

**COngenital heart disease and the Diagnostic yield with Exome sequencing (CODE Study):
prospective cohort study and systematic review**

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Short Title: Exome sequencing in congenital cardiac anomalies

Keywords: cardiac; congenital heart disease; exome sequencing; fetus; prenatal diagnosis; next generation sequencing

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/uog.22072

CONTRIBUTION

What are the novel findings of this work?

This is the first systematic review assessing the incremental yield of antenatal exome sequencing over chromosome microarray/karyotype in prenatally diagnosed congenital heart disease.

What are the clinical implications of this work?

Dependent on the presence of robust pathways, exome sequencing may be considered in prenatal congenital heart disease, with particular consideration for not just those with extra-cardiac abnormalities but in those of an isolated nature.

ABSTRACT

OBJECTIVES: To determine the yield of antenatal exome sequencing (ES) over chromosome microarray (CMA) / conventional karyotyping in; (i) any prenatally diagnosed congenital heart disease (CHD); (ii) isolated CHD; (iii) multi-system CHD and; (iv) CHD by phenotypic subgroup.

METHODS: A prospective cohort study of 197 trios undergoing ES following CMA/karyotype because CHD was identified prenatally and a systematic review of the literature was performed. MEDLINE, EMBASE and CINAHL (2000–Oct 2019) databases were searched electronically. Selected studies included those with; (i) >3 cases; (ii) initiation of testing based upon a prenatal phenotype only and; (iii) where CMA/karyotyping was negative.

PROSPERO No. CRD42019140309

RESULTS: In our cohort ES gave an additional diagnostic yield in; (i) all CHD; (ii) isolated CHD and; (iii) multi-system CHD of 12.7% (n=25/197), 11.5% (n=14/122) and 14.7% (n=11/75) (p=0.81). The pooled incremental yields for the aforementioned categories from 18-studies (n=636) were 21% (95% CI, 15-27%), 11% (95% CI, 7-15%) and 37% (95% CI, 18%-56%) respectively. This did not differ significantly when sub-analyses were limited to studies including >20 cases. In instances of multi-system CHD in the primary analysis, the commonest extra-cardiac anomalies associated with a pathogenic variant were those affecting the genitourinary system 44.2% (n=23/52). Cardiac shunt lesions had the greatest incremental yield, 41% (95% CI, 19-63%), followed by right-sided lesions 26% (95% CI, 9-

43%). In the majority of instances pathogenic variants occurred *de novo* and in autosomal dominant (monoallelic) disease genes (68/96; 70.8%). The commonest monogenic syndrome identified was Kabuki syndrome (n=19/96; 19.8%).

CONCLUSIONS: Despite the apparent incremental yield of prenatal exome sequencing in congenital heart disease, the routine application of such a policy would require the adoption of robust bioinformatic, clinical and ethical pathways. Whilst the greatest yield is with multi-system anomalies, consideration may also be given to performing ES in the presence of isolated cardiac abnormalities.

INTRODUCTION

Congenital heart disease (CHD) complicates 1% of live-born neonates and is associated with significantly high rates of perinatal morbidity and mortality.^{1,2} Prenatal detection of CHD and establishment of a unifying genetic diagnosis can inform prenatal management, optimise post-natal outcome and aid in the counselling of parents in both index and subsequent pregnancies.³ Of all prenatally diagnosed CHD, 2/3 tends to be isolated while 1/3 can be associated with extra-cardiac anomalies (ECAs).⁴ Aneuploidy is present in between 28-45% of prenatally diagnosed CHD, with at least one ECA present in as many as 98% of such cases.³ Copy number variation (CNV) can be present in a further 2-25%.³ The additional proportion of CHD caused by monogenic Mendelian disorders is traditionally thought to be ~5% although results vary.³ Since the introduction of exome sequencing (ES), large prospective studies suggest that this proportion is greater.^{5,6} It has been proposed that a significant number of identified variants in CHD within the pediatric population are *de novo* in nature, most notably when there are co-existing neurodevelopmental and ECAs.^{7,8} There are a paucity of studies which have formally assessed the diagnostic yield offered from ES over standard chromosome microarray(CMA)/karyotype in prenatally diagnosed CHD and there is no evidence to suggest which phenotypic CHD sub-types have the greatest diagnostic yield.^{9,10,11} Hence, the objectives of this prospective cohort study, systematic review and meta-analysis were to determine the yield of ES over CMA/karyotype in; (i) any prenatally diagnosed CHD; (ii) isolated CHD; (iii) CHD associated with ECAs and; (iv) CHD dependent on phenotypic subgroup.

METHODS

Extended PAGE Cohort

CODE assessed the extended cohort of the published Prenatal Assessment of Exomes and Genomes (PAGE) study which included 850 trios (fetus and parents) that underwent ES analysis when a fetal structural anomaly was detected on ultrasound.⁵ This prospective extended cohort study recruited between October 2014 and May 2018 across 34 fetal medicine centres in England and Scotland, using the West Midlands Genetic Research Laboratory (WMGRL) as their laboratory hub and then through the Wellcome Trust Sanger Institute (for exome sequencing).⁵ Eligibility criteria included; (i) prenatal detection of an anomaly after 11-weeks' gestation including an increased nuchal translucency (NT) ($\geq 4\text{mm}$); (ii) an invasive test having been performed; (iii) informed written consent obtained from both parents for testing and both were >16 -years and; (iv) negative CMA or karyotype testing. Study methodology is as documented in the original published study but briefly utilized a standard ES approach with variant interpretation based upon a targeted virtual gene panel for developmental disorders encompassing 1628 genes.⁵ Phenotypes of all cases were classified using Human Phenotype Ontology (HPO) terms and those which were cardiac related were selected. Following manual review of free-text descriptions, miscoded terms and small muscular ventricular-septal defects (VSDs) were removed. CHD was initially further classified into 'isolated' and 'multi-system' with a HPO approach to coding additional ECAs, including fetal growth restriction, single umbilical artery and nuchal thickening but not an elevated first trimester NT. Cardiac phenotypes were described by fetal medicine

specialists and sonographers and confirmed by fetal cardiologists using the Viewpoint® Version 5.6.16 GE Healthcare, 2012 and were subsequently coded using the American Heart Association/American College of Cardiology (AHA/ACC) criteria as; (i) shunt lesions; (ii) left-sided obstructive lesions; (iii) right-sided lesions and; (iv) complex lesions.¹² Two clinicians reviewed each classification for concordance (F.M. and M.D.K). Pathogenic variants and variants of uncertain significance (VUS) where the American College of Medical Genetics classification had been agreed upon at the clinical review panel were included in the final list of variants.¹³ Incidental findings (IFs) were not reported. The study was approved by the Research and Development offices and Research Ethics Committees at each institution and obtained ethical approval from the Research and Development offices and Research Ethics Committees at the West Midlands – South Birmingham (ref: 13/WM/1219) and each institution.

Data Sources

A systematic review was conducted in a standardized fashion in line with PRISMA guidance.¹⁴ A systematic electronic search of MEDLINE, CINAHL, EMBASE and clinicaltrials.gov was performed from January 2000 (as ES was not available prior to this) until October 2019. MeSH keywords with word variations of the terms ‘exome sequencing’ and ‘prenatal’ were used in an attempt to capture as many relevant studies as possible. Alternative terms for ES included ‘exome sequencing, whole’; ‘exome sequencing, complete’; ‘whole genome sequencing’ and ‘sequence analysis, DNA’. Alternative terms for

prenatal included 'fetal'; 'fetus' and 'antenatal'. Experts were also contacted and bibliographies of all relevant papers were searched. Studies not in the English language were translated. The search strategy is available from the corresponding author on request. This systematic review was registered prospectively with PROSPERO No. CRD42019140309.

Eligibility criteria for study selection and data extraction

All study abstracts were screened by two reviewers (F.M. and M.D.K.) and full text articles were subsequently reviewed where further information was required. Studies were selected if; (i) they included three or more cases of CHD undergoing ES; (ii) testing was initiated based upon a prenatal ultrasound-based phenotype and; (iii) CMA/ karyotype testing was negative. In cases where ES was initiated postnatally, these were only included where testing was based upon the prenatal phenotype. Data extracted from studies where obtainable included: ultrasound phenotype, ES approach, genomic variants, source of fetal DNA, turnaround time for testing, fetal outcome, maternal age and gestation at testing. An ES result was deemed positive only if it was graded IV to V 'likely pathogenic' or 'pathogenic' and determined to be causative of the phenotype. VUS and IFs were reported separately.¹³

Quality assessment and data synthesis

The incremental yield or risk difference of ES over CMA/karyotype was calculated for each study with 95% confidence intervals and as a meta-analysis for; (i) all CHD; (ii) subgroup

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analyses of isolated and multisystem CHD with only studies included in the latter when the presence or absence of CHD were available from the data. Cases were stratified as per the aforementioned cohort study. Risk differences from each study were pooled using a random effects model throughout to estimate the overall yield and the yield for isolated and multi-system CHD using RevMan version 5.3.4 (Review Manager, The Cochrane Collaboration, Copenhagen, Denmark) via a previously published method which facilitated calculation of the incremental yield with adjustment for 'zero' values from negative CMA testing which was applicable to all included studies.¹⁵ Findings were displayed as forest plots with corresponding 95% confidence intervals. Heterogeneity was assessed graphically and statistically (Higgins' I^2) and a sub-analysis was performed including studies with >20 cases to determine if results differed significantly. Publication bias was assessed graphically using funnel plots (also generated by RevMan version 5.3.4 and demonstrated as Supplementary Figure 1a-c). Quality assessment of studies was assessed using a modified Standards for Reporting of Diagnostic Accuracy (STARD) criteria. The quality criteria deemed most important to optimise accuracy were; (i) if trio analysis was performed; (ii) ACMG criteria for variant interpretation and; (iii) Sanger validation of variants.¹³ Due to the limited number of studies available, beyond the pre-defined inclusion criteria, quality assessment could not be incorporated into the analysis so as to optimise the number of cases included.^{13,16,17}

RESULTS

Extended PAGE Cohort

Of 850 fetuses undergoing trio ES with prenatally detected structural anomalies, there were n=197 (23.2%) CHD cases in total, of which 61.9% (n=122) were isolated and 38.1% (n=75) associated were with ECAs. Where documented (n=190), the source of fetal DNA was; a) chorionic villi 15.8% (n=30); b) amniocytes 81.1% (n=154) or; c) lymphocytes 3.2% (n=6). G-banding karyotype was performed 3.0% (n=6) of cases, with CMA in the remainder. The diagnostic yield of ES in each group (excluding VUS) was 12.7% (n=25/197) all CHD, 11.5% (n=14/122) isolated CHD and 14.7% (n=11/75) in multisystem CHD respectively ($p=0.81$). In instances of multi-system CHD with a pathogenic variant, the commonest systems affected were those affecting growth, the nervous system and face (all 45.5% n=5/11). There were not enough cases to identify a dominant sub-classification of CHD hence this was explored further in the systematic review. The overall incidence of VUS was 5.1%.

Systematic review and meta-analysis

In all instances where a study was suitable for inclusion but data was incomplete, the corresponding author was contacted (n=6), of which three responded and two provided complete data.^{6,18} Authors of the second largest included study, the Petrovski, *et al.* Columbia University-based study, provided a completed dataset on their CHD cohort as an extended version of their original study.⁶ In addition to both the extended PAGE cohort

study and the extended Petrovski, *et al.* study⁶, a further 16 studies met the overall selection criteria, leading to a total of 18 studies, as demonstrated in Figure 1.^{5,6, 9-11, 18-30} Table 1 outlines the study characteristics and Figure 2 outlines the overall quality assessment of all studies included. There was one study where ES was targeted using a CHD panel while the remainder used a whole ES approach.⁹ Not all studies broke CHD down into isolated/multi-system or distinctive phenotypes as demonstrated or described the cardiac phenotype [Table 1].

Combined cohort outcomes

18-studies were included, encompassing n=636 CHD cases undergoing ES, of which n=529 stated whether CHD was isolated or associated with ECAs. Hence, 54.4% (n=288/529) of cases were isolated and 45.6% (n=241/529) multi-system CHD. Where available, the mean maternal age and gestation at the time of testing was 30 (+/-3.5 SD) years and 22 (+/-4.7) weeks. The primary genetic test performed prior to ES was CMA 98.0% (n=623/636) with the predominant source of fetal DNA from amniocytes 54.6% (n=322/590). Of the n=18 studies included, information regarding the originally recruited cohort prior to CMA/karyotype results were stated for n=5 studies.^{5,6,9,11,24} These revealed that there was an abnormal CMA/karyotype in 21.0% (n=1109/5285) of cases. Where stated (n=261), the median turnaround time for ES was 42 (range 7-82) days and pregnancy outcome was reported in n=341, of which livebirth 47.8% (n=163) and termination of pregnancy 46.3%

(n=158) were the commonest outcomes. Where reported, the pooled incremental yields of VUS and IFS were 26% (95% CI, 14-39% p=0.0001) and 8% (95% CI, 0-17% p=0.0001).

Incremental yield of pathogenic variants

The pooled incremental yields (excluding VUS) from all 18-studies are illustrated in the forest plots for (i) all ; (ii) isolated and; (iii) multi-system CHD [Figure 3(a-c)]. In the cases of (ii) and (iii) 13 and 15-studies included relevant cases for inclusion. Incremental yields for the aforementioned groups were 21% (95% CI, 15-27% p=0.0006), 11% (95% CI, 7-15% p<0.00001) and 37% (95% CI, 18%-56% p<0.00001) respectively. The sub-analysis of studies with >20-cases (n=8) is demonstrated in Supplementary Figures 2a-c with corresponding funnel plots (Supplementary Figures 3a-c). Findings did not differ significantly from the primary analysis, apart from multi-system CHD, where the incremental yield was greater at 49% (95% CI, 17-80% p=0.003). Where gestational age was recorded in isolated CHDs the incremental yield for those diagnosed after 15-weeks' gestation was greater than for all cases at 24% (95% CI, 7%-41%, p=0.002, I²=68%). In instances of multi-system CHD in the primary analysis, the commonest ECAs associated with a pathogenic variant were those affecting the genitourinary system 44.2% (n=23/52), nervous system 34.6% (n=18/52) and face 34.6% (n=18/52). In multisystem CHDs, where a pathogenic variant was detected and the specific ECA was documented (82.7%, n=43/52), there was one instance (2.3%, n=1/43) where a 'minor ECA' was present (single umbilical artery), with the remainder being major or affecting two or more systems.

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On classification as per AHA/ACC criteria for all CHD, shunt lesions (septal anomalies and total anomalous pulmonary venous drainage) had the greatest pooled incremental yield of pathogenic variants 41% (95% CI, 19-63% p=0.003), followed by right-sided 26% (95% CI, 9-43%, p=0.001), complex 23% (95% CI, 9-36%, p=0.001) and left-sided obstructive lesions 18% (95% CI, 0-35% p=0.02). Where documented, pathogenic variants are described in Supplementary Table 1. Where pathogenic variants were documented (n=96/111; 86.5%), the commonest genetic syndromes identified were those of Kabuki syndrome (n=19/96; 19.8%), CHARGE (Coloboma-Heart defects-Atresia choanae-Retardation of growth-genital abnormalities-ear abnormalities) syndrome (n=8/96; 8.3%), Noonan syndrome (n=6/96; 6.3%) and Primary Ciliary Dyskinesia (n=6/96; 6.3%). In syndromes where CHD was typically described as being multi-system in nature, in 54.1% (n=20/37) of such syndromes only an isolated CHD was detected prenatally e.g. Adams-Oliver, CHARGE, Kabuki and Simpson-Golabi-Behmel syndrome. In the majority of instances pathogenic variants occurred *de novo* and in autosomal dominant (monoallelic) disease genes (68/96; 70.8%) [Supplementary Table 1].

DISCUSSION

This is the first systematic review assessing the yield of antenatal ES in prenatally diagnosed CHD in which CMA/karyotype was negative. The results of this study show an apparent incremental yield of ES in CHDs, particularly for shunt lesions and multi-system CHD. Most pathogenic variants occurred *de novo* in monoallelic disease genes with a high incidence of Kabuki syndrome. The majority were reported in syndromes which typically present with ECAs yet presented with an isolated CHD.

The diagnostic yield from our cohort study was modest compared to other studies in the meta-analysis. This is potentially secondary to several factors; (i) bias in case selection – smaller series may have had an element of selection bias only selecting cases with positive results;³¹ (ii) the proportion of multi-system CHD – the greater the proportion, the higher the overall yield and; (iii) the sequencing approach used e.g. targeted or whole exome; the series from Hu *et al.* (n=44 CHD cases)⁹ revealed a high diagnostic yield when a targeted 77 cardiac panel approach was used (n=7; 15.9%). Of the 77 genes, only 5 genes were not included in the PAGE study panel, none of which were found to be causative in the Hu, *et al* study.⁹ While use of targeted gene panels potentially provide a greater yield in a shorter time frame, users must exert caution as they are primarily based upon postnatal and not prenatal phenotypes.³¹

The greater incremental yield with ES associated with multi-system vs. isolated CHD is similar to the pattern seen with aneuploidy and CNV, as is the case with shunt lesions and left-sided obstructive lesions.¹⁵ Shunt lesions tend to be associated with ECAs which is probably why the diagnostic yield with ES in this group is most significantly enriched.^{3,4} The predominance of *de novo* variants in monoallelic disease genes is also in keeping with published evidence.^{3,7,8,32} It is interesting that the most common syndromes unveiled in this study were those of Kabuki and CHARGE. Kabuki syndrome has a highly variable phenotype.³³ There is limited evidence with regards the prenatal presentation and the high incidence as seen in this study has not been previously reported, although an overall association with postnatally diagnosed left-sided CHD has been established.³³⁻³⁵ Both CHARGE and Kabuki syndromes are caused by pathogenic variants in genes encoding proteins implicated in chromatin function and gene regulation.³⁶ There is a potential link between these syndromes with an association between DNA methylation targets in their gene-specific signatures.³⁶ This reflects that epigenetic dysregulation is the commonest pathway responsible for the greatest proportion of CHD where pathogenic variants were uncovered in this series.³⁶

The strength of this study is the robust and systematic methodology utilised so that all available studies were included to limit selection bias. International collaboration between the two groups publishing the two largest series of prenatal congenital anomalies and ES has optimised the numbers. By excluding studies where phenotypes were based on

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postnatal examination, our study is specific for prenatal ES testing focusing on ultrasound detected CHD. The quality of included studies based upon pre-specified criteria was optimal due to the high number which had an ES approach to testing, variant interpretation based upon ACMG criteria and Sanger sequencing validation which meant most had a uniform and hence comparable approach.¹³

The main study limitation was high heterogeneity. This was likely caused by differing platforms used, as well as small-study effects reflected in asymmetry within the funnel plots. However, limiting the inclusion of studies to those with >20 cases didn't show a significant difference in incremental yield. There is currently no recognised classification system for prenatal CHD hence we selected an adult-based system.¹² This meant that rare CHD associated with high instances of perinatal demise could not be appropriately classified. Alternative classification systems were considered and experts were consulted, however the categories included were too broad which mean that due to a restricted number of cases where the phenotype was described, relevant associations would not be identified.^{37,38}

The challenges of ES in prenatally diagnosed CHD include; (i) the limited phenotype available from ultrasound imaging. Although concordance is generally high, more information is typically gathered from detailed postnatal examination^{1,39,40}; (ii) whether targeted panels or a whole ES approach should be used and; (iii) that CHD tends to be a highly heterogenous group of anomalies with multi-gene and multifactorial pathologies which may not be

unveiled with genomic testing.³ Further novel gene discovery may lie in epigenomic or genomic changes encoding proteins involved in chromatin re-modelling, the RAS signalling pathway, ciliary function and sarcomere architecture.² A further challenge with ES in pregnancy is the time constraint which it poses. Several studies made an *a priori* decision to report the results after the end of the pregnancy and thus the clinical/laboratory pathways were not accelerated to achieve real time results to individual members of the study. However, several fetal ES studies have reported delivering results in a timely fashion to inform pregnancy management,²⁸ and a rapid fetal ES service will shortly be introduced in the English National Health Service for the diagnosis of monogenic disorders. As well as turnaround time, the clinical utility of ES in CHD is dependent not just on the prospective targeting of phenotypes but also robust bioinformatics filtering within accredited genomic laboratories and detailed analysis by clinical multidisciplinary review groups to assess and determine causative variants. Pre-test counselling must be accurate, clear and comprehensive with consideration given to ethical challenges. Without such robust bioinformatics and clinical screening of variants, prenatal ES should not be offered or used in clinical practice.^{41,42}

In conclusion, despite the apparent incremental yield of prenatal ES in CHD, the routine application of such a policy would require the adoption of robust bioinformatic, clinical and ethical pathways. Whilst the highest yield is with multi-system anomalies, consideration may also be given to performing ES in the presence of isolated

CHDs. Further work is required to explore the benefits and challenges of delivering targeted or whole exome analysis. Clinical guidelines must be introduced to ensure that testing is correctly implemented.

ACKNOWLEDGEMENTS

The PAGE study was supported by a Health Innovation Challenge from the UK Department of Health and Wellcome Trust (no. HICF-R7-396). We are grateful to Jane Fisher from Antenatal Results and Choices and to Michael Parker of The Ethox Centre, Nuffield Department of Population Health and Wellcome Centre for Ethics and Humanities for their valuable input into the study. We are also grateful to the members of the PAGE study clinical review panel. LSC is partially funded by the National Institute for Health Research (NIHR) Biomedical Research Centre at Great Ormond Street Hospital and ERM acknowledges support from NIHR Cambridge Biomedical Research Centre (an NIHR Senior Investigator Award). The University of Cambridge has received salary support with regard to ERM from the UK National Health Service (NHS) in the east of England through the Clinical Academic Reserve. The views expressed are those of the authors and not necessarily those of the NIHR, NHS, or Department of Health.

CONFLICT OF INTEREST

RYE and JL reports grants from the Health Innovation Challenge Fund during the conduct of the PAGE study. DJM reports grants for travel expenses from Congenica to attend educational symposia during the conduct of the PAGE study. MEH reports grants from the Wellcome Trust and the UK Government Department of Health during the conduct of the study and personal fees from Congenica, outside the submitted work. MDK is a member of

Illumina's International Perinatal Advisory Group but receives no payment for this. ERM has received travel expenses, accommodation and consultant fees for participating in an Illumina International Advisory Group after completion of the PAGE study. MDK is funded through the Department of Health, Wellcome Trust and Health Innovation Challenge Fund (award number HICF-R7-396) for the PAGE and PAGE2 research studies complete August 2019. LSC was partially funded by the same group in relation to PAGE. RJW receives funding from Illumina and NIH for research. All other authors declare no competing interests.

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Figure legends

Figure 1 Flowchart demonstrating included studies

Figure 2 Quality assessment for studies in the systematic review (n=18) using modified STARD criteria

Figure 3 Forest plots of incremental yield by exome sequencing over karyotype/microarray in fetuses with prenatally detected cardiac anomalies in (a) all; (b) isolated and; (c) multi-system cardiac anomalies. Only first author of each study is given. [CMA = chromosome microarray; M-H = Mantel-Haenszel].

Figure S1 Funnel plots of ALL studies reporting on incremental yield of exome sequencing over microarray/karyotyping in fetuses with congenital heart anomalies (CHAs)

- **Figure S1a** All CHD
- **Figure S1b** Isolated CHAs
- **Figure S1c** Multisystem CHAs

Figure S2 Forest plots of studies with >20 cases reporting on reporting on incremental yield of exome sequencing over microarray/karyotyping in fetuses with congenital heart disease (CHD)

- **Figure S2a** All CHD
- **Figure S2b** Isolated CHD
- **Figure S2c** Multisystem CHD

Figure S3 Funnel plots of studies with >20 cases reporting on incremental yield of exome sequencing over microarray/karyotyping in fetuses with congenital heart disease (CHD)

- **Figure S3a** All CHD
- **Figure S3b** Isolated CHD
- **Figure S3c** Multisystem CHD

Table legends

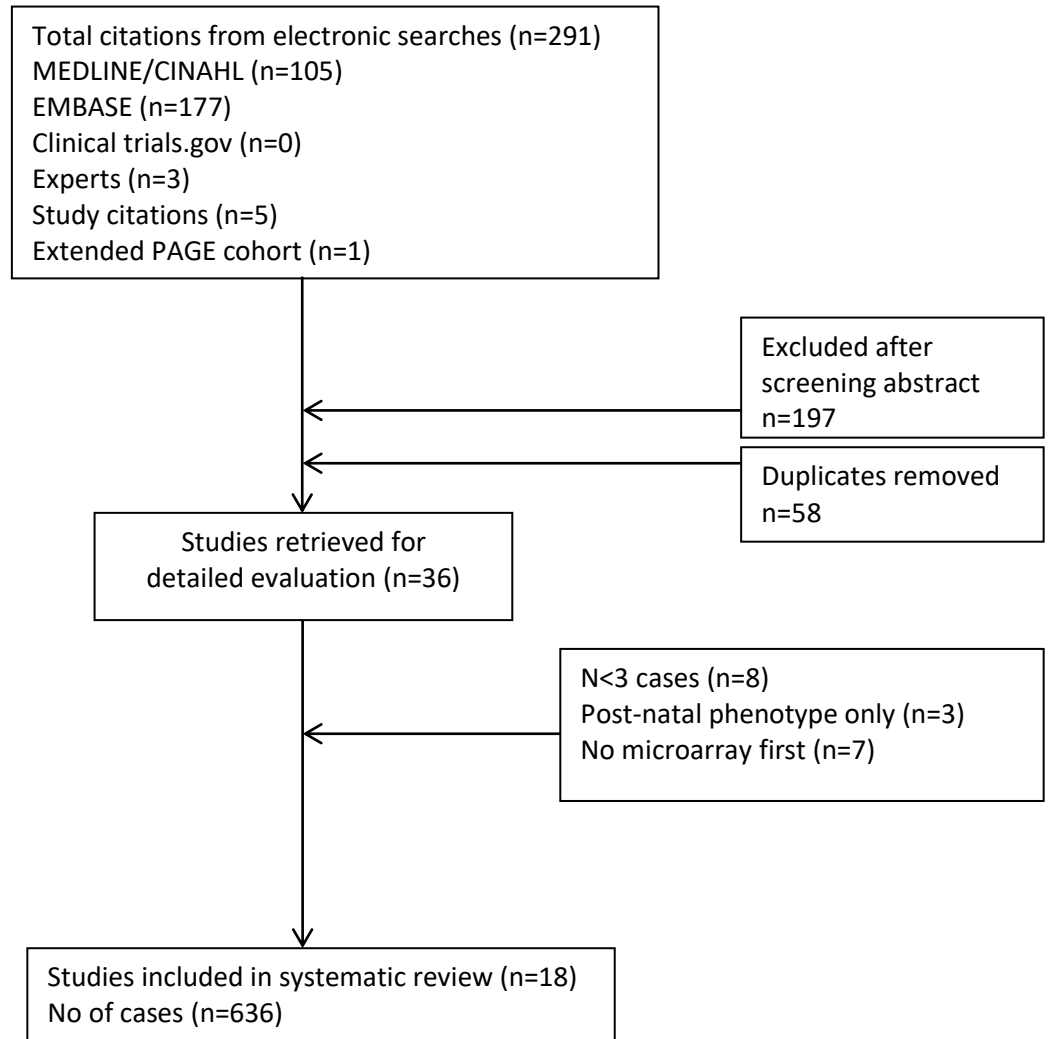
Table 1 Study characteristics and rates of pathogenic variants and variant of uncertain significance [CE=Clinical Exome; N/S = not-stated; WES=Whole exome sequencing *coverage not stated]

Table S1 Diagnostic variants identified from the systematic review

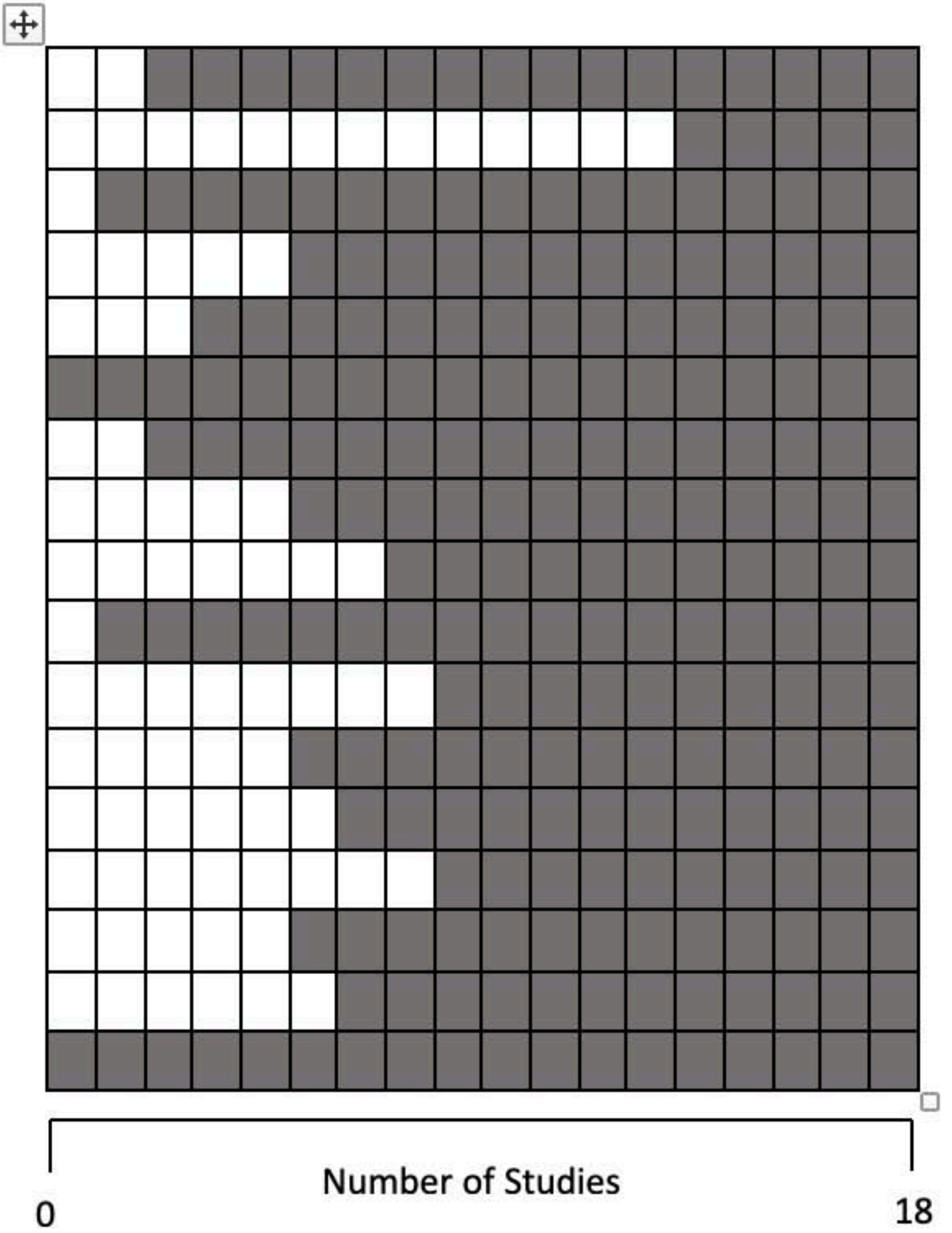
Table 1- Study characteristics and rates of pathogenic variants and variant of uncertain significance [CE=Clinical Exome; N/S = not-stated; WES=Whole exome sequencing *coverage not stated]

Study	ES Approach	Number of Cardiac anomalies		
		All cardiac	Isolated cardiac	Multi-system cardiac
Arabi <i>et al.</i> * ²⁶	WES Trio 20,000 gene panel 60-140X coverage	4	2	2
Boissel <i>et al.</i> ²⁰	WES Trio 110X coverage Agilent capture + Illumina HiSeq 2000 or 2500	11	2	9
Carss <i>et al.</i> ²¹	WES Trio 103X coverage Agilent capture + Illumina HiSeq	3	2	1
Daum <i>et al.</i> * ²²	WES Mainly proband only Agilent capture+ Illumina HiSeq 2500	5	1	4
De Koning <i>et al.</i> ³⁰	WES Trio 1128 genes 80X coverage Agilent capture + NextSeq 500	10	2	8
Duury <i>et al.</i> * ²³	WES Mainly proband only TruSeq Exome + Illumina HiSeq 1000 or Illumina Nextera Rapid Exome kit + HiSeq 2500	3	1	2
Fu <i>et al.</i> ²⁴	WES Mainly proband only 120X coverage Agilent capture+ Illumina HiSeq 2500	34	29	5
Hu <i>et al.</i> ⁹	CE Proband only 77 genes NimbleGen SeqCap EZ targeted capture Illumina HiSeq 2500 98.9% coverage of targeted region	44	N/S	N/S
Leung <i>et al.</i> ¹⁸	WES Trio 100X coverage TruSeq Rapid Exome Library Prep Kit Illumina sequencing	7	4	3
Murd <i>et al.</i> ⁵	WES Trio 1628 genes Agilent capture + Illumina Hi-Seq 2500 98.3% of the bait regions covered at a minimum depth of 5X	197	122	75

Normand <i>et al.</i> ²⁸	WES Trio Coverage 150X Roche NimbleGen capture Illumina Genome Analyzer IIx platform or HiSeq 2000	37	N/S	N/S
Petrovski <i>et al.</i> ⁶	WES Trio Nimblegen SeqCap EZ capture + Illumina HiSeq 2500 Average read coverage 89.3 reads Bioinformatic signatures	143	50	93
Stals <i>et al.</i> ²⁵	WES Parents only 80X coverage Agilent capture + Illumina HiSeq 2500 or NextSeq500 Only include het rare (MAF<0.001) variants in same gene in both parents	8	2	6
San <i>et al.</i> ^{*11}	WES Trio Agilent capture + Illumina HiSeq 4000 or Novaseq	66	55	11
Vora <i>et al.</i> ^{*29}	CE and WES Trio Illumina Hi-Seq 2500	3	0	3
Westerfield <i>et al.</i> ²⁷	WES Trio 130X coverage Roche NimbleGen capture + Illumina Genome Analyzer IIx or HiSeq 2000	5	0	5
Westphal <i>et al.</i> ¹⁰	WES Trio 20,000 genes 150X coverage	30	16	14
Vytes <i>et al.</i> ¹⁹	WES Trio 140X coverage Agilent capture + Illumina HiSeq 2000 or 2500	26	N/S	N/S



- Aim of article explained
- Specific cardiac phenotype study
- Source of patients described
- Number of patients >5
- Eligibility criteria described
- Description of NGS approach
- ACMG classification used
- Trio analysis
- Sanger validation
- Description of test protocol
- Clinical patient background described
- Cardiac phenotype described
- VUS reported
- Incidental findings reported
- Evaluation of sensitivity
- Study limitations described
- Study Implications described



No Yes

