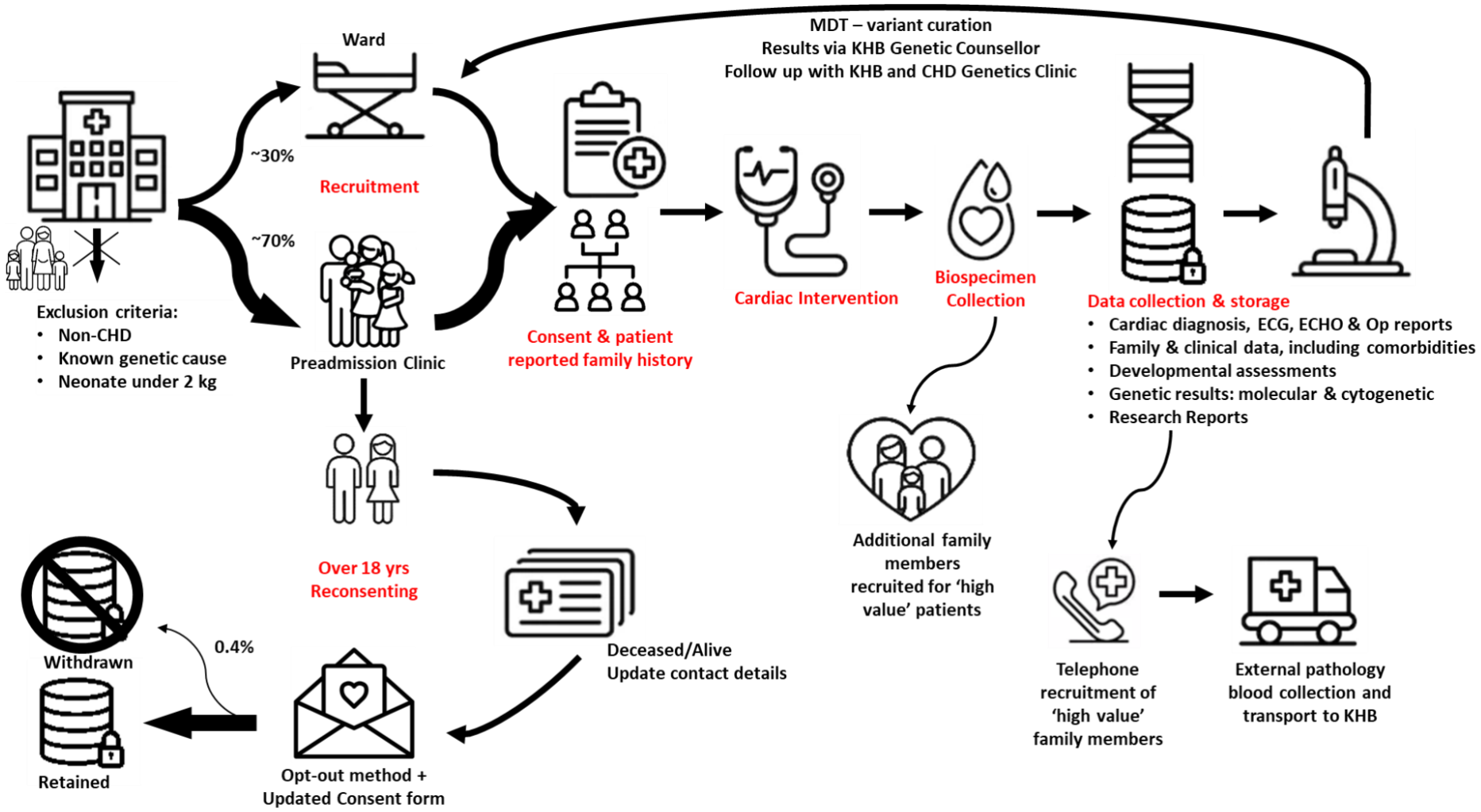
SUPPLEMENTARY MATERIAL

Supplementary Figure S1: Kids Heart BioBank processes including, recruitment, biospecimen processing and data collection.



Supplementary Table S1: Biobanking and recruitment costs per participant. Costs are standardised to 2023 values and reported in Australian dollars.

Recruitment Activity	Cost (AU$)/Participant
Face-to-face recruitment (20 minutes)	12.06
Database entry and pedigree refinement (30 minutes)	18.09
Sample collection (10 minutes)	6.03
Sample processing (in-house hospital provided)	30
Coding and collating medical information (60 minutes)	36.17
Storage (cryogenic racks, boxes and microcentrifuge tubes)	0.9
Total costs	**103.25**

Supplementary Table S2: Reasons for Kids Heart BioBank non-consent as reported by parents/legal guardians.

Reasons For Non-Consent (2006 - 2022)		
Reason	**Inpatient Cases (%)**	**Outpatient Cases (%)**
Too much happening/overwhelmed	32 (16.7)	17 (8.9)
Need more time; no time to discuss with partner; partner does not consent	10 (5.2)	26 (13.5)
Concerned about blood loss (during procedure)	11 (5.7)	8 (4.2)
Assessed by Clinical Genetic services - current genetic investigations adequate	3 (1.6)	5 (2.6)
Language barrier	1 (0.5)	10 (5.2)
Accepts child has CHD and want to move on with their lives	2 (1.0)	7 (3.6)
Indefinite storage of DNA/Privacy concerns	1 (0.5)	4 (2.1)
Respecting wishes of child e.g., child did not want to participate or wanting child to decide when they are an adult	0 (0.0)	5 (2.6)
Participating in other research	1 (0.5)	2 (1.0)
Other*	0 (0.0)	6 (3.1)
Reason not recorded	7 (3.6)	34 (17.7)
Total	68 (35.4)	124 (64.6)

*Other reasons for non-consent: prior negative experience with genetic testing, distrust in research and concerns on how the DNA might be misused despite ethical oversight.

Supplementary Table S3: Research genetic testing outcomes of Kids Heart BioBank probands including variant details and references.

Note: Variant classifications reflect published literature and research reports at the time and have not been re-evaluated in accordance with updated ACMG guidelines.
Testing technologies utilised include targeted CHD panels, exome and genome sequencing.

Pathogenicity n=135 (% total probands)	Proband Category (%)*	Genetic Variants	Cited in Reference
Pathogenic 20 (14.8)	**Family History 9 (45.0)** · Singleton: 1 (11.1) · Trio: 8 (88.9)	TBX5 p.Y40X; chr 10q22.3-q23.2del; NOTCH1 p.N1955Kfs*26; NOTCH1 del(chr9:136537696-136560250); SPTB p.M625fs; CFC1 p.A175Rfs*56; GATA4 p.R302Q; DLL4 p.P255S; BCOR p.S830Cfs*6; TFAP2β R285Q	(1-4)
	Consanguinity 2 (10.0) · Singleton: 1 (50.0) · Trio: 1 (50.0)	HAAO p.W186*(hom); KIAA0586 c.4268-1G>A(hom)	(3, 5)
	Sporadic 5 (35.0) · Singleton: 0 (0.0) · Trio: 5 (100.0)	PBX1 p.R184P; 22q11del; KMT2C p.Q1880Afs*9; JAG1 p.P810L; NODAL p.R307*	(2-4, 6)
	ECA 4 (20.0) · Singleton: 1 (25.0) · Trio: 3 (75.0)	PTPN11 p.P491S; PTPN11 p.Y62D; INVS p.N1061Kfs*20(hom); SETD5 p.R618*	(2)
Likely Pathogenic 19 (14.1)	**Family History 15 (78.9)** · Singleton: 4 (26.7) · Trio: 11 (73.3)	TBX5 p.D166G; NODAL p.Y48Wfs*5 (x2); NF1 p.L1187R; ACTC1 p.T68I; UPF2 p.I869S; NOTCH1 p.A2036Pfs*3; NOTCH1 p.G200R; USP34 p.A288V; FLT4 p.G781Vfs*18; TEK p.R915H; TLL1 p.T860A; GATA6 p.P532Hfs*100; ELN c.950-3C>G; TIE1 p.V448Cfs*9	(1-4)
	Consanguinity 0 (0.0) · Singleton: 0 (0.0) · Trio: 0 (0.0)		
	Sporadic 2 (10.55) · Singleton: 0 (0.0) · Trio: 2 (100.0)	TEAD2 pR399*; ZFP36L2 p.R211H	(2, 3)
	ECA 2 (10.55) · Singleton: 1 (50.0) · Trio: 1 (50.0)	NFE2L2 p.L30P; CNOT1 p.H514Q	(2)

VUS 34 (25.2)	**Family History 22 (64.7)** · Singleton: 5 (22.7) · Trio: 17 (77.3)	ZFPM2 p.I227V; CHD7 p.N700D; KMT2C p.E2798Ffs*11; KMT2C p.Q4753L; BIN1 p.N1467W; DNAH5 p.W4271C; KDM5A p.R1467W; NOTCH1 p.N718S; NOTCH1 p.G1091S; NOTCH1 p.R1279C; SMAD5; DCHS1 p.P1109L; GATA4 p.T384T; PRSS23 p.T78Nfs*75; GDF1 p.E140D(hom); HMCN1 p. R5595*; WDR1 p.S114N; JARID2 p.P362G; NODAL p.P53Rfs*23; CHD4 p.P1811L; JAG1 p.P248=; TMEM2 p.L322V; PRMT5; MYOCD D160N	(1-3, 7)
	Consanguinity 1 (2.9) · Singleton: 0 (0.0) · Trio: 1 (100.0)	GDF1 p.V366M(hom)	
	Sporadic 7 (20.6) · Singleton: 0 (0.0) · Trio: 7 (100.0)	WDR90; MYH6 p.R244H; MYH6 p.L1932*; HNRNPK p.S420L; SEMA3D p.S64*; SMAD6 p.G29Afs*35; HAND1 chr5:154478752 G>A, ACVR1 chr2:157769997C>G	(3)
	ECA 4 (11.8) · Singleton: 1 (25.0) · Trio: 3 (75.0)	KIF7 p.E408M; DCHS1; PTPN23; KMT2D p.G4441W	
Uninformative 62 (45.9)	**Family History 17 (27.4)** · Singleton: 6 (35.3) · Trio: 11 (64.7)		(1-3)
	Consanguinity 0 (0.0) · Singleton: 0 (0.0) · Trio: 0 (0.0)		
	Sporadic 38 (61.3) · Singleton: 0 (0.0) · Trio: 38 (100.0)		(2, 3)
	ECA 7 (11.3) · Singleton: 0 (0.0) · Trio: 7 (100.0)		(2, 3)

*Proportion of probands as a percentage of pathogenicity category.
P=pathogenic; LP=likely pathogenic; hom=homozygous; VUS=variant of uncertain significance.

Supplementary Table S4: Peer-reviewed publications supported by Kids Heart BioBank participants and/or associated data.

Genetic/Genomic Technology	Manuscript Title	KHB Participants (%)	Year	Reference
Single Gene	Mutations in cardiac T-box factor gene *TBX20* are associated with diverse cardiac pathologies, including defects of septation and valvulogenesis and cardiomyopathy	71.7	2007	(8)
	GATA4 mutations in 357 unrelated patients with congenital heart malformation	81.5	2010	(9)
	Investigation of association between PFO complicated by cryptogenic stroke and a common variant of the cardiac transcription factor *GATA4*	43.2	2011	(10)
	Somatic mutations in *NKX2–5*, *GATA4*, and *HAND1* are not a common cause of Tetralogy of Fallot or Hypoplastic Left Heart	100.0	2011	(11)
	Association between C677T polymorphism of methylene tetrahydrofolate reductase and congenital heart disease: meta-analysis of 7,697 cases and 13,125 controls	4.9	2013	(12)
	Analysis of *DICER1* in familial and sporadic cases of Transposition of the Great Arteries	63.6	2018	(13)
Genome-wide association studies	Phenotype-specific effect of chromosome 1q21.1 rearrangements and GJA5 duplications in 2436 congenital heart disease patients and 6760 controls	3.2	2012	(14)
	Contribution of global rare copy-number variants to the risk of sporadic congenital heart disease	16.7	2012	(15)
	Genome-wide association study of multiple congenital heart disease phenotypes identifies a susceptibility locus for atrial septal defect at chromosome 4q16	16.5	2013	(16)
	Genome-wide association study identifies loci on 12q24 and 13q32 associated with Tetralogy of Fallot	9.5	2013	(17)
	Common genetic variants contribute to risk of Transposition of the Great Arteries	6.5	2022	(18)
Targeted Gene Panels	Targeted Next-Generation sequencing identifies pathogenic variants in familial congenital heart disease	100.0	2014	(1)

	Genetic burden and associations with adverse neurodevelopment in neonates with congenital heart disease	100.0	2018	(19)
Whole Exome/Genome	The promises and challenges of exome sequencing in familial, non-syndromic congenital heart disease	100.0	2017	(7)
	De novo, deleterious sequence variants that alter the transcriptional activity of the homeoprotein PBX1 are associated with intellectual disability and pleiotropic developmental defects	12.5	2017	(20)
	A screening approach to identify clinically actionable variants causing congenital heart disease in exome data	100.0	2018	(2)
	Identification of clinically actionable variants from genome sequencing of families with congenital heart disease	90.7	2019	(3)
	Whole Exome Sequencing reveals the major genetic contributors to non-syndromic Tetralogy of Fallot	9.5	2019	(21)
	Whole genome sequencing in Transposition of the Great Arteries and associations with clinically relevant heart, brain and laterality genes	50.0	2022	(22)
	Insights into the genetic architecture underlying complex, critical congenital heart disease	100.0	2022	(23)
Functional Studies	NAD deficiency, congenital malformations, and niacin supplementation	25.0	2017	(24)
	Functional genomics and gene-environment interaction highlight the complexity of congenital heart disease caused by Notch pathway variants	92.1	2019	(4)
	Functional characterization of a novel *PBX1 de novo* missense variant identified in a patient with syndromic congenital heart disease	100.0	2020	(6)
Methodologies	A universal and robust integrated platform for the scalable production of human cardiomyocytes from pluripotent stem cells	100.0	2015	(25)
	Large-scale production of cardiomyocytes from human pluripotent stem cells using a highly reproducible small molecule-based differentiation protocol	100.0	2016	(26)
Data and Bioinformatics	CHDgene: A curated database for congenital heart disease genes	Many*	2022	(27)
	ConanVarVar: A versatile tool for the detection of large syndromic copy number variation from whole-genome sequencing data	Many*	2023	(28)
	Examination of validity of identifying congenital heart disease from hospital discharge data without a gold standard: Using a data linkage approach.	Many*	2023	(29)
	Using novel data linkage of congenital heart disease biobank data with administrative health data to identify cardiovascular outcomes to inform genomic analysis.	Many*	2023	(30)

Reviews and Clinical studies	Progress and challenges in the genetics of congenital heart disease	N/A	2005	(31)
	Congenital heart disease: current knowledge about causes and inheritance	N/A	2012	(32)
	Parents' perceptions of genetics services for congenital heart disease: The role of demographic, clinical, and psychological factors in determining service attendance	N/A	2014	(33)
	Bioengineering and stem cell technology in the treatment of congenital heart disease	N/A	2015	(34)
	Genetic counselling in parents of children with congenital heart disease significantly improves knowledge about causation and enhances psychosocial functioning	N/A	2015	(35)
	Advances in the genetics of congenital heart disease: A clinician's guide	N/A	2017	(36)
	Current practice of genetic testing and counselling in congenital heart disease: An Australian perspective	N/A	2020	(37)
	Why and how did this happen?: Development and evaluation of an information resource for parents of children with CHD	100.0	2020	(38)
	A new era of genetic testing in congenital heart disease: A review	N/A	2022	(39)
	Biological and structural phenotypes associated with neurodevelopmental outcomes in congenital heart disease.	N/A	2023	(40)

*Exact number of Kids Heart BioBank contributing to these efforts unknown.
KHB= Kids Heart BioBank.

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