NHS Lothian
South East Scotland Genetic Laboratories

CONNECTIVE TISSUE DISORDERS GENE PANEL - TECHNICAL INFORMATION

Design: The connective tissue disorders gene panel was designed as part of a custom probe set from Twist Bioscience (TE-97534584) to cover 66 genes associated with connective tissue disorders, including Ehlers-Danlos syndrome. This panel provides horizontal coverage of >99% for the coding regions and flanking intronic sequences (+/- 15bp) for the 66 genes listed below. Due to phenotypic overlap, additional genes associated with familial thoracic aortic aneurysm and dissection (TAAD) were added to the custom probe design. Analysis of these genes can be requested alongside requests for connective tissue disorders.

Method: Library preparation and target enrichment was performed using the custom designed probe set (Twist Bioscience) and DNA Prep with Enrichment (Illumina). Sequencing was performed using a 150bp paired-end sequencing kit on a MiSeq (Illumina). All stages of the workflow were performed according to the manufacturer's instructions.

Coverage criteria: For each sample reported, >95% of the target regions were covered to a minimum depth of 20 reads (20X). Pseudogene regions, such as those associated with TNXB and NOTCH2, may interfere with the sequence alignment process which may increase the risk of false negative results. DNA is forwarded to the Sheffield genetics laboratory for extended testing if one pathogenic/likely pathogenic TNXB sequence variant is detected. Specific details of coverage and depth for individual tests are available from the laboratory on request.

Variant identification and interpretation: Sequence data were mapped and variants identified using GenomeAnalysisToolKit (GATK) and NextGENe (Softgenetics) with hg19 (GRCh37) human genome as the reference. Variants identified were subsequently classified according to recent ACGS Best Practice Guidelines for Variant Classification using all available evidence. Any clinically significant variants were confirmed by Sanger sequencing.

Variant reporting: Variants were reported according to HGVS guidelines using the MANE Select transcript, unless otherwise stated. Variants categorised as non-pathogenic, likely non-pathogenic or low evidence variants of uncertain clinical significance were not included in the clinical report. Details of these variants are available from the laboratory on request.

Genes included:

ABCC6, ACTA2, ADAMTS2, AEBP1, ALDH18A1, ALPL, ATP6V0A2, ATP6V1A, ATP7A, B3GALT6, B4GALT7, BGN, BMP1, C1R, C1S, CBS, CHST14, COL12A1, COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, COL6A1, COL6A2, COL6A3, CRTAP, DSE, EFEMP1, EFEMP2, ELN, ENPP1, FBLN5, FBN1, FBN2, FKBP10, FKBP14, FLNA, GORAB, IFITM5, LOX, LRP5, LTBP2, LTBP4, MYLK, NOTCH1, NOTCH2, OLA1, PLOD1, PLOD2, PPIB, PRDM5, PYCR1, RIN2, ROBO3, SKI, SLC2A10, SLC39A13, SMAD2, SMAD3, SP7, TGFB2, TGFB3, TGFBR1, TGFBR2, TNXB, ZNF469

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Additional TAAD genes (available on request):
ABL1, ARIH1, ASPH, FOXE3, IPO8, MFAP5, MYH11, PMEPA1, PRKG1, SECISBP2, SMAD4, THSD4

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