SUPPLEMENTAL MATERIAL
Supplemental methods

Data access
The data that support the findings of this study are available from the corresponding author upon reasonable request. The filtered CNV calls have been made publicly available (10.25375/uct.11953749). Data from consortia were accessed subject to the applicable data-sharing agreements.

Patient recruitment
CHD patients were prospectively recruited into the PROTEA clinical registry (PROTEA Aim 1) via convenience sampling by clinical members of the Children’s Heart Disease Research Unit, University of Cape Town. Recruitment was performed at Red Cross War Memorial Children’s Hospital or Groote Schuur Hospital, Cape Town, South Africa. No population-based case finding was performed. To minimize selection bias, recruitment to the clinical registry was systematic and all patients referred to the Western Cape cardiology service were screened via folder review (for prevalent cases) and echocardiogram (for all incident and certain prevalent cases). All patients found to have structural CHD and fitting the inclusion and exclusion criteria were invited to participate in Aim 1. All patients with structural CHD as confirmed by echocardiogram were considered eligible for inclusion in PROTEA Aim 1, while those with isolated conduction or functional abnormalities, patent foramen ovale, peripheral pulmonary stenosis or patent ductus arteriosus in premature infants were excluded. Participants were selected from the clinical registry for genetic analysis (PROTEA Aim 2) via both purposive and convenience sampling. Participants scheduled for cardiac catheterization and cardiac surgery were preferentially selected, so that phlebotomy could be performed under anesthesia and any associated pain or distress minimized.

This PROTEA sub-study analysis consisted of 90 CHD patients for whom DNA was available at the time of investigation, and who met the inclusion and exclusion criteria above. In addition, patients with previously recognized specific genetic diagnoses or known chromosomal aberrations, that had been detected by standard techniques, were excluded. Patients were recruited at all ages and were eligible for the study if they presented with an isolated heart defect or CHD with additional extra-cardiac anomalies (ECAs). The Human Research Ethics Committee of the University of Cape Town approved all procedures (reference no. 389/2019). Written informed consent to participate in this study was obtained from all participants, or from
their legal guardians or next of kin where appropriate, as well as a peripheral blood sample for DNA extraction.

Genotyping and filtering
Genotyping was performed using the Affymetrix CytoScan HD platform (Affymetrix, Santa Clara, CA USA). CMA data that did not pass quality control criteria (mean absolute pairwise difference \(\leq 0.25\), SNP quality control \(\geq 15.0\), waviness-standard deviation \(\leq 0.12\)) were excluded from further analysis. Germline CNVs were called using Affymetrix Power Tools software v2.11.0 and based on human genome build hg19. CNVs with > 70% overlap with centromeres, telomeres or regions of segmental duplication were excluded, as were CNVs of low quality, as assessed using Affymetrix Chromosome Analysis Suit (confidence < 0.85, marker count < 50, mean marker distance < 15 kb). CNVs > 100 kb in size were extracted from the data and assessed, as described below, for their frequencies and overlap with a CHD gene panel. Mosaic CNVs were not investigated in this study.

CNV control populations
Two published control datasets were used: UK Biobank healthy controls (n = 472,378), and a healthy pediatric African population from Tanzania (n = 3,463). The UK Biobank was used as described previously. CNVs occurring in < 0.01 of either of the control populations were considered rare and analyzed further.

CHD gene list
Large, rare CNVs were then compared to a list of CHD-associated genes (Supplemental Table II). To identify CNVs occurring in gene regions known to be associated with CHD, all CNVs that overlapped with one or more CHD genes listed in the Genomics England PanelApp gene panel for non-syndromic CHD were investigated (available at https://panelapp.genomicsengland.co.uk/panels/212/). The Genomics England PanelApp is a publicly available crowdsourcing tool that represents a consensus of causative genes for many diseases. The genes in each panel are rated according to a traffic-light system, in which green represents genes with a high level of evidence for the gene-disease association, amber represents those with a moderate level of evidence, and red represents genes with minimal evidence. A list of the CHD genes in the panel alongside their genetic rating is presented in Supplemental Table II. These classifications are used to guide clinical interpretation, so all classifications (green, amber, red) were considered in this investigation to maximize discovery.
The PanelApp list was curated in-house with additional known and suspected genes for syndromic and non-syndromic CHD, that have been associated with CHD in at least one published paper. CNVs overlapping any of these genes, from PanelApp or the in-house list, were prioritized for further investigation and assessed using ACMG criteria for pathogenicity.19

Identification of candidate CHD genes
Once CNVs overlapping known CHD-associated genes were analyzed, candidate CHD genes were identified by filtering the remaining genes using ExAC probability of loss-of-function intolerance (pLI) ≥ 0.8.54 To further refine this list, genes were considered candidates for CHD if expression in the embryonic mouse heart was reported in the Gene Expression Database (http://www.informatics.jax.org/expression), and if they had a recorded cardiac phenotype or caused embryonic lethality when mutated in mice, according to the Mouse Genome Informatics database (http://www.informatics.jax.org/).
### Supplemental Tables

Supplemental Table I. CNVs of interest identified in the cohort by chromosomal microarray analysis

| Patient ID | Chromosomal coordinates | Event                  | Size (kb) | Encompassed genes* |
|------------|--------------------------|------------------------|-----------|---------------------|
| 2_00013    | 5:176598618-176727216    | Partial gene duplication | 599.82    | **NSD1**, **RPL21P60**, **PRMT1P1** |
| 2_00013    | 5:177356690-180679497    | Duplication            | 3,322.81  | LOC100128340, **SUDS3P1**, **PROP1**, **FAM153C**, LOC100288656, **RPL19P9**, **N4BP3**, **RMND5B**, **NHP2**, LOC645853, **GMCL1P1**, **HNRPAB**, **PHYKPL**, **COL23A1**, **MRPL50P3**, **RN7SL646P**, **CLK4**, **RN7SKP70**, **VN2R2P**, ZNF354A, **AACSP1**, LOC100129457, **ZNF354B**, **RNU1-39P**, **ZFP2**, LOC1004222593, **PIGFP1**, ZNF454, **GRM6**, LOC100288803, ZNF879, ZNF354C, **ADAMTS2**, LOC100128622, LOC100289470, **RUFY1**, **PRDX2P3**, LOC101928445, **HNRNPH1**, C5orf60, LOC100502572, LOC105377763, **CBY3**, **HMGB3P22**, **CANX**, **MAML1**, LOC100884169, **LTCS4**, **MGAT4B**, **MIR1229**, **SQSTM1**, **MRNIP**, **RN7SKP150**, LOC100996419, **TBC1D9B**, **RPS15AP18**, **RNF130**, **MIR340**, LOC646058, **RASGEF1C**, **RPS8P7**, **MAPK9**, LOC100419721, **GFPT2**, **RNU6-525P**, **CNOT6**, **LOC100329129**, **SCGB3A1**, **FLT4**, LOC100420514, **OR2A1P1**, **RNU1-17P**, **OR2Y1**, **MGAT1**, **HEIH**, **LINC00847**, **ZFP62**, **BTNL8**, **RPS29P12**, LOC100128762, LOC100419853, LOC646227, **BTNL3**, **RNU6-1035P**, **BTNL9**, **MIR8089**, **RPL13P10**, **TRNAV2**, **TRNAL34**, **FOXO1B**, **TRNAL24**, **TRNAV9**, LOC729707, **OR2V1**, **OR2V2**, **TRNAV5**, **TRL-AAG7-1**, **TRNAV3**, **TRNAV4**, **TRNAL4**, **TRNAL32**, **TRNAV10**, **TRNAP3**, **TRNAP2**, **TRNAP3** |
| Start | End | Type | Length | Genes |
|-------|-----|------|--------|-------|
| 2_00018 | 11:61206220-63379913 | Deletion | 2,173.69 | SDHAF2, RN7SL23P, PPP1R32, MIR4488, LRRC10B, SYT7, LOC101927495, RPLP0P2, DAGLA, MYRF, DKFZP434K028, TMEM258, MIR611, FEN1, FADS1, MIR1908, FADS2, FADS3, MIR6746, RAB3L1, RNU6-1243P, BEST1, FTH1, RPS2P37, LOC100507521, INCENP, EEF1DP8, SCGB1D1, SCGB2A1, SCGB1D2, SCGB2A2, SCGB1D4, NPM1P35, ASRGL1, RCC2P6, SCGB1A1, AHNAK, EEF1G, MIR6747, TUT1, MTA2, EML3, ROM1, B3GAT3, GANAB, INT5S, C11orf98, LBHD1, METTL12, SNORA57, UQCC3, UBXN1, LRRN4CL, BSCL2, HNRNPUL2-BSCL2, GNG3, HNRNPUL2, TTC9C, ZBTB3, POLR2G, TAF6L, TMEM179B, MIR6748, TMEM223, NXF1, MIR6514, STX5, RNU6-118P, LOC105369332, WDR74, RNU2-2P, SNHG1, SNORD22, SNORD31, SNORD30, SNORD29, SNORD28, SNORD27, SNORD26, SNORD25, SLC3A2, CHRM1, RN7SL259P, SLC22A6, SLC22A8, LOC100127954, LOC644436, SLC22A24, RPL29P22, SLC22A25, CCND2P1, SLC22A10, SLC22A9, HRASLS5, LOC100533643, LGALS12, TMSB4XP5, RARRES3, HRASLS2, PLA2G16 |
| 2_00025 | 22:18916828-19784699 | Deletion | 877.87 | PRODH, DGCR5, LOC100287576, LOC100506454, DGCR9, DGCR10, DGCR2, DGCR11, LOC100129262, TSSK1A, DGCR14, TSSK2, GSC2, LINC01311, SLC25A1, CLTCL1, RPL34P35, DVL1P1, KRT18P62, HIRA, RN7SL168P, MRPL40, C22orf39, |
| 2_00025 | 22:19790229-21083936 | Deletion | 1.293:71 | LOC100506503, UFD1L, CDC45, CLDN5, LINC00895, LOC100129254, LOC100420103, SEPT5, SEPT5-GP1BB, GP1BB, **TBX1**, GNB1L, RPL7AP70
| 2_00025 | 22:21050078-21800471 | Deletion | 373.28 | GNB1L, C22orf29, TXNRD2, RPL8P5, COMT, MIR4761, ARVCF, TANGO2, MIR185, DGCR8, MIR3618, MIR1306, TRMT2A, MIR6816, RANBP1, ZDHHC8, CCDC188, LOC284865, LINC00896, RTN4R, MIR1286, LOC440792, DGCR6L, LOC729444, FAM230A, GGTLC3, TMEM191B, PI4KAP1, LOC100506613, RN7SKP131, RIMBP3, SUSD2P2, CA15P2, PPP1R26P2, GGTLT5P, LINC01660, PPP1R26P3, LOC101927859, USP41, ZNF74, RNU6-225P, SCARF2, KLHL22, LOC100420177, RNY1P9, RN7SL812P, KRT18P5, MED15, SMPD4P1, IGLL4P, LOC100421121, SLC9A3P2, ABHD17AP4, POM121L4P, BCRP5, TMEM191A, PI4KA
| 2_00036 | 11:66832101-66967055 | Duplication | 134.95 | RHOD, KDM2A
| 2_00039 | 6:15283550-15522252 | Partial gene duplication | 752.77 | JARID2, RNU6-522P, RNU6-645P
| 2_00097 | 5:180035579-180206228 | Partial gene duplication | 170.65 | **FLT4**, LOC100420514, OR2A11P, RNU1-17P, OR2Y1
| 2_00132 | 3:120032413-124764110 | Duplication | 4,731.70 |
| --- | --- | --- | --- |
| LLRC58, FSTL1, MIR198, RPL34P9, NDUF54, HGD, RABL3, MTCO1P29, MTCO2P29, GTF2E1, NAP1L1P3, LINC02049, STXB5P5L, MIR5682, LOC100419755, LOC100422044, POLQ, RPL7AP11, ARGFX, FBXO40, HCLS1, RN7SL172P, RNU4-62P, GOLGB1, IQCB1, EAF2, SLC15A2, ILDR1, CD86, CASR, HNRNPA1P23, CTA, CCDC58, FAM162A, WDR5B, LOC102723582, KPNAP1, PARP9, DTX3L, PARP15, LOC100421636, EIF4BP8, PARP14, HSPBAP1, DIRC2, LINC02035, SEMA5B, PDIA5, MIR7110, SEC22A, ADCY5, HACD2, MYLK-AS1, MYLK, MYLK-AS2, CCDC14, ROPN1, KALRN, MIR5002, RPL7P15, MIR6083, RNU6-143P, LOC100421444, UMP5, MIR544B, RPS26P22, ITGB5, ENO1P3, MUC13, HEG1 |

| 2_00162 | 22:21092382-21465662 | Deletion | 750.39 |
| --- | --- | --- | --- |
| PI4KA, SERPIND1, SNAP29, CRKL, LINC01637, AIFM3, LZTR1, THAP7, THAP7-AS1, TUBA3FP, P2RX6, SLC7A4, MIR649, P2RX6P, LLRC58, TUBA3GP, BCRP2 |

| 2_00168 | 8:8093066-11877967 | Deletion | 3,784.90 |
| --- | --- | --- | --- |
| FAM86B3P, ALG11L13P, PRAG1, RN7SL178P, CLDN23, MFHAS1, RNU6-682P, MRPS18CP2, RPL10P19, ER11, MIR4660, PPP1R3B, LOC101929128, RNU6-1151P, LOC157273, RNU6-526P, LOC100420404, TNKS, MIR597, LINC00599, MIR124-1, MSRA, LOC100420053, LINC-0001, PRSS52P, RNU6-729P, PRSS55, RPL1L1, MIR4286, C8orf74, RNAS5P252, SOX7, PINX1, MIR1322, LOC101929229, XKR6, MIR598, LOC10192926, RPL17P29, RPL19P13, MTMR9, LOC100505734, SLC35G5, TDH, FAM167A-AS1, RN7SL293P, RNU6-1084P, FAM167A, BLK, LINC00208, GATA4, SNORA99, C8orf49, NEIL2, SUB1P1, FDFT1, CTSB, OR7E158P, OR7E161P, DEFB136, DEFB135, DEFB134, OR7E160P, LOC100421446 |
| 2_00192 | 15:22770422-23282799 | Deletion | 512.38 | ELMO2P1, TUBGCP5, CYFIP1, NIPA2, NIPA1, LOC283683, LOC729900, LOC100132817, PDCD6IPP1, LOC100133165, LOC101269108, WHAMMP3, GOLGA8IP, RN7SL495P, HERC2P2 |

* Genes in bold are in the CHD gene list (Supplemental Table II), while genes that are underlined were identified as CHD candidate genes.
Supplemental Table II. List of CHD-associated genes used in filtering of CMA data

| Gene symbol | Phenotype                                         | Source                      |
|-------------|---------------------------------------------------|-----------------------------|
| ABL1        | CHD and skeletal malformations syndrome           | PanelApp (green)            |
| ACTC1       | ASD                                               | PanelApp (amber)            |
| ACVR1       | Fibrodyplasia ossificans progressiva              | In-house gene list          |
| ACVR2B      | Heterotaxy                                        | PanelApp (green)            |
| ADAMTS10    | Weill-Marchesani syndrome                         | In-house gene list          |
| ALDH1A2     | TOF                                               | PanelApp (red)              |
| ANKRD1      | DCM, total anomalous pulmonary venous return      | In-house gene list          |
| ANKRD11     | KBG syndrome                                      | In-house gene list          |
| ARID1A      | Coffin-Siris syndrome                             | In-house gene list          |
| ARID1B      | Coffin-Siris syndrome                             | In-house gene list          |
| ATP7A       | Menkes disease                                    | In-house gene list          |
| B3GAT3      | Multiple joint dislocations, short stature, craniofacial dysmorphism, with or without CHD | In-house gene list          |
| BCOR        | Syndromic microphthalmia                          | In-house gene list          |
| BMPR2       | Familial pulmonary hypertension                    | In-house gene list          |
| BRAF        | LEOPARD syndrome                                  | In-house gene list          |
| BVES        | Limb-girdle muscular dystrophy                    | In-house gene list          |
| CACNA1C     | Long QT syndrome, Brugada syndrome                 | In-house gene list          |
| CDK13       | CHD, dysmorphic facial features and intellectual development disorder | In-house gene list          |
| CDKL5       | Encephalopathy                                    | In-house gene list          |
| CFAP53      | Heterotaxy                                        | PanelApp (green)            |
| CFC1        | Heterotaxy                                        | PanelApp (green)            |
| CHD4        | Sifrim-Hitz-Weiss syndrome                         | In-house gene list          |
| CHD7        | CHARGE syndrome                                   | In-house gene list          |
| CHUK        | Cocoon syndrome                                   | In-house gene list          |
| CITED2      | ASD, VSD                                          | PanelApp (red)              |
| COX7B       | Linear skin defects with multiple congenital anomalies | In-house gene list          |
| CREBBP      | Rubinstein-Taybi syndrome                          | In-house gene list          |
| CRELD1      | AVSD with heterotaxy                               | PanelApp (amber)            |
| CRKL        | TOF                                               | PanelApp (red)              |
| DDX11       | Warsaw breakage syndrome                           | In-house gene list          |
| DDX59       | Orofaciodigital syndrome                           | In-house gene list          |
| Gene   | Phenotype                                                                 | PanelApp Status |
|--------|---------------------------------------------------------------------------|-----------------|
| DISP1  | TOF                                                                       | PanelApp (red)  |
| DLL4   | Adams-Oliver syndrome                                                     | In-house gene list |
| DOCK6  | Adams-Oliver syndrome                                                     | In-house gene list |
| EFTUD2 | Mandibulofacial dysostosis                                                | In-house gene list |
| EHMT1  | Kleefstra syndrome                                                        | In-house gene list |
| ELN    | Supravalvular aortic stenosis                                             |                  |
| EVC    | Ellis-van Creveld syndrome                                                |                  |
| EVC2   | Ellis-van Creveld syndrome                                                |                  |
| EYA1   | Branchiootic syndrome                                                    |                  |
| FANCL  | Fanconi anemia                                                            |                  |
| FBN1   | Marfan syndrome, MASS syndrome, geleophysic dysplasia                     |                  |
| FGFR2  | Apert syndrome                                                            |                  |
| FLNA   | Valvular dysplasia                                                        | PanelApp (green) |
| FLT4   | TOF                                                                       | PanelApp (red)  |
| FOXA2  | None                                                                      |                  |
| FOXC1  | Axenfeld-Rieger syndrome                                                  |                  |
| FOXC2  | Lymphedema-distichiasis syndrome                                          |                  |
| FOXH1  | VSD, TGA                                                                  | PanelApp (red)  |
| FOXL1  | HLHS                                                                      | PanelApp (red)  |
| GATA3  | Hypoparathyroidism, sensorineural deafness, and renal dysplasia           |                  |
| GATA4  | ASD, AVSD, TOF, VSD                                                      | PanelApp (green) |
| GATA5  | TOF                                                                       | PanelApp (amber) |
| GATA6  | TOF                                                                       | PanelApp (green) |
| GDF1   | TOF, DORV, TGA, heterotaxy                                               | PanelApp (green) |
| GJA1   | HLHS                                                                      | PanelApp (red)  |
| GJA5   | Familial atrial fibrillation                                              |                  |
| GPC3   | Simpson-Golabi-Behmel syndrome                                            |                  |
| GPC5   | TOF                                                                       |                  |
| HAND1  | HLHS                                                                      |                  |
| HAND2  | TOF                                                                       |                  |
| HEY2   | AVSD, tricuspid atresia, aortic aneurysm                                  |                  |
| HIBCH  | 3-hydroxyisobutryl-CoA hydrolase deficiency                               |                  |
| HOXA1  | Bosley-Salih-Alorainy syndrome                                            |                  |
| Gene  | Description                                      | List                  |
|-------|--------------------------------------------------|-----------------------|
| HRAS  | Costello syndrome                               | In-house gene list    |
| INVS  | Infantine nephronophthisis                       | In-house gene list    |
| IRX4  | VSD                                             | PanelApp (red)        |
| JAG1  | TOF, Alagille syndrome                           | PanelApp (green)      |
| KANSL1| Koolen-De Vries syndrome                         | In-house gene list    |
| KAT6A | Arboleda-Tham syndrome                           | In-house gene list    |
| KAT6B | Genitopatellar syndrome, SBBYSS syndrome         | In-house gene list    |
| KCNH2 | Long QT syndrome                                 | In-house gene list    |
| KDM6A | Kabuki syndrome                                  | In-house gene list    |
| KDR   | Capillary infantile hemangioma                   | In-house gene list    |
| KMT2A | Wiedemann-Steiner syndrome                        | In-house gene list    |
| KMT2D | Kabuki syndrome                                  | In-house gene list    |
| KRAS  | Noonan syndrome, craniofaciocutaneous syndrome   | In-house gene list    |
| LEFTY2| Heterotaxy                                       | PanelApp (amber)      |
| MAP2K1| Cardiofaciocutaneous syndrome                    | In-house gene list    |
| MAP2K2| Cardiofaciocutaneous syndrome                    | In-house gene list    |
| MED13L| TGA                                             | PanelApp (red)        |
| MEIS2 | Cleft palate, cardiac defects and mental retardation | In-house gene list |
| MESP1 | TOF, VSD                                        | In-house gene list    |
| MMP21 | Heterotaxy                                       | PanelApp (green)      |
| MYBPC3| LVNC, HCM, DCM                                   | In-house gene list    |
| MYH11 | Familial thoracic aortic aneurysm                | In-house gene list    |
| MYH6  | ASD, Shone complex                                | PanelApp (green)      |
| MYH7  | LVNC, HCM, DCM                                   | In-house gene list    |
| MYOM2 | TOF                                             | PanelApp (red)        |
| NFATC1| TOF                                             | PanelApp (red)        |
| NHS   | Nance-Horan syndrome                             | In-house gene list    |
| NIPBL | Cornelia de Lange syndrome                       | In-house gene list    |
| NKX2-5| ASD, VSD, TOF, HLHS, atrioventricular conduction block, atrioventricular valve defects | PanelApp (green) |
| NKX2-6| TrA                                             | PanelApp (red)        |
| NODAL | Heterotaxy                                       | PanelApp (green)      |
| NOTCH1| Aortic valve disease                             | PanelApp (amber)      |
| NOTCH2| Alagille syndrome                                | PanelApp (green)      |
| Gene   | Description                                                                 | List                |
|--------|-----------------------------------------------------------------------------|---------------------|
| NPHP3  | Meckel syndrome, nephronophthisis, renal-hepatic-pancreatic dysplasia        | In-house gene list  |
| NPHP4  | Nephronophthisis                                                            | In-house gene list  |
| NR2F2  | Multiple CHDs                                                               | PanelApp (green)    |
| NRAS   | Noonan syndrome                                                             | In-house gene list  |
| NRPS   | TOF                                                                         | In-house gene list  |
| NSD1   | Sotos syndrome                                                              | In-house gene list  |
| PEX7   | Peroxisome biogenesis disorder                                              | In-house gene list  |
| PITX2  | Axenfeld-Rieger syndrome                                                    | In-house gene list  |
| PLXNA2 | TOF                                                                         | In-house gene list  |
| PLXND1 | TrA                                                                         | PanelApp (red)      |
| PQBP1  | Renpenning syndrome                                                         | In-house gene list  |
| PRDM6  | PDA                                                                         | In-house gene list  |
| PRKD1  | CHDs and ectodermal dysplasia                                               | In-house gene list  |
| PTPN11 | Noonan syndrome, LEOPARD syndrome                                           | In-house gene list  |
| RAB23  | Carpenter syndrome                                                          | In-house gene list  |
| RAD21  | Cornelia de Lange syndrome                                                  | In-house gene list  |
| RAF1   | Noonan syndrome, LEOPARD syndrome                                           | In-house gene list  |
| RA1    | Smith-Magenis syndrome                                                      | In-house gene list  |
| RBM8A  | Thrombocytopenia-absent radius syndrome                                     | In-house gene list  |
| RIT1   | Noonan syndrome                                                             | In-house gene list  |
| RPL11  | Diamond-Blackfan anaemia                                                    | In-house gene list  |
| RPSA   | Congenital asplenia                                                         | PanelApp (red)      |
| SALL1  | Townes-Brocks syndrome                                                      | In-house gene list  |
| SALL4  | Duane-radial ray syndrome                                                   | In-house gene list  |
| SEMA3D | TOF                                                                         | PanelApp (red)      |
| SEMA3E | CHARGE syndrome                                                             | In-house gene list  |
| SF3B4  | Acrofacial dysostosis                                                       | In-house gene list  |
| SHOC2  | Noonan-like syndrome                                                        | In-house gene list  |
| SHROOM3| Heterotaxy                                                                  | PanelApp (amber)    |
| SMAD3  | Loeys-Dietz syndrome                                                        | In-house gene list  |
| SMAD4  | Myhre syndrome                                                              | In-house gene list  |
| SMAD6  | Aortic valve disease                                                        | PanelApp (red)      |
| SMARCA4| Coffin-Siris syndrome                                                       | In-house gene list  |
| SMARCB1| Coffin-Siris syndrome                                                       | In-house gene list  |
| Gene   | Syndrome/Condition                                      | Category            |
|--------|--------------------------------------------------------|---------------------|
| SMC1A  | Cornelia de Lange syndrome                            | In-house gene list  |
| SMC3   | Cornelia de Lange syndrome                            | In-house gene list  |
| SNX8   | TOF                                                    | In-house gene list  |
| SON    | ZTTK syndrome                                          | In-house gene list  |
| SOS1   | Noonan syndrome                                        | In-house gene list  |
| STK11  | Peutz-Jeghers syndrome                                | In-house gene list  |
| STRA6  | Syndromic microphthalmia                              | In-house gene list  |
| TAB2   | Non-syndromic CHDs                                     | PanelApp (green)    |
| TBX1   | TOF                                                    | PanelApp (red)      |
| TBX20  | ASD                                                    | PanelApp (amber)    |
| TBX5   | Holt-Oram syndrome                                     | PanelApp (green)    |
| TDGF1  | Forebrain defects                                      | In-house gene list  |
| TRAF7  | Cardiac, facial, and digital anomalies with developmental delay | PanelApp (green)    |
| TFAP2B | Char syndrome, PDA                                     | In-house gene list  |
| TGFBR1 | Loeys-Dietz syndrome                                   | In-house gene list  |
| TLL1   | ASD                                                    | PanelApp (red)      |
| TMEM260| Structural heart defects and renal anomalies syndrome  | In-house gene list  |
| TTC37  | Trichohepatoenteric syndrome                           | In-house gene list  |
| UBR1   | Johanson-Blizzard syndrome                             | In-house gene list  |
| VEGFA  | TOF                                                    | In-house gene list  |
| ZFPM2  | TOF                                                    | PanelApp (red)      |
| ZIC3   | Heterotaxy                                             | PanelApp (green)    |

**Abbreviations:**
- ASD – atrial septal defect
- AVSD – atrioventricular septal defect
- CHD – congenital heart disease
- DCM – dilated cardiomyopathy
- DORV – double outlet right ventricle
- HCM – hypertrophic cardiomyopathy
- HLHS – hypoplastic left heart syndrome
- LVNC – left ventricular noncompaction
- PDA – patent ductus arteriosus
- TGA – transposition of the great arteries
- TOF – Tetralogy of Fallot
- TrA – truncus arteriosus
- VSD – ventricular septal defect
Supplemental Table III. Additional CNVs identified in the proband with Klinefelter syndrome

| Chromosomal coordinates | CN | Size (kb) | Encompassed genes |
|-------------------------|----|----------|-------------------|
| 1:849467-923023         | 3  | 73.56    | LOC284600, LOC100130417, SAMD11, NOC2L, KLHL17, PLEKHN1, PERM1 |
| 2:48833179-48908728     | 3  | 75.55    | STON1-GTF2A1L, GTF2A1L, TPT1P11 |
| 5:158840054-158997220   | 3  | 157.17   | LINC01845 |
| 10:135357401-135427143  | 3  | 69.74    | SYCE1, SPRNP1, OR6L1P |
| 11:4436061-4474701      | 3  | 38.64    | OR51R1P, OR52P2P, OR52K2 |
| 14:106270454-106849677  | 3  | 579.22   | IGH, IGHD, IGHM, LOC100288273, MIR4539, MIR4507, MIR4538, MIR4537, IGJ6, IGJ3P, IGJ5, IGJ4, IGJ3, IGJ2P, IGJ2, IGJ1, IGHD7-27, IGJ1P, IGHD1-26, IGHD6-25, IGHD5-24, IGHD4-23, IGHD3-22, IGHD2-21, IGHD1-20, IGHD6-19, IGHD5-18, IGHD4-17, IGHD3-16, IGHD2-15, IGHD1-14, IGHD6-13, IGHD5-12, IGHD4-11, IGHD3-10, IGHD3-9, IGHD2-8, IGHD1-7, IGHD6-6, IGHD5-5, IGHD4-4, IGHD3-3, IGHD2-2, FAM30A, IGHD1-1, IGHV6-1, IGHVII-1-1, ADAM6, RPS8P1, IGHV1-2, IGHVIII-2-1, IGHV1-3, IGHV4-4, IGHV2-5, IGHVIII-5-1, IGHVIII-5-2, IGHV3-6, IGHV3-7, IGHV1-8, IGHV3-9, IGHV2-10, IGHV3-11, IGHVIII-11-1, IGHV1-12, IGHV3-13, IGHVIII-13-1, IGHV1-14, IGHV3-15, IGHVII-15-1, IGHV3-16, IGHVIII-16-1, IGHV1-17, SLC20A1P2, IGHV1-18, IGHV3-19, IGHV3-20, IGHVII-20-1, IGHV3-21, IGHV3-22, IGHVII-22-1, IGHVIII-22-2, IGHV3-23, IGHV1-24, HOMER2P2, LINC00226, IGHV3-25, IGHVIII-25-1, IGHV2-26, IGHVIII-26-1, IGHVII-26-2, IGHV7-27, |
| Chromosome | Region | Bin | GC % | Genes |
|------------|--------|-----|------|-------|
| 15:43888262-43968546 | 1 | 80.29 | CKMT1B, STRC, RNU6-554P, CATSPER2, RNU6-610P, PDIA3P2, PPIP5K1P1 |
| 16:17360916-17427230 | 1 | 66.32 | XYLT1 |
| 17:44213434-44292742 | 3 | 79.31 | KANSL1, KANSL1-AS1 |
| 19:15780652-15820326 | 4 | 39.68 | CYP4F12 |