

## COngenital heart disease and the Diagnostic yield with Exome sequencing (CODE Study)

Mone, Fionnuala; Eberhardt, Ruth Y; Morris, R. Katie; Hurles, Matthew E; McMullen, Dominic; Maher, Eamonn; Lord, Jenny ; Chitty, Lynn; Giordano, Jessica; Wapner, Ronald J; Kilby, Mark

DOI:  
[10.1002/uog.22072](https://doi.org/10.1002/uog.22072)

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*Document Version*  
Peer reviewed version

*Citation for published version (Harvard):*  
Mone, F, Eberhardt, RY, Morris, RK, Hurles, ME, McMullen, D, Maher, E, Lord, J, Chitty, L, Giordano, J, Wapner, RJ & Kilby, M 2020, 'COngenital heart disease and the Diagnostic yield with Exome sequencing (CODE Study): a prospective cohort study and systematic review', *Ultrasound in Obstetrics and Gynecology*. <https://doi.org/10.1002/uog.22072>

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

Journal:	<i>Ultrasound in Obstetrics and Gynecology</i>
Manuscript ID	UOG-2020-0020.R2
Wiley - Manuscript type:	Systematic Review or Meta-Analysis
Date Submitted by the Author:	n/a
Complete List of Authors:	Mone, Fionnuala; Birmingham Women's and Children's NHS Foundation Trust, Eberhardt, Ruth; Wellcome Sanger Institute Morris, R. Katie; University of Birmingham, Institute of Metabolism and Systems Research, College of Medical & Dental Sciences Hurles, Matthew; Wellcome Sanger Institute McMullen, Dominic; West Midlands Regional Genetics Laboratory, Birmingham Women's NHS Foundation Trust, Edgbaston, Birmingham, UK, Maher, Eamonn; University of Cambridge, Department of Medical Genetics; NIHR Biomedical Research Centre, Cambridge; Cambridge University Hospitals NHS Foundation Trust, Clinical Genetics Lord, Jenny; Wellcome Sanger Institute Chitty, Lyn; Great Ormond Street Hospital For Children NHS Foundation Trust, London North Genomic Laboratory Hub ; UCL Great Ormond Street Institute of Child Health Giordano, Jessica; Columbia University Medical Center, Institute for Genomic Medicine; Columbia University Vagelos Medical Center, Division of Maternal-Fetal Medicine Wapner, Ron; Columbia University Medical Center, Institute for Genomic Medicine; Columbia University Vagelos Medical Center, Division of Maternal-Fetal Medicine Kilby, Mark; Birmingham Women's and Children's NHS Foundation Trust, West Midlands Fetal Medicine Centre; University of Birmingham, Institute of Metabolism and Systems Research, College of Medical & Dental Sciences
Keywords:	Cardiac, Exome sequencing, Fetus, Prenatal diagnosis, Next generation sequencing, Congenital heart disease
Manuscript Categories:	Obstetrics

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Manuscripts

1 Congenital heart disease and the Diagnostic yield with Exome sequencing (CODE Study): A  
2 prospective cohort study and systematic review

3  
4 SHORT TITLE: Exome sequencing in congenital cardiac anomalies

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6 F. MONE<sup>1</sup>, R.Y. EBERHARDT<sup>2</sup>, R.K. MORRIS<sup>1,3</sup>, M.E HURLES<sup>2</sup>, D.J. McMULLAN<sup>4</sup>; E.R. MAHER<sup>5</sup>,  
7 J. LORD<sup>2</sup>, L.S. CHITTY<sup>6</sup>, J.L.GIORDANO<sup>7</sup>, R.J. WAPNER<sup>7</sup>, MD KILBY<sup>1,3</sup> and the CODE Study  
8 Collaborators

9  
10 <sup>1</sup>West Midlands Fetal Medicine Centre, Birmingham Women's and Children's National  
11 Health Service (NHS) Foundation Trust, Birmingham, UK; <sup>2</sup>Wellcome Sanger Institute,  
12 Hinxton, UK; <sup>3</sup>Institute of Metabolism and Systems Research, College of Medical & Dental  
13 Sciences, University of Birmingham, Edgbaston, Birmingham, UK; <sup>4</sup>West Midlands Regional  
14 Genetics Service, Birmingham Women's and Children's National Health Service (NHS)  
15 Foundation Trust, Birmingham, UK; <sup>5</sup> Department of Medical Genetics, University of  
16 Cambridge, Cambridge, UK; NIHR Cambridge Biomedical Research Centre, Cambridge, UK;  
17 Department of Clinical Genetics, Cambridge University Hospitals NHS Foundation Trust,  
18 Cambridge, UK; <sup>6</sup>London North Genomic Laboratory Hub, Great Ormond Street NHS  
19 Foundation Trust and UCL Great Ormond Street Institute of Child Health, London UK;  
20 <sup>7</sup>Institute for Genomic Medicine, Columbia University Medical Center, New York, NY, USA;  
21 Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Columbia  
22 University Vagelos Medical Center, New York, NY, USA

23  
24 CODE Study Collaborators:

25 A.S.Y KAN and B.H.Y CHUNG - Department of Obstetrics and Gynaecology, Queen Mary  
26 Hospital, The University of Hong Kong, Hong Kong, Hong Kong Special Administrative  
27 Region, China.

28  
29 Corresponding author: Dr Fionnuala Mone. Fetal Medicine Centre, Birmingham Women's  
30 and Children's NHS Foundation Trust, Edgbaston, Birmingham B15 2TG, UK  
31 E: [fionnuala.mone@nhs.net](mailto:fionnuala.mone@nhs.net) T: +44-121-472-1377

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34  
35 KEY WORDS : CARDIAC; CONGENITAL HEART DISEASE; EXOME SEQUENCING; FETUS;

36 PRENATAL DIAGNOSIS; NEXT GENERATION SEQUENCING

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46 CONTRIBUTION

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48 What are the novel findings of this work?

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

52

53 What are the clinical implications of this work?

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

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## 61 ABSTRACT

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63

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

68 METHODS: A prospective cohort study of 197 trios undergoing ES [following](#)  
69 CMA/karyotype because CHD was identified prenatally and a systematic review of the  
70 literature was performed. MEDLINE, EMBASE and CINAHL (2000–Oct 2019) databases were  
71 searched electronically. Selected studies included those with; (i) >3 cases; (ii) initiation of  
72 testing based upon a prenatal phenotype only and; (iii) where CMA/karyotyping was  
73 negative. PROSPERO No. CRD42019140309

74 RESULTS: In our cohort ES gave an additional diagnostic yield in; (i) all CHD; (ii) isolated CHD  
75 and; (iii) multi-system CHD of 12.7% (n=25/197), 11.5% (n=14/122) and 14.7% (n=11/75)  
76 (p=0.81). The pooled incremental yields for the aforementioned categories from 18-studies  
77 (n=636) were 21% (95% CI, 15-27%), 11% (95% CI, 7-15%) and 37% (95% CI, 18%-56%)  
78 respectively. This did not differ significantly when sub-analyses were limited to studies  
79 including >20 cases. In instances of multi-system CHD in the primary analysis, the  
80 commonest extra-cardiac anomalies associated with a pathogenic variant were those  
81 affecting the genitourinary system 44.2% (n=23/52). Cardiac shunt lesions had the greatest  
82 incremental yield, 41% (95% CI, 19-63%), followed by right-sided lesions 26% (95% CI, 9-  
83 43%). In the majority of instances pathogenic variants occurred *de novo* and in autosomal

84 dominant (monoallelic) disease genes (68/96; 70.8%). The commonest monogenic  
85 syndrome identified was Kabuki syndrome (n=19/96; 19.8%).

86 CONCLUSIONS: Despite the apparent incremental yield of prenatal exome sequencing  
87 in congenital heart disease, the routine application of such a policy would require the  
88 adoption of robust bioinformatic, clinical and ethical pathways. ~~In the setting of robust~~  
89 ~~bioinformatic, clinical and ethical pathways, prenatal exome sequencing should be~~  
90 ~~considered when cardiac abnormalities are detected.~~ Whilst the greatesthighest diagnostic  
91 yield is with multi-system anomalies, consideration may should be also be given to  
92 performingoffering ES in the presence of isolated cardiac abnormalities.

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## 96 INTRODUCTION

97

98 Congenital heart disease (CHD) ~~occurs in~~ complicates 1% of live-born ~~infants~~ neonates and is  
99 associated with significantly high rates of perinatal morbidity and mortality.<sup>1,2</sup> Prenatal  
100 detection of CHD and establishment of a unifying genetic diagnosis can inform prenatal  
101 management, optimise post-natal outcome and aid in the counselling of parents in both  
102 index and subsequent pregnancies.<sup>3</sup> Of all prenatally diagnosed CHD, 2/3 tends to be  
103 isolated while 1/3 can be associated with extra-cardiac anomalies (ECAs).<sup>4</sup> Aneuploidy is  
104 present in between 28-45% of prenatally diagnosed CHD, with at least one ECA present in as  
105 many as 98% of such cases.<sup>3</sup> Copy number variation (CNV) can be present in a further 2-  
106 25%.<sup>3</sup> The additional proportion of CHD caused by monogenic Mendelian disorders is  
107 traditionally thought to be ~5% although results vary.<sup>3</sup> Since the introduction of exome  
108 sequencing (ES), large prospective studies suggest that this proportion is greater.<sup>5,6</sup> It has  
109 been proposed that a significant number of identified variants in CHD within the pediatric  
110 population are *de novo* in nature, most notably when there are co-existing  
111 neurodevelopmental and ECAs.<sup>7,8</sup> There are a paucity of studies which have formally  
112 assessed the diagnostic yield offered from ES over standard chromosome  
113 microarray(CMA)/karyotype in prenatally diagnosed CHD and there is no evidence to  
114 suggest which phenotypic CHD sub-types have the greatest diagnostic yield.<sup>9,10,11</sup> Hence,  
115 the objectives of this prospective cohort study, systematic review and meta-analysis were to  
116 determine the yield of ES over CMA/karyotype in; (i) any prenatally diagnosed CHD; (ii)  
117 isolated CHD; (iii) CHD associated with ECAs and; (iv) CHD dependent on phenotypic  
118 subgroup.



119

## 120 METHODS

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## 122 Extended PAGE Cohort

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124 CODE assessed the extended cohort of the published Prenatal Assessment of Exomes and  
125 Genomes (PAGE) study which included 850 trios (fetus and parents) that underwent ES  
126 analysis when a fetal structural anomaly was detected on ultrasound.<sup>5</sup> This prospective  
127 extended cohort study recruited between October 2014 and May 2018 across 34 fetal  
128 medicine centres in England and Scotland, using the West Midlands Genetic Research  
129 Laboratory (WMGRL) as their laboratory hub and then through the Wellcome Trust Sanger  
130 Institute (for exome sequencing).<sup>5</sup> Eligibility criteria included; (i) prenatal detection of an  
131 anomaly after 11-weeks' gestation including an ~~elevated~~ increased nuchal translucency (NT)  
132 ( $\geq 4$ mm); (ii) an invasive test having been performed; (iii) informed written consent obtained  
133 from both parents for testing and both were  $>16$ -years and; (iv) negative CMA or karyotype  
134 testing. Study methodology is as documented in the original published study but briefly  
135 utilized a standard ES approach with variant interpretation based upon a targeted virtual  
136 gene panel for developmental disorders encompassing 1628 genes.<sup>5</sup> Phenotypes of all cases  
137 were classified using Human Phenotype Ontology (HPO) terms and those which were  
138 cardiac related were selected. Following manual review of free-text descriptions, miscoded  
139 terms ~~and cases of 'single umbilical artery' or 'lymphatic malformations' were removed~~  
140 ~~from the analysis, and~~ as were small muscular ventricular-septal defects (VSDs) were  
141 removed. CHD was initially further classified into 'isolated' and 'multi-system' with a HPO

142 approach to coding additional ECAs, including fetal growth restriction, single umbilical artery  
143 and nuchal thickening but not an elevated first trimester NT. Cardiac phenotypes were  
144 described by fetal medicine specialists and sonographers and confirmed by fetal  
145 cardiologists using the Viewpoint® Version 5.6.16 GE Healthcare, 2012 and were  
146 subsequently coded using the American Heart Association/American College of Cardiology  
147 (AHA/ACC) criteria as; (i) shunt lesions; (ii) left-sided obstructive lesions; (iii) right-sided  
148 lesions and; (iv) complex lesions.<sup>12</sup> Two clinicians reviewed each classification for  
149 concordance (F.M. and M.D.K). Pathogenic variants and variants of uncertain significance  
150 (VUS) where the American College of Medical Genetics classification had been agreed upon  
151 at the clinical review panel were included in the final list of variants.<sup>13</sup> Incidental findings  
152 (IFs) were not reported. The study was approved by the Research and Development offices  
153 and Research Ethics Committees at each institution and obtained ethical approval from the  
154 Research and Development offices and Research Ethics Committees at the West Midlands –  
155 South Birmingham (ref: 13/WM/1219) and each institution.

156

157

## 158 Data Sources

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

165 complete'; 'whole genome sequencing' and 'sequence analysis, DNA'. Alternative terms for  
166 prenatal included 'fetal'; 'fetus' and 'antenatal'. Experts were also contacted and  
167 bibliographies of all relevant papers were searched. Studies not in the English language  
168 were translated. The search strategy is available from the corresponding author on request.  
169 This systematic review was registered prospectively with PROSPERO No. CRD42019140309.

170

171 Eligibility criteria for study selection and data extraction

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

182

183 Quality assessment and data synthesis

184 The incremental yield or risk difference of ES over CMA/karyotype was calculated for each  
185 study with 95% confidence intervals and as a meta-analysis for; (i) all CHD; (ii) subgroup  
186 analyses of isolated and multisystem CHD with only studies included in the latter when the  
187 presence or absence of CHD were available from the data. Cases were stratified as per the

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

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## 214 RESULTS

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## 216 Extended PAGE Cohort

217 Of 850 fetuses undergoing trio ES with prenatally detected structural anomalies, there were

218 n=197 (23.2%) CHD cases in total, of which 61.9% (n=122) were isolated and 38.1% (n=75)

219 associated were with ECAs. Where documented (n=190), the source of fetal DNA was; a)

220 chorionic villi 15.8% (n=30); b) amniocytes 81.1% (n=154) or; c) lymphocytes 3.2% (n=6). G-

221 banding karyotype was performed 3.0% (n=6) of cases, with CMA in the remainder. The

222 diagnostic yield of ES in each group (excluding VUS) was 12.7% (n=25/197) all CHD, 11.5%

223 (n=14/122) isolated CHD and 14.7% (n=11/75) in multisystem CHD respectively (p=0.81). In

224 instances of multi-system CHD with a pathogenic variant, the commonest systems affected

225 were those affecting growth, the nervous system and face (all 45.5% n=5/11). There were

226 not enough cases to identify a dominant sub-classification of CHD hence this was explored

227 further in the systematic review. The overall incidence of VUS was 5.1%. ~~0.06 per CHD~~

228 ~~respectively.~~

229

## 230 Systematic review and meta-analysis

231 In all instances where a study was suitable for inclusion but data was incomplete, the

232 corresponding author was contacted (n=6), of which three responded and two provided

233 complete data.<sup>6,18</sup> Authors of the second largest included study, the Petrovski, *et al.*

234 Columbia University-based study, provided a completed dataset on their CHD cohort as an  
235 extended version of their original study.<sup>6</sup> In addition to both the extended PAGE cohort  
236 study and the extended Petrovski, *et al.* study<sup>6</sup>, a further 16 studies met the overall  
237 selection criteria, leading to a total of 18 studies, as demonstrated in Figure 1.<sup>5,6, 9-11, 18-30</sup>  
238 Table 1 outlines the study characteristics and Figure 2 outlines the overall quality  
239 assessment of all studies included. There was one study where ES was targeted using a CHD  
240 panel while the remainder used a whole ES approach.<sup>9</sup> Not all studies broke CHD down into  
241 isolated/multi-system or distinctive phenotypes as demonstrated or described the cardiac  
242 phenotype [Table 1].

243

#### 244 Combined cohort outcomes

245 18-studies were included, encompassing n=636 CHD cases undergoing ES, of which n=529  
246 stated whether CHD was isolated or associated with ECAs. Hence, 54.4% (n=288/529) of  
247 cases were isolated and 45.6% (n=241/529) multi-system CHD. Where available, the mean  
248 maternal age and gestation at the time of testing was 30 (+/-3.5 SD) years and 22 (+/-4.7)  
249 weeks. The primary genetic test performed prior to ES was CMA 98.0% (n=623/636) with  
250 the predominant source of fetal DNA from amniocytes 54.6% (n=322/590). Of the n=18  
251 studies included, information regarding the originally recruited cohort prior to  
252 CMA/karyotype results were stated for n=5 studies.<sup>5,6,9,11,24</sup> These revealed that there was  
253 an abnormal CMA/karyotype in 21.0% (n=1109/5285) of cases. Where stated (n=261), the  
254 median turnaround time for ES was 42 (range 7-82) days and pregnancy outcome was  
255 reported in n=341, of which livebirth 47.8% (n=163) and termination of pregnancy 46.3%

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

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261 Incremental yield of pathogenic variants

262 The pooled incremental yields (excluding VUS) from all 18-studies are illustrated in the

263 forest plots for (i) all ; (ii) isolated and; (iii) multi-system CHD [Figure 3(a-c)]. In the cases of

264 (ii) and (iii) 13 and 15-studies included relevant cases for inclusion. Incremental yields for

265 the aforementioned groups were 21% (95% CI, 15-27% p=0.0006), 11% (95% CI, 7-15%

266 p<0.00001) and 37% (95% CI, 18%-56% p<0.00001) respectively. The sub-analysis of studies

267 with >20-cases (n=8) is demonstrated in Supplementary Figures 2a-c with corresponding

268 funnel plots (Supplementary Figures 3a-c). Findings did not differ significantly from the

269 primary analysis, apart from multi-system CHD, where the incremental yield was greater at

270 49% (95% CI, 17-80% p=0.003). Where gestational age was recorded in isolated CHDs the

271 incremental yield for those diagnosed after 15-weeks' gestation was greater than for all

272 cases at 24% (95% CI, 7%-41%, p=0.002, I<sup>2</sup>=68%). In instances of multi-system CHD in the

273 primary analysis, the commonest ECAs associated with a pathogenic variant were those

274 affecting the genitourinary system 44.2% (n=23/52), nervous system 34.6% (n=18/52) and

275 face 34.6% (n=18/52). In multisystem CHDs, where a pathogenic variant was detected and

276 the specific ECA was documented (82.7%, n=43/52), there was one instance (2.3%, n=1/43)

277 where a 'minor ECA' was present (single umbilical artery), with the remainder being major

278 or affecting two or more systems.

279

280 On classification as per AHA/ACC criteria for all CHD, shunt lesions (septal anomalies and  
281 total anomalous pulmonary venous drainage) had the greatest pooled incremental yield of  
282 pathogenic variants 41% (95% CI, 19-63% p=0.003), followed by right-sided 26% (95% CI, 9-  
283 43%, p=0.001), complex 23% (95% CI, 9-36%, p=0.001) and left-sided obstructive lesions  
284 18% (95% CI, 0-35% p=0.02). Where documented, pathogenic variants are described in  
285 Supplementary Table 1. Where pathogenic variants were documented (n=96/111; 86.5%),  
286 the commonest genetic syndromes identified were those of Kabuki syndrome (n=19/96;  
287 19.8%), CHARGE (Coloboma-Heart defects-Atresia choanae-Retardation of growth-genital  
288 abnormalities-ear abnormalities) syndrome (n=8/96; 8.3%), Noonan syndrome (n=6/96;  
289 6.3%) and Primary Ciliary Dyskinesia (n=6/96; 6.3%). In syndromes where CHD was typically  
290 described as being multi-system in nature, in 54.1% (n=20/37) of such syndromes only an  
291 isolated CHD was detected prenatally e.g. Adams-Oliver, CHARGE, Kabuki and Simpson-  
292 Golabi-Behmel syndrome. In the majority of instances pathogenic variants occurred *de*  
293 *novo* and in autosomal dominant (monoallelic) disease genes (68/96; 70.8%)  
294 [Supplementary Table 1].

295

296

297



## 299 DISCUSSION

300 This is the first systematic review assessing the yield of antenatal ES in prenatally diagnosed  
301 CHD in which ~~CMA~~chromosome microarray/karyotype testing was negative. The results of  
302 this study ~~show an apparent incremental yield of ES in CHD~~support the use of ES in the  
303 ~~investigation of prenatally detected CHD. The diagnostic yield is~~particularly high for shunt  
304 lesions and multi-system CHD. Most pathogenic variants occurred *de novo* ~~and~~ in  
305 ~~autosomal dominant (monoallelic)~~ disease genes with a high incidence of Kabuki  
306 ~~syndromesyndrome. The majority were . A high number of pathogenic variants were~~  
307 reported in syndromes which typically present with ECAs yet presented with an isolated  
308 CHD.

309

310 The diagnostic yield from our ~~own~~cohort study ~~(12.7% all CHD)~~ was modest compared to  
311 other studies ~~included~~ in the meta-analysis ~~(range 0-40% all CHD)~~. This is potentially likely to  
312 ~~be~~secondary to several factors; (i) bias in case selection – ~~while some studies in the review~~  
313 ~~such as PAGE and Petrovski, et al.<sup>5,6</sup> presented both positive and negative ES results,~~ smaller  
314 series may have had an element of selection bias only selecting cases with where there  
315 ~~were~~positive results;<sup>31</sup> (ii) the proportion of multi-system CHD – the greater the proportion,  
316 ~~n of these then~~the higher the overall yield and; (iii) the sequencing approach used e.g.  
317 targeted or whole exome; the series from Hu *et al.* (n=44 CHD cases)<sup>9</sup> revealed a high  
318 diagnostic yield when a targeted 77 cardiac ~~gene~~panel approach was used (n=7; 15.9%). Of  
319 the 77 genes, only 5 genes were not included in the PAGE study panel, none of which were  
320 found to be causative ~~of CHD~~ in the Hu, *et al* study.<sup>9</sup> While use of targeted gene panels  
321 potentially have potential to provide a greater yield in a shorter time frame, users must

322 exert caution as they are primarily based upon postnatal and not prenatal phenotypes  
323 ~~which can differ from the prenatal phenotype where the diagnosis may be less definitive.~~<sup>31</sup>

324

325 The greater incremental yield with ES associated with multi-system vs. isolated CHD is  
326 similar to the pattern seen with aneuploidy and CNV, as is the case with shunt lesions and  
327 left-sided obstructive lesions.<sup>15</sup> Shunt lesions tend to be associated with ECAs which is  
328 probably why the diagnostic yield with ES in this group is most significantly enriched.<sup>3,4</sup> The

329 predominance of *de novo* variants ~~occurring in autosomal dominant (monoallelic)~~ disease  
330 genes is also in keeping with published published evidence.<sup>3,7,8,32</sup> It is interesting that the

331 most common syndromes unveiled in this study were those of Kabuki and CHARGE. Kabuki

332 syndrome has a highly variable phenotype ~~with characteristic facies, abnormal growth,~~  
333 ~~developmental delay and cardiac and renal anomalies.~~<sup>33</sup> There is limited evidence with

334 regards the prenatal presentation and the high incidence as seen in this study has not been  
335 previously reported, although an overall association with postnatally diagnosed left-sided

336 CHD cardiac lesions has been established.<sup>33-35</sup> Both CHARGE and Kabuki syndromes are

337 caused by pathogenic variants in genes encoding proteins implicated in chromatin function  
338 and gene regulation.<sup>36</sup> ~~DNA methylation profiles are altered in both disorders<sup>36</sup> and~~

339 ~~epigenetic dysregulation was the commonest pathway linked to genetically characterised~~

340 CHD in our own series and in the systematic review. ~~There~~ There is a potential link between

341 these syndromes with an association between DNA methylation targets in their gene-  
342 specific signatures.<sup>36</sup> This reflects that epigenetic dysregulation is the commonest pathway

343 responsible for the greatest proportion of CHD where pathogenic single gene variants were

344 uncovered in this series.<sup>36</sup>

345

346 The strength of this study is the robust and systematic methodology utilised so that all

347 available studies ~~of both a positive and negative nature~~ were included to limit selection bias.348 International collaboration between the two groups publishing the two largest series ~~to~~349 ~~date~~ of prenatal congenital anomalies and ES has optimised the numbers ~~included~~. By350 excluding studies where phenotypes were based on ~~a~~ postnatal examination, our study is

351 specific for prenatal ES testing focusing on ultrasound detected CHD. The quality of

352 included studies based upon pre-specified criteria was optimal due to the high number ~~of~~353 ~~studies~~ which had an ES approach to testing, variant interpretation based upon ACMG354 criteria ~~and with~~ Sanger sequencing validation which meant ~~that most many of the studies~~355 ~~included~~ had a uniform and hence comparable approach.<sup>13</sup>

356

357

358 The main study limitation ~~of the analysis~~ was high heterogeneity, ~~notably in the multi-~~359 ~~system group~~. This was likely caused by differing platforms used, as well as small-study360 effects, ~~as~~ reflected in asymmetry within the funnel plots. However, limiting the inclusion of361 studies to those with >20 cases did ~~n~~'ot show a significant difference in incremental yield.362 There is currently no recognised classification system for prenatal CHD ~~hence and in our~~363 ~~study~~, we selected an adult-based ~~classification~~ system.<sup>12</sup> This meant that rare CHD364 associated with high instances of perinatal ~~or in utero~~ demise ~~e.g. heterotaxy~~ could not be

365 appropriately classified. Alternative classification systems were considered and experts

366 were consulted, however ~~it was felt that~~ the categories included were too broad which

367 mean that due to a restricted number of cases where the phenotype was described,

368 relevant associations would not be identified.<sup>37,38</sup>

369

370 The challenges of ES in prenatally diagnosed CHD include; (i) the limited phenotype available  
371 from ultrasound imaging. Although ~~the~~ concordance is generally high, more information is  
372 typically gathered from detailed post-natal examination.<sup>1,39,40</sup>; (ii) whether targeted panels  
373 or a whole ES approach should be used and; (iii) that CHD tends to be a highly heterogenous  
374 group of anomalies with multi-gene and multifactorial pathologies which may not be  
375 unveiled with genomic testing.<sup>3</sup> Further novel gene discovery may lie in epigenomic or  
376 genomic changes encoding proteins involved in chromatin re-modelling, the RAS signalling  
377 pathway, ciliary function and sarcomere architecture.<sup>2</sup> A further challenge with ES in  
378 pregnancy is the time constraint which it poses. ~~Turn-around time for prenatal ES was of~~  
379 ~~limited value from the systematic review.~~ Several studies made an *a priori* decision to  
380 report the results after the end of the pregnancy and thus the clinical/laboratory pathways  
381 ~~were~~ not accelerated to achieve real time results to individual members of the study.  
382 However, several fetal ES studies have reported delivering results in a timely fashion to  
383 inform pregnancy management,<sup>28</sup> and a rapid fetal ES service will shortly be introduced in  
384 the English National Health Service for the diagnosis of monogenic disorders. As well as  
385 turnaround time, the clinical utility of ES in CHD ~~(as with other structural anomalies)~~ is  
386 dependent not just on the prospective targeting of phenotypes but also robust  
387 bioinformatics filtering within accredited ~~molecular-genomic~~ laboratories and ~~then~~  
388 detailed analysis by clinical multidisciplinary review groups to assess and determine ~~assess~~  
389 ~~variants and decide if they are~~ causative variants of the phenotype. ~~In addition,~~ Pre-test  
390 counselling must be accurate, clear and comprehensive with consideration given to ethical  
391 challenges. Without such robust bioinformatics and clinical screening of variants, prenatal  
392 ES should ~~not~~ be offered or used in clinical practice.<sup>41,42</sup>

393

394 In conclusion, despite the apparent incremental yield of prenatal ES in CHD, the  
395 routine application of such a policy would require the adoption of robust  
396 bioinformatic, clinical and ethical pathways. Whilst the highest yield is with multi-system  
397 anomalies, consideration may also be given to performing ES in the presence of isolated  
398 CHDs. In conclusion, ES should be considered in CHD. Whilst the highest diagnostic yield is  
399 in cases with multisystem abnormalities, consideration should be given to offering it when  
400 CHD is isolated. Further work is required to explore the benefits and challenges of delivering  
401 targeted or whole exome analysis. Clinical guidelines must be introduced to ensure that  
402 testing is correctly implemented.

## 404 ACKNOWLEDGEMENTS

405

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

418

## 419 CONFLICT OF INTEREST

420 RYE and JL reports grants from the Health Innovation Challenge Fund during the conduct of  
421 the PAGE study. DJM reports grants for travel expenses from Congenica to attend  
422 educational symposia during the conduct of the PAGE study. MEH reports grants from the  
423 Wellcome Trust and the UK Government Department of Health during the conduct of the  
424 study and personal fees from Congenica, outside the submitted work. MDK is a member of  
425 Illumina's International Perinatal Advisory Group but receives no payment for this. ERM has  
426 received travel expenses, accommodation and consultant fees for participating in an  
427 Illumina International Advisory Group after completion of the PAGE study. MDK is funded

428 through the Department of Health, Wellcome Trust and Health Innovation Challenge Fund  
429 (award number HICF-R7-396) for the PAGE and PAGE2 research studies complete August  
430 2019. LSC was partially funded by the same group in relation to PAGE. RJW receives  
431 funding from Illumina and NIH for research. All other authors declare no competing  
432 interests.

433

434

435

ur Peer Review

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## 629 LEGENDS FOR ILLUSTRATIONS

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631 Figure 1 - Flowchart demonstrating included studies

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

633 STARD criteria

634 Figure 3 - Forest plots of incremental yield by exome sequencing over karyotype/microarray

635 in fetuses with prenatally detected cardiac anomalies in (a) all; (b) isolated and; (c) multi-

636 system cardiac anomalies. Only first author of each study is given. [CMA = chromosome

637 microarray; M–H = Mantel–Haenszel].

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Study	ES Approach	Number of Cardiac anomalies		
		All cardiac	Isolated cardiac	Multi-system cardiac
Aarabi <i>et al.</i> * <sup>26</sup>	WES Trio 20,000 gene panel 60-140X coverage	4	2	2
Boissel <i>et al.</i> <sup>20</sup>	WES Trio 110X coverage Agilent capture + Illumina HiSeq 2000 or 2500	11	2	9
Carss <i>et al.</i> <sup>21</sup>	WES Trio 103X coverage Agilent capture + Illumina HiSeq	3	2	1
Daum <i>et al.</i> * <sup>22</sup>	WES Mainly proband only Agilent capture+ Illumina HiSeq 2500	5	1	4
De Koning <i>et al.</i> <sup>30</sup>	WES Trio 1128 genes 80X coverage Agilent capture + NextSeq 500	10	2	8
Drury <i>et al.</i> * <sup>23</sup>	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> <sup>24</sup>	WES Mainly proband only 120X coverage Agilent capture+ Illumina HiSeq 2500	34	29	5
Hu <i>et al.</i> <sup>9</sup>	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> <sup>18</sup>	WES Trio 100X coverage TruSeq Rapid Exome Library Prep Kit Illumina sequencing	7	4	3
Lord <i>et al.</i> <sup>5</sup>	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> <sup>28</sup>	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> <sup>6</sup>	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> <sup>25</sup>	WES Parents only 80X coverage Agilent capture + Illumina HiSeq 2500 or NextSeq500	8	2	6

	Only include het rare (MAF<0.001) variants in same gene in both parents			
Sun <i>et al.</i> * <sup>11</sup>	WES Trio Agilent capture + Illumina Hiseq 4000 or Novaseq	66	55	11
Vora <i>et al.</i> * <sup>29</sup>	CE and WES Trio Illumina Hi-Seq 2500	3	0	3
Westerfield <i>et al.</i> <sup>27</sup>	WES Trio 130X coverage Roche NimbleGen capture + Illumina Genome Analyzer IIx or HiSeq 2000	5	0	5
Westphal <i>et al.</i> <sup>10</sup>	WES Trio 20,000 genes 150X coverage	30	16	14
Yates <i>et al.</i> <sup>19</sup>	WES Trio 140X coverage Agilent capture + Illumina HiSeq 2000 or 2500	26	N/S	N/S

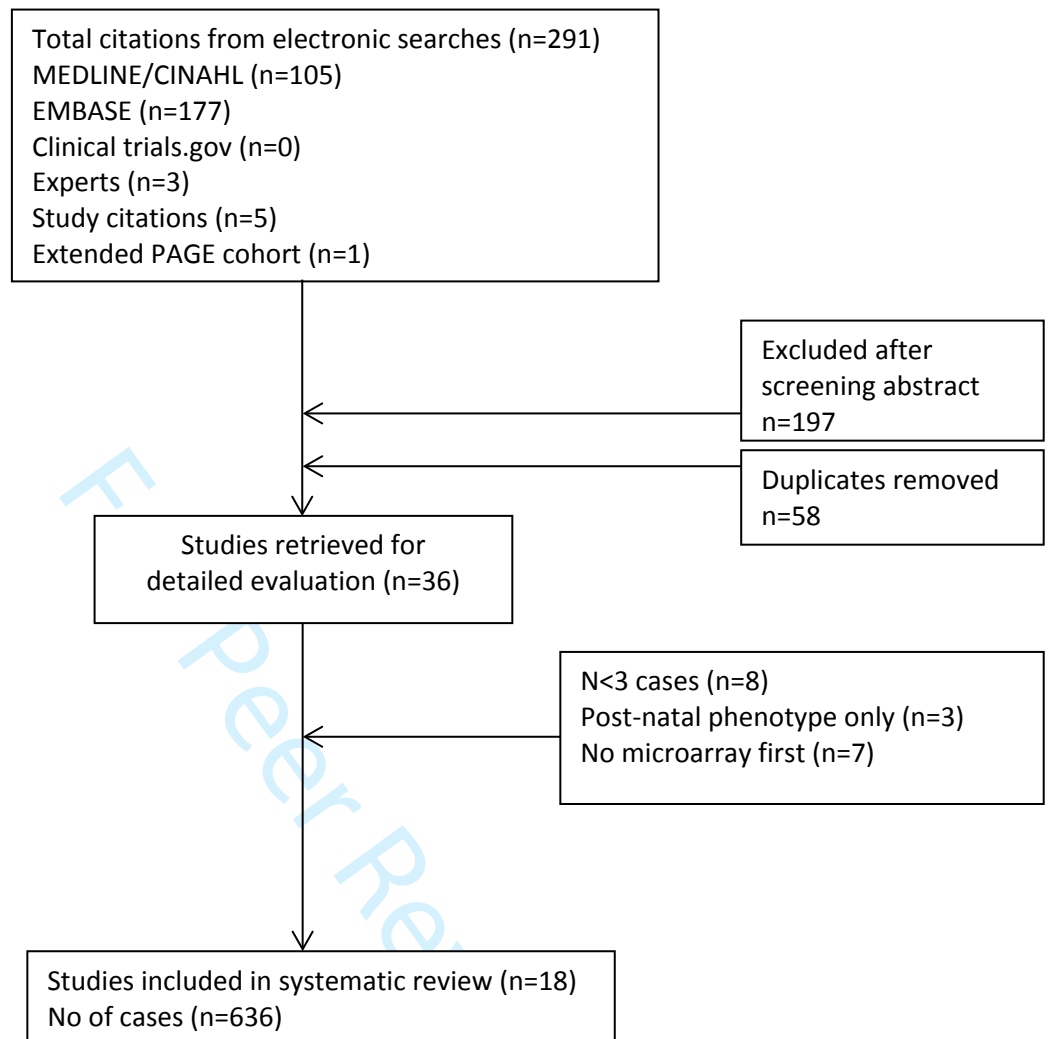
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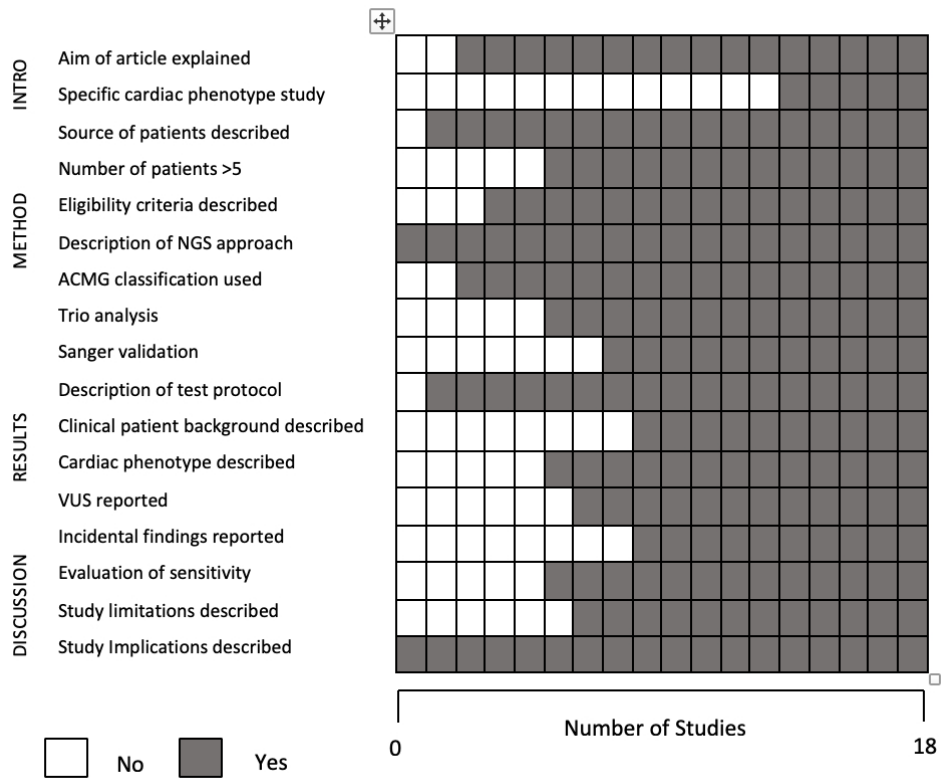
655 Table 1- Study characteristics and rates of pathogenic variants and variant of uncertain

656 significance [CE=Clinical Exome; N/S = not-stated; WES=Whole exome sequencing \*coverage

657 not stated]

658





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

351x295mm (72 x 72 DPI)

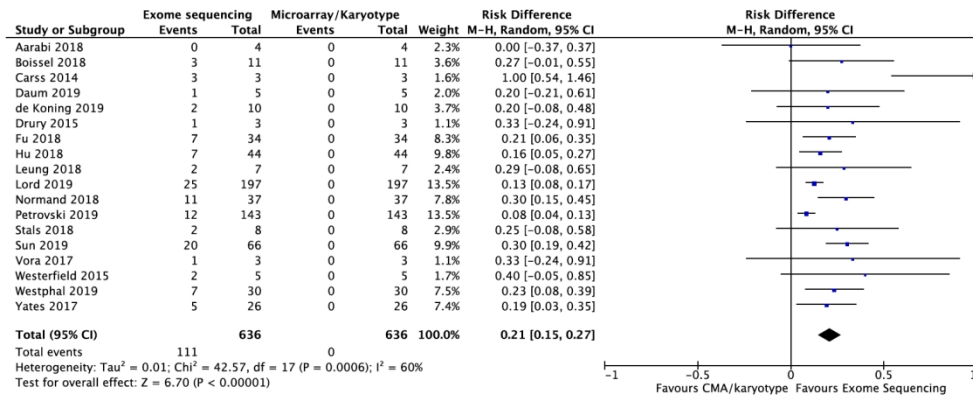


Figure 3 - Forest plots of incremental yield by exome sequencing over karyotype/microarray in fetuses with prenatally detected cardiac anomalies in (a) all; (b) isolated and; (c) multi-system cardiac anomalies. Only first author of each study is given. [CMA = chromosome microarray; M-H = Mantel-Haenszel].

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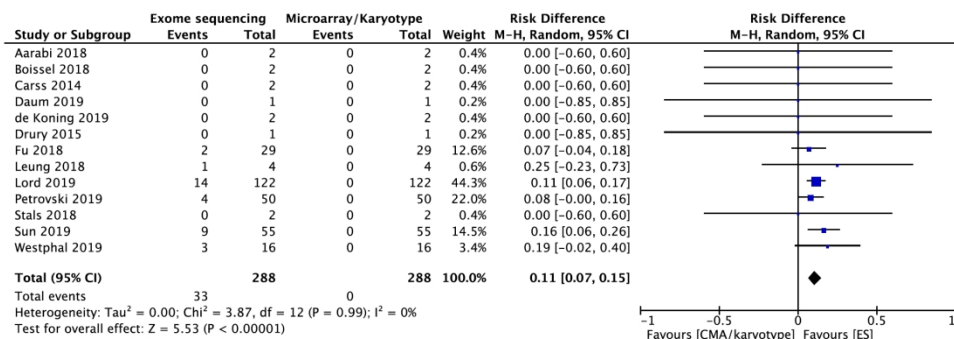


Figure 3 - Forest plots of incremental yield by exome sequencing over karyotype/microarray in fetuses with prenatally detected cardiac anomalies in (a) all; (b) isolated and; (c) multi-system cardiac anomalies. Only first author of each study is given. [CMA = chromosome microarray; M-H = Mantel-Haenszel].

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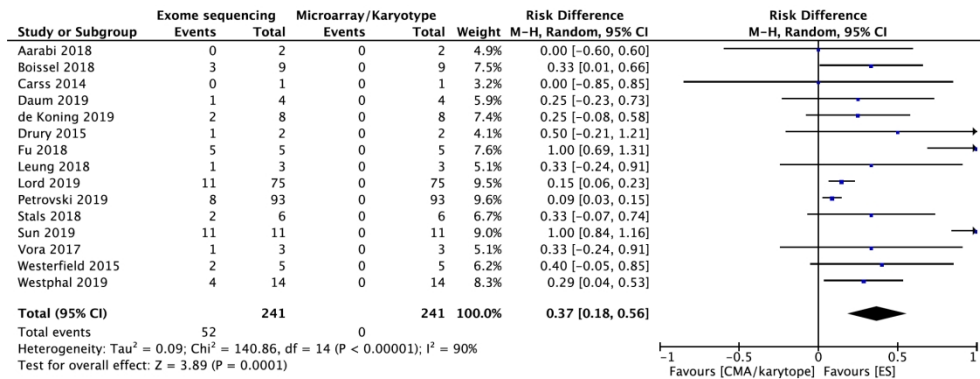


Figure 3 - Forest plots of incremental yield by exome sequencing over karyotype/microarray in fetuses with prenatally detected cardiac anomalies in (a) all; (b) isolated and; (c) multi-system cardiac anomalies. Only first author of each study is given. [CMA = chromosome microarray; M-H = Mantel-Haenszel].

746x326mm (72 x 72 DPI)

ID	Cardiac Phenotype	Additional systems	AHA/ACC Class	Variant	Zygoty	Monoallelic (M) or Biallelic (B)	Clinical syndrome	Reference
SR-001	ASD, PLSVC	Brain, Face, GU	1	CHD7 c.2362C>T (p.Gln788Ter*)	Het	M	CHARGE	22
SR-007	PA dilatation, PLSVC	Extremities, Face	3	TGFBR1 c.605_606insGAGAACTATTGT (p.A202delinsARTIV)	Het	M	Loeys-Dietz syndrome 1	20
ST-008	VSD	GU, Thorax, GI	1	FRAS1 c.370C>T (p.R124X)	Hom	B	Fraser 1	20
SR-009	TOF	GI	3	CHD7 c.5428C>T (p.R1810X)	Hom	M	CHARGE	20
SR-019	COA	Skeleton, Thorax	2	C5orf42 c. 8167C > T (p.Gln2723*) + c.8628C > T (p.Ser2876Ser)	Comp het	B	Oral facial digital type VI	23
SR-024	TOF	Extremities, Face	3	ASPH (p.X226E)	Hom	B	Traboulsi	24
SR-025	Single atrium, single ventricle, PS, RA isomerism		4	DNAH11 c.3426-1G>A	Hom	B	PCD 7, with or without situs inversus	24
SR-026	TGA	GU, Skeleton	4	NEK8 IVS10-1G>A	Hom	B	Renal–hepatic–pancreatic dysplasia 2 [615415]/nephronophthisis 9	24
SR-027	TOF	Face	3	IL11RA (Q159X)	Hom	B	Cariosynostosis and dental anomalies	24
SR-028	VSD		1	ANKRD11 (p.S1271X)	Het	M	KBG	24
SR-029	VSD	Brain	1	MRPS22 IVS5+1G>A (p.Q337X)	Comp het	B	Combined oxidative phosphorylation deficiency 5	24
SR-030	Univentricular	Brain	4	AH11 (p.E1086G)	Hom	B	Joubert syndrome 3	24
SR-059	Heterotaxy		4	DNAH11 c.13288G>A p.(Gly4430Glu) and c.8533_8536delinsATCCG	Comp het	B	PCD 7, with or without situs inversus	18
SR-060	PA		3	CHD7 c.2957+1G>A	Het	M	CHARGE	18
SR-066	TOF		3	CHD7 c.2550_2554delGA GAA (p.K850Nfs*6)	Het	M	CHARGE	9
SR-067	ASD, VSD		1	CITED2 c.574_579delAGC GGC (p.S192_G193del)	Het	M	ASD 8, VSD2	9
SR-068	Single atrium, single ventricle, AA		4	MYH6 c.2168+1G>A	Het	M	ASD 3; cardiomyopathy, dilated, 1EE; cardiomyopathy, familial hypertrophic, 14; sick sinus syndrome	9
SR-069	Cardiac anomaly	GU	6	KMT2D c.11248C>T (p.Q3750*)	Het	M	Kabuki 1	9
SR-070	Extracorporeal heart, VSD	GU	5	ZFPM2 c.2107A>C (p.M703L)	Het	M	Diaphragmatic hernia 3; TOF	9
SR-071	VSD	GU	1	KMT2D c.12140_12168del GGCCGTTAGCAAT AGGAACTACCCCTGAG (p.G4047Vfs*5)	Het	M	Kabuki 1	9
SR-072	Cardiac anomaly	Skeleton	6	JAG1 c.1078 T>G (p.C360G)	Het	M	TOF, Alagille syndrome	9



ID	Cardiac Phenotype	Additional systems	AHA/ACC Class	Variant	Zygoty	Monoallelic (M) or Biallelic (B)	Clinical syndrome	Reference
SR-115	HPV, MGA, TA, VSD.	GI	4	MYH7 c.1727A>G (p.His576Arg)	Comp het	B	Hypertrophic cardiomyopathy 1	10
SR-117	AVSD, DV agenesis	Brain, Face, Skin	1	PTPN11 c.214G>A (p.Ala72Thr)	Het	M	Noonan 1	10
SR-119	DORV, PAPVC	GI	4	DNAI1 c.1003G>T (p.Val335Phe) and c.1543G>A (p.Gly515Ser)	Comp Het	B	PCD, 1, with or without situs inversus	10
SR-126	SYPKA, left SVC, PA, VSD	Face, Extremities, Skin	3	Microdeletion 9q34.3 (approx. chr9:139252466-139418430, including NOTCH1)	Het	M	Adams-Oliver 5	10
SR-127	PA, SYPKA, VSD		3	c.385G>A (p.Glu129Lys)	Het	M	Tetralogy of Fallot	10
SR-128	PA, UV, VSD	GI, Skin	3	c.1372C>T (p.Arg458*) and c.281G>C, (p.Arg94Pro)	Comp Het	B	Heterotaxy, visceral 7	10
SR-130	AA, HRV, MGA		4	PUM1 c.1738C>T (p.Arg580*)	Het	M		10
SR-133	HLHS		4	KMT2D c.11093dup (p.Phe3699Leufs*14)	Het	M	Kabuki 1	10
SR-149	Hypertrophic cardiomyopathy	Brain, Skin, Thorax	5	MRPS22 p.[(Arg170His)];[?] c.[509G>A];[878+1G>T]	Comp Het	B	Combined oxidative phosphorylation deficiency 5	25
SR-150	Hypertrophic cardiomyopathy	GU, Thorax	5	FRAS1 c.[5530-2A>C];[6010G>A] (p.[?];[Gly2004Ser])	Comp Het	B	Fraser 1	25
SR-151 †	VSD, overriding aorta,	Brain, Extremities, Face, GI, Spine	1	PORCN c.90G>A (p.Trp30Ter)	Het	M		5
SR-152*	TR, ECF, PA atresia, HAA, aberrant retro-oesophageal left subclavian artery, dilated left ventricular chamber	Face, Skin, Spine	4	NRAS c.34G>C (p.Gly12Arg)	Het	M	Noonan 6	5
SR-153*	ECF, TR	GU, Skeleton, Skull	5	TCTN2 c.1506-2A>G	Hom	B	Joubert 24	5
SR-154*	Dilated heart, pericardial effusion	GI, Growth	5	COQ9 c.730C>T (p.Arg244Ter)	Hom	B	Coenzyme 10 deficiency	5
SR-155 †	TOF	Brain, GI, Growth, Skin, Extremities	3	FGFR3 c.749C>G (p.Pro250Arg)	Het	M	Thanatophoric dysplasia	5
SR-156*	Truncus arteriosus	Brain, Face, Extremities	4	CHD7 c.988C>T (p.Gln330Ter)	Het	M	CHARGE	5
SR-157*	Cardiac anomaly	Skeleton	6	EVC2 c.3637_3638insTT (p.Trp1213PhefsTer11)	Hom	B	Ellis-van Creveld	5
SR-158*	Bilateral SVCs	Extremities, Skeleton	5	FLNB c.4750G>C (p.Ala1584Pro)	Het	M		5
SR-159*	TOF	Brain, Extremities, Face, Growth, GU	3	RAB23 c.434T>A (p.Leu145Ter)	Hom	B	Carpenter	5

ID	Cardiac Phenotype	Additional systems	AHA/ACC Class	Variant	Zygoty	Monoallelic (M) or Biallelic (B)	Clinical syndrome	Reference
SR-160*	Anomalous pulmonary vessel connection, VSD	Face	1	CHD7 c.757del (p.Val253CysfsTer52)	Het	M	CHARGE	5
SR-161*	TGA, R aortic arch		4	SOS1 c.796_797insAAG (p.Thr266delinsLysAla)	Het	M	Noonan 4	5
SR-162*	Rhabdomyomas		5	PKD1/TSC2 41.2kb deletion	Het	M	Tuberous sclerosis 2	5
SR-163 †	HLHS		4	KMT2D c.11848C>T (p.Gln3950Ter)	Het	M	Kabuki 1	5
SR-165*	AVSD		1	DNAH11 stopped gain	Hom	B	PCD 7, with or without situs inversus	5
SR-166*	AVSD		1	GATA4 frameshift variant	Het	M		5
SR-167*	AS		2	RIT1 c.335G>C (p.Gly112Ala)	Het	M	Noonan 8	5
SR-168*	AVSD		1	ANKRD11 c.5957_5958del (p.Arg1986IlefsTer45)	Het	M	KBG	5
SR-169*	Cardiac anomaly		6	NR2F2 c.745T>C (p.Trp249Arg)	Het	M	Congenital heart defects, multiple types	5
SR-170*	Right atrial isomerism		4	CCDC103 c.461A>C (p.His154Pro)	Hom	B	PCD	5
SR-172*	Cardiac anomaly		6	KMT2D c.673+1G>A	Het	M	Kabuki 1	5
SR-173*	Cardiac anomaly		6	CHD7 c.656dup (p.Leu220ProfsTer67)	Het	M	CHARGE	5
SR-338 †	TOF		3	GPC3 c.677del (p.Thr226IlefsTer8)		M	Simpson-Golabi-Behmel 1	5
SR-341*	Cardiac anomaly		6	TAB2 c.1407_1408del (p.Pro470GlnfsTer2)	Het	M	Congenital heart defects, non-syndromic 2	5
SR-347 †	TOF		3	DNAH5 frameshift variant	Hom	B	PCD 3, with or without situs inversus	5
SR-351	VSD	GU, thorax	1	NIPBL c.459-2A>G	Het	M	Cornelia de Lange type 1	27
SR-354	VSD	Extremities, GU, Skin, Thorax	1	WDR19 c.275>G (p.L92X) and c.880G>A (p.G294R)	Comp Het	B	Short rib thoracic dysplasia, 5, with or without polydactyly	27
SR-357	MGA	Extremities, GU Skull	4	DYNC2H1 c.10594C>T (p.Arg3532Ter) and c.8012T>C (p.Met2671Thr)	Com Het	B	Short rib polydactyly, 3, with or without polydactyly	29
SR-361	DORV and RAA	GU	4	CHD7 c.7890T>A (p.Cys2360*)	Het	M	CHARGE	30
SR-370	left heart obstruction (Shone's complex)	Growth, GU	2	KTM2D c.207T>A (p.Cys69*)	Het	M	Kabuki 1	30
SR-374	Complex cardiac anomaly		4	KMT2D c.6617dupC (p.A2207fs)	Het	M	Kabuki 1	28
SR-375	Complex cardiac anomaly	GU	4	KMT2D c.1967delT (p.L656fs)	Het	M	Kabuki 1	28
SR-376	Complex cardiac anomaly	GU	4	KMT2D c.15680_15693dup (p.I5232fs)	Het	M	Kabuki 1	28
SR-377	Complex cardiac anomaly	GU	4	KMT2D c.5705C>T (p.R10903X)	Het	M	Kabuki 1	28
SR-378	Cardiac anomaly	Skeleton	6	COL1A2 c.2576G>A (p.G859D)	Het	M	OI types 2-4 and Ehlers Danlos type 7B and cardiac valvular	28
SR-379	Cardiac anomaly	Brain, GU, Skeleton	6	DVL1 c.1519delT (p.W507fs)	Het	M	Robinow autosomal dominant 2	28

ID	Cardiac Phenotype	Additional systems	AHA/ACC Class	Variant	Zygoty	Monoallelic (M) or Biallelic (B)	Clinical syndrome	Reference
SR-411	Cardiomyopathy	Brain, Skin	5	MRPS22 c.768_769 and p.R170H	Comp Het	B	MRPS22-related mitochondrial dysfunction	19
SR-412	Cardiomegaly	Skin	5	CYP11A1 (p.R120X)	Hom	B	Adrenal insufficiency, congenital, with 46XY sex reversal, partial or complete	19
SR-413	Cardiac axis deviation	Brain, Extremities	5	FANCB c.987_990del	Hemi	M	Fanconi anaemia, complementation group B	19
SR-414	Cardiac anomaly	Brain, Extremities, Face, Skull	6	AMER1 c.705delT	Hemi	M	Osteopathia striata with cranial sclerosis	19
SR-415	Cardiac anomaly	Brain, Skin	6	RIT1 p.F82C	Het	M	Noonan 8	19
SR-416	Cardiac anomaly	Brain	6	PIK3R2 p.K564E	Het	M	Megalencephaly-poly-microgyria-polydactyly-hydrocephalus	19
SR-437	COA	Face, GI, GU	2	KMT2D c.8430dupA (p.Gln2811Thrfs*34)	Het	M	Kabuki 1	11
SR-438	HLHS		4	KMT2D c.15920_15921+2delC (p.Leu5380Alafs*36)	Het	M	Kabuki 1	11
SR-439	Aortic valve atresia		2	KMT2D c.8074_8075delCG (p.Arg2692Alafs*31)	Het	M	Kabuki 1	11
SR-440	HLHS		4	KMT2D c.1845_1846del (p.Leu617Phefs*5)	Het	M	Kabuki 1	11
SR-441	Mitral atresia	Face	2	KMT2D c.6595delT (p.Tyr2199Ilefs*65)	Het	M	Kabuki 1	11
SR-442	HLHS		4	KMT2D c.8159G>A (p.Trp2720*)	Het	M	Kabuki 1	11
SR-443	HLHS		4	KTM2D c.16489_16491del (p.Ile5497del)	Het	M	Kabuki 1	11
SR-444	COA		2	NOTCH1 c.3643+1G>A	Het	M	Adams-Oliver 5	11
SR-445	HLHS		4	NOTCH1 c.4015-2A>G mat	Het	M	Adams-Oliver 5	11
SR-446	AS		2	NOTCH1 c.4837C>T (p.Gln1613*)	Het	M	Adams-Oliver 5	11
SR-447	HLHS		4	NOTCH1 c.2452dupC (p.Leu818Profs*10)	Het	M	Adams-Oliver 5	11
SR-448	COA	Thorax	2	MYRF c.789delC (p.Ser264Alafs*8)	Het	M	Cardiac-urogenital syndrome	11
SR-449	HLHS	Thorax	4	CRB2c.2029C>T (p.Arg677Cys) and c.3076_3077insTGGCGCGCCCCGCGCGCGCCCC (p.Arg1038Alafs*45)	Het	B		11
SR-545	Rhabdomyomas	Brain	5	TSC2 Chr 16, 2120571, C→T 1831C→T, Arg611Trp	Het	M	Tuberous sclerosis 2	6
SR-546	VSD	GU	1	PKD1 Chr 4, 88983135, ACT→A 2101_2102delTC, Ser701ArgfsX9	Het	M	MPKD	6
SR-557	COA	Growth, GU, Spine	2	KMT2D Chr 12, 49443635, TAG→T 3734_3735delCT, Ser1245TyrfsX4	Het	M	Kabuki 1	6
SR-561	VSD		1	MYL2 484G→A (p.Gly162Arg)	Het	M	Familial hypertrophic cardiomyopathy	6

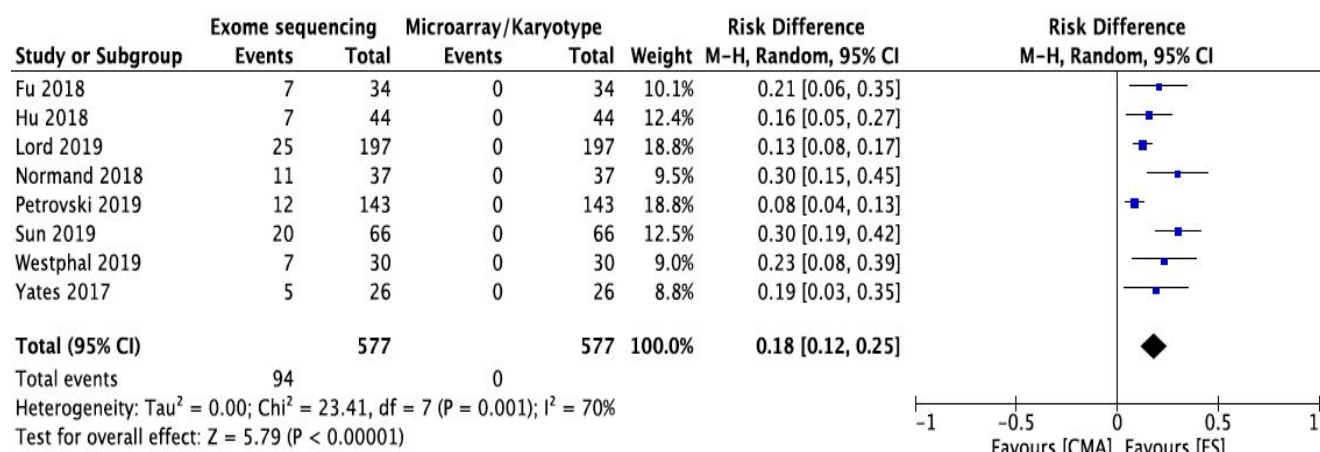
ID	Cardiac Phenotype	Additional systems	AHA/ACC Class	Variant	Zygoty	Monoallelic (M) or Biallelic (B)	Clinical syndrome	ID
SR-569	TGA	Brain	4	COL4A1 Chr 13, 110830552, C→G 2485G→C (p.Gly829Arg)	Het	M		6
SR-576	Rhabdomyomas		5	TSC2 Chr 16, 2138293, CCGGCTCCGCCACATCAAG→C 5037_5054delC_A, His1679_Arg1684del	Het	M	Tuberous sclerosis 2	6
SR-592	TOF		3	NIPBL variant	Het	M	Cornelia de Lange 1	6
SR-598	Cardiac anomaly	Neck/Skin	6	NR2F2 variant	Het	M	Congenital heart defects, multiple, type 4	6
SR-612	Cardiac anomaly	Face, Skeleton, Thorax	6	SCN2A variant	Het	M	Epileptic encephalopathy, early infantile, 11	6
SR-613	Cardiac anomaly		6	LZTR1 variant	Comp Het	B	Noonan 2	6
SR-635	TOF	GU	3	KMT2D variant	Het	M	Kabuki 1	6

Table S1 – Diagnostic variants identified from the systematic review

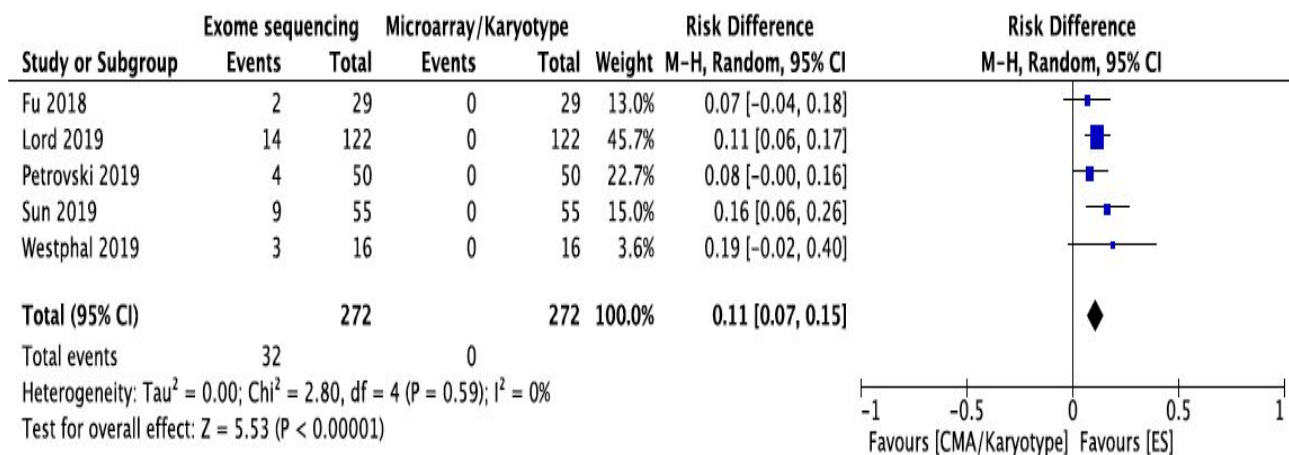
[Abbreviations; AA = aortic atresia; ADPKD; Autosomal dominant polycystic kidney disease; AHA/ACC = American Heart Association/American College of Cardiology; AS = aortic stenosis; ASD = atrial septal defect; AVSD = atrial-ventricular septal defect; B = Biallelic; COA = coarctation of the aorta;; DOLV = Double outlet left ventricle; DORV = double outlet right ventricle; DV = ductus venosus; ECF = echogenic cardiac focus; GI = Gastrointestinal; GU = Genitourinary; HAA = hypoplastic aortic arch; HLHS = hypoplastic left heart; HPV; hypoplastic pulmonary veins; HRHS = hypoplastic right heart; HRV = hypoplastic right ventricle; MGA = malposition of great arteries; PA = pulmonary atresia; PAPVC = partial anomalous pulmonary venous connection; PCD = Primary Ciliary Dyskinesia; PLSVC = partial left superior vena cava; PS = pulmonary stenosis; SVC = superior vena cava; TA = tricuspid atresia; TGA = transposition of the great arteries; TOF=Tetralogy of Fallot; TR = tricuspid regurgitation; UV = univentricular; VSD = ventricular septal defect] \*previously reported in PAGE study publication; † unreported in PAGE study publication. AHA/ACC Criteria 1= Shunt lesions; 2= left-sided obstructive lesions; 3= right-sided lesions and; 4= complex lesions; 5=miscellaneous; 6=uncategorised).

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)

S2a – All CHD

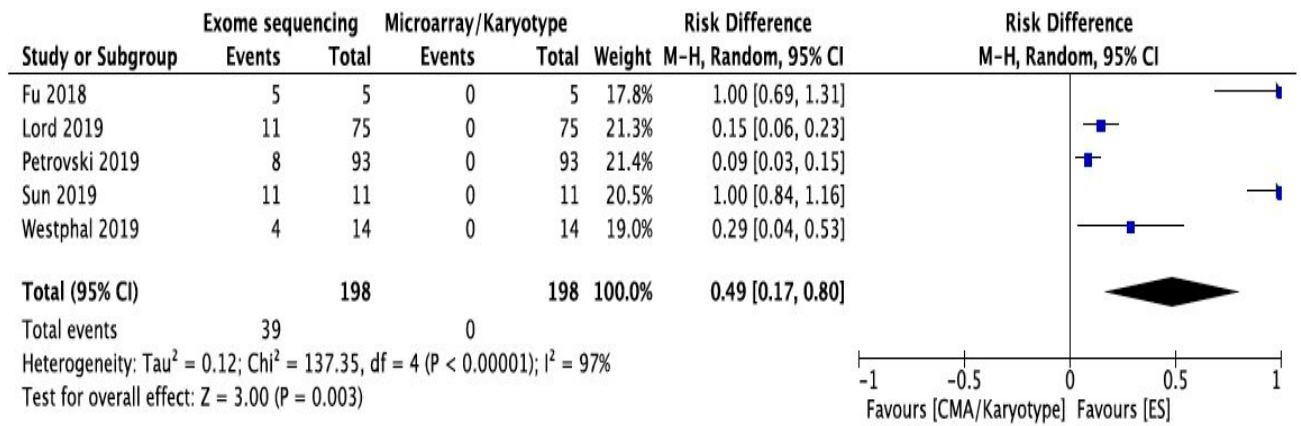


S2b – Isolated CHD



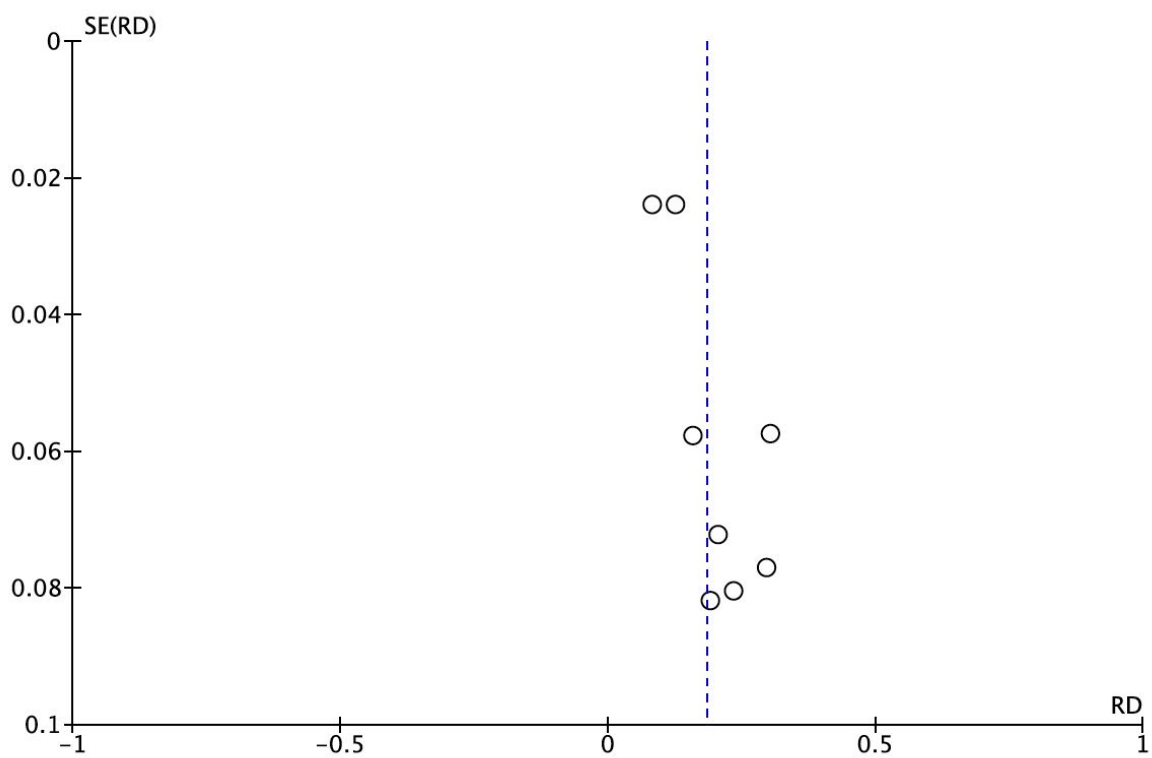
Peer Review

## S2c – Multi-system CHD



For Peer Review

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)



S3a – All CHD



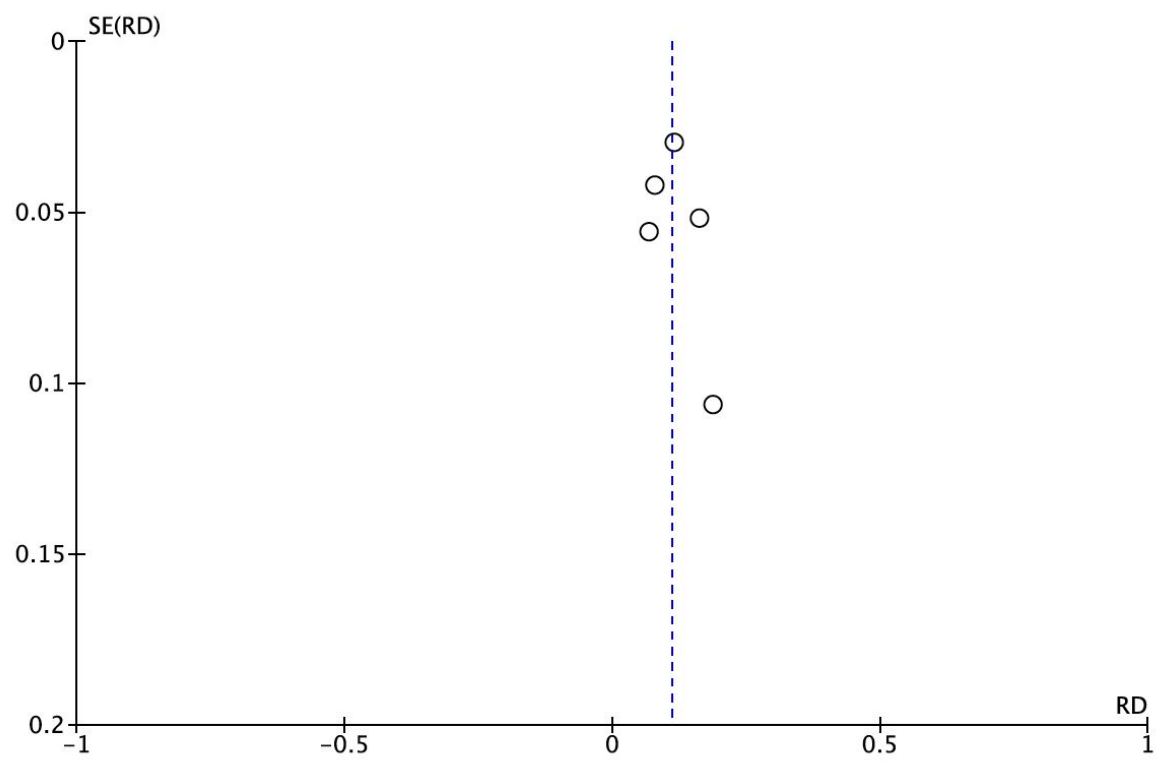
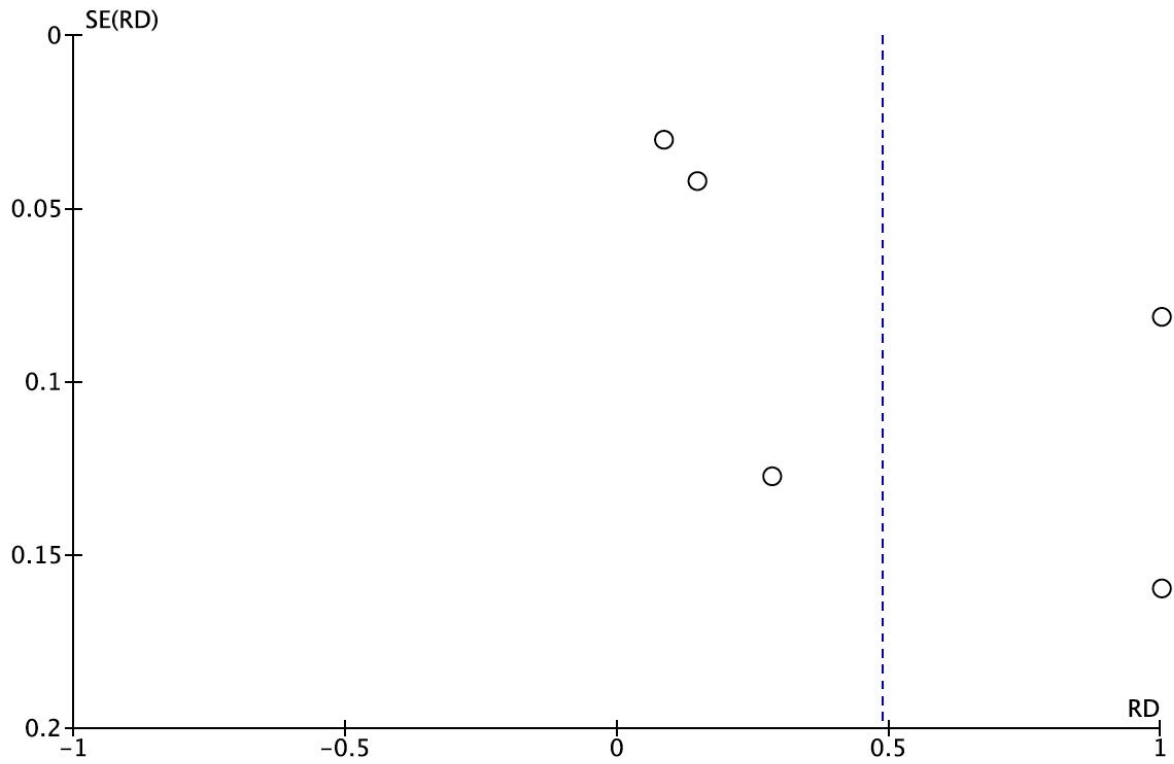


Figure S3b – Isolated CHD

Peer Review



S3c – Multi-system CHD

Peer Review



# PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
<b>ABSTRACT</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
<b>INTRODUCTION</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
<b>METHODS</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6-7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7-8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7-8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$ ) for each meta-analysis.	7-8



# PRISMA 2009 Checklist

Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7-8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7-8
<b>RESULTS</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Fig 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	10
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Fig 3, 14
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Fig 3
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10-11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10-11
<b>DISCUSSION</b>			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12-14
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
<b>FUNDING</b>			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	16

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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