCTGC     | 613                       |
| Exon-7.4     | TACAGAACCTTCAGCCTC     | AGTTTCTGGAGAAATAGTCG      | 610                       |
| Exon-7.5     | TTAGTACCTTCTGTTCCATC   | AATAATCCTGAGCCTAAATC      | 599                       |
| Exon-7.6     | TTTACATCATCTTTGAGTCC   | TCTAATAGCAGCATTGG         | 658                       |
| Exon 8.1 UTR | GGAATTTGTCTTGGTAGTTC   | TCGAAAAGTATGTATCATCAG     | 635                       |
| Exon 8.2 UTR | TTTGCTGATGTATTTCTG     | AAAATTGAGGCCATATC         | 584                       |
| Exon 8.2 UTR | GCTCTAGAACAGATTATAAAGG | TGGATCTGTTTCTTCACTAC      | 644                       |
| Exon-8.1     | GATTTGAATCCTTCTTTGTG   | CTCATGGAATGGGACTG         | 633                       |
| Exon-8.2     | TTACATGGAAGCCTGAG      | GTGATTATCCTGCTAGTGTC      | 598                       |
| Exon-8.3     | AATTACAGAAGGCTCTGG     | CCCTGCTCCATAAAGAC         | 597                       |
| Exon-8.4     | TACATTAGAAAATTTGGGG    | CTACTGGGTCTTCTGAG         | 582                       |
| Exon-8.5     | AGAAGAAACGGTAATGATG    | TTCAGTAAATGTCTGTGAATC     | 592                       |
| Exon-8.6     | ATTGAAAGTGAAACAACATC   | CCACATTTTCCATTCTG         | 595                       |
| Exon-8.7     | AAGTGGAACAAATCAATAAC   | ATCCATCAGCAGTAACAG        | 621                       |
| Exon-8.8     | GAAGTGGATATTGTTGATTC   | TCAGTTAAAGAAAGGTTGAC      | 596                       |
| Exon-8.9     | TGGAATGCAAACAGATATAG   | AGTAGCAACTTAGATAGGG       | 580                       |
| Exon-8.10    | AAACATGCTGGTCCCTC      | AAACATCTTGATTGCTTCTC      | 582                       |
| Exon-8.11    | GAAGATGATGGTAAACCTG    | AATAATATGAAACTCTCCATTG    | 680                       |
| Exon-9       | CTAAATTGCTATGGTAAAGC   | TTGAAATCTCTGATTGATTC      | 322                       |
| Exon-10      | AATTTAACTGGCTGTCTTG    | GAAATGTGAATTTCCACTG       | 318                       |
| Exon-11      | GACCAAATTTTATGAATCAG   | TTAAGTAATTCACAGATGAGG     | 433                       |
| Exon-12      | AATCCAGCCAGTAAAGAG     | AGTCTAATGCACCCTCTG        | 287                       |
| Exon-13      | GAAATTGTTGAGTCTATTCC   | GTCTCAAACAATGAATTTG       | 342                       |
| Exon-14      | TTGGTACCATAAAGAAAGAG   | CACACAATATTACTTGCTCC      | 387                       |
| Exon-15.1    | CTAGACACCTTCATTTTACG   | AATCATCTTATTTACATGGC      | 576                       |
| Exon-15.2    | TTTAGTTTTCTATTTGCCTC   | TATGAAATGCATTGATCG        | 593                       |
| Exon-15.3    | AGGCCTGAATGGAGGACTTT   | ACAGGAAGAAATGCCACAC       | 587                       |
| Exon-15.4    | TCCTCACACAATTTGGAATCA  | TCCTTCTAAGCCAAAGGAGGT     | 475                       |

Primer designs for VCAN: Primers cover all coding and UTR (untranslated) regions of the gene including intron/exon boundaries. Primers were designed using Exon Primer (Helmholtz Center Munich) and Primer3 (<http://ihg2.helmholtz-muenchen.de/ihg/ExonPrimer.html>).

**Table S3. Primers for COL2A1 Sequencing.**

| Exon        | Forward Primer         | Reverse Primer        | Product size (base pairs) |
|-------------|------------------------|-----------------------|---------------------------|
| Exon-1      | CTTGGTCTAGGGCTCTC      | ACTTTGCAAGTTCAGTATTC  | 904                       |
| Exon-2      | GCATTTGAATTCTGTCAAG    | ACCAGCATCTATGGGAG     | 392                       |
| Exons-3-5   | ATGAGTGTTTGAGTGACAAG   | AGAACATGGTAAGATGACAG  | 579                       |
| Exons-6/7   | GGTAAGAGAGGGGAGAAATC   | CAGGTAAGTGCAAGCAG     | 505                       |
| Exon-8      | CTCAGTAGAGTTCTTTGTATCC | TATCTGTAAAGCAGAGGTTG  | 266                       |
| Exons-9/10  | TGCCACAGAGTAACTTC      | ACTGTGGGAAAGAGCTG     | 434                       |
| Exon-11     | CCAAACTACAATGTCAAAG    | AGTCTTGCTCCTCAAGATAC  | 259                       |
| Exons-12/13 | GAGTCTGAAGGAGGTGAG     | AAGAGAAAATGTTCAATCAG  | 745                       |
| Exons-14/15 | GAGAGAAAAGCACTTTGG     | ACAGAATATGAACTTTGCAC  | 585                       |
| Exon-16     | ATCCTCCCTCTCCTCTG      | TTGTTTCGAGGGTCAAG     | 242                       |
| Exon-17     | GTCCTTCGTTTTCTGTAAG    | GTTGCACCCAATACACC     | 226                       |
| Exon-18     | AGGAGTGTGAGGTGTCG      | AGCAGGTGGTTGTTGAG     | 232                       |
| Exons-19/20 | GTGCATGTGCATAATTTAG    | CATGAGAAGAAGGGAGG     | 641                       |
| Exons-21/22 | AGATGGAATTCCTGGAG      | CATAATCTGAAAGGACCC    | 593                       |
| Exon-23     | GCTACTTCCTTCATACTCTG   | GACTTGACCAGAACACG     | 279                       |
| Exons-24-26 | ACCTCTGAGAATCCTGC      | AAAATTCACAGTACTTCAGG  | 678                       |
| Exon-27     | TCAATCCTAGATGCTGAG     | ACTCATCACTGTCCCTG     | 341                       |
| Exon-28     | GATACTTTGCTTTATCTTGG   | ACAGAGATCAACACTCAATAC | 300                       |
| Exons-29/30 | AGTACCTTTGCCAATC       | GACCCAAAGAAAGGAAG     | 705                       |
| Exons-31/32 | GTCTGCCCTATACTGTGC     | GAAGGTCCAGGGAGAAG     | 526                       |
| Exons-33/34 | GTCCTATGCTCCTGCTC      | ACAGAAACCTTCATCACC    | 649                       |
| Exons-35/36 | ACCACAGCAAATTCCTC      | AGGGTGACAGTGGTGAG     | 616                       |
| Exons-37/38 | TGCACAGTAACACAGGC      | GGTTCTATTAGTATGGAGGC  | 757                       |
| Exon-39     | CTAATAGAACCATCATGTCC   | TCCTTCTACCAACATGG     | 363                       |
| Exon-40     | TAGTGCCAAGAAAGCTG      | GTTTCCACAGTCAGCAC     | 416                       |
| Exon-41     | CTTCCATACCAGGCTC       | CTCAGAGGAGTGAAGGC     | 260                       |
| Exons-42/43 | AGAGGAAACTGCTGTAC      | CAAGGGACAGTCTGAG      | 652                       |
| Exon-44     | GCTTCTACCTCCCTCAG      | ATTGTACCTAGGCTTTCAG   | 378                       |
| Exons-45/46 | TCAAAGTGTGAGTGAGTTG    | CTCCTTAGTCCAGAGACTG   | 545                       |
| Exons-47/48 | CAGTCTCTGGACTAAGGAG    | CTAGAAACTGCTTAGGGTG   | 546                       |
| Exon-49     | GAGGAGGAAGTGAAAG       | ATTACTGAGTGAGGACCC    | 293                       |
| Exon-50     | GACTGAGCATGTGAAGAAC    | CTAAAAGAGGCCCTGAG     | 322                       |
| Exon-51     | CTAGTACATTCTAGCAAATGG  | TTCTCTGCTGTGAAATAAG   | 521                       |
| Exon-52     | TCTCTGTCTTTTCAGTCAG    | AGGTGCTAAAAGCTTCC     | 364                       |
| Exon-53     | CCTGAACCATGAACTC       | ACTTTAGGACCTGACAGC    | 430                       |
| Exon-54.1   | TGTGGTTCAACCTTGTG      | ACCCGAAGGTCTTACAG     | 506                       |
| Exon-54.2   | TGACCTGATGTCCATTC      | TCAACAGAAGAAGTCCTTAG  | 437                       |

Primer designs for COL2A1: Primers cover all coding and UTR (untranslated) regions of the gene including intron/exon boundaries. Primers were designed using Exon Primer (Helmholtz Center

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Munich) and Primer3 (<http://ihg2.helmholtz-muenchen.de/ihg/ExonPrimer.html>).

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**Table S4. Primers for COL2A1 cDNA expression analysis**

| cDNA Tissue Expression Primers | Primer Sequence      | Product Size (base pairs) |
|--------------------------------|----------------------|---------------------------|
| COL2A1-exon1- Forward          | CGCTGTCCTTCGGTGCA    | 510 or 303*               |
| COL2A1-exon8- Reverse          | TCAAATCCTCCAGCCATCTG |                           |

\*Depending on the presence or absence of the exon 2 in the amplified cDNA sequence, the product can be 510 or 303 bp long.

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**Figure S1.** COL2A1 cDNA tissue expression analysis.

