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Array comparative genomic hybridization and fetal congenital heart defects: a systematic review and meta-analysis

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KEYWORDS: array comparative genomic hybridization; congenital heart defects; copy number variants; prenatal diagnosis

ABSTRACT

Objective *Array comparative genomic hybridization (aCGH) is a molecular cytogenetic technique that is able to detect the presence of copy number variants (CNVs) within the genome. The detection rate of imbalances by aCGH compared to standard karyotyping and 22q11 microdeletion analysis by fluorescence* in-situ *hybridization (FISH), in the setting of prenatally-diagnosed cardiac malformations, has been reported in several studies. The objective of our study was to perform a systematic literature review and meta-analysis to document the additional diagnostic gain of using aCGH in cases of congenital heart disease (CHD) diagnosed by prenatal ultrasound examination, with the aim of assisting clinicians to determine whether aCGH analysis is warranted when an ultrasonographic diagnosis of CHD is made, and to guide counseling in this setting.*

Methods *Articles in PubMed, EMBASE and Web of Science databases from January 2007 to September 2014 describing CNVs in prenatal cases of CHD were included. Search terms were: 'array comparative genomic hybridization', 'copy number variants' and 'fetal congenital heart defects'. Articles regarding karyotyping or 22q11 deletion only were excluded.*

Results *Thirteen publications (including 1131 cases of CHD) met the inclusion criteria for the analysis. Meta-analysis indicated an incremental yield of 7.0% (95% CI, 5.3–8.6%) for the detection of CNVs using aCGH, excluding aneuploidy and 22q11 microdeletion cases. Subgroup results showed a 3.4% (95% CI, 0.3–6.6%) incremental yield in isolated CHD cases, and 9.3% (95% CI, 6.6–12%) when extracardiac malformations were present. Overall, an incremental yield of 12% (95% CI, 7.6–16%) was found when 22q11 deletion cases were included. There was an additional yield of 3.4% (95% CI, 2.1–4.6%) for detecting variants of unknown significance (VOUS).*

Conclusions *In this review we provide an overview of published data and discuss the benefits and limitations of using aCGH. If karyotyping and 22q11 microdeletion analysis by FISH are normal, using aCGH has additional value, detecting pathogenic CNVs in 7.0% of prenatally diagnosed CHD, with a 3.4% additional yield of detecting VOUS.* *Copyright © 2014 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Congenital heart disease (CHD) is the leading cause of non-infectious neonatal mortality, affecting up to 1% of newborns. For most cases of CHD, surgical repair or palliation is now possible, with a good outcome[1]. In some cases, however, the prognosis is dominated by the presence of chromosomal or extracardiac malformations[2–4]. In the prenatal setting, the incidence of chromosomal anomalies is reported to be as high as 18–22% of all CHD cases, most being trisomies 21 and 18 and 22q11 microdeletion[5–7]. Furthermore, fetuses with CHD carry a residual risk of additional genetic anomalies including microdeletion or microduplication syndromes such as Williams–Beuren and Potocki–Lupski, or monogenetic

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anomalies such as Noonan syndrome[8,9]. Providing information about the association of CHD with additional anomalies is important when counseling future parents. Assessing the presence of a pathogenic copy number variant (CNV) is crucial for prognostic purposes, given that the risk of non-iatrogenic neurological impairment is increased even in apparently isolated CHD[10]. Prenatal diagnosis of genetic conditions can also influence treatment plans[2,4]. In certain types of severe CHD, the interval between delivery and the necessary surgical procedure can be short, highlighting the importance of prenatal testing.

Cytogenetic fetal karyotyping used to be the gold standard of prenatal genetic testing. Karyotyping is able to detect aneuploidy and large chromosomal rearrangements of up to 5–10 megabases (Mb). Array comparative genomic hybridization (aCGH) is a cytogenetic molecular technique that detects the presence of CNVs within the genome with increased resolution, much higher than that of conventional karyotyping, depending on the probe spacing and platform used.

Reports detailing the incremental yield of aCGH in the prenatal setting are rapidly emerging[11–22]. Most published reports include large cohorts, but describe the incremental yield for a variety of indications. Subgroup analysis of (different types of) CHD, the most common structural abnormality detected in the prenatal setting, is rarely reported. In this review, we describe the incremental yield of aCGH in prenatally-diagnosed CHD. Our goal was to assist clinicians in determining whether aCGH is warranted once the diagnosis of a fetal CHD is made, and to guide them as they counsel future patients in this setting.

METHODS

A literature review was performed conforming to the Database of Abstracts of Reviews of Effects (DARE) criteria[23]. We conducted a systematic search of articles available on the PubMed, EMBASE and Web of Science databases from January 2007 to September 2014, using search terms: 'array comparative genomic hybridization', 'copy number variants', 'prenatal' or 'fetal malformations' and 'congenital heart defects', with related search terms (complete search string is available in Appendix S1). There was no language restriction to our search.

The extracted articles were evaluated for relevance by two independent researchers (F. J. and M. H.). Eligible titles were identified and further screened based on the abstract. Non-English abstracts were assessed by an appropriate native speaker. Only original research articles discussing the yield of array analysis in the prenatal setting were reviewed for the full text. If a (sub)group of CHD could be identified in the published data, the article was included. Genetic locus association studies in familial occurrence of CHD and case reports were excluded. We analyzed the references of eligible articles for further inclusions. Data on inclusion criteria, patient characteristics (type of CHD, presence of multiple malformations), array resolution, methods of CNV interpretation and postnatal confirmation of the heart defect were extracted from the publications. Details of all reported aCGH anomalies were assessed by two authors independently (F. J. and J. C.) to evaluate clinical significance. Raw data of one publication[16]

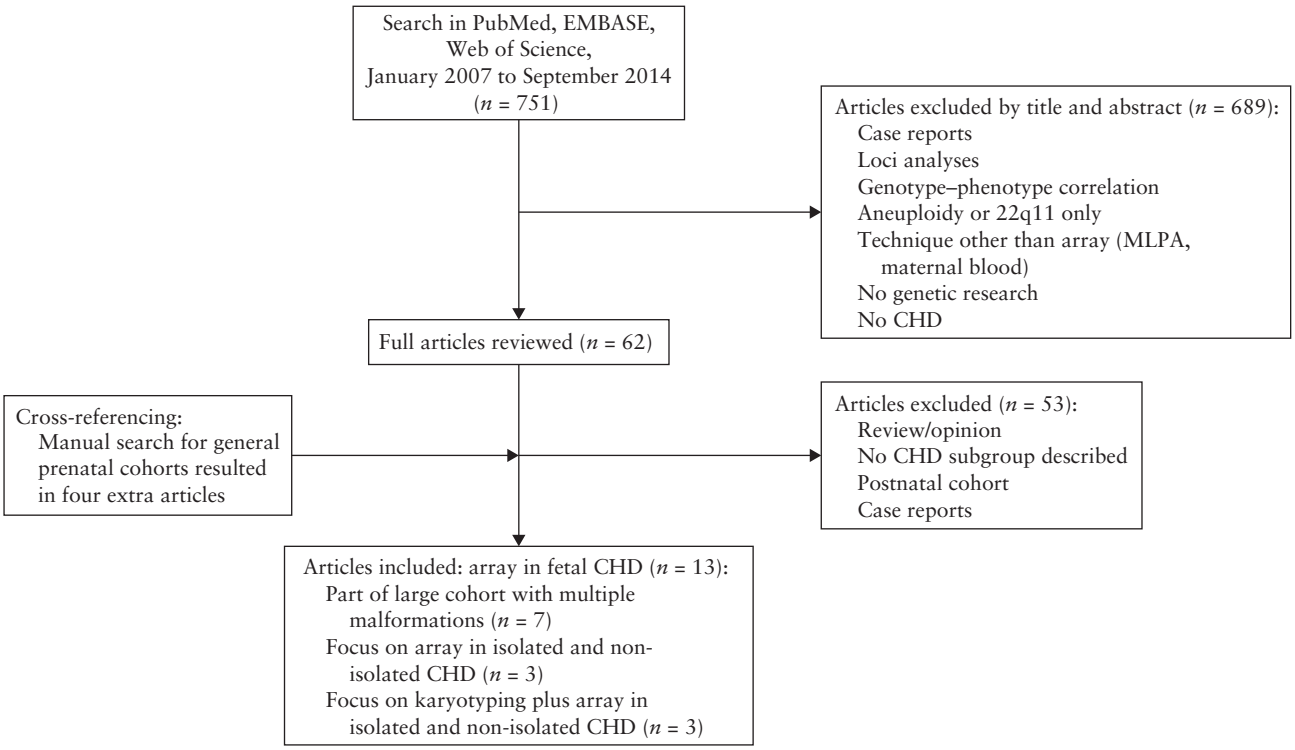

Figure 1 Flow-chart showing inclusion of studies in the review. CHD, congenital heart defects; MLPA, multiplex ligation-dependent probe amplification.

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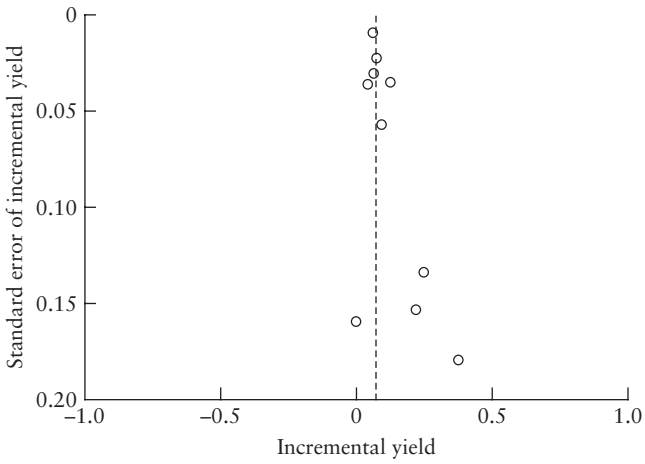

Figure 2 Funnel plot of studies reporting on the incremental yield of isolated and non-isolated congenital heart defect cases combined, detected by array comparative genomic hybridization, over karyotyping and 22q11 fluorescent *in-situ* hybridization.

were provided by A. F. Incremental yield of aCGH was defined as the yield over karyotyping only, or over karyotyping and 22q11 microdeletion analysis by fluorescence *in-situ* hybridization (FISH) combined. The incremental yields from each study were pooled to estimate an overall and subgroup incremental yield of aCGH using RevMan version 5.3.4 (Review Manager, The Cochrane Collaboration, Copenhagen, Denmark) and 95% CIs were computed. Studies with fewer than 20 cases were excluded from the meta-analysis. Statistical heterogeneity was examined using Higgins I^2 (quantitative) test. To take into account the low statistical power of tests of heterogeneity, we considered statistically significant heterogeneity using Cochran's Q test with a $P < 0.1$ or I^2 greater than 30%. A random-effects model was used when there was significant heterogeneity. We assessed publication bias graphically using funnel plots and the study quality based on the factors we considered most likely to threaten study validity (Table S1).

Table 1 Summary of findings from the 13 studies included in this review of incremental yield of array comparative genomic hybridization (aCGH) over karyotyping only or karyotyping and fluorescent *in-situ* hybridization (FISH) in cases of prenatal congenital heart defects (CHD)

Study	*Study design*	*Inclusion criteria for original study*	n*	*Non-isolated CHD (n (%))*	*Criteria for subsequent aCGH*	*aCGH resolution*	*Description of CNV interpretation*	*Postnatally confirmed CHD*
Tyreman[12]	R	Various US abnormalities	34	NS	Normal karyotype and/or normal FISH 22q11	NS (~50 kb)	Yes	NS
Schmid[30]	P	Fetal CHD	12	9 (75)§	Normal FISH 22q11 and karyotype	Various: 1 Mb backbone/ targeted 200 kb, 50 kb, 1 kb	No	NS
Shaffer[16]	P	Various US abnormalities	580	343 (59)¶	Normal karyotype	Various	No	No
Lee[15]	P	Various indications for genetic sampling	50	NS	None (no aneuploidy in CHD group)	Various platforms, 0.5–0.1 Mb	Yes††	NS
Faas[14]	P	Various US abnormalities	10	0	Normal QF-PCR	150 kb loss/ 200 kb gain	No	No
Bao[27]	P	Fetal CHD	7	NS	Complex CHD	50 kb	Yes	NS
Mademont-Soler[7]	R	Fetal CHD or cardiac markers	51‡	23 (45)§	Normal FISH 22q11 and karyotype	100 kb	Yes	Yes
Hillman[25]	P	Various US abnormalities	41	0	Normal QF-PCR	> 2 Mb backbone/ targeted > 200 Mb	Yes	Some
Vestergaard[13]	P	Various US abnormalities	9	NS	Normal karyotype in 50% of original sample	~80 kb	Yes	NS
Yan[28]	P	Fetal CHD	76	27 (36)**	Normal FISH 22q11 and karyotype	300 kb	Yes	Yes
Liao[26]	P	Fetal CHD†	99	30 (30)§	Normal karyotype	100 kb	Yes	NS
Donnelly[24]	P	Various US abnormalities	154	88 (57)§	Normal karyotype	NS (two platforms)	Yes††	NS
Chen[29]	R	Conotruncal CHD	8	NS	Normal FISH 22q11 and karyotype	< 1 kb	Yes	Yes

Only first author is given for each reference. *Number of CHD cases that underwent aCGH. †Excluding minor CHD, unless additional malformations were present. ‡45 CHD, 6 cardiac markers. §Fetuses with additional soft markers or minor malformations included in non-isolated group. ¶Fetuses with non-structural abnormalities (soft markers, growth anomalies) included in isolated group. **No specifications provided for 'associated abnormalities'. ††Provided in a related paper. CNV, copy number variants; NS, not stated; P, prospective; QF-PCR, quantitative fluorescent polymerase chain reaction; R, retrospective; US, ultrasound.

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Table 2 Summary of results of array comparative genomic hybridization (aCGH) in fetuses with isolated or non-isolated congenital heart defects (CHD) and no aneuploidy, found in the studies included in the review

	VOUS		*pCNV including 22q11*		*pCNV excluding 22q11*		
Study	n	*Incremental yield (95% CI)*	n	*Incremental yield (95% CI)*	n	*CHD*	*Additional malformations (n)*
Tyreman[12]	1/32	0.03 (−0.05 to 0.11)	5/34§	0.15 (0.02 to 0.27)	3/32	2 HLHS, 1 VSD	1/3
Schmid[30]	3/12*	0.25 (−0.01 to 0.51)†	—	—	3/12	2 VSD, 1 CAT	3/3
Shaffer[16]	19/569	0.03 (0.02 to 0.05)	46/580	0.08 (0.06 to 0.10)	35/569	11 HLHS, 5 TOF, 14 VSD, 5 other	30/35
Lee[15]	0/45	0.00 (−0.04 to 0.04)	6/49¶	0.12 (0.03 to 0.22)	2/45¶	1 TOF, 1 VSD	1/2
Bao[27]	1/5	0.20 (−0.21 to 0.61)†	NS	—	0/5	—	—
Mademont-Soler[7]	0/45	0.00 (−0.04 to 0.04)	—	—	2/45	1 TOF, 1 unbalanced AVSD	2/2
Vestergaard[13]	0/9	0.00 (−0.19 to 0.19)†	2/9¶	0.22 (−0.08 to 0.52)†	2/9¶	2 TOF	No
Yan[28]	4/76	0.05 (0.00 to 0.11)	—	—	5/76	1 TOF, 1 HLHS, 1 DORV & MA, 1 VSD & PLSVC, 1 PS & VSD	2/5
Liao[26]	5/94	0.05 (0.00 to 0.10)	17/99**	0.17 (0.10 to 0.25)	12/94**	4 VSD, 1 TOF, 3 minor CHD, 2 other	5/12
Donnelly[24]	5/146	0.03 (0.00 to 0.07)	19/154	0.12 (0.07 to 0.18)	11/146	2 VSD, 2 CoA, 3 HLHS, 1 AVSD, 3 other	7/11
Chen[29]	NS	—	—	—	3/8‡‡	1 TOF, 1 TGA, 1 iAA	NS
Pooled result		0.034 (0.021 to 0.046)‡ $I^2 = 0\%$		0.12 (0.08 to 0.16)†† $I^2 = 53\%$			

Only first author is given for each reference. Numbers indicate patients, as multiple array malformations can occur within one patient. Yield of aCGH excluding 22q11 fluorescence *in-situ* hybridization (FISH) analysis is plotted in forest plot (Figure 3). *At resolution 50–200 kb. †Not included in data pooling. ‡Fixed effects model. §22q11 microdeletion was already excluded by FISH in some cases. ¶Abnormal karyotype not completely excluded; following review of the study of Lee *et al.*[15] we excluded one abnormal karyotype but could not be sure that abnormal karyotypes did not remain. **Minor cardiac malformations included as CHD. ††Random effects model. ‡‡Conotruncal malformations only. AVSD, atrioventricular septal defect; CAT, common arterial trunk; CoA, coarctation of aorta; DORV, double outlet right ventricle; HLHS, hypoplastic left heart syndrome; iAA, interrupted aortic arch; MA, mitral atresia; NS, not stated; pCNV, pathological copy number variants; PLSVC, persistent left superior vena cava; PS, pulmonary stenosis; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VOUS, variants of unknown significance; VSD ventricular septal defect.

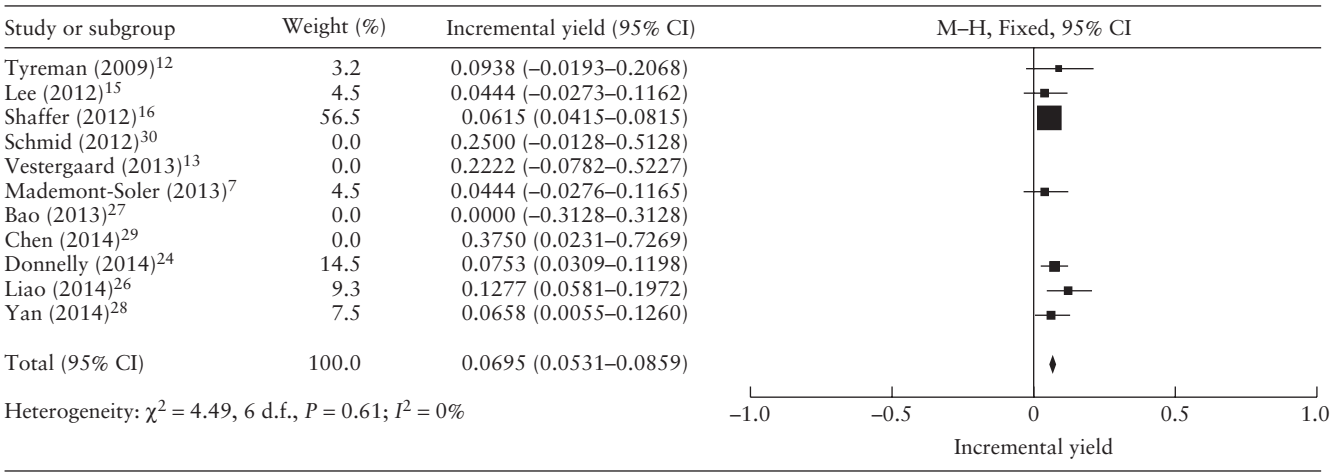

Figure 3 Forest plot of incremental yield by array comparative genomic hybridization in fetuses with either isolated or non-isolated congenital heart defects, after exclusion of aneuploidy or 22q11 microdeletion. Studies with fewer than 20 cases of CHD were not included in the meta-analysis. M–H, Mantel–Haenszel.

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Table 3 Summary of results of array comparative genomic hybridization (aCGH) in fetuses with isolated congenital heart defects (CHD) and no aneuploidy, found in the studies included in the review

		pCNV including 22q11		*pCNV excluding 22q11*	
Study	*VOUS (n)*	n	*Incremental yield (95% CI)*	n	*CHD*
Schmid[30]	1/7	—	—	0/7	—
Shaffer[16]	9/236	6/237†	0.03 (0.00 to 0.05)	5/236†	4 HLHS, 1 NS
Faas[14]	0/9	1/10	0.10 (−0.14 to 0.34)‡	0/9	—
Mademont-Soler[7]	NS	—	—	0/28§	—
Hillman[25]	1/37	4/41	0.10 (0.00 to 0.20)	0/37	—
Yan[28]	3/49	—	—	3/49	1 DORV & MA, 1 VSD & PS, 1 TOF
Liao[26]	NS	10/69	0.14 (0.06 to 0.23)	7/66	2 VSD, 1 AVSD, 1 SV & TGA, 1 iAA, 2 minor CHD
Donnelly[24]	1/64	6/66	0.09 (0.02 to 0.16)	4/64	1 VSD, 1 CoA, 2 NS
*Pooled result**			0.08 (0.01 to 0.16) $I^2 = 82\%$		

Only first author is given for each reference. Numbers indicate patients, as multiple array malformations can occur within one patient. Yield of aCGH excluding 22q11 fluorescence *in-situ* hybridization analysis is plotted in forest plot (Figure 4). *Random effects model. †44 cases with additional non-structural defects included. ‡Not included in data pooling. §Including unknown number of cases with cardiac markers. AVSD, atrioventricular septal defect; CoA, coarctation of aorta; DORV, double outlet right ventricle; HLHS, hypoplastic left heart syndrome; iAA, interrupted aortic arch; MA, mitral atresia; NS, not stated; pCNV, pathological copy number variant; PS, pulmonary stenosis; SV, single ventricle; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VOUS, variants of unknown significance; VSD, ventricular septal defect.

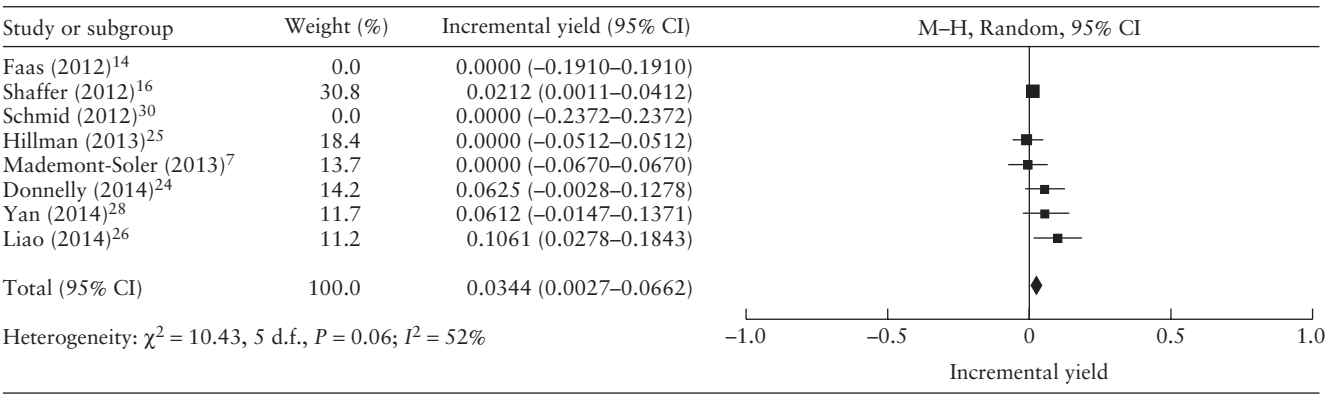

Figure 4 Forest plot of incremental yield by array comparative genomic hybridization in fetuses with isolated congenital heart defects alone, after exclusion of aneuploidy or 22q11 microdeletion. Studies with fewer than 20 cases of CHD were not included in the meta-analysis. M–H, Mantel–Haenszel.

RESULTS

The search revealed 751 studies, of which 13 primary articles (including 1131 CHD cases) met the final inclusion criteria (Figure 1)[7,12–16,24–30]. We encountered no overlapping populations among the studies selected. The funnel plot suggesting publication bias is shown in Figure 2. Table 1 displays characteristics of the studies, including study design, resolution of array and number of assessed cases. Details of the included studies and the meta-analysis are provided in Tables 2–4 and Figures 3 and 4. There were 120 aCGH anomalies and these varied between 0.85 kilobases (kb)[29] and 14.9 Mb[7] in size and are listed in Table S2. The pooled results from the seven studies that included ≥ 20 cases (Table 2) indicate that the incremental yield of aCGH detecting CNVs, after karyotyping and 22q11 FISH analysis, was 7.0% (95% CI, 5.3–8.6%) (Figure 3). The incremental yield over karyotyping alone, including the yield of 22q11 microdeletions, is also summarized in Table 2: pooled results of five studies indicate an incremental yield of 12% (95% CI, 7.6–16%) for isolated and non-isolated CHD cases combined. One study was excluded from this

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Table 4 Summary of results of array comparative genomic hybridization (aCGH) in fetuses with non-isolated congenital heart defects (CHD) and without aneuploidy, found in the studies included in the meta-analysis

		pCNV including 22q11		*pCNV excluding 22q11*		
Study	*VOUS* (n)	n	*Incremental yield (95% CI)*	n	*Incremental yield (95% CI)*	*CHD*
Schmid[30]	2/5	—	—	3/5§	0.60 (−0.15 to 1.05)	2 VSD, 1 CAT
Shaffer[16]	10/343	40/343	0.12 (0.08–0.15)	30/333	0.09 (0.06 to 0.12)	7 HLHS, 5 TOF, 14 VSD, 4 other
Mademont-Soler[7]	NS	—	—	2/23¶‡	0.09 (−0.05 to 0.22)	1 TOF, 1 unbalanced AVSD
Yan[28]	1/27	—	—	2/27	0.07 (−0.04 to 0.19)	1 HLHS, 1 VSD & PLSVC
Liao[26]	NS	7/30†‡	0.23 (0.08–0.39)	5/28†‡	0.18 (0.03 to 0.33)	1 CoA, 1 PS, 1 VSD, 1 PLSVC
Donnelly[24]	4/82	13/88‡	0.15 (0.07–0.22)	7/82‡	0.09 (0.02 to 0.15)	1 VSD, 1 CoA, 3 HLHS, 1 AVSD, 1 NS
*Pooled result**			0.13 (0.10–0.16) $I^2 = 19\%$		0.09 (0.07 to 0.12) $I^2 = 0\%$	

Only first author is given for each reference. Numbers indicate patients, as multiple array malformations can occur within one patient.
*Random effects model. †Minor cardiac malformations included as CHD. ‡Fetuses with additional soft markers included. §Not included in data pooling. ¶Including unknown number of cases with cardiac markers. AVSD, atrioventricular septal defect; CAT, common arterial trunk; CoA, coarctation of aorta; HLHS, hypoplastic left heart syndrome; NS, not stated; pCNV, pathological copy number variants; PLSVC, persistent left superior vena cava; PS, pulmonary stenosis; TOF tetralogy of Fallot; VOUS, variants of unknown significance; VSD, ventricular septal defect.

sub-group analysis owing to a small sample size[13]. The additional yield of aCGH detecting variants of unknown significance (VOUS) was 3.4% (95% CI, 2.1–4.6%).

The incremental yield of aCGH varied depending on the presence or absence of extracardiac malformations and/or soft markers. Most authors reported on subgroups of isolated and non-isolated CHD. However, whether additional minor malformations and soft markers were included in the isolated or non-isolated groups varied. For isolated CHD, pooled results from six studies that included ≥ 20 cases (Table 3) indicated that the incremental yield of aCGH after karyotyping and 22q11 FISH analysis was 3.4% (95% CI, 0.3–6.6%) (Figure 4). Two studies were excluded from this sub-group analysis owing to small sample size[14,30]. Statistical tests for variation in outcomes between studies showed significant heterogeneity. For non-isolated CHD, summarized and pooled results are shown in Table 4. Forest plots of all subgroups are shown in Figure S1.

Two studies reported on subgroups of specific cardiac lesions. Combined results from these two studies were not eligible for meta-analysis. Shaffer *et al.*[16] reported separately on groups with $n > 20$ in their cohort: hypoplastic left heart syndrome (HLHS), tetralogy of Fallot (TOF), ventricular septal defect (VSD) and dextrocardia/situs inversus (D/SI). In 42 isolated cases of HLHS, aCGH had a yield of 10% ($n = 4$), and all anomalies were < 10 Mb in size. No aCGH anomalies were detected in 18 fetuses with isolated TOF, 38 with isolated VSDs or 21 with isolated D/SI. In the subgroup of fetuses with multiple structural malformations, the yield of clinically significant CNVs was higher, but this also included anomalies > 10 Mb in size. Significant findings were found in 7/26 (26.9%) cases with non-isolated HLHS, 5/25 (20.0%) with non-isolated TOF, 14/94 (14.9%) with non-isolated VSD and 1/27 (3.7%) with non-isolated D/SI.

Donnelly *et al.*[24] analyzed subgroups of malformations visible on the four-chamber view, outflow tract malformations, TOF and heterotaxy. They did not, however, elaborate on the specific method of subgrouping. In the category of isolated outflow tract malformations (aortic stenosis, coarctation or interruption, transposition of the great arteries, common arterial trunk) an incremental yield of 30% ($n = 3$) was found; 22q11 deletions were not among these three CNVs. The incremental yield excluding 22q11 deletions in the other subgroups could not be extracted.

DISCUSSION

Considering the association of genetic anomalies with CHD, and the implications for both prenatal and postnatal management, obtaining the most accurate and detailed genetic information in the prenatal setting is important for both patients and care-providers. In this systematic review, aCGH yielded additional clinically valuable information in 7.0% (95% CI, 5.3–8.6%) of fetal CHD cases, even after karyotyping and 22q11 FISH analysis were normal. This includes both causative aCGH anomalies as well as incidental, but clinically relevant, findings such as a high risk for neurodevelopmental delay. The additional yield of VOUS was 3.4% (95% CI, 2.1–4.6%).

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In particular, there were more pathogenic CNVs (estimated at 9.3% (95% CI, 6.6–12.0%)) when extracardiac defects were present. This yield appears lower when compared with published reports of aCGH in the postnatal setting, which describe yields of 17–53% in CHD with extracardiac malformations, neurodevelopmental delay and/or dysmorphic features[31–38]. This discrepancy can be attributed to non-comparable cohorts. There may be ascertainment bias of cases in the postnatal groups that already present with neurodevelopmental delay or dysmorphic features.

When analyzing isolated CHD, an incremental yield of 3.4% (95% CI, 0.3–6.6%) was found. In postnatal cohorts of isolated CHD with normal karyotype and 22q11 microdeletion analysis, the yield appears to be somewhat lower, at 0–4%[34,39–43]. This small difference may be due to the limitation of prenatal ultrasound examination in detecting dysmorphic features and other subtle expressions of syndromal anomalies[44].

It seems that VSDs (mainly perimembranous[45]) with extracardiac malformations, conotruncal malformations (TOF, interrupted arch) and left ventricular outflow tract malformations are common in prenatal cases that yield pathogenic aCGH results. Even transposition of the great arteries and heterotaxy, which are not considered to be associated with chromosomal anomalies detected by karyotyping, were found to have pathogenic aCGH results. However, the reported CHD with aCGH anomalies are highly heterogeneous, and subgroups of different types of CHD are not large enough to analyze separately. Moreover, the categorization of CHD is not consistent between the different reports, which inhibits calculation of the yield per specific CHD. Therefore, our recommendation is to offer aCGH for all types of CHD.

In addition to submicroscopic anomalies < 5–10 Mb in size, aCGH also yields anomalies > 10 Mb. For example, in one study karyotyping failed to detect a large 14.9-Mb deletion that was subsequently detected by aCGH[7]. Shaffer *et al.*[16] reported separately that the yield was > 10 Mb. This emphasizes that karyotyping does not detect 100% of anomalies > 10 Mb in size, and aCGH may be a more reliable method of detecting these mutations[46].

The possibility of aCGH replacing 22q11 FISH analysis in the prenatal setting of CHD merits consideration. The reported prevalence of 22q11 microdeletions in fetal CHD is as high as 7%[7], with aortic arch and conotruncal malformations having the highest yields[9,47]. 22q11 FISH analysis is, therefore, already an important part of the diagnostic genetic work-up in cases of isolated and non-isolated CHD. An important benefit of aCGH over FISH analysis for 22q11 microdeletions was noted by Chen *et al.*[29], reporting on two deletions in the 22q11 region that were not detected by FISH.

The limitation of our review is that pooled results are predominantly influenced by the report from Shaffer *et al.*[16], which has considerable uncertainty regarding confirmation of diagnosis. Furthermore, publications show large variability in the size of the cohort, platforms used, patient characteristics and classification of extracardiac malformations. This results in high rates of statistical heterogeneity, especially in the isolated CHD subgroup. The process of interpreting CNV as pathogenic or benign is not always described and seems to vary significantly between the different groups. Larger prospective cohorts, focusing further on different types of CHD, are therefore warranted. Questions regarding the optimal probe spacing, platform and resolution, as well as the method of CNV categorization into benign or pathogenic, remain important.

There are some limitations of aCGH to be considered. First of all, the detection of VOUS could lead to challenges in counseling and parental anxiety. Combining data from large cohorts and linking certain aCGH anomalies with specific anatomic malformations could, however, increasingly reduce the frequency and clinical ambiguity of VOUS, for care-providers and their patients. Also, comparison with parental aCGH results can aid in detecting VOUS, which are inherited from presumably healthy parents, and are therefore less likely to be pathogenic. From our review, it appears that studies that routinely performed aCGH of both parents encountered a lower frequency of VOUS. Secondly, clinicians should be aware that single-gene disorders are also associated with CHD and they will not be detected by aCGH. These remain to be screened-for individually on a case-by-case indication, until whole genome sequencing is available in the prenatal setting. Moreover, triploidies, chromosomal inversions and balanced translocations will not be detected by aCGH. Considerations for karyotype replacement by aCGH should therefore include an additional rapid method of aneuploidy/triploidy detection (RAD) such as quantitative fluorescent polymerase chain reaction[17]. Detection of balanced translocations and inversions does not seem a solid reason to perform complete karyotyping, as those chromosomal rearrangements will be detected if accompanied by a small deletion. Furthermore, if they are truly balanced, they most probably do not cause CHD.

For pretest counseling purposes, results can be summarized as follows: the chance of finding an aCGH anomaly in cases of prenatal CHD (including 22q11 microdeletion) is approximately 14% in total: 3% VOUS, 4% 22q11 microdeletion and 7% other pathogenic CNVs. In cases of isolated CHD with normal karyotype and 22q11 microdeletion analysis by FISH, the yield of additional aCGH has not yet been firmly established, but may be approximately 3%. In non-isolated cases, this yield is more evident, at approximately 9%. In our opinion, given the available data, aCGH should be considered in cases of prenatally diagnosed fetal cardiovascular malformations, even if the lesion is apparently isolated based on prenatal imaging. As the common aneuploidies are most frequently associated with CHD, especially in cases of additional extracardiac malformations, aCGH can be considered if RAD results are normal, in order to reduce healthcare utilization and costs. However, local practices, gestational age and regulations on pregnancy termination may lead providers to consider RAD and aCGH concurrently.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Appendix S1 Complete search string

Table S1 Quality assessment

Table S2 List of encountered aCGH anomalies

Figure S1 Forest plots of all subgroup analyses

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