This health technology assessment is based on research conducted by RTI International under contract to the Washington Health Care Authority (HCA) (Contract No. K1959). The findings and conclusions in this document are those of the authors, who are responsible for its contents; the findings and conclusions do not necessarily represent the views of the Washington HCA. Therefore, no statement in this report should be construed as an official position of Washington HCA.

The information in this report is intended to help the Washington HCA make well-informed coverage determinations and thereby improve the quality of health care services. This report is not intended to be a substitute for the application of clinical judgment. Anyone who makes decisions concerning the provision of clinical care should consider this report in the same way as any medical reference and in conjunction with all other pertinent information (i.e., in the context of available resources and circumstances presented by individual patients).

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None of the investigators has any affiliations or financial involvement that conflicts with the material presented in this report.

Acknowledgments

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List of Abbreviations

ASD autism spectrum disorder
CGH comparative genomic hybridization
CMA chromosomal microarray (also called genomic microarray)
CNV copy number variant
DD developmental disability
FISH fluorescent in situ hybridization
GDD global developmental delay
HTA health technology assessment
ID intellectual disability
MCA multiple congenital anomalies
UK United Kingdom
US United States
WES whole exome sequencing
Executive Summary

Structured Abstract

**Purpose:** To review the safety, efficacy, and cost of chromosomal microarrays and whole exome sequencing when used for the diagnosis and management of children with developmental and intellectual disabilities, autism spectrum disorder, or multiple congenital anomalies.

**Data Sources:** PubMed from January 2000 through September 2017; trial registry, payor coverage databases, websites for the United States Food and Drug Administration, professional societies, and organizations that conduct health technology assessments, and bibliographies of relevant articles.

**Study Selection:** We selected English-language studies using a priori criteria. We included studies that evaluated chromosomal microarray or whole exome sequencing if they addressed the safety, diagnostic yield, impact on management or health outcomes, or cost or cost-effectiveness when used in children with developmental and intellectual disabilities, autism spectrum disorder, or multiple congenital anomalies. Studies focused on prenatal use, analytic validity or ethics of testing were excluded. Diagnostic yield studies were excluded if the testing was performed prior to 2009 or used obsolete testing platforms, or the studies were conducted outside of the United States.

**Data Extraction:** One research team member extracted data and a second checked for accuracy. Two investigators independently assessed risk of bias of included studies.

**Data Synthesis:** We included a total of 18 studies. One study provided evidence on a safety issue; specifically, discrimination resulting from abnormal chromosomal microarray results. Five primary research studies and one health technology assessment provided evidence related to diagnostic yield and the types of clinical conditions for which it is most useful. The pooled summary estimate of diagnostic yield from the five primary research studies was 8.8% (95% CI, 8.4% to 9.3%). The median diagnostic yield for chromosomal microarray testing among patients with global developmental delay with or without intellectual disability in the health technology assessment was 13.6% [interquartile range, 9.5% to 17.2%] across 55 applicable studies included in this health technology assessment; and was 19% among the 21 studies published in 2012 or later. The diagnostic yield in the one primary research study that reported on whole exome sequencing was 27% (95% CI not reported).

Seven studies evaluated the impact of chromosomal microarray testing on the clinical management of children. Between 27.1% to 93.8% of children with a pathogenic variant on testing, which was 3.6% and 6.7% of all cases tested, had a change in management prompted by their results. We did not identify any studies that reported the impact of testing on health outcomes. We identified five eligible studies reporting cost outcomes, all specific to chromosomal microarray testing and diagnostic yield. Costs per array ranged from $271 to $1,575 (in 2010 US dollars). The cost per additional diagnosis ranged from $-88,819 to $12,296 (in 2010 US dollars). No studies reported cost-effectiveness with respect to health outcomes.

We graded the strength of evidence as **very low** for safety, impact on clinical management, and cost and cost-effectiveness, and as **low** for diagnostic yield. These grades were resulted from
observational study designs and depending on the outcome (safety, efficacy, or cost) serious concerns in one or more domains including risk of bias, inconsistency, indirectness, or imprecision.

Limitations: The risk of bias of individual studies varied, and study reporting limited our ability to assess risk of bias for some included studies. Studies assessing diagnostic yield and impact on management, and cost were clinically and methodologically heterogenous. The evidence base was very limited for assessing safety, and none of the cost studies we identified evaluated cost-effectiveness related to health outcomes or were conducted in the United States.

Conclusions: Chromosomal microarray identifies a pathogenic or likely pathogenic variant in nearly 9% of all children referred for testing and in 5% of those referred because of autism spectrum disorders; these findings are based on a low strength of evidence. The results of chromosomal microarray tests generate changes in management in over half of children who are identified as having a pathogenic or likely pathogenic variant; this finding is based on very low strength of evidence. The evidence is very limited with respect to the safety of testing and we identified no evidence related to the impact of testing on health outcomes. The cost per additional diagnosis for chromosomal microarray testing as a first-line diagnostic test varies between $-88,819 and $12,296 (in 2010 US dollars); this finding is based on very low strength of evidence.

Background

Condition Description
Chromosomes, the genetic structures of a cell, are constructed of deoxyribose nucleic acid (DNA) and the proteins and other elements that protect, regulate, and package the DNA. Humans normally have 23 pairs of chromosomes, with half inherited from each parent. During cell replication, chromosomes are sometimes lost or broken and rearranged. Rearrangements vary in size and complexity, and may be balanced, with no loss of DNA, or unbalanced with loss or gain of DNA.

Disease Burden
Unbalanced chromosomal rearrangements that are present at conception or that occur during fetal development have profound consequences for the developing fetus, resulting in fetal death, structural defects, genetic diseases, or intellectual impairment.1 Chromosomal abnormalities occur in 43.8 per 10,000 births that survive to 20 weeks gestation or later.2 Trisomies 21 (Down syndrome), 18, and 13; 45, X (Turner syndrome), and other sex chromosome abnormalities account for most abnormalities. Excluding these, the prevalence of more rare abnormalities is 7.4 per 10,000 births.2 Small pathogenic duplications or deletions, called copy number changes or variants (CNV), occur in 1 of 270 pregnancies.3 The consequences of CNVs depend on the size and location within the genome.

Technology Description
Chromosomal Microarrays (CMA)
Karyotyping and fluorescent in situ hybridization (FISH) have traditionally been used to identify unbalanced chromosomal rearrangements. In the early 2000s, genome-wide microarrays for chromosomal analysis, commonly known as chromosomal microarray (CMA), which use
comparative genomic hybridization (CGH) or single nucleotide polymorphism (SNP) arrays to evaluate the number of copies of portions of the chromosomes, were introduced as an adjunct to karyotype and FISH testing for chromosome abnormalities.

Although CMA can identify aneuploidies and large rearrangements, its strength lies in identifying small deletions and duplications. CGH can identify rearrangements as small as 150 base pairs, whereas karyotyping can detect no fewer than approximately 5,000 base pairs. For this reason, professional societies now recommended CMA be the first test used to diagnose chromosomal abnormalities in children with multiple congenital anomalies (MCAs) or developmental or intellectual disabilities (DD/IDs). However, CMA cannot identify balanced rearrangements or low-level mosaicism so karyotyping may still be required in some cases.

The platforms used for CGH have changed since their introduction. The first genome-wide platforms used bacterial artificial chromosome (BAC) probes that could detect deletions or duplications of approximately 1,000,000 base pairs (1 Mb). Around 2007, arrays based on small synthesized oligonucleotides (‘oligo’) began replacing BAC arrays. Oligonucleotide probes are smaller, and oligo-based arrays have many more probes, enabling detection of smaller CNVs. Many current CMA testing platforms use a combination of labeled SNPs and oligo-based probes to assess genetic bases or sequences throughout the genome.

Whole Exome Sequencing
Whole exome sequencing (WES) may be used with or instead of chromosomal microarray or other chromosomal analysis. WES is the sequencing of the protein coding regions of the genome. Multiple large regions of the genome are sequenced simultaneously (i.e., in parallel using a technique referred to as next generational ‘next gen’ sequencing). In addition to the detection of CNVs, WES provides the base pair sequence for the exons, allowing the detection of single nucleotide changes within specific genes. Thus, some have suggested that for certain conditions and syndromes, WES may be more efficient for testing multiple specific genes than sequential testing for single gene disorders, particularly when a genetic cause is suspected but the phenotype is not typical for any specific single gene disorder.

Policy Context
The State of Washington Health Care Authority selected testing with CMA and WES as a topic based on medium, high, and high concerns for safety, efficacy, and cost, respectively. Several practice guidelines have been issued that call for CMA to replace G-banded karyotype as the first-tier test for diagnosis of individuals with DD, ID, or MCA, and for the clinical evaluation of autism spectrum disorder (ASD). These guidelines, combined with the increasing prevalence of autism, could greatly increase orders for CMA. The purported increased diagnostic yield of chromosomal abnormalities by CMA compared to karyotype underlies these guidelines. However, the circumstances in which these tests are most useful and their contribution to the medical and educational management and ultimate health outcomes of affected children are unclear.

Regulatory Status
CMA and WES are considered laboratory-developed tests and are not regulated by the United States (U.S.) Food and Drug Administration (FDA). Clinical laboratories that conduct these tests must comply with regulatory standards for high complexity testing within the Clinical
Laboratory Improvement Act. Thus, these tests are generally only available through commercial diagnostic testing laboratories or hospital-based laboratories. FDA approval is required when a company markets and sells a kit for CMA or WES testing. The FDA has approved one CMA kit for marketing in the United States, the Affymetrix CytoScan® Dx assay (Affymetrix, Inc., Santa Clara, CA). The FDA-approved indications for this kit include postnatal detection of CNVs associated with DD, ID, MCA, or dysmorphic features. This assay was FDA-approved on January 21, 2014.

**Practice Guidelines and Payer Coverage**

Several practice guidelines or policy statements (*Table ES-1*) endorse the use of CMA in place of karyotype as a first-line test in the evaluation of children with DD, ID, ASD, or MCA, particularly when dysmorphic features are present or signs, symptoms and initial non-genetic testing are not consistent with a single gene disorder.

<table>
<thead>
<tr>
<th>Organization</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>International Standard Cytogenomic Consortium</td>
<td>2010</td>
</tr>
<tr>
<td>National Institute for Health and Care Excellence (UK)</td>
<td>2011</td>
</tr>
<tr>
<td>American College of Medical Genetics and Genomics</td>
<td>2013</td>
</tr>
<tr>
<td>American Academy of Pediatrics</td>
<td>2014</td>
</tr>
<tr>
<td>American Academy of Neurology</td>
<td>2015</td>
</tr>
</tbody>
</table>

The Centers for Medicare and Medicaid Services (CMS) has no national coverage determination for the use of CMA or WES. *Table ES-2* summarizes selected payer coverage determinations for CMA and WES testing. Among payers, good alignment exists for the criteria under which CMA is covered. Typically, it is covered as first-line diagnostic for DD, ID, ASD when relevant biochemical and metabolic diseases have been ruled out and the clinical presentation is not specific to a well-delineated genetic syndrome and the results of CMA could impact the clinical management of the child.
Table ES-2. Payer coverage for CMA and WES Testing

<table>
<thead>
<tr>
<th>Payer</th>
<th>CMA Testing</th>
<th>WES Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aetna17</td>
<td>Covered for specific indications</td>
<td>Not covered</td>
</tr>
<tr>
<td>Blue Cross (Premera)18</td>
<td>Covered for specific indications</td>
<td>Covered for specific indications</td>
</tr>
<tr>
<td>Regence Blue Shield Regence19,20</td>
<td>Covered for specific indications</td>
<td>Not covered</td>
</tr>
<tr>
<td>Cigna21,22</td>
<td>Covered for specific indications</td>
<td>Covered for specific indications</td>
</tr>
<tr>
<td>Humana23</td>
<td>Covered for specific indications</td>
<td>Not covered</td>
</tr>
<tr>
<td>Kaiser Permanente24</td>
<td>Covered for specific indications</td>
<td>Not covered</td>
</tr>
<tr>
<td>Medicare Fee for Service</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Medicaid15,25,26</td>
<td>Not all states have policies; those that do typically cover for specific indications</td>
<td>Unknown</td>
</tr>
<tr>
<td>UnitedHealthcare27</td>
<td>Covered for specific indications</td>
<td>Covered for specific indications</td>
</tr>
</tbody>
</table>

Research Questions

Figure ES-1 provides the analytic framework and Table ES-3 provides the final research questions and study selection criteria related to the population, intervention, comparator, outcomes, time period, and setting used to conduct this HTA.

Figure ES-1. Analytic Framework for Chromosomal Microarray and Whole Exome Sequencing in Children with Developmental or Intellectual Disability, Autism, or Multiple Congenital Anomalies

Abbreviations: ASD=autism spectrum disorder; CMA=chromosomal microarray; CQ=cost question; DD=developmental disability; EQ=efficacy question; ID=intellectual disability; MCA=multiple congenital anomalies; SQ=safety question; WES=whole exome sequencing
Table ES-3. Research Questions and Scoping Parameters for Chromosomal Microarray or Whole Exome Sequencing in Children with Intellectual Disability, Autism, or Birth Defects

<table>
<thead>
<tr>
<th>Research Questions</th>
<th>Safety</th>
<th>Efficacy</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety</td>
<td>SQ1. What, if any, safety issues do CMA and WES pose beyond those associated with phlebotomy?</td>
<td>EQ1. How often do CMA or WES return an informative result (i.e. diagnostic yield)? EQ2. For what types of conditions is CMA or WES most useful? EQ3. Does the diagnosis of a chromosomal disorder change the child’s management? EQ4. Do children with congenital defects, autism, intellectual disability or developmental disability tested with CMA or WES have better health outcomes?</td>
<td>CQ1. What is the cost and cost-effectiveness of genetic diagnostic testing for these conditions with CMA or WES?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Populations; Interventions; Comparators, Outcomes; Time Period; Setting</th>
<th>Populations</th>
<th>Intervention</th>
<th>Comparator</th>
<th>Safety Outcomes</th>
<th>Efficacy Outcomes</th>
<th>Cost Outcomes</th>
<th>Time Period</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Populations</td>
<td>Children diagnosed with congenital defects, autism, intellectual disability or developmental disability without known syndrome or specific genetic abnormality.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td>1. CMA testing with currently available platforms, obsolete and superseded platforms will be excluded. 2. WES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparator</td>
<td>EQ1, EQ2, SQ1: descriptive and may not have comparator groups. EQ3: Management before and after diagnosis; management of similarly affected undiagnosed children EQ4 and CQ1: No genetic diagnostic testing or genetic diagnostic testing did not include CMA or WES.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safety Outcomes</td>
<td>SQ1. Harms reported as related to testing other than those associated with phlebotomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost Outcomes</td>
<td>CQ1: Cost of assay, cost per diagnosis, cost per additional diagnosis, cost per quality-adjusted life year, cost per disability-adjusted life year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time Period</td>
<td>2009 to 2017 for EQ1 and EQ2, 2000-2017 for all others</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Setting</td>
<td>Clinical genetic laboratories, medical genetic clinics, general and specialty pediatric clinics; non-US studies were excluded for EQ1 and EQ2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CMA = chromosomal/genomic microarray; CQ = cost question; EQ = efficacy question; SQ = safety question; US = United States; WES = whole exome sequencing.

What is Excluded from This HTA
This HTA will not address the analytic validity of CMA or WES, as this testing is available within Clinical Laboratory Improvement Act-licensed laboratories as a laboratory-developed test and analytic validity is assumed based on meeting those standards.28 This HTA will also not address the use of CMA or WES to identify or monitor chromosomal changes in tumor cells or
its use for prenatal testing, or the use of WES to identify mutations within single genes. The review will not assess the clinical utility of incidental findings not related to the health conditions for which the tests were ordered and will also not address the ethical considerations of these findings. Because of the large volume of studies identified for the first efficacy question (EQ1) related to diagnostic yield and the rapidly evolving technology in use for CMA testing, we excluded studies conducted outside of the United States and studies that used obsolete testing platforms (e.g., bacterial artificial chromosome) or conducted testing prior to 2009 from EQ1.

Methods

Data Sources and Search
We searched MEDLINE® (via PubMed) and a clinical trials registry (clinicaltrials.gov) for relevant English-language studies published in 2000 or later. We searched the FDA website, selected payer and health care professional society websites, and other organizations that conduct and disseminate HTAs. In addition, we reviewed the reference lists of relevant studies, practice guidelines, and other HTAs on this topic to identify any relevant articles not identified through the electronic search. The detailed search strategy is provided in Appendix A of the Full Report.

Study Selection
We screened titles and abstracts and full-text articles based on the study selection criteria listed in Table ES-3. We included all study designs except case reports. A single team member screened titles/abstracts following an initial set of 20 independent, dual reviews with the entire team to assess interrater reliability. The principal investigator reviewed all abstracts excluded for “ineligible intervention” and a sample of titles/abstracts excluded for other reasons to ensure continued consistency in application of study selection criteria. One senior team member screened each full-text article for inclusion, and the principal investigator confirmed the decisions.

Data Abstraction and Quality Assessment
One team member extracted relevant study data into a structured abstraction form. The principal investigator reviewed the abstractions for accuracy and consistency. Two senior team members conducted independent risk of bias assessment on all included studies and met to reconcile any disagreements, in consultation with the principal investigator if needed. Because of the diverse types of studies included in this HTA, we adapted signaling questions from the QUADAS-2 instrument, a risk of bias assessment for diagnostics test studies, and items from the RTI item bank for observational studies.29,30 The signaling questions assessed the major sources of bias including selection bias (both how study population was selected and attrition/missing data), confounding, and measurement/information bias. We used the ROBIS instrument to assess the risk of bias for systematic reviews.31

Data Synthesis and Analysis
Study characteristics and results were qualitatively synthesized for each research question in tabular and narrative formats. For cost outcomes, we adjusted all reported outcomes to 2010 US dollars (Appendix B).32,33 To determine whether quantitative synthesis was appropriate, we assessed the number of studies and the clinical and methodological heterogeneity present based on established guidance.34,35 We required three or more publications with similar approach and the same outcome measure to calculate a summary estimate. We estimated summary effects
using a fixed effects model if the test for heterogeneity was nonsignificant and a random effects model if the test for heterogeneity was significant using OpenMetaAnalyst (For Windows 8, 64-bit) and the method of Hedges and Olkin to estimate between-study variance.\(^{36}\) We graded the strength of evidence for each research question using GRADE, which assesses the strength of evidence based on domains relating to risk of bias, inconsistency, imprecision, indirectness, and other considerations, such as reporting bias.\(^{37}\) Under GRADE, the strength of evidence can be graded as very low, low, moderate, or high.

**Results**

**Literature Search**
We identified and screened 2,717 unique citations. We excluded 2,375 after title and abstract review. We reviewed the full-text of 348 articles, and excluded 330 for the reasons listed in Figure 2 of the Full Report. We included 18 studies. One provided evidence on safety issues (SQ1), seven provided evidence on diagnostic yield (EQ1 and EQ2), seven on changes in management (EQ3), and five on costs (CQ1). No studies provided information on health outcomes (EQ4). Individual study characteristics for all included studies are summarized in Full Report Appendix C, Table C-1. The list of studies we screened at the full text stage, but which were excluded from the review, is provided in Appendix D. Note that studies may have been excluded based on more than one reason but we report only one reason. Individual risk of bias assessments for all included studies are reported in Appendix E.

**Safety**

SQ1. What, if any, safety issues do CMA and WES pose beyond those associated with phlebotomy?

One study\(^{38}\) provided evidence on safety issues that arise in chromosomal microarray (CMA) testing. Table ES-4 summarizes the study characteristics and key outcomes related to discrimination resulting from testing. We graded the strength of evidence for this research question as very low (Table ES-5). We did not identify any studies reporting on safety outcomes related to whole exome sequencing (WES) testing.
Table ES-4. Summary of Findings for the Safety of Testing with Chromosomal Microarray or Whole Exome Sequencing

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Study Population; Sample Size</th>
<th>Primary Outcomes</th>
<th>Key Results</th>
<th>Risk ofBias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamilton (2015)(^{38})</td>
<td>Children referred for CMA testing noted as being in foster care; N=6</td>
<td>Adoption request for child in foster care withdrawn after report of CNV associated with autism</td>
<td>1 of 4 cases with abnormal results experienced discrimination</td>
<td>High</td>
</tr>
</tbody>
</table>

Abbreviations: CMA=chromosomal microarray; CNV=copy number variant.

Table ES-5. Strength of Evidence for Findings Related to the Safety of Testing with Chromosomal Microarray or Whole Exome Sequencing

<table>
<thead>
<tr>
<th>No. of Studies; Subjects</th>
<th>Study Design</th>
<th>Risk of Bias</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Precision</th>
<th>Reporting Bias</th>
<th>Strength of Evidence Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>2; 27</td>
<td>Observational</td>
<td>Serious(^a)</td>
<td>Unable to assess</td>
<td>Not serious</td>
<td>Unable to assess</td>
<td>Not serious</td>
<td>Very Low</td>
</tr>
</tbody>
</table>

\(^a\) Enrolment in foster care not routinely collected, only available if noted on test requisition. High risk of selection bias.

Efficacy

**EQ1. How often do CMA or WES return an informative result (i.e., diagnostic yield)?**

Five primary studies\(^{39-43}\) and one health technology assessment (HTA)\(^{28}\) provided evidence related to diagnostic yield (EQ1) and the types of clinical conditions for which CMA is most useful (EQ2). One study provided evidence related to diagnostic yield of WES testing.\(^{44}\) **Table ES-6** summarizes the study characteristics and findings of included studies. The pooled summary estimate of diagnostic yield from the five primary research studies we identified was 8.8% (95% CI, 8.4% to 9.3%). The individual study estimates of diagnostic yield in these studies ranged from 7.3%\(^{39}\) to 14.9%.\(^{42}\) The median diagnostic yield for CMA testing among patients with global developmental delay (DD) with or without intellectual disability (ID) in the HTA was 13.6% [interquartile range, 9.5% to 17.2%] across 55 applicable studies in this HTA, and was 19% among the 21 studies published in 2012 or later.\(^{28}\) The diagnostic yield in the one study that reported on WES testing was 27% (95% CI, NR).\(^{44}\) We graded the strength of evidence for this research question as low (**Table ES-7**).
Table ES-6. Summary of Findings for the Diagnostic Yield of Testing with Chromosomal Microarray or Whole Exome Sequencing

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Study Population; Sample Size; Test</th>
<th>Diagnostic Yield [Detection of A Pathogenic Variant] N (%)</th>
<th>Risk of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowling (2017)</td>
<td>Clinic-based family recruitment of children with mild to severe ID, age ≥ 2; N=632; WES</td>
<td>100 (27.1%)</td>
<td>Low</td>
</tr>
<tr>
<td>Coulter (2011)</td>
<td>Patients of children’s hospital; N=1,792, CMA</td>
<td>131 (7.3%)</td>
<td>Unclear</td>
</tr>
<tr>
<td>Henderson (2014)</td>
<td>Laboratory-based series of patients; N=1,780; CMA</td>
<td>227 (12.7%)</td>
<td>Low</td>
</tr>
<tr>
<td>Ho (2016)</td>
<td>Laboratory-based series of patients with neurodevelopmental disorders; N=10,351; CMA</td>
<td>890 (8.6%)</td>
<td>Low</td>
</tr>
<tr>
<td>Roberts (2014)</td>
<td>Laboratory-based series of patients with mixed phenotypes of ID/MCA; N=215; CMA</td>
<td>32 (14.9%)</td>
<td>Low</td>
</tr>
<tr>
<td>Stobbe (2014)</td>
<td>Clinic-based study of adults with autism; N=25; CMA</td>
<td>2 (12.0%)</td>
<td>Low</td>
</tr>
</tbody>
</table>

Abbreviations: CMA=chromosomal microarray; CNV=copy number variant; ID=intellectual disability; MCA=multiple congenital anomalies; VUS=variant of undetermined significance; WES=whole exome sequencing.

Table ES-7. Strength of Evidence for Findings Related to the Diagnostic Yield (EQ1) of Testing with Chromosomal Microarray or Whole Exome Sequencing

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of Studies; Subjects; Study Design</th>
<th>Risk of Bias</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Precision</th>
<th>Other considerations</th>
<th>Strength of Evidence Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic yield of CMA Range 8.6% to 19%</td>
<td>5; 14,163; Observational</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Definition of outcome</td>
<td>Low</td>
</tr>
<tr>
<td>Diagnostic yield of WES 27%</td>
<td>1; 632; Observational</td>
<td>Not serious</td>
<td>NA, single study</td>
<td>Not serious</td>
<td>Serious*</td>
<td>None</td>
<td>Very low</td>
</tr>
</tbody>
</table>

Abbreviations: CMA=chromosomal microarray; NA=not applicable; WES=whole exome sequencing.

* No confidence intervals or other estimates of precision provided.

**EQ2. For what types of conditions is CMA or WES most useful?**
Few studies reported diagnostic yield by patient characteristics or specific diagnosis. However, three studies, Stobbe et al.,43 Ho et al.,41 and Roberts et al.42 reported the diagnostic yield of CMA among patients whose indication for testing was ASD. The pooled summary estimate of diagnostic yield among these children was 5.4% (95% CI, 4.8% to 6.0%).

**EQ3. Does the diagnosis of a chromosomal disorder change the child’s management?**
Seven studies39,40,45-49 evaluated the impact of CMA testing on the clinical management of children with ASD, DD, ID, or MCA. These studies varied in design and outcomes measured. Table ES-8 summarizes study characteristics and findings. Across this body of evidence between 27.1% to 93.8% of children with a pathogenic variant on CMA testing had a change in management prompted by their CMA results (i.e. a management change occurred because of the new information provided by the CMA results). This represents between 3.6% and 6.7% of all
cases tested. Hayeems et al. found that patients with a pathogenic variant were 36% (RR 1.36 [95% CI, 1.21 to 1.53]) more likely to have changes in management than patients with a benign variant on CMA testing. We graded the strength of evidence for this research question as very low (Table ES-9). We identified no studies reporting on the impact of clinical management resulting from WES testing.

Table ES-8. Summary of Findings for the Impact of Chromosomal Microarray Testing on Clinical Management

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Study Population; Sample Size</th>
<th>Outcome Definition</th>
<th>Result</th>
<th>Risk of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coulter (2011)</td>
<td>Retrospective clinic-based cohort of all children with CMA Total tested: 1,792 Total with pathogenic or VUS: 235 Eligible for follow-up study: 194</td>
<td>At least one management change (surveillance start/stopped, referral, diagnostic testing) due to pathogenic CNV.</td>
<td>65 patients with management change (53.7% of follow-up study; 3.6% of all tested)</td>
<td>Cannot determine</td>
</tr>
<tr>
<td>Ellison (2012)</td>
<td>Retrospective laboratory-based cohort. Total tested: 46,298 Clinically actionable CNV: 1,996</td>
<td>Patients with clinically actionable CNV (known microdeletion or duplication syndrome, increased cancer susceptibility, deleted genes associated with genetic disease requiring follow-up).</td>
<td>1,996 cases with clinically actionable CNV (4.3%)</td>
<td>High</td>
</tr>
<tr>
<td>Hayeems (2015)</td>
<td>Retrospective clinic-based cohort of all children with CMA testing followed at tertiary pediatric hospital; N=752</td>
<td>Average number of recommendations (surveillance, referral, diagnostic testing, medication indication/contraindication, family testing) due to pathogenic CNV</td>
<td>Mean 2.35 recommendations per patient</td>
<td>Low</td>
</tr>
<tr>
<td>Henderson (2014)</td>
<td>Retrospective laboratory-based cohort of all children with CMA testing Tested: N=1,780 Pathogenic CNV: 227 Follow-up available: 187</td>
<td>At least one management change (surveillance, referral, diagnostic testing, medical/surgical procedure, medication indication, contraindication) due to pathogenic CNV</td>
<td>102 cases with management change (54.5% of follow-up study; 5.7% of total tested)</td>
<td>Low</td>
</tr>
</tbody>
</table>
### Table ES-8. Summary of Findings for the Impact of Chromosomal Microarray Testing on Clinical Management (continued)

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Study Population; Sample Size</th>
<th>Outcome Definition</th>
<th>Result¹</th>
<th>Risk of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riggs (2014)⁴⁷</td>
<td>Retrospective case series of syndromes diagnosable by CMA; N=28,526 Pathogenic and likely pathogenic: 4,125</td>
<td>At least one management change (referral, diagnostic testing, surgical/intervention procedures, surveillances, medication, contraindication, lifestyle changes) recommended for pathogenic CNV</td>
<td>1,908 (46.3% of cases with recommended change in management; 6.7% of all tested)</td>
<td>High</td>
</tr>
<tr>
<td>Saam (2008)⁴⁸</td>
<td>Retrospective case series of patients with abnormal CNV; N=48</td>
<td>At least one management change (referral, screening, stop screening) due to pathogenic CNV</td>
<td>13 cases with change in management (27.1%)</td>
<td>Cannot determine</td>
</tr>
<tr>
<td>Tao (2014)⁴⁹</td>
<td>Retrospective case series of children with ID/DD, ASD, or MCA; N=327</td>
<td>At least one management change (surveillance, referral, diagnostic testing, medical/surgical procedure, medication indication, contraindication, lifestyle recommendation) due to pathogenic CNV</td>
<td>28 cases with recommended change in management (75.7%)</td>
<td>Low</td>
</tr>
</tbody>
</table>

Abbreviations: CMA=chromosomal microarray; CNV=copy number variant; DD=developmental disability; ID=intellectual disability; MCA=multiple congenital anomalies; VUS=variant of undetermined significance

¹ Confidence intervals were not reported by study authors unless specified.

### Table ES-9. Strength of Evidence for Findings Related to the Impact of Testing with Chromosomal Microarray on Clinical Management (EQ3)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of Studies; Subjects; Study Design</th>
<th>Risk of Bias</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Precision</th>
<th>Other Considerations</th>
<th>Strength of Evidence Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of patients with abnormal results that have a change in management range 27.1 to 93.8%</td>
<td>7; 658; Observational</td>
<td>Serious⁴</td>
<td>Serious⁵</td>
<td>Serious⁶</td>
<td>Serious⁷</td>
<td>None</td>
<td>Very low</td>
</tr>
</tbody>
</table>

⁴ Potential for recall bias when changes in management are collected by physician interview, lack of detail in determining clinical actionability by retrospective review, potential conflict of interest due to goal of promoting reimbursement for CMA.

⁵ Wide range of findings among studies.

⁶ Three studies measured actionability based on published guidelines and recommendations, not actual changes in management for tested patients.

⁷ None of the studies provided confidence intervals or other measures of precision. Sample sizes were small to moderate.
**EQ4. Do children with congenital defects, autism, intellectual disability, or developmental disability tested with CMA or WES have better health outcomes?**

We did not identify any studies that reported on health outcomes among children tested with CMA or WES, either as single-arm studies or compared to patients not tested or tested with other platforms.

**Cost and Cost-Effectiveness**

**CQ1. What is the cost and cost-effectiveness of genetic diagnostic testing for these conditions with CMA or WES?**

We identified five eligible studies reporting cost, cost per patient, cost per diagnosis, or cost per additional diagnosis. All identified studies were specific to CMA testing; no studies evaluated WES testing or reported cost per quality-adjusted or disability-adjusted life year. Study findings are summarized in Table ES-10 by phenotype. Costs per array varied across studies and by testing platforms; these costs ranged from $271 to $1,575 (in 2010 US dollars). These costs reflect the cost per array, which was only one of several costs used to estimate overall costs of CMA testing compared to no CMA testing. The cost per additional diagnosis ranged from $-88,819 to $12,296 (in 2010 US dollars). We graded the strength of evidence for this research question as very low.

**Table ES-10. Summary of Findings of Studies Evaluating Cost Outcomes of Chromosomal Microarray Testing, Outcomes Reported in 2010 US Dollars**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Cost Per Patient or Diagnosis (95% CI)</th>
<th>Difference in Cost (95% CI)</th>
<th>Cost per additional diagnosis (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outcome</strong></td>
<td><strong>CMA Testing</strong></td>
<td><strong>No CMA Testing</strong></td>
<td></td>
</tr>
<tr>
<td>Intellectual Disability</td>
<td>2 (NA)</td>
<td>Cost per diagnosis</td>
<td>$2,919 (2,671 to 3,188)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cost per diagnosis</td>
<td>$6,269 (NR)</td>
</tr>
<tr>
<td>Developmental Delay</td>
<td>1 (114)</td>
<td>Cost per patient</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cost per diagnosis</td>
<td>NR</td>
</tr>
<tr>
<td>Intellectual disability or developmental delay or both</td>
<td>2 (1,636)</td>
<td>Cost per patient</td>
<td>$2,536 (NR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cost per diagnosis</td>
<td>Range $4,381 to $7,757</td>
</tr>
</tbody>
</table>

Abbreviations: CMA=chromosomal microarray; CI=confidence interval; NA=not applicable; NR=not reported.
a Both studies were conducted using decision analyses using hypothetical cohorts; thus sample size is not applicable.

b Assumes that CMA testing increases diagnostic yield from 19.2% to 27.5%

c Assumes that CMA testing increases diagnostic yield from 8% to 18%, cost per diagnosis is 2440 with a 15% absolute increase in diagnostic yield.

d Depending on which kinds of follow-up testing after karyotype used.

e Calculated based on data provided in the study.

f When using local hospital laboratory for testing.

g When using commercial laboratory for testing.

Table ES-11. Strength of Evidence for Findings Related to the Cost-Effectiveness of Chromosomal Microarray Testing Compared to No Testing (CQ1)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of Studies; Subjects; Study Design</th>
<th>Risk of Bias</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Imprecision</th>
<th>Other Considerations</th>
<th>Strength of Evidence Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost per additional diagnosis range $88,919 to $12,296</td>
<td>5; 1,750g Observational and decision analyses</td>
<td>Not serious</td>
<td>Seriousb</td>
<td>Seriousc</td>
<td>Very seriousd</td>
<td>None</td>
<td>Very low</td>
</tr>
</tbody>
</table>

a Total sample size from three retrospective cohort studies; two additional studies generated outcomes based on decision analyses among hypothetical cohorts.

b Clinical and methodological heterogeneity likely explains most of inconsistency in results, though it is unclear to what extent these factors can explain the degree of inconsistency noted.

c Cost per additional diagnosis is a surrogate outcome; this outcome presumes that additional diagnoses would leave to changes in management that ultimately would lead to improved health outcomes.

d Few studies provided confidence intervals around estimates; optimal information size criteria likely not met by any included studies.

Discussion

Summary of the Evidence

The strength of the evidence for all included research questions was very low (safety, impact on management, and costs) or low (diagnostic yield). We identified no eligible studies addressing the impact of chromosomal microarray (CMA) or whole exome sequencing (WES) testing on patient health outcomes (EQ4). Key findings include:

- Safety: The only safety concern that we identified based on one included study is discrimination because of the test results. The body of evidence was not sufficient to determine the frequency with which these issues may arise in CMA testing compared to other types of genetic tests. We graded the strength of the evidence related to safety as very low. We identified no studies that reported safety outcomes related to WES testing.

- Diagnostic yield: In studies that conducted testing in the United States (US) in 2009 or later, CMA testing identified pathogenic or likely pathogenic variants in 8.8% (95% CI, 8.4% to 9.3%) of children tested for any reason, and 5.4% (95% CI, 4.8% to 6.0%) of
children referred for autism spectrum disorder (ASD). A previous health technology assessment (HTA) by Grant et al. that included US and non-US studies found that among studies published in 2012 or later, the diagnostic yield averaged 19% for global developmental delay or intellectual disability (ID) and 12% for ASD. We graded the strength of the evidence related to diagnostic yield of CMA testing as low. One primary research study of WES reported a diagnostic yield of 27% (95% CI, NR) and we graded the strength of this evidence as very low.

- Impact on clinical management: CMA results prompted changes in clinical management in 27% to 94% of patients with a pathogenic variant, which was 3.6% to 6.7% of all patients tested. We graded the strength of this evidence as very low. We identified no studies reported on change in management related to WES testing.

- Costs: The cost per additional diagnosis across this body of evidence ranged in 2010 US dollars from $-88,819 to $12,296. No studies reported on cost per quality-adjusted or disability-adjusted life year. We graded the strength of the evidence on costs as very low.

**Limitations and Applicability of the Evidence Base**

Almost all studies we included focused on CMA. Clinical use of WES is still new, and the body of evidence regarding its impact is limited. Across the body of evidence for all research questions, study design, study population, and outcome measurement details were often sparse, resulting in our inability to assess the risk of bias for some studies. Most of the studies reporting on diagnostic yield included some cases for indications other than our population of interest. In addition, prior diagnostic testing received by the cases varied. Diagnostic yield may differ among more homogenous case series.

One aspect of evaluating the risk of bias not addressed in existing instruments we used are the financial or intellectual conflicts of interest of the study authors. Authors of several included studies stated that a goal of the research was to provide evidence of clinical utility to get CMA covered by payors, potentially providing a strong incentive for analytic decisions that would increase the estimate of diagnostic yield or impact on management. Studies evaluating the impact of testing on management were small, so each included only a small portion of known microduplication or microdeletion syndromes. The clinical features of these syndromes and the appropriate management actions vary accordingly, and are likely an explanation for the large heterogeneity of estimates on impact on management.

The body of evidence related to cost and cost-effectiveness is limited by the lack of studies conducted in the US, the absence of a societal perspective in any of the analyses, and the absence of cost per quality-adjusted or disability-adjusted life year outcomes. Further, this body of evidence is limited by extreme clinical and methodological heterogeneity, which most likely explains the inconsistency in cost per additional diagnosis. The precise role of these tests in the overall sequence and approach to diagnostic evaluation in children with DD, ID, and ASD has also evolved; thus, the cost of the diagnostic journey with or without CMA testing reflected in the included studies may no longer be relevant to current clinical practice.
Limitations of this HTA
We did not include studies published in languages other than English and only searched two US-based electronic databases. We used a single reviewer to screen most titles/abstracts, which may have led to studies inappropriately excluded. For the research question related to diagnostic yield (EQ1), we restricted eligibility to studies with CMA conducted in the US in 2009 or later that used current testing platforms to reduce heterogeneity and provide results more applicable to what is in current clinical use. We did not assess analytic validity or reproducibility or conduct an in-depth analysis or synthesis of the cases, breakpoints, or other information related to CNV findings that were presented by study authors. In addition, our review was limited to the use of WES to detect chromosomal abnormalities.

Conclusion
Chromosomal microarray identifies a pathogenic or likely pathogenic variant in nearly 9% of all children referred for testing and in 5% of those referred because of autism spectrum disorders; these findings are based on a low strength of evidence. The results of chromosomal microarray tests generate changes in management in over half of children who are identified as having a pathogenic or likely pathogenic variant; this finding is based on very low strength of evidence. The evidence is very limited with respect to the safety of testing and we identified no evidence related to the impact of testing on health outcomes. The cost per additional diagnosis for chromosomal microarray testing as a first-line diagnostic test varies between $-88,819 and $12,296 (in 2010 US dollars); this finding is based on very low strength of evidence.
Full Technical Report

Background

Condition Description
Chromosomes, the genetic structures of a cell, are constructed of deoxyribose nucleic acid (DNA) and the proteins and other elements that protect, regulate, and package the DNA. Humans normally have 23 pairs of chromosomes, with half inherited from each parent. During cell replication, chromosomes are sometimes lost or gained, or broken and rearranged. Rearrangements vary in size and complexity, and may be balanced, with no loss of genetic material, or unbalanced with loss or gain of DNA.

Disease Burden
Unbalanced chromosomal rearrangements that are present at conception or that occur during fetal development have profound consequences for the developing fetus, resulting in fetal death, structural defects, genetic diseases, or intellectual impairment. Chromosomal abnormalities occur in 43.8 per 10,000 births that survive to 20 weeks gestation or later. Trisomies 21, 18, and 13; 45, X, and other sex chromosome abnormalities account for most abnormalities. Excluding these, the prevalence of more rare abnormalities is 7.4 per 10,000 births. Small pathogenic duplications or deletions, called copy number changes or variants (CNVs), occur in 1 of 270 pregnancies. The consequences of CNVs depend on the size and location within the genome. Studies examining the prevalence of chromosomal abnormalities have focused on the prenatal period, the prevalence at birth, or the prevalence among individuals with specific structural defects or developmental disabilities. The number of living children or adults with a chromosomal abnormality is unknown. Although the life expectancy for individuals with a chromosomal abnormality may be significantly shortened by birth defects and other conditions, the life span of affected individuals has increased in recent years.

Technology Description
Chromosomal Microarrays (CMA)
Karyotyping and fluorescent in situ hybridization (FISH) have traditionally been used to identify unbalanced chromosomal rearrangements. In the early 2000s, genome-wide microarrays for chromosomal analysis, commonly known as chromosomal microarray (CMA), which use comparative genomic hybridization (CGH) or single nucleotide polymorphism (SNP) arrays to evaluate the number of copies of portions of the chromosomes, were introduced as an adjunct to karyotype and FISH testing for chromosome abnormalities. CGH uses probes fixed to glass plates. Patient and control DNA samples are tagged with fluorescent markers and hybridized to the probes. Computer analysis uses the intensity and color of fluorescence to determine how many copies of each chromosomal region are present. For SNP arrays, individual base pairs throughout the genome that vary within the normal population are tagged with different fluorescent dyes. The number of alleles and whether the individual has the same allele on both chromosome or different alleles can be determined by analysis of the color and intensity of the bound fluorescent dyes.
Although CMA can identify aneuploidies and large rearrangements, its strength lies in identifying small deletions and duplications. CGH can identify rearrangements as small as 150 base pairs, whereas karyotyping can detect no fewer than approximately 5,000 base pairs. For this reason, professional societies now recommended CMA be the first test used to diagnose chromosomal abnormalities in children with multiple congenital anomalies (MCAs) or developmental or intellectual disabilities (DD/IDs). As a result, CMA has increasingly replaced karyotyping and FISH as the initial test for postnatal diagnosis of chromosomal abnormalities. However, CMA cannot identify balanced rearrangements or low-level mosaicism so karyotyping may still be required in some cases.

The platforms used for CGH have changed since their introduction. The first genome-wide platforms used bacterial artificial chromosome (BAC) probes that could detect deletions or duplications of approximately 1,000,000 base pairs (1 Mb). Around 2007, arrays based on small synthesized oligonucleotides (‘oligo’) began replacing BAC arrays. Oligonucleotide probes are smaller, and oligo-based arrays have many more probes, enabling detection of smaller CNVs.

CMA is more expensive than karyotyping. Greenwood Genetics Center, a nonprofit organization that provides clinical genetic services and diagnostic testing, charges $602 for routine resolution karyotyping, $794 for high-resolution karyotyping, and $1,950 for chromosomal analysis by CMA. The laboratory recommends karyotyping in conjunction with the CMA (charge $620) if not completed previously. A hospital-based genetics laboratory located in a midwestern academic medical center charges $1,905 for CMA testing. Several commercial diagnostic laboratories also provide this testing, but prices are not publicly available.

Whole Exome Sequencing
Whole exome sequencing (WES) may also be used with or instead of karyotyping or other chromosomal analysis. WES is the sequencing of the protein coding regions of the genome. Multiple large regions of the genome are sequenced simultaneously (i.e., in parallel using a technique referred to as next generational ‘next gen’ sequencing). In addition to the detection of CNVs, WES provides the base pair sequence for the exons, allowing the detection of single nucleotide changes within specific genes. Thus, some have suggested that for certain conditions and syndromes, WES may be more efficient for testing for abnormalities in multiple specific genes than sequential testing for single gene disorders, particularly when a genetic cause is suspected but the phenotype is not typical for any specific single gene disorder.

Policy Context
The State of Washington Health Care Authority selected testing with CMA and WES as a topic based on medium, high, and high concerns for safety, efficacy, and cost, respectively. Several practice guidelines have been issued that call for CMA to replace G-banded karyotype as the first-tier test for diagnosis of individuals with DD, ID, MCA, and for the clinical evaluation of autism spectrum disorder (ASD). These guidelines, combined with the increasing prevalence of autism, could greatly increase orders for CMA. The purported increased diagnostic yield of
chromosomal abnormalities by CMA compared to karyotype underlies these guidelines.\textsuperscript{10,11} However, the circumstances in which these tests are most useful and their contribution to the medical and educational management and ultimate health outcomes of affected children are unclear.
Washington State Agency Utilization Data

**Populations:**

The *Genomic Micro-array and Single Exome Sequencing* analysis includes member utilization and cost data from the following agencies: PEBB/UMP (Public Employees Benefit Board Uniform Medical Plan) and HCA Medicaid (formerly Fee-for-Service) and the Managed Care (MCO) Medicaid programs. Neither the Department of Labor and Industries (LNI) workers’ compensation plan, nor PEBB Medicare experienced any paid claim activity during the four years examined.

The analysis period was four (4) calendar years, 2013 - 2016. Primary population inclusion criteria included experiencing at least one of the CPT/HCPCS codes from Table I. Individuals with denied claims were excluded from the analysis.

**Methods**

Lab services/units were calculated based on an individual experiencing a paid provider-patient face-to-face, on a specific date *and* including at least one of the CPT codes from Table I. Data evaluation included examining utilization by member; and by average claims’ cost incurred by a member. Total claims were not analyzed for all services provided on the date of lab service. A high level of cost variability, based on site of service (inpatient/outpatient), and simultaneous or subsequent procedures including births, would have skewed the findings.

**Table I. CPT Descriptions**

<table>
<thead>
<tr>
<th>CPT</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81228</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number variants (e.g., bacterial artificial chromosome [BAC] or oligo-based comparative genomic hybridization [CGH] microarray analysis)</td>
</tr>
<tr>
<td>81229</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities</td>
</tr>
<tr>
<td>81415</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities</td>
</tr>
<tr>
<td>81416</td>
<td>Exome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator exome (e.g., parents, siblings) (List separately in addition to code for primary procedure)</td>
</tr>
</tbody>
</table>
Table II. Definitions for Utilization and Cost Tables

<table>
<thead>
<tr>
<th></th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unique Patients</td>
<td>Non-duplicated members seen as patients by year, reported by agency</td>
</tr>
<tr>
<td>Encounters</td>
<td>Defined as a single patient-provider face-to-face on a specific date.</td>
</tr>
<tr>
<td>Average Encounters/Patient</td>
<td>Total encounters/total unique members</td>
</tr>
<tr>
<td>Total Dollars Paid</td>
<td>Paid dollars for all specified CPT codes</td>
</tr>
<tr>
<td>Dollars Paid by Encounter-Mean</td>
<td>Paid dollars for services received on the date of the treatment</td>
</tr>
<tr>
<td>Mean</td>
<td>Sum of all values, divided by the number of observations</td>
</tr>
</tbody>
</table>

Utilization Analysis – Genomic Micro-array and single exome sequencing

<table>
<thead>
<tr>
<th></th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PEBB/ UMP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unique patients</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Encounters</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Average encounters/ Patient</td>
<td>1</td>
<td>1122</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total dollars paid</td>
<td>$540</td>
<td>$492</td>
<td>$12,080</td>
<td>$28,054</td>
<td>$41,166</td>
</tr>
<tr>
<td>Dollars paid by encounter - Mean</td>
<td>$540</td>
<td>$492</td>
<td>$2,013</td>
<td>$1,650</td>
<td>$1,647</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medicaid MCO and Medicaid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unique patients</td>
<td>122</td>
<td>314</td>
<td>574</td>
<td>685</td>
<td>1677</td>
</tr>
<tr>
<td>Encounters</td>
<td>134</td>
<td>335</td>
<td>599</td>
<td>749</td>
<td>1817</td>
</tr>
<tr>
<td>Average encounters/ Patient</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total dollars paid</td>
<td>$14,683</td>
<td>$169,085</td>
<td>$257,922</td>
<td>$302,643</td>
<td>$744,333</td>
</tr>
<tr>
<td>Dollars paid by encounter - Mean</td>
<td>$110</td>
<td>$505</td>
<td>$431</td>
<td>$404</td>
<td>$410</td>
</tr>
</tbody>
</table>

1 Medicaid MCO accounts for 95% of all Paid Dollars and 93% of all Services.

Medicaid MCO and Medicaid HCA Detail

<table>
<thead>
<tr>
<th></th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCO Max of Paid Dollars per CPT</td>
<td>$1,537</td>
<td>$5,842</td>
<td>$2,775</td>
<td>$7,500</td>
</tr>
<tr>
<td>MCO Median of Paid Dollars</td>
<td>$711</td>
<td>$424</td>
<td>$105</td>
<td>$21</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LNI</strong></td>
<td>No encounters</td>
</tr>
<tr>
<td><strong>Medicare/ PEBB</strong></td>
<td>No encounters</td>
</tr>
</tbody>
</table>
Regulatory Status
At the current time, CMA and WES are considered laboratory-developed tests and are not regulated by the United States (U.S.) Food and Drug Administration (FDA). Clinical laboratories that conduct these tests must comply with regulatory standards for high complexity testing within the Clinical Laboratory Improvement Act. Thus, these tests are generally only available through commercial diagnostic testing laboratories (e.g., LabCorp, Ambry Genetics, Lineagen, CombiMatrix) or hospital-based laboratories. FDA approval is required when a company markets and sells a kit for CMA or WES testing. The FDA has approved one CMA kit for marketing in the United States, the Affymetrix CytoScan® Dx assay (Affymetrix, Inc., Santa Clara, CA).13 The FDA-approved indications for this kit include postnatal detection of CNVs associated with DD, ID, MCA, or dysmorphic features. This assay was FDA-approved as a Class II test on January 21, 2014.

Practice Guidelines
In 2010, the International Standard Cytogenomic Array (ISCA) Consortium released a consensus statement that CMA should replace G-banded karyotype as a first-tier test for the diagnosis of individuals with DD/IDs or MCAs.10

In 2011, the National Institute for Health and Care Excellence (NICE) in the United Kingdom issued a clinical guideline related to ASD in children recommending that CMA testing should not be routinely done on all children with autism, but only in those with dysmorphic features or ID.14

In 2013, the American College of Medical Genetics and Genomics (ACMG) recommended that CMA replace G-band karyotype for the clinical evaluation of ASDs.11

In 2014 Clinical Report from the American Academy of Pediatrics (AAP) Committee on Genetics, CMA is considered the first-tier diagnostic test in all children with global DD/ID for whom the causal diagnosis is not known.61 The AAP also considers CMA as a standard for diagnosis of patients with ASDs and MCAs. The AAP Committee on Genetics considers WES an emerging technology for the future and has no current practice guideline related to its use.61

In a 2015 medical coverage policy, the American Academy of Neurology (AAN) considers CMA to be reasonable and medically necessary for diagnosing children with DD/ID or ASD when relevant biochemical and metabolic testing is negative, relevant targeted genetic testing is negative, the results of testing could impact the clinical management of the patient, and face-to-face genetic counseling with a trained and experienced health care professional has been provided.62 The AAN’s practice guideline for evaluation of children with global DD (2003) is currently being updated.63 In a 2016 statement, the AAN acknowledges the rapidly changing landscape of WES testing and costs, yet indicates the following may be indications for WES: undiagnosed neurologic disorder with nonspecific or clinically heterogenous phenotype; expert evaluation with detailed clinical history, comprehensive neurological examination, and complete family history; complete evaluation for common causes not requiring genetic testing, and negative initial genetic testing (e.g., high-yield single gene or multigene testing, chromosomal microarray testing) based on clinical evaluation as appropriate.16
Other Related HTAs


Selected Payer Coverage Determinations

*Table 1* summarizes the Centers for Medicare and Medicaid Services (CMS) and selected other payer coverage determinations for CMA and WES. Among payers, good alignment exists for the criteria under which CMA is covered. Typically, it is covered as first-line diagnostic for global DD/ID or ASD when relevant biochemical and metabolic diseases have been ruled out and the clinical presentation is not specific to a well-delineated genetic syndrome and the results of CMA could impact the clinical management of the child.

<table>
<thead>
<tr>
<th>Payer</th>
<th>CMA Testing</th>
<th>WES Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aetna</td>
<td>Covered for specific indications</td>
<td>Not covered</td>
</tr>
<tr>
<td>Blue Cross (Premera)</td>
<td>Covered for specific indications</td>
<td>Not covered</td>
</tr>
<tr>
<td>Regence Blue Shield</td>
<td>Covered for specific indications</td>
<td>Not covered</td>
</tr>
<tr>
<td>Cigna</td>
<td>Covered for specific indications</td>
<td>Covered for specific indications</td>
</tr>
<tr>
<td>Humana</td>
<td>Covered for specific indications</td>
<td>Not covered</td>
</tr>
<tr>
<td>Kaiser Permanente</td>
<td>Covered for specific indications</td>
<td>Not covered</td>
</tr>
<tr>
<td>Medicare Fee for Service</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Medicaid</td>
<td>Not all states have coverage policies; those that do typically cover for specific indications</td>
<td></td>
</tr>
<tr>
<td>United Healthcare</td>
<td>Covered for specific indications</td>
<td>Covered for specific indications</td>
</tr>
</tbody>
</table>

**CMS**

Medicare has no national coverage determination for the use of CMA or WES. Local coverage determinations vary.

**Aetna**

Aetna considers CMA medically necessary and covered for diagnosing genetic abnormalities in children with MCAs, DD/ID, or ASD when relevant biochemical testing for metabolic diseases is negative; when targeted genetic testing if or when indicated by clinical and family history is negative; when the clinical presentation is not specific to a well-delineated genetic syndrome, and when the results of testing could impact the clinical management of the child.¹⁷ This coverage policy was last affirmed September 22, 2017.
Aetna considers WES testing to be experimental and investigational and, thus, not a covered benefit. This coverage policy was last affirmed on August 8, 2017.

**Blue Cross (Premera)**

Premera considers CMA medically necessary as first-line testing of individuals with nonsyndromic DD/ID or ASD or two or more congenital anomalies not specific to a well-delineated genetic syndrome. Premera considers testing using next-generation sequencing to be investigational. This coverage policy was effective August 25, 2017.

Premera considers WES medically necessary for (1) the evaluation of unexplained congenital or neurodevelopmental disorders in children when the patient has been evaluated by a clinician with expertise in clinical genetics and counseled about the potential risks of genetic testing, (2) there is a potential for change in management and clinical outcome as a result of testing, and (3) a genetic etiology is considered the most likely explanation for the phenotype despite previous genetic testing or when prior genetic testing failed to yield a diagnosis and the individual is faced with invasive procedures or testing as the next diagnostic step. This coverage policy was effective February 1, 2017.

**Blue Shield (Regence)**

Regence considers CMA medically necessary in children as first- or second-line assessment in children with apparent nonsyndromic cognitive DD/ID, ASD, or MCAs not specific to a well-delineated genetic syndrome. Further, Regence considers testing using next-generation sequencing to be investigational and not a covered benefit. This policy was last affirmed April 2017.

Regence considers all applications of WES to be investigational and not a covered benefit. This coverage policy was last effective August 1, 2017.

**Cigna**

Cigna considers CMA medically necessary when phenotypic characteristics of a specific genetic disorder are absent for patients with ASD and nonsyndromic global DD/ID. Testing is also considered medically necessary when MCAs are present and cannot be ascribed to a specific genetic syndrome. In addition to these indications, testing must be recommended by independent board-certified or eligible medical geneticists, certified genetic counselors, or certified genetic nurses. The health professionals recommending testing (1) cannot be employed by a commercial genetic testing laboratory, (2) must have evaluated the individual, including a three-generation pedigree, and (3) must intend to engage in post-test follow-up counseling. This coverage policy was effective November 15, 2016.

Cigna considers WES medically necessary when a genetic etiology is the most likely explanation for the phenotype demonstrated, no other cause can explain symptoms, and the clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available; and the differential diagnosis and/or phenotype would require testing of multiple genes such that WES testing would be more practical than separate genetic tests or preclude the need for multiple and/or invasive procedures. In addition, the individual must have been evaluated by a board-certified medical geneticist or other physician specialist with expertise in the conditions and relevant genes for which testing is being considered, and WES results are
expected to directly impact clinical decision-making and clinical outcome for the individual being tested. Pre- and post-test genetic counseling is required. This coverage policy was effective November 15, 2016.

**Humana**
Humana covers CMA for the evaluation of children diagnosed with ASD, unexplained global DD/ID and the absence of a clinically identifiable single gene disorder, clinical syndrome, and no family history of chromosomal rearrangement or multiple miscarriages. Humana also covers CMA for the evaluation of multiple anomalies, the combination of which are not suggestive of a specific syndrome. This coverage policy was effective July 27, 2017.

Humana considers WES experimental/investigational, and this testing is not covered. This coverage policy was effective August 18, 2017.

**Kaiser Permanente**
Kaiser considers CMA medically necessary for the evaluation of ID for individuals with significant dysmorphic features or congenital anomalies, when results are expected to affect clinical management, and when genetic counseling by a health care professional with appropriate genetic training and experience has been conducted. The source documentation did not include an effective date for this coverage policy.

Kaiser does not cover WES. The source documentation did not include an effective date for this coverage policy.

**Medicaid**
Some states have Medicaid coverage policies related to CMA testing. In Indiana, CMA testing is covered as a first-line test in postnatal evaluation of children with unexplained intellectual disability, development delay, or ASD. Similarly, CMA testing is also covered in North Carolina. In Massachusetts, testing is covered with prior authorization.

**United**
United Healthcare considers CMA medically necessary for evaluating patients with multiple anomalies not specific to a well-delineated genetic syndrome and that cannot be identified by a clinical evaluation alone, nonsyndromic DD/ID, and ASDs. This coverage determination was effective August 1, 1017.

United Healthcare considers WES medically necessary when the patient’s clinical presentation is nonspecific, does not fit a well-defined syndrome for which a targeted gene test exists, and testing has been recommended by a board-certified medical geneticist, neonatological, neurologist, or developmental pediatrician with specific expertise in the conditions for which testing is being considered, and the results are expected to directly influence management and clinical outcome. Additional medically necessary conditions are: the patient’s clinical presentation and family history strongly suggest a genetic cause; the patient has a confident clinical diagnosis of a genetic condition where there is significant genetic heterogeneity, and WES would be more practical approach than multiple individual genetic tests; or the patient likely has a genetic disorder and has had multiple targeted gene tests that failed to identify the underlying cause. This coverage determination was effective November 1, 2017.
Research Questions and Analytic Framework

The draft research questions for this Health Technology Assessment (HTA) were posted from August 16, 2017 to August 29, 2017 and received one public comment requesting inclusion of fragile X testing along with consideration of chromosomal microarray (CMA) and whole exome sequencing (WES) testing. This item was not added to the scope to keep the HTA focused on CMA and WES testing for the diagnosis of chromosomal abnormalities. Figure 1 provides the analytic framework and Table 2 provides the final research questions and study selection criteria related to the population, intervention, comparator, outcomes, time period, and setting.

Figure 2. Analytic Framework for Chromosomal Microarray and Whole Exome Sequencing in Children with Intellectual Disability, Autism, or Birth Defects

Abbreviations: ASD=autism spectrum disorder; CD=congenital defects; CMA=chromosomal microarray; CQ=cost question; DD=developmental disability; EQ=efficacy question; ID=intellectual disability; SQ=safety question; WES=whole exome sequencing
Table 2. Research Questions and Scoping Parameters for Chromosomal Microarray or Whole Exome Sequencing in Children with Intellectual Disability, Autism, or Birth Defects

<table>
<thead>
<tr>
<th>Research Questions</th>
<th>Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>SQ1</td>
<td>What, if any, safety issues do CMA and WES pose beyond those associated with phlebotomy?</td>
</tr>
</tbody>
</table>

| Efficacy               | EQ1. How often do CMA or WES return an informative result (i.e., diagnostic yield)?  |
|                       | EQ2. For what types of conditions is CMA or WES most useful?                      |
|                       | EQ3. Does the diagnosis of a chromosomal disorder change the child’s management?   |
|                       | EQ4. Do children with congenital defects, autism, intellectual disability or developmental disability tested with CMA or WES have better health outcomes? |

| Cost                   | CQ1. What is the cost and cost-effectiveness of genetic diagnostic testing for these conditions with CMA or WES? |

| Populations; Interventions; Comparators, Outcomes; Time Period; Setting |
|--------------------------|-------------------------------------------------------------------------|
| Populations              | Children diagnosed with congenital defects, autism, intellectual disability or developmental disability without known syndrome or specific genetic abnormality |
| Intervention             | 1. CMA testing with currently available platforms, obsolete and superseded platforms will be excluded. |
|                          | 2. WES testing                                                          |
| Comparator               | EQ1, EQ2, SQ1: descriptive and may not have comparator groups.           |
|                          | EQ3: Management before and after diagnosis following testing; management of similarly affected untested/undiagnosed children |
|                          | EQ4 and CQ1: No genetic diagnostic testing or genetic diagnostic testing did not include CMA or WES. |
| Safety Outcomes          | SQ1. Harms reported as related to testing other than those associated with phlebotomy |
| Efficacy Outcomes        | EQ1 and EQ2: Diagnostic yield or earlier diagnosis                      |
|                          | EQ3. Change in medical or educational interventions                     |
|                          | EQ4. Mortality during infancy or childhood                               |
|                          | EQ4. Development of comorbidities                                       |
|                          | EQ4. Functional achievement                                              |
| Cost Outcomes            | CQ1: Cost of assay, cost per diagnosis, cost per additional diagnosis, cost per quality-adjusted life year, cost per disability-adjusted life year |
| Time Period              | 2010 to 2017 for EQ1 and EQ2, 2000-2017 for all others                 |
| Setting                  | Clinical genetic laboratories, medical genetic clinics, general and specialty pediatric clinics; non-US studies were excluded for EQ1 and EQ2. |

Abbreviations: CMA=chromosomal/genomic microarray; CQ=cost question; EQ=efficacy question; SQ=safety question; US=United States; WES=whole exome sequencing.

What is Excluded from This HTA

This HTA will not address the analytic validity of CMA or WES, as this testing is available within Clinical Laboratory Improvement Act (CLIA)-licensed laboratories as a laboratory-developed test and analytic validity is assumed based on meeting CLIA standards.28 This HTA will also not address the use of CMA or WES to identify or monitor chromosomal changes in tumor cells or its use for prenatal testing, or the use of WES to identify mutations within single genes. The review will not assess the clinical utility of incidental findings not related to the
health conditions for which the tests were ordered and will also not address the ethical considerations of these findings. Because of the large volume of studies identified for the first efficacy question (EQ1) related to diagnostic yield and the rapidly evolving technology in use for CMA testing, we excluded studies from EQ 1 that were conducted outside of the US and studies conducted prior to 2009 or that used obsolete testing platforms (e.g., bacterial artificial chromosome).

Methods

Data Sources and Searches
We searched MEDLINE® (via PubMed) and a clinical trials registry (clinicaltrials.gov) for relevant English-language studies published in 2000 or later. We searched the FDA website, selected payer and health care professional society websites, the US Agency for Healthcare Research and Quality, and other organizations that conduct and disseminate HTAs. In addition, we reviewed the reference lists of relevant studies, practice guidelines, and other HTAs on this topic to identify any relevant articles not identified through the electronic search. The detailed search strategy is provided in Appendix A.

Study Selection
Study selection criteria for this HTA were as follows:

Population
For all research questions, we included studies that reporting testing individuals (children or adults) with autism, cerebral palsy, global developmental delay (GDD), developmental disability (DD), intellectual disability (ID), autism spectrum disorder, including Asperger’s syndrome, mental retardation, mental deficiency, congenital defects, or birth defects. We excluded studies focused on testing among populations with syndromes known to be associated with single gene disorders or among populations with single, specific birth defects.

Intervention and Comparator
For all research questions, we included studies that reported on the use of chromosomal microarray (CMA) testing (including the specific terms such as genome-wide array, genomic array, chromosomal array, chromosomal microarray, comparative genomic hybridization, deoxyribose nucleic acid (DNA) sequencing, or molecular karyotype) or whole exome sequencing (WES testing). Further, we required studies to be using these tests to identify imbalanced rearrangements, translocations, duplications, deletions, copy number variants (CNVs), or chromosomal aberrations and limited study selection for diagnostic yield (EQs1 and 2) to studies using current testing platforms. Studies that focused on fragile X testing, mutations in a single gene or a single gene panel or single gene sequencing, epigenetic testing, or whole genomic sequencing were excluded. Further, studies that used CMA or WES to identify mutations, trinucleotide repeats, or aneuploidy were excluded. For the research questions on comparative efficacy related to management and health outcomes (EQ3 and EQ4), we selected studies with comparison groups that were historical (management before/after testing) or concurrent controls (no CMA or WES testing or testing that did not include CMA or WES).
Outcomes
For the research question on safety (SQ1), we included studies that reported on harms or adverse effects of testing with CMA or WES. We excluded studies solely reporting harms associated with phlebotomy, and did not include harms or adverse effects associated with incidental findings or ethical issues associated with this testing. For the research questions on diagnostic yield (EQ1 and EQ2), we included studies that reported on the proportion of CMA and WES testing that identified a known or likely pathogenic variant among all tested children or among children or adults with the conditions of interest. For EQ3, we included studies that reported on time to diagnosis and medical or educational interventions resulting from the results of testing. For EQ4, we included studies that reported mortality, morbidity, or functional achievement. For the research question on cost (CQ1), we included studies that reported on the cost of testing, cost per patient, cost per diagnosis, cost per additional diagnosis, or cost per quality-adjusted life year or disability-adjusted life year measures from either a payer or societal perspective. Studies that did not report at least one eligible outcome were excluded.

Time Period
For EQ1 and EQ2 we selected studies that conducted testing in 2009 or later. Studies published in 2000 or later were eligible for all other research questions.

Settings
We included studies conducted in clinical settings or where the CMA or WES was being evaluated as a clinical test; we excluded studies focused on test development or that occurred strictly within a research setting without any connection to clinical practice. For EQ1 and EQ2, we excluded studies conducted outside of the U.S.

Study Design
We included all study designs except case reports.

We screened titles and abstracts and full-text articles based on these study selection criteria. Team members were trained on study selection criteria, and all team members independently screened an initial set of 20 titles and abstracts. Because we had excellent concordance among screeners on the initial set, a single team member screened the remaining titles/abstracts. The principal investigator reviewed all abstracts excluded for “ineligible intervention” and a sample of titles/abstracts excluded for other reasons to ensure continued consistency in application of study selection criteria by team members. A senior team member screened each full-text article for inclusion, and the principal investigator confirmed the decisions.

Data Abstraction and Quality Assessment
One team member extracted relevant study data into a structured abstraction form. The principal investigator reviewed the abstractions for accuracy and consistency. Two senior team members conducted independent risk of bias assessment on all included studies and met to reconcile any disagreements, in consultation with the principal investigator if needed. Because of the diverse types of studies included in this HTA, we adapted signaling questions from the QUADAS-2 instrument, a risk of bias assessment for diagnostics test studies, and items from the RTI item bank for observational studies.²⁹,³⁰ The signaling questions assessed the major sources of bias including selection bias (both how study population was selected and attrition/missing data),
confounding, and measurement/information bias. We used the ROBIS instrument to assess the risk of bias for systematic reviews.31

Data Synthesis and Analysis
Study characteristics and results were qualitatively synthesized for each research question in tabular and narrative formats. For cost outcomes, we adjusted all reported outcomes in foreign currency to US dollars based on the US Department of Treasury mid-year exchange rate for the year reported by study authors and then used the chain-weighted consumer price index (CPI) to adjust to 2010 US dollars (Appendix B).32,33 To determine whether quantitative synthesis was appropriate, we assessed the number of studies and the clinical and methodological heterogeneity present based on established guidance.34,35 We required three or more publications with similar approach and the same outcome measure to calculate a summary estimate. We estimated summary effects using a fixed effects model if the test for heterogeneity was nonsignificant and a random effects model if the test for heterogeneity was significant using OpenMetaAnalyst (For Windows 8, 64-bit) using the method of Hedges and Olkin to estimate between-study variance.36 We graded the strength of evidence for each research question using GRADE, which assesses the strength of evidence based on domains relating to risk of bias, inconsistency, imprecision, indirectness, and other considerations, such as reporting bias.37 Under GRADE, the strength of evidence can be graded as very low, low, moderate, or high.

Results

Literature Search
Figure 2 depicts the study flow diagram. We identified and screened 2,717 unique citations. We excluded 2,375 after title and abstract review. We reviewed the full-text of 348 articles, and excluded 330 for the reasons listed in Figure 2. We included a total of 18 studies. One provided evidence on safety issues (SQ1), seven provided evidence on diagnostic yield (EQ1 and EQ2), seven on changes in management (EQ3), and five on costs (CQ1). No studies provided information on health outcomes (EQ4). Individual study characteristics for all included studies are summarized in Appendix C, Table C-1. The list of studies we screened at the full text stage, but which were excluded from the review, is provided in Appendix D. Note that studies may have been excluded based on more than one reason but we report only one reason. Individual risk of bias assessments for all included studies are reported in Appendix E.
Figure 3. Study Flow Diagram

Number of citations identified through database searches: 2912

Number of titles/abstracts screened after duplicates removed: 2717

Number of full-text articles identified through handsearches assessed for eligibility: 6

Number of full-text articles assessed for eligibility: 348

Number of full-text articles excluded: 2375

Number of full-text articles excluded: 330

By reason:
- Ineligible population: 29
- Ineligible intervention: 38
- Ineligible setting: 50
- Ineligible outcome: 7
- Ineligible country (EQ1/EQ2 only): 85
- Ineligible platform: 89
- Ineligible publication type: 14
- EQ1/EQ2 CMA prior to 2009: 3
- No research question: 6
- Duplicates/superseded: 9

Number of studies included for SQ1 (Safety): 1

Number of studies included for EQ1 and EQ2 (Diagnostic Yield): 7

Number of studies included for EQ3 (Changes in Management): 7

Number of studies included for EQ4 (Health Outcomes): 0

Number of studies included for CQ1 (Cost): 5

Safety

SQ1. What, if any, safety issues do CMA and WES pose beyond those associated with phlebotomy? One study provided evidence on safety issues that arise in chromosomal microarray (CMA) testing. Individual study characteristics and findings are included in Appendix C, Table C-1 and C-2 respectively; individual study risk of bias assessments are in Appendix E, Table E-1. We did not identify any studies reporting on safety outcomes related to whole exome sequencing (WES) testing.

Study Characteristics

The sole study that reported on a safety issues was related to CMA testing. Hamilton et al. reported on a study of 6 children in the United Kingdom that evaluated the consequences of CMA testing among children in foster care.
**Findings**

**Discrimination and social consequences.** Of six children whose referral for CMA testing noted that they were in foster care, four had abnormal or ambiguous results. In one of the cases of abnormal results, the application to adopt the child from foster care was withdrawn by the prospective adopters because of the chromosome abnormality that was identified with CMA testing. The 18-month-old boy had mild speech and motor delay, but showed no evidence of social disability. CMA testing detected a microduplication at 15.11.2, a variant suspected to cause behavioral difficulties and/or autism. The authors caution that data from untargeted genetic testing such as CMA or WES may be considered in ways detrimental to tested children.

**Summary and Strength of Evidence: Safety**

The findings are summarized in Table 3 and the strength of evidence is summarized in Table 4. The one included study has a very small sample and a high probability of selection bias, since four of six children identified as being in foster care had an abnormal CMA result. The study demonstrates that there are safety issues to be considered in CMA or WES testing, but the body of evidence is insufficient to estimate the frequency with which these issues arise or to compare the frequency to other types of genetic testing. We graded the strength of this body of evidence as very low because of serious study limitations in the study and the inability to evaluate inconsistency and imprecision domains.

**Table 3. Summary of Findings for the Safety of Testing with Chromosomal Microarray or Whole Exome Sequencing**

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Study Population; Sample Size</th>
<th>Primary Outcomes</th>
<th>Key Results</th>
<th>Risk of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamilton (2015)</td>
<td>Children referred for CMA testing noted as being in foster care; N=6</td>
<td>Adoption request for child in foster care withdrawn after report of CNV associated with autism</td>
<td>1 of 4 cases with abnormal results experienced discrimination</td>
<td>High</td>
</tr>
</tbody>
</table>

Abbreviations: CMA=chromosomal microarray; CNV=copy number variant.

**Table 4. Strength of Evidence for Findings Related to the Safety of Testing with Chromosomal Microarray or Whole Exome Sequencing**

<table>
<thead>
<tr>
<th>No. of Studies; Subjects</th>
<th>Study Design</th>
<th>Risk of Bias</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Precision</th>
<th>Reporting Bias</th>
<th>Strength of Evidence Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1; 6</td>
<td>Observational</td>
<td>Serious*</td>
<td>Unable to assess</td>
<td>Not serious</td>
<td>Unable to assess</td>
<td>Not serious</td>
<td>Very Low</td>
</tr>
</tbody>
</table>

* Enrollment in foster care not routinely collected, only available if noted on test requisition. High risk of selection bias.

**Efficacy**

**EQ1. How often do CMA or WES return an informative result (i.e., diagnostic yield)?**

Five primary studies and one health technology assessment provided evidence related to diagnostic yield (EQ1) and the types of clinical conditions for which CMA is most useful (EQ2). One study provided evidence related to diagnostic yield of WES testing. Study characteristics and findings are summarized in Table 5. Appendix C, Tables C-1 and C-3 provide detailed individual study characteristics and findings, respectively; individual study risk of bias assessments are provided in Appendix E, Table 1.
**Study Characteristics**

Grant et al. conducted a health technology assessment (HTA) of chromosomal microarray testing that was published in 2015 and that included a thorough review on the diagnostic yield of chromosomal microarray in patients with global developmental delay (GDD), intellectual disability (ID), or autism spectrum disorders (ASDs). This review included 67 studies published prior to June 24, 2015 that provided evidence on the diagnostic yield of CMA or its impact on clinical management decisions or patient outcomes. They included case series or cohorts of at least 20 patients with GDD with or without ID, or ASD with or without negative karyotype results. We assessed this risk of bias of this HTA as “unclear”, primarily because it did not assess the risk of bias for studies included in its synthesis.

In addition to the Grant et al. HTA, we included four primary research studies published from 2010 to 2015 that were included in the Grant et al. HTA, and one study published in 2016, giving us a total of five primary research studies that provided evidence on the diagnostic yield of CMA. We also included one study of the diagnostic yield of WES. Three studies used oligonucleotide CMA, two used SNP-oligo CMA, and two studies used high-resolution SNP CMA. The patient populations of the studies were mixed. Four studies of CMA testing reported findings for patients with congenital anomalies, DD, ID, or both DD and ID. Stobbe et al. reported on CMA results among adults with ASD. The single study reporting on diagnostic yield of WES testing reported findings for children with ID.

The methods for classifying a variant as pathogenic or likely pathogenic varied across studies. Coulter et al. classified a variant as pathologic if it was 1) associated with a known microduplication or deletion syndrome, 2) a deletion of genes that are known to cause disease when haploinsufficient, 3) or a large (size unspecified) duplication or deletion. A variant was classified as possibly pathogenic if it overlapped a known syndromic region, or contained genes suspected of causing disease, with the potential unmasking of recessive alleles. Henderson et al. defined variants as pathologic if they were 1) associated with a known microduplication or deletion syndrome, 2) encompassed or interrupted genes associated with disease, 3) included numerous genes and were not found in healthy individuals, or 4) were a region of homozygosity greater than 10 Mb. Ho et al. classified variants as pathologic if they were found in less than 1% of the general population and there were at least two independent peer-reviewed reports that haploinsufficiency or triplosensitivity of the region or gene(s) caused clinical symptoms similar to those of the patient. Roberts et al. classified variants as pathologic if they had previously been associated with ASD or learning disability. They evaluated variants using the University of California at Santa Cruz (UCSC) Genome Browser, Database of Genomic Variants (DGV), Online Mendelian Inheritance in Man (OMIM), DECIPHER, dbVar, and CombiTrak, an internal database. Stobbe classified variants as likely pathogenic if a prior case report including well-defined breakpoints and well-specified phenotype linked the variant to autism or if the duplication or deletion included a gene for which there was compelling and specific evidence that it was associated with autism. Bowling et al. defined variants or mutations as pathogenic if they resulted in loss-of-function (LOF) of genes where LOF is a known disease mechanism; if the mutation was a missense mutation known or computationally predicted to cause disease; if the mutation or variant was de novo and predicted to be damaging or to cause LOF in a gene known to cause dominantly inherited disease; if the individual had compound heterozygotes for two recessive alleles in a gene associated with recessive genetic disease, both alleles were
predicted to be damaging, and the population frequency of each is low enough to plausible given the incidence of disease. We rated Coulter et al. 39 as having an unclear risk of bias, primary because of incomplete reporting on their microarray platform. The other five studies were rated as having a low risk of bias.

Our exclusion from diagnostic yield of studies conducted outside of the US substantially reduced the number of studies we included compared to the number included in the Grant et al. HTA28 Of the 31 studies published in 2010 or later that were included in the Grant et al. HTA, 24 were not conducted in the U.S., and five studies were excluded from our review because of obsolete platforms or testing was conducted prior to 20009(Appendix D).

**Findings**

**Diagnostic Yield of CMA Testing.** The median diagnostic yield for CMA testing among patients with GDD with or without ID in the Grant et al. HTA was 13.6% [interquartile range (IQR), 9.5 to 17.2%] across 55 applicable studies in this HTA, and was 19% among the 21 studies published in 2012 or later.28 Diagnostic yield of CMA among patients with GDD increased by 1% per year on average. For patients with ASD, the median diagnostic yield among 12 relevant studies was 8.4% (IQR,7.2% to 17.3%) and was 12.3% among the four studies published in 2012 or later.

**Table 5** summarizes the study characteristics and findings related to diagnostic yield among the five primary research studies on CMA testing that we included. The pooled summary estimate of diagnostic yield, from these studies including known and likely pathogenic variants, was 8.8% (95% CI, 8.4% to 9.3%) (Figure 3). The individual study estimates ranged from 7.3%39 to 14.9%.42 The studies were heterogeneous (p < 0.001), so a random effects model was used to calculate the pooled summary estimate.

**Table 5. Summary of Findings for the Diagnostic Yield of Testing with Chromosomal Microarray or Whole Exome Sequencing**

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Study Population; Sample Size, Test</th>
<th>Diagnostic Yield [Detection of A Pathogenic Variant] N (%)</th>
<th>Risk of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowling (2017)</td>
<td>Clinic-based family recruitment of children with mild to severe ID, age ≥ 2; N=632, WES</td>
<td>100 (27%)</td>
<td>Low</td>
</tr>
<tr>
<td>Coulter (2011)</td>
<td>Patients of children’s hospital; N=1,792, CMA</td>
<td>131 (7.3%)</td>
<td>Unclear</td>
</tr>
<tr>
<td>Henderson (2014)</td>
<td>Laboratory-based series of patients; N=1,780, CMA</td>
<td>227 (12.7%)</td>
<td>Low</td>
</tr>
<tr>
<td>Ho (2016)</td>
<td>Laboratory-based series of patients with neurodevelopmental disorders; N=10,351, CMA</td>
<td>890 (8.6%)</td>
<td>Low</td>
</tr>
<tr>
<td>Roberts (2014)</td>
<td>Laboratory-based series of patients with mixed phenotypes of ID/MCA; N=215, CMA</td>
<td>32 (14.9%)</td>
<td>Low</td>
</tr>
<tr>
<td>Stobbe (2014)</td>
<td>Clinic-based study of adults with autism; N=25, CMA</td>
<td>2 (12.0%)</td>
<td>Low</td>
</tr>
</tbody>
</table>

Abbreviations: CMA=chromosomal microarray; CNV= copy number variant; ID= intellectual disability; MCA=multiple congenital anomalies; VUS=variant of undetermined significance; WES=whole exome sequencing.
Figure 4. Summary Pooled Estimate for Diagnostic Yield of Chromosomal Microarray for All Included Phenotype

Diagnostic Yield of WES Testing. One study (Bowling et al.44) examined the diagnostic yield

Bowling et al.44 evaluated use of WES among 371 cases with DD or ID aged 2 years or older. A pathogenic or likely pathogenic mutation or CNV was identified in 100 (27.1%) cases, of which 92 (24.8% of all cases) were single gene mutations and eight (2.2% of all cases) were CNVs.

Summary and Strength of Evidence: Efficacy of Diagnostic Yield

The pooled average diagnostic yield from the five primary research studies we included was 8.8% (95% CI, 8.4% to 9.3%), lower than the diagnostic yield reported by the Grant et al. HTA (19% for GDD [with or without ID] among studies published in 2012 or later. The studies included in this body of evidence are predominantly consecutive case series from individual clinical laboratories. Most studies included a mix of phenotypes, including DD, ID, and ASD, and multiple congenital anomalies (MCAs). The definition of pathogenic variant used in the studies varied, partially because the tools for assessing pathogenicity have evolved. Although all the definitions used are valid, the differing definitions may account for some of the variability in the findings. These studies are likely applicable to diagnostic yield in clinical practice; however, they may have included some patients with indications that did not fit our inclusion criteria. Although most of these studies have a low risk of bias, because of observational study designs the strength of evidence cannot be graded any higher than low.

Only one study44 provided evidence on the diagnostic yield of WES testing, which was reported as 27.1%. No confidence intervals were provided for this observational study, thus we graded the strength of evidence for WES testing as very low.
Table 6. Strength of Evidence for Findings Related to the Diagnostic Yield (EQ1) of Testing with Chromosomal Microarray or Whole Exome Sequencing

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of Studies; Subjects; Study Design</th>
<th>Risk of Bias</th>
<th>Inconsistency</th>
<th>Indirectness</th>
<th>Precision</th>
<th>Other considerations</th>
<th>Strength of Evidence Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic yield of CMA Range 7.3% to 19%</td>
<td>5; 14,795; Observational</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Not serious</td>
<td>Definition of outcome</td>
<td>Low</td>
</tr>
<tr>
<td>Diagnostic yield of WES 27%</td>
<td>1; 632; Observational</td>
<td>Not serious</td>
<td>NA, single study</td>
<td>Not serious</td>
<td>Serious(^a)</td>
<td>None</td>
<td>Very low</td>
</tr>
</tbody>
</table>

Abbreviations: CMA=chromosomal microarray; NA= not applicable; WES=whole exome sequencing.

\(^a\) No confidence intervals or other estimates of precision provided.

**EQ2. For what types of conditions is CMA or WES most useful?** Although some studies reported on diagnostic yield by patient characteristics or specific diagnosis, most factors were only examined in a single study. Thus, we were unable to evaluate the diagnostic yield for most individual phenotypes. However, three studies, Stobbe et al., Ho et al., and Roberts et al. reported the diagnostic yield of CMA among patients whose indication for testing was ASD. The summary estimate of diagnostic yield was 5.4% (95% CI, 4.8% to 6.0%) as depicted in Figure 4. We used a fixed effects model to calculate the summary estimate because the test for heterogeneity was nonsignificant (p> 0.05). As with the estimate for all phenotypes, our estimated diagnostic yield is lower than that of Grant et al. who found an average diagnostic yield of 12.3% for ASD among studies published after 2012.28

**Figure 5. Evidence on Diagnostic Yield of Chromosomal Microarray for Autism Spectrum Disorders**

**EQ3. Does the diagnosis of a chromosomal disorder change the child’s management?**

Seven studies evaluated the impact of CMA testing on the management of children with ASD, DD, ID, or MCA. Study characteristics and findings are summarized in Table 7. Appendix C, Table C-1 and Table C-4, provide detailed individual study characteristics and results, respectively; Appendix E, Table 1 provides individual study risk of bias assessments.
Study Characteristics

Included studies used two different approaches to addressing this research question. Two studies\textsuperscript{45,47} evaluated queried databases to identify CMA-tested cases with abnormal variants that had implications for medical management.\textsuperscript{45,47} Ellison\textsuperscript{45} and five additional studies\textsuperscript{39,40,46,48,49} measured actual actions taken as a result of the CMA results, four\textsuperscript{39,40,46,49} by medical record review and two\textsuperscript{45,47} by physician survey. Five\textsuperscript{39,40,45,47,48} of the studies were conducted in the U.S., although the databases that Riggs et al.\textsuperscript{47} and Ellison et al.\textsuperscript{45} used could have contained cases from other countries. Hayeems et al.\textsuperscript{46} was conducted in Canada, and Tao et al.\textsuperscript{49} in Hong Kong. The studies were conducted between 2008 and 2013, and included patients tested between 2004 and 2013. Three\textsuperscript{40,46,49} of the studies were rated as low risk of bias, two as unclear risk of bias\textsuperscript{39,48} and two as high risk of bias.\textsuperscript{45,47} The reasons for the ratings were lack of detail on testing methodology\textsuperscript{39} or variant classification\textsuperscript{39,45,47} or potential recall bias.\textsuperscript{45,48} We did not identify any studies that evaluated the impact of WES testing on clinical management.

Except for Hayeems et al.,\textsuperscript{46} these studies limited their population to patients diagnosed with a pathogenic or likely pathogenic variant and only counted management changes attributable to the CMA results. Hayeems et al.\textsuperscript{46} included all patients with detected CNV, and compared management changes, in total and the number attributable to the CMA results, for patients with pathogenic or likely pathogenic variants to management changes for patients with benign variants.

The methods for classifying a variant as pathogenic varied among the studies. Hayeems et al.\textsuperscript{46} and Saam et al.\textsuperscript{48} relied primarily on the clinical laboratory report. If the laboratory report did not classify the variant, Hayeems classified it according to the American College of Medical Genetics (ACMG) guidelines.\textsuperscript{11} Tao et al. also used the ACMG guidelines to classify variants, referring to internal and public databases of variants to aid in the classification. Coulter et al.\textsuperscript{39} and Henderson et al.\textsuperscript{40} classified as pathogenic known microduplication or microdeletion syndromes, large duplications or deletions, and deletions that encompassed genes known to cause disease when haploinsufficient\textsuperscript{39} or associated with genetic disease.\textsuperscript{40} Henderson et al. also classified as pathogenic large (>10 Mb) regions of homozygosity.\textsuperscript{40}

Ellison et al.\textsuperscript{45} reviewed the results of all patients with CMA testing in Signature Genetics’ laboratory database to identify those with abnormal results that were clinically actionable. They defined a clinically actionable variant as one that was associated with a microduplication or microdeletion syndrome with features that require specific follow-up (40 variants), that were associated with increased cancer susceptibility (27 variants), or that involved known dosage sensitive genes that cause genetic disease requiring specific follow-up (38 variants).\textsuperscript{45}

Riggs et al. identified genes covered by the International Standards for Cytogenomic Arrays (ISCA) Consortium 180k array design that were associated with a syndrome described in Gene Reviews, DECIPHER (https://decipher.sanger.ac.uk/syndromes), or the ISCA Consortium’s known pathogenic list (http://www.ncbi.nlm.nih.gov/dbvar/studies/nstd45/), a total of 205 syndromes for a total of 235 phenotypes.\textsuperscript{47} They excluded 49 phenotypes as not diagnosable by CMA. Of the 186 phenotypes diagnosable by CMA, 146 (79%) had specific medical management recommendations based on professional guidelines (level 1) or peer-reviewed publications (level 2) and were classified as clinically actionable.
All studies defined management change to include specialist referrals, diagnostic testing, changes in medical surveillance or screening, surgical or intervention procedures, and prescribed or contraindicated medications specifically related to the CMA results. Riggs et al.\(^47\) and Tao et al.\(^49\) also included recommendations for lifestyle changes. Saam et al.\(^48\) included changes in counseling on recurrence risk and improved access to services.

**Findings**

From 27.1% to 93.8% of cases with a pathogenic variant had a change in management prompted by their CMA results. Table 7 summarizes the findings related to the impact on clinical management of CMA testing for the seven primary research studies we included. Among the three studies that measured management changes using medical record review, 53.7% to 75.7% of cases with a pathogenic variant had at least one management change prompted by their CMA results. The cases with management changes represented 3.6% to 6.7% of all cases tested, which is similar to the proportion of clinically actionable variants identified by Ellison et al.\(^45\) and Riggs et al.\(^47\) Of the 46,298 patients with CMA results in their laboratory database, 1,996 (4.3%) had variants defined by Ellison et al. as clinically actionable.\(^45\) Of 28,526 cases in the ISCA database, 1,908 (6.8%), were clinically actionable.\(^47\) Almost half, 48% of the pathogenic variants in the ISCA database were clinically actionable. Hayeems et al.\(^46\) found that patients with a pathogenic variant received an average of 2.4 medical management recommendations directly related to their CMA results and were 36% (RR, 1.36 [95% CI, 1.21 to 1.53]) more likely to have changes in management than patients with a benign variants on CMA testing.

Recommended changes to medical surveillance for cancer or other conditions resulted from CMA test results in up to 50% of cases. CMA may identify an increased risk of cancer susceptibility among the population of children with ID, DD, MCA, or ASD in three ways: 1) increased cancer susceptibility that is a known feature of the syndrome that also causes the phenotype for which they were tested (i.e., Beckwith-Wiedemann or Rubinstein-Taybi syndrome), CNVs that involve a cancer susceptibility gene as well as other genes that are likely to cause the phenotype, and 3) CNVs that are unlikely to have produced the observed clinical phenotype that involve a cancer susceptibility gene (secondary finding). CMA may also find that a presumed diagnosis that indicates an increased risk of cancer is incorrect, negating the need for frequent cancer screening.
### Table 7. Summary of Findings for the Impact of Chromosomal Microarray Testing on Clinical Management

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Study Population; Sample Size</th>
<th>Outcome Definition</th>
<th>Result1</th>
<th>Risk of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coulter (2011)39</td>
<td>Retrospective clinic-based cohort of all children with CMA Total tested: 1,792 Total with pathogenic or VUS: 235 Eligible for follow-up study: 194</td>
<td>At least one management change (surveillance start/stopped, referral, diagnostic testing) due to pathogenic CNV.</td>
<td>65 (53.7% of follow-up study; 3.6% of all tested)</td>
<td>Cannot determine</td>
</tr>
<tr>
<td>Ellison (2012)45</td>
<td>Retrospective laboratory-based cohort. Total tested: 46,298 Clinically actionable CNV: 1,996</td>
<td>Patients with clinically actionable CNV (known microdeletion or duplication syndrome, increased cancer susceptibility, deleted genes associated with genetic disease requiring follow-up).</td>
<td>1,996 (4.3%)</td>
<td>High</td>
</tr>
<tr>
<td>Hayeems (2015)46</td>
<td>Retrospective clinic-based cohort of all children with CMA testing followed at tertiary pediatric hospital; N=752</td>
<td>Average number of recommendations (surveillance, referral, diagnostic testing, medication indication/contraindication, family testing) due to pathogenic CNV</td>
<td>Mean 2.35 recommendations per patient</td>
<td>Low</td>
</tr>
<tr>
<td>Henderson (2014)40</td>
<td>Retrospective laboratory-based cohort of all children with CMA testing Tested: N=1,780 Pathogenic CNV: 227 Follow-up available: 187</td>
<td>At least one management change (surveillance, referral, diagnostic testing, medical/surgical procedure, medication indication, contraindication) due to pathogenic CNV</td>
<td>102 (54.5% of follow-up study; 5.7% of total tested)</td>
<td>Low</td>
</tr>
<tr>
<td>Riggs (2014)47</td>
<td>Retrospective case series of syndromes diagnosable by CMA; N=28,526 Pathogenic and likely pathogenic: 4,125</td>
<td>At least one management change (referral, diagnostic testing, surgical/intervention procedures, surveillances, medication, contraindication, lifestyle changes) due to pathogenic CNV</td>
<td>1,908 (46.3% of pathogenic; 6.7% of all tested)</td>
<td>High</td>
</tr>
<tr>
<td>Saam (2008)48</td>
<td>Retrospective case series of patients with abnormal CNV; N=48</td>
<td>At least one management change (referral, screening, stop screening) due to pathogenic CNV</td>
<td>13 (27.1%)</td>
<td>Cannot determine</td>
</tr>
<tr>
<td>Tao (2014)69</td>
<td>Retrospective case series of children with ID/DD, ASD, or MCA; N=327</td>
<td>At least one management change (surveillance, referral, diagnostic testing, medical/surgical procedure, medication indication, contraindication, lifestyle recommendation) due to pathogenic CNV</td>
<td>28 (75.7%)</td>
<td>Low risk of bias</td>
</tr>
</tbody>
</table>

Abbreviations: CMA=chromosomal microarray; CNV=copy number variant; DD=developmental disability; ID=intellectual disability; MCA=multiple congenital anomalies; VUS=variant of undetermined significance

1 Confidence intervals were not reported by study authors unless specified.
Except for Saam et al., the studies reviewed here limited their consideration of the impact of CMA testing to short-term clinical management of the proband. Saam et al. considered two additional applications of CMA testing results to management: increased access to services and more accurate estimation of recurrence risk. CMA diagnosis provided easier access to services for 25% of the cases and more accurate estimation of recurrence risk for family counseling in 35% of the cases.

**Summary and Strength of Evidence: Efficacy for Impact of Testing on Clinical Management**

The proportion of cases with a pathogenic variant that had a change in management prompted by CMA testing ranged from 27.1% to 93.8%. However, the body of evidence comprised exclusively of observational study designs also had serious concerns in all four domains, and we graded the strength of the evidence as very low (Table 8). As discussed above, four of seven studies were rated as having an unclear or high risk of bias due to unreported study information or potential recall bias. Only three studies measured impact directly by documented management changes in the medical record. The estimates of impact were inconsistent.

**Table 8. Strength of Evidence for Findings Related to the Impact of Testing with Chromosomal Microarray on Clinical Management (EQ3)**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of Studies; Subjects; Study Design</th>
<th>Risk of Bias</th>
<th>Inconsistency</th>
<th>Indirect-ness</th>
<th>Precision</th>
<th>Other Considerations</th>
<th>Strength of Evidence Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of patients with abnormal results that have a change in management range 27.1 to 93.8%</td>
<td>7; 658; Observational</td>
<td>Serious&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Serious&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Serious&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Serious&lt;sup&gt;d&lt;/sup&gt;</td>
<td>None</td>
<td>Very low</td>
</tr>
</tbody>
</table>

<sup>a</sup> Potential for recall bias when changes in management collected by physician interview, lack of detail in determining clinical actionability by retrospective review, potential conflict of interest due to goal of promoting reimbursement for CMA.

<sup>b</sup> Wide range of findings among studies.

<sup>c</sup> Three studies measured actionability based on published guidelines and recommendations, not actual changes in management for tested patients.

<sup>d</sup> None of the studies provided confidence intervals or other measures of precision. Sample sizes were small to moderate.

**EQ4. Do children with congenital defects, autism, intellectual disability, or developmental disability tested with CMA or WES have better health outcomes?**

We did not identify any studies that reported on health outcomes among children tested with CMA or WES, either as single-arm studies or compared to patients not tested or tested with other platforms.
Cost and Cost-Effectiveness
CQ1. What is the cost and cost-effectiveness of genetic diagnostic testing for these conditions with CMA or WES?

We identified five eligible studies reporting cost, cost per patient, cost per diagnosis, or cost per additional diagnosis.50-54 All identified studies were specific to CMA testing; no studies evaluated WES testing or reported cost per quality-adjusted or disability-adjusted life year. Study characteristics and findings are summarized in Table 9. Appendix C, Tables C-1 and C-5A, provide individual study characteristics and findings respectively. Appendix E, Tables E-1 and E-2, provide individual study risk of bias assessments.

Study Characteristics
The populations included across the five studies varied; two focused exclusively on children with ID53,54 one focused exclusively on children with DD,51 and two included a mixed population of children with ID, DD, or both.50,52 Studies evaluated the use of CMA as a first-line test for diagnostic evaluation, in comparison to karyotype as a first-line test in three studies,52-54 and in comparison to a variety of metabolic, genetic, imaging, or tissue biopsy testing in two studies.50,51 All studies reported cost-related outcomes from a payer perspective, and studies were highly varied as to what type of costs were included, and the currency year for which outcomes were reported. Because of this heterogeneity, we did not quantitatively synthesize findings. The authors conducted these studies from 2005 to 2009. Three studies used a retrospective cohort design with sample sizes ranging from 46 to 1590,50-52 while two studies used decision analysis with a hypothetical cohort of participants.53,54 Three studies were conducted in the United Kingdom50,52,54 and two were conducted in Canada.51,53 We rated all included studies for the cost outcomes as having a low risk of bias.

Findings
Costs per array varied across studies and by testing platforms; these costs ranged from $271 to $1,575 (in 2010 US dollars). These costs reflect the cost per array, which was only one of several costs used to estimate overall costs of CMA testing compared to no CMA testing. The wide variation in costs per array can largely be attributed to the use of different testing platforms, in different years and the use of commercial laboratories (higher cost per array) compared with hospital-based laboratories (lower cost per array).

The cost per patient and cost per diagnosis estimated with CMA testing compared to no CMA testing is summarized in Table 9 and is organized by phenotypes evaluated. In this table, we converted and reported all outcomes in 2010 US dollars for comparison across studies. Appendix C, Table C-5B, provides this same data in the currency and year reported by study authors. Studies reported cost per patient tested, cost per diagnosis rendered, and cost per additional diagnosis rendered, which is the outcome that measures the incremental cost for the additional diagnoses rendered by CMA testing compared to no CMA testing. A negative value for this outcome suggests that first-line CMA testing saves money compared to no CMA testing. A positive value for this outcome suggests that testing does not save money, but could be cost-effective depending on the amount a decisionmaker is willing to pay for a higher diagnostic yield.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>No. of Studies (No. of Participants)</th>
<th>Cost Per Patient or Diagnosis (95% CI)</th>
<th>Difference in Cost (95% CI)</th>
<th>Cost per additional diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CMA Testing</td>
<td>No CMA Testing</td>
<td></td>
</tr>
<tr>
<td>Intellectual Disability</td>
<td>2 (NA\textsuperscript{a})</td>
<td>$2,919\textsuperscript{b} (2,671 to 3,188)</td>
<td>$2,707 (2,448 to 2,990)</td>
<td>$213 (168 to 256)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$6,269\textsuperscript{c} (NR)</td>
<td>Range $4,280 to $9,966\textsuperscript{d}</td>
<td>Range -3,697 to $1,988\textsuperscript{d}</td>
</tr>
<tr>
<td>Developmental Delay</td>
<td>1 (114)\textsuperscript{\textit{y}1}</td>
<td>Cost per patient</td>
<td>NR</td>
<td>-$101 (98% CI, -$186 to -$168\textsuperscript{f})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cost per diagnosis</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range $4,381 to $7,757\textsuperscript{50}</td>
<td>NR\textsuperscript{50}</td>
<td>$4,381 (NR)\textsuperscript{50}</td>
</tr>
</tbody>
</table>

Abbreviations: CMA=chromosomal microarray; CI=confidence interval; NA=not applicable; NR=not reported.

\textsuperscript{a} Both studies were conducted using decision analyses using hypothetical cohorts; thus, the sample size is not applicable.

\textsuperscript{b} Assumes that CMA testing increases diagnostic yield from 19.2% to 27.5%.

\textsuperscript{c} Assumes that CMA testing increases diagnostic yield from 8% to 18%, cost per diagnosis is 2440 with a 15% absolute increase in diagnostic yield.

\textsuperscript{d} Depending on which kinds of follow-up testing after karyotype used.

\textsuperscript{e} Calculated based on data provided in the study.

\textsuperscript{f} When using local hospital laboratory for testing.

\textsuperscript{g} When using commercial laboratory for testing.
Cost Per Additional Diagnosis: Intellectual Disability. Regier et al. and Wordsworth et al. studied children with ID using decision analyses conducted with hypothetical cohorts.53,54 The cost per diagnosis was higher in both arms of one study54 compared with the other study.53 However, the study with lower costs per diagnosis estimated a higher cost per additional diagnosis ($2,592 [95% CI $1,586 to $5,188]) compared with the other study (range estimated to be between $-370 to $199 per additional diagnosis). These studies did not use similar cost inputs and assumed different diagnostic yield estimates, both of which explain the variation in estimates.

Cost Per Additional Diagnosis: DD. Trakadis et al., studied cost outcomes among children with DD.51 In this retrospective cohort of 114 children, eight additional diagnoses were made as a result of CMA testing compared with no CMA testing. This study estimated that the difference in cost per patient tested (CMA tested versus not CMA tested) was -$101 (98% CI, -$186 to -$16) when array testing was performed by a local hospital laboratory and $402 (98% CI, $227 to $577) when testing was performed by a commercial laboratory. This translated to a cost per additional diagnosis of $1,317 (local hospital laboratory) to $12,296 (commercial laboratory).

Cost Per Additional Diagnosis: Mixed Populations. Finally, Newman et al. and Sagoo et al. studied cost outcomes among children with ID, DD, or both. Although the costs per patient tested were much higher in both arms in one study50 compared with the other study,52 the difference in cost per patient (CMA tested versus not tested) between studies was similar (-$687 and -$344, respectively). Newman et al. reported a cost per additional diagnosis of $4,381 while Sagoo et al. reported a cost per additional diagnosis of $-88,819. The difference in findings between these two studies can largely be explained by a large difference in cost per diagnosis favoring CMA testing in the Sagoo et al. study since the absolute increase in diagnostic yield in Sagoo et al. was minimal (11.05% in non-CMA tested arm, 11.44% in CMA tested arm).

Summary and Strength of Evidence: Cost and Cost Effectiveness

The cost per additional diagnosis across this body of evidence ranged from $-88,819 to $12,296 (in 2010 US dollars). Table 10 summarizes the strength for evidence about the cost-effectiveness of diagnostic evaluations that use first-line CMA testing compared with those that do not use first-line CMA testing. Although this body of evidence did not have any serious concerns for risk of bias, we identified serious concerns related to inconsistency and imprecision. Further, we rated the indirectness the outcome reported (costs per additional diagnosis) as having very serious concerns given that this measure is a surrogate outcome and not a direct reflection of patient health outcomes. Because of these concerns, we graded the certainty of this estimate as very low.
The strength of the evidence for all included research questions was very low (safety, impact on clinical management, and costs) or low (diagnostic yield). We identified no eligible studies addressing the impact of chromosomal microarray (CMA) or whole exome sequencing (WES) testing on patient health outcomes. Key findings include:

- **Safety**: The only safety concern that we identified based on one included study is discrimination because of the test results. The body of evidence was not sufficient to determine the frequency with which these issues may arise in CMA testing compared to other types of genetic tests. We graded the strength of the evidence related to safety as very low. We identified no studies that reported safety outcomes related to WES testing.

- **Diagnostic yield**: In studies that conducted testing in the U.S. in 2009 or later, CMA testing identified pathogenic or likely pathogenic variants in 8.8% (95% CI, 8.4% to 9.3%) of children tested for any reason, and 5.4% (95% CI, 4.8% to 6.0%) of children referred for an autism spectrum disorder (ASD). A previous health technology assessment (HTA) by Grant et al. that included US and international studies found that among studies published in 2012 or later, the diagnostic yield averaged 19% for global developmental disability (GDD) with or without intellectual disability (ID) and 12% for ASD. This HTA also reported that diagnostic yield increased 1% per year on average between 2004 and 2015. We graded the strength of the evidence for diagnostic yield of CMA testing as low. One primary research study of WES reported a diagnostic yield of 27% (95% CI, NR) and we graded the strength of evidence on diagnostic yield of WES testing as very low.
• Impact on clinical management: CMA results prompted changes in clinical management in 27% to 94% of patients with a pathogenic variant, which was 3.6% to 6.7% of all patients tested. Considering only studies that measured management changes by medical record review, CMA results changed clinical management in 54% to 76% of patients. We graded the strength of this evidence as very low. We identified no studies reported on change in management related to WES testing.

• Costs: The cost per additional diagnosis across this body of evidence ranged from $-88,819 to $12,296 (in 2010 US dollars). No studies reported on cost per quality-adjusted or disability-adjusted life year. We graded the strength of evidence on costs as very low.

Additional Context for Interpreting Findings
Our review revealed some aspects of CMA and WES testing that, though outside the scope of our systematic review, may add to the interpretation of our results.

Diagnostic Yield of CMA in General Population
CMA testing among biobank samples drawn from the general population of Estonia and tied to a database with phenotype information found 56 (0.7%) of 7,877 enrollees had a DECIPHER-listed pathogenic variant.71 The study did not include a clinical evaluation, but 70% of the 56 individuals with a pathogenic variant reported clinical features consistent with their genetic findings.

False Negatives
As reported by D’Amours et al.,65 some array software may return false positive results by incorrectly calling a copy number variant (CNV) that does not exist, or falsely identifying an inherited variant as de novo. None of the studies included in this review reported the number of false negative results, which is the failure to identify a chromosomal abnormality identified by karyotype or fluorescent in situ hybridization (FISH). Bi et al.7 reported that CMA failed to detect an abnormality in 0.24% of all cases tested by CMA and karyotype. CMA missed 6 of 43 cases of mosaicism and 29 of 30 balanced rearrangements; thus, testing with CMA may not identify low-level mosaicism or most balanced translocations.

False positives
D’Amours et al.65 compared CMA results for 21 children with DD or ID across four high-resolution SNP arrays and the moderate-resolution oligo-array in use for clinical diagnosis at the time of the study. All four SNP arrays identified pathogenic abnormalities in six (28.6%) patients; the oligo-array only identified three (14.3%) of the six abnormalities. Three of the four SNP platforms identified a total of 17 false copy number variants (CNVs). The false positive rate among these arrays ranged from 0% to 5.8%. The false positives resulted from false calls by the platform software or incorrect assignment as de novo because the software did not detect the parental CNV. The authors concluded that high-resolution SNP arrays increase diagnostic yield, but that different platforms vary significantly regarding false positive CNV identification and breakpoint accuracy.
Incidental Findings
Ethical issues concerning secondary findings from genetic sequencing have been widely discussed, resulting in American College of Medical Genetics (ACMG) guidelines on reporting clinically actionable findings. Bowling et al. found 55 (9%) pathogenic or likely pathogenic genetic variants unrelated to DD or ID among 605 tested parents of affected children. Nine variants (1.5%) were related to a disorder that the parent self-reported, 12 (2.0%) were one of 56 genes ACMG identified as having potentially clinically actionable variants, and 28 parents were carriers for an autosomal recessive disorder. One couple both carried an allele for the same autosomal recessive disorder, giving them a 25% risk of having a child with the disorder.

Impact on Management and Outcomes
Although we did not identify any eligible studies that evaluated the impact of CMA and WES testing on health outcomes, Saam et al. reported that CMA testing increased access to services, such as public health insurance, among children with a pathogenic variant. Children referred for CMA testing have complex medical and developmental conditions. As reported in our results section, Hayeems et al. found 80% of children with a reportable variant and 62% of children with a benign variant received medical recommendations after their CMA testing. Improved access to insurance or to early invention services may improve long-term health or social outcomes for children with DD, ID, or ASD.

Limitations of the Evidence Base
The body of evidence on safety, impact on management changes, and cost was limited in size, risk of bias, and applicability, limiting our ability to draw strong conclusions for these research questions. Further, almost all studies we included focused on CMA. Clinical use of WES is still new, and the body of evidence regarding its impact is limited.

Most of the studies reporting on diagnostic yield included some cases for indications other than our population of interest. In addition, prior diagnostic testing received by the cases varied. Diagnostic yield may differ among more homogenous case series. This factors may explain some of the difference between our summary estimate of 9% and the median diagnostic yield of 19% among studies published since 2012 that was observed by Grant et al. Differences in methods of calling pathogenic variants or access to family history or parent samples may contribute to the differences in diagnostic yield.

Studies evaluating the impact of testing on management were small, so each included only a small portion of known microduplication or microdeletion syndromes. The clinical features of these syndromes and the appropriate management actions vary accordingly, and are likely an explanation for the large heterogeneity of estimates on impact on management we observed across studies. Further, the estimated proportion of cases whose management is impacted by their CMA results may not apply across the population of children for whom CMA is ordered because most included studies were limited to patients followed by a single institution. The two studies that included a broader population of patients measured outcomes by physician survey or interview, which could be subject to recall bias, reporting bias, and response bias.

The body of evidence related to cost and cost-effectiveness is limited by the lack of studies conducted in the U.S. and the absence of a societal perspective in any of the analyses. Given large differences in the access to health care and its financing between the U.S. and non-U.S.
countries, it is not clear whether the findings observed in the studies we identified apply to U.S. settings. Further, this body of evidence is limited by extreme clinical and methodological heterogeneity, which most likely explains the inconsistency in costs per additional diagnosis that we observed. Because CMA and WES tests have rapidly evolved over the past decade, the genetic assay costs used in the included studies may no longer be accurate. Further, the precise role of these tests in the overall sequence and approach to diagnostic evaluation in children with DD, ID, and ASD has also evolved: the cost of the diagnostic journey with or without CMA testing reflected in the included studies may no longer be relevant to current clinical practice.

Across the body of evidence for all research questions, study design, study population, and outcome measurement details were often sparse, resulting in our inability to assess the risk of bias for some studies. One aspect of evaluating the risk of bias not addressed in existing instruments we used are the financial or intellectual conflicts of interest of the study authors. Authors of several included studies stated that a goal of the research was to provide evidence of clinical utility to get CMA covered by payors, potentially providing a strong incentive for analytic decisions that would increase the estimate of diagnostic yield or impact on management.

**Limitations of this HTA**

We did not include studies published in languages other than English and only searched two US-based electronic databases. These were pragmatic decisions but may have resulted in missing relevant studies; however, we conducted extensive hand searches of the reference lists of included studies and believe the possibility of missing a study that would have altered the findings to be low. For pragmatic reasons, we used a single reviewer to screen most titles/abstracts, which may have led to studies inappropriately excluded. However, we had an excellent concordance initial set of 20 independently dual-reviewed set of titles/abstracts; the principal investigator checked all studies excluded for ineligible intervention and randomly checked a subset of other excluded studies to minimize this possibility.

For the research question related to diagnostic yield (EQ1), we restricted eligibility to U.S. studies that conducted CMA testing in 2009 or later that used current testing platforms to reduce heterogeneity and provide results more applicable to what is in current clinical use. We did not assess analytic validity or reproducibility and did not conduct an in-depth analysis or synthesis of the cases, breakpoints, or other information related to CNV findings that were presented by study authors.

In addition, our review was limited to the use of WES to detect chromosomal abnormalities. Although WES can detect chromosomal abnormalities, it is primarily used to detect pathogenic mutations or small insertions or deletions within single genes after chromosomal abnormalities have been ruled out by CMA.

**Ongoing Research and Future Research Needs**

We found no registered studies of research on this topic in clinical trials.gov registry. We identify the following future research needs:

- We need randomized clinical trials or well-designed observational studies to evaluate comparative strategies for using CMA or WES as part of the diagnostic evaluation of children with DD, ID, ASD, or MCA. Clinical genetic testing will likely continue to
incorporate WES and whole genome sequencing as replacements for single gene sequencing and mutation panels.

- Karyotype is still the first-line test for the prenatal diagnosis of chromosomal abnormalities, but CMA is increasingly being emphasized as the first-tier test for prenatal diagnosis. In our search for this HTA, we identified many studies focused on the use in prenatal testing, and a systematic review focused on prenatal use of would synthesize the efficacy, safety, and costs when these tests are used in the prenatal context.

Conclusion

Chromosomal microarray identifies a pathogenic or likely pathogenic variant in nearly 9% of all children referred for testing and in 5% of those referred because of autism spectrum disorders; these findings are based on a low strength of evidence. The results of chromosomal microarray tests generate changes in management in over half of children who are identified as having a pathogenic or likely pathogenic variant; this finding is based on very low strength of evidence. The evidence is very limited with respect to the safety of testing and we identified no evidence related to the impact of testing on health outcomes. The cost per additional diagnosis for chromosomal microarray testing as a first-line diagnostic test varies between $-88,819 and $12,296 (in 2010 US dollars); this finding is based on very low strength of evidence.
References


Appendix A. Search Strategy

PubMed searched from 1/1/2000-9/18/2017

**Condition being Diagnosed (#1)**


Yield: 174,578

**Laboratory Testing Methods (Genetic Microarray or Whole Exome Sequencing) (#2)**


Yield: 131,811

**Clinical Population (Children with Intellectual Disability, Autism, or Birth Defects) OR Non-Clinical Population (#3)**


Genetic microarray and whole exome sequencing: Draft evidence report
("general"[Title/Abstract] OR "unselected"[Title/Abstract] OR "normal"[Title/Abstract] OR "disease-free"[Title/Abstract])) Filters: Publication date from 2000/01/01; English

Yield: 311,528

**Combining #1, #2, #3**

#4 (#1 AND #2 AND #3) Filters: Publication date from 2000/01/01; English 4318

#5 #4 NOT ("Animals"[Mesh] NOT "Humans"[Mesh]) OR "Comment"[Publication Type] OR "Editorial"[Publication Type] OR "Case Reports"[Publication Type]) Filters: Publication date from 2000/01/01; English

Total Yield: 2,264

**ClinicalTrials.Gov Search from inception to 10/11/2017**

Terms: Microarray, limit Child

Total Yield: 101

Terms: Whole Exome, limit Child

Total Yield: 4

**Other Data**

The following websites were searched using the terms genetic or chromosomal microarray, whole exome, WES, and CMA to identify information relevant to this health technology assessment.

US Food and Drug Administration
Centers for Medicare and Medicaid Services
Aetna
UnitedHealth
Humana
BlueCross BlueShield (Premera and Regence)
Kaiser Permanente
National Institute for Health and Care Excellence (UK)
US Agency for Healthcare Research and Quality
American Academy of Pediatrics
American Academy of Neurology
American College of Medical Genetics and Genomics
Appendix B. Additional Methods

The following exchanges rates were used to convert foreign costs reported to US dollars:

<table>
<thead>
<tr>
<th>Year</th>
<th>US $</th>
<th>British Pound</th>
<th>Canadian$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>1</td>
<td>0.605</td>
<td>-</td>
</tr>
<tr>
<td>2006</td>
<td>1</td>
<td>0.534</td>
<td>-</td>
</tr>
<tr>
<td>2007</td>
<td>1</td>
<td>-</td>
<td>1.069</td>
</tr>
<tr>
<td>2010</td>
<td>1</td>
<td>-</td>
<td>1.047</td>
</tr>
<tr>
<td>2013</td>
<td>1</td>
<td>0.66</td>
<td>-</td>
</tr>
</tbody>
</table>


The following chain-weighted, average year consumer price indices were used to adjust all reported costs to 2010 dollars.

<table>
<thead>
<tr>
<th>Year</th>
<th>Annual Average CPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>113.7</td>
</tr>
<tr>
<td>2006</td>
<td>117.0</td>
</tr>
<tr>
<td>2007</td>
<td>119.957</td>
</tr>
<tr>
<td>2010</td>
<td>125.615</td>
</tr>
<tr>
<td>2013</td>
<td>133.592</td>
</tr>
</tbody>
</table>

### Appendix C. Evidence Tables

#### Table C-1. Characteristics of Included Studies

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Key Questions</th>
<th>Population, Age Group</th>
<th>Study Design</th>
<th>Genetic Test Type</th>
<th>Variant Classification:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowling (2017)</td>
<td>SQ1, EQ1, EQ2</td>
<td>Intellectual Disabilities, Children North Alabama Children’s Specialists, Huntsville, AL, USA</td>
<td>Cross-sectional Clinic-based, family recruitment</td>
<td>WGS/WES</td>
<td>Benign: Allele frequency higher than observed frequency of disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NR</td>
<td>INC: Clinical relationship with co-investigator, mild to severe ID, ≥ 2 years old, weight ≥ 19.8 lbs Exc: Abnormal CMA 371</td>
<td>SNP</td>
<td>Pathologic: Loss-of-function where known disease mechanism; Missense mutation known or computationally predicted to mechanism of disease; de novo and predicted to be damaging or LOF in dominant disease gene; Recessive or compound heterozygous in gene with known recessive genetic disease, at frequencies low enough to plausible for disease, predicted to be damaging VUS: Variant is de novo and computationally predicted to be damaging; is very rare, predicted to be damaging, and exist in compound heterozygous or recessive states; impacts a gene with a specific, plausible biological connection to disease; impacts a gene predicted to be intolerant of variation; conflicting evidence.</td>
</tr>
<tr>
<td>Coulter (2011)</td>
<td>EQ3</td>
<td>Mixed, Mixed Children's hospital USA 7/1/2009 - 7/1/2010 2009-2010</td>
<td>Retrospective cohort No sampling. 2 missing, no clear reason for exclusion. Inc: CMA result of pathogenic or possible pathogenic CNV during study period; Exc: Down Sx known or suspected (8); VUS with missing parental studies (31/104); Total tested: 1792 Total with pathogenic or VUS: 235</td>
<td>Microarray</td>
<td>Benign: Previously reported in unaffected individuals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NR</td>
<td>NR - testing done prior to study</td>
<td>NR</td>
<td>Pathologic: Known microduplication or deletion sx; deletion of genes known to cause disease when haploinsufficient; large duplications or deletions Possible pathogenic: CNV that overlap known sx, contain genes suspected of causing disease, possible unmasking of autosomal recessive</td>
</tr>
<tr>
<td>Author (Year)</td>
<td>Study Design</td>
<td>Genetic Test Type</td>
<td>Variant Classification:</td>
<td></td>
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<tr>
<td>Ellison (2012)45</td>
<td>Retrospective case series, in silico review</td>
<td>Microarray</td>
<td>Benign: NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EQ3</td>
<td></td>
<td></td>
<td>Pathologic: Associated with 1) established microdeletion or duplication syndrome with features that require specific follow-up, 2) increased cancer susceptibility, 3) phenotypes with obvious medical follow-up caused by CNV in dosage sensitive genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamilton (2015)38</td>
<td>Cross-sectional</td>
<td>Microarray</td>
<td>NR</td>
<td></td>
<td></td>
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<tr>
<td>SQ1</td>
<td></td>
<td></td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hayeems (2015)46</td>
<td>Retrospective cohort</td>
<td>Microarray</td>
<td>All: Based on laboratory report. If not specified in report, assigned based on ACMG guidelines and verified by expert review.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EQ3</td>
<td></td>
<td>Agilent</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>75 kb</td>
<td>Blood</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Henderson (2014)46</td>
<td>Retrospective, pre-post</td>
<td>Microarray</td>
<td>Benign: Does not involve disease genes, reported in healthy populations, seen in &gt; 1% of parents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EQ3, EQ1</td>
<td>All eligible</td>
<td>Other</td>
<td>Pathologic: Known syndromes, encompassed or interrupted disease associated gene, included numerous genes and not found in healthy individuals. &gt; 10 Mb region of homozygosity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Benign/VUS variant, No follow-up available</td>
<td>Illumina Omni1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMA tested, 1780 Pathogenic variant, 227 Clinical follow-up available, 187</td>
<td>NR</td>
<td>Blood</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total in analysis: 194, 121 pathogenic CNV and 73 with VUS

VUS: CNV not in literature or available databases and does not include genes known to be related to disease.
<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Population, Age Group Other Clinical Characteristics Setting</th>
<th>Study Design Sample Selection Inclusion / Exclusion Criteria Sample Size</th>
<th>Genetic Test Type Platform Resolution Specimen Type</th>
<th>Variant Classification: Benign; Pathologic; Unknown Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ho (2016)41</td>
<td>Mixed, Mixed Clinical testing laboratory, USA 7/2012-9/2016 4.2 Years</td>
<td>Cross-sectional Clinic-based Consecutive series of patients with neurodevelopmental disorders 10351</td>
<td>Microarray Affymetrix Ultra-high resolution Affymetrix CytoscanHD® platform plus 88,435 custom probes yielding 2.8 million probes Other</td>
<td>Benign: In databases of benign variants (DGV) Pathologic: CNVs of &lt;1% population frequency and at least two independent reports that haploinsufficiency or triplosensitivity of the region or gene(s) is causative of clinical features VUS: Preliminary evidence for a causative role or areas of absence of heterozygosity (AOH) that may increase risk of autosomal recessive or imprinting conditions.</td>
</tr>
<tr>
<td>Newman (2007)50</td>
<td>Mixed, Children Regional genetics clinical service. United Kingdom NR 2005</td>
<td>Cohort (retrospective) Clinic-based INC: phenotype with undiagnosed ID/DD with or without dysmorphic features with normal karyotype EXC: None noted 46</td>
<td>Microarray Bacterial artificial chromosomes (BAC) 1-Mb Blood</td>
<td>NR</td>
</tr>
<tr>
<td>Riggs (2014)47</td>
<td>Mixed, Mixed In silico. USA In ISCA database March 2012 NA</td>
<td>Cross-sectional, survey NSGC or ACMG membership INC: Pathogenic, clinically actionable phenotypes Evidence in database: 4125 CNVs, 28,526 cases</td>
<td>Microarray NR NR-Test results retrieved from database NR</td>
<td>On ISCA array (384 genes), Well-described sx (GeneReviews, DECIPHER, ISCA) (153 genes, 235 phenotypes), diagnosable by CMA (186)</td>
</tr>
<tr>
<td>Roberts (2014)42</td>
<td>Mixed, Mixed Clinical genetics clinic, USA 2009-2012</td>
<td>Cross-sectional Clinic-based</td>
<td>Microarray Oligonucleotide</td>
<td>Benign: NR Pathologic: Previously reported as associated with ASD or learning disability.</td>
</tr>
<tr>
<td>Author (Year)</td>
<td>Key Questions</td>
<td>Population, Age Group</td>
<td>Other Clinical Characteristics</td>
<td>Setting</td>
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</tr>
<tr>
<td>**Sagoo (2015)**52</td>
<td><strong>Cost</strong></td>
<td>ID/DD, Mixed</td>
<td>Clinical genetics service; United Kingdom</td>
<td>2006-2013</td>
</tr>
<tr>
<td>**Sagoo (2015)**52</td>
<td><strong>Cost</strong></td>
<td>ID/DD, Mixed</td>
<td>Clinical genetics service; United Kingdom</td>
<td>2006-2013</td>
</tr>
<tr>
<td>Author (Year)</td>
<td>Key Questions</td>
<td>Population, Age Group</td>
<td>Setting</td>
<td>Time Period of Study</td>
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<td>----------------------</td>
</tr>
<tr>
<td><strong>Stobbe (2014)</strong>&lt;sup&gt;43&lt;/sup&gt; EQ1, EQ2</td>
<td>Autism, Adults Autism Genetics Clinic, USA 7/1/09-4/30/12 2009-2012</td>
<td>Retrospective cohort Clinic-based EXC: Patient tested with fragile X. 36 aCGH: 25</td>
<td>Microarray Oligonucleotide 135k nucleotide probes (NimbleGen CGX-3 v1.0; Roche NimbleGen, Madison, WI) Blood</td>
<td>Likely Benign: No genes in the interval, reported in databases of variants in general population but not common. Likely pathogenic: Prior case report with well-defined breakpoints &amp; phenotype, or gene in CNV compelling &amp; specific for the phenotype. VUS: Genes in CNV, but dose sensitivity unknown, or multiple contradictory publications or databases (DGV, db VAR, ISCA, DECIPHER, OMIN, UCSC Genome Bioinformatics, European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations, and literature search.</td>
</tr>
<tr>
<td><strong>Tao (2014)</strong>&lt;sup&gt;49&lt;/sup&gt; EQ3</td>
<td>Mixed, Children Genetics laboratory. Hong Kong 1/2011-5/2013 2011-2013</td>
<td>Retrospective, pre-post Clinic-based EXC: Clinically recognized syndrome, patients who had prenatal CMA testing or parents who chose not to receive the test results 327</td>
<td>Microarray Oligo-SNP (Nimblegen CGX-135K) Probe spacing: 140 kb Blood</td>
<td>Classification by ACMG practice guidelines. Used Signature Genomics database, Tsan Yuk Hospital internal database, DGV, ISCA, DECIPHER, OMIN.</td>
</tr>
<tr>
<td><strong>Trakadis (2011)</strong>&lt;sup&gt;51&lt;/sup&gt; Cost</td>
<td>DD, Children Academic pediatric neurology practice; Canada 2006-2009 2006-2009</td>
<td>Cohort (retrospective) Clinic-based INC: Final diagnosis of DD, age &lt; 6.5 years, had complete history and physical exam, and laboratory testing completed as part of diagnostic assessment</td>
<td>Microarray Bacterial artificial chromosomes (BAC) and oligonucleotides (for N=6 subjects) NR for BAC, 105K for oligonucleotide Blood</td>
<td>NR</td>
</tr>
</tbody>
</table>

Genetic microarray and whole exome sequencing: Draft evidence report
<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Key Questions</th>
<th>Population, Age Group</th>
<th>Study Design</th>
<th>Genetic Test Type</th>
<th>Variant Classification:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wordsworth (2011)**</td>
<td>ID, Children</td>
<td>Clinical genetics laboratories; United Kingdom</td>
<td>Decision Analysis</td>
<td>Microarray</td>
<td>Benign; Pathologic; Unknown Significance</td>
</tr>
<tr>
<td></td>
<td>Cost</td>
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<td>Agilent</td>
<td>NA</td>
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</tbody>
</table>

ACMG=American College of Medical Genetics, CNV=copy number variant, CGX=DD=developmental delay, DECIPHER=Database of genomic variation and Phenotype in Humans using Ensembl Resources, EXC=Excluded, ID=intellectual disabilities, GCAD=Genoglyphix Chromosome Aberration Database, INC=Included, ISCA=International Standards for Cytogenomic Arrays, NA=not applicable, NR=not reported, NSGC=National Society of Genetic Counselors, OMIN=Online Mendelian Inheritance in Man, SNP=single nucleotide polymorphism, Sx=syndrome, VUS=variant of unknown significance, UCSC=University of California at Santa Cruz, WES=whole exome sequencing, WGS=whole genome sequencing.
Table C-2. Individual Study Findings Related to Safety of Chromosomal Microarray or Whole Exome Sequencing Testing (SQ1)

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Subgroup</th>
<th>CMA/WES Tested</th>
<th>Safety Issue</th>
<th>Total</th>
<th>Experienced issue N (%)</th>
<th>Characteristics of affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamilton (2015)\textsuperscript{38}</td>
<td>Withdrawn adoption application due to chromosomal abnormality: 1</td>
<td>Total cohort: 6 (16.7%)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

CMA=chromosomal microarray, CNV=copy number variant, SNP=single nucleotide polymorphism, SQ=safety question, WES=whole exome sequencing

Table C-3. Individual Study Findings Related to Safety of Chromosomal Microarray or Whole Exome Sequencing Testing (EQ1)

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Analytic Method</th>
<th>Subgroup</th>
<th>Sample Size</th>
<th>Diagnostic Result N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowling (2017)\textsuperscript{44}</td>
<td>Descriptive: Count, percents</td>
<td>ID/DD, Probands</td>
<td>371</td>
<td>Pathogenic: 100 (27%)</td>
</tr>
<tr>
<td>Bowling (2017)\textsuperscript{44}</td>
<td>Descriptive: Count, percents</td>
<td>Probands, CNV only</td>
<td>371</td>
<td>Pathogenic: 42 (11.3%)</td>
</tr>
<tr>
<td>Bowling (2017)\textsuperscript{44}</td>
<td>Descriptive: Count, percents</td>
<td>Families with no affected relatives</td>
<td>93</td>
<td>Pathogenic: 35 (37.6%)</td>
</tr>
<tr>
<td>Bowling (2017)\textsuperscript{44}</td>
<td>Descriptive: Count, percents</td>
<td>No affected 1st degree relatives ≥1 2nd/3rd degree affected</td>
<td>85</td>
<td>Pathogenic: 22 (26.0%)</td>
</tr>
<tr>
<td>Bowling (2017)\textsuperscript{44}</td>
<td>Descriptive: Count, percents</td>
<td>1st affected first degree relative)</td>
<td>123</td>
<td>Pathogenic: 24 (20%)</td>
</tr>
<tr>
<td>Coulter (2011)\textsuperscript{39}</td>
<td>Descriptive: Count, percents</td>
<td>All</td>
<td>1792</td>
<td>Pathogenic: 131 (7.3%)</td>
</tr>
<tr>
<td>Henderson (2014)\textsuperscript{46}</td>
<td>Counts, percentages</td>
<td>All</td>
<td>1780</td>
<td>Pathogenic: 227 (12.7%)</td>
</tr>
<tr>
<td>Ho (2016)\textsuperscript{41}</td>
<td>Descriptive: Count, percents</td>
<td>All</td>
<td>10351</td>
<td>Pathogenic: 890 (8.6%)</td>
</tr>
<tr>
<td>Ho (2016)\textsuperscript{41}</td>
<td>Descriptive: Count, percents</td>
<td>Non-ASD</td>
<td>4657</td>
<td>Pathogenic: 583 (12.5%)</td>
</tr>
<tr>
<td>Ho (2016)\textsuperscript{41}</td>
<td>Descriptive: Count, percents</td>
<td>Any ASD</td>
<td>5694</td>
<td>Pathogenic: 307 (5.4%)</td>
</tr>
<tr>
<td>Author (Year)</td>
<td>Analytic Method</td>
<td>Subgroup</td>
<td>Sample Size</td>
<td>Diagnostic Result</td>
</tr>
<tr>
<td>---------------</td>
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</tr>
<tr>
<td><strong>Ho (2016)</strong>&lt;sup&gt;41&lt;/sup&gt;</td>
<td>Descriptive: Count, percents</td>
<td>ASD+other indication</td>
<td>2844</td>
<td>Pathogenic: 184 (6.5%)&lt;br&gt;Benign: 2108 (74.1%)&lt;br&gt;VUS: 552 (19.4%)</td>
</tr>
<tr>
<td><strong>Ho (2016)</strong>&lt;sup&gt;41&lt;/sup&gt;</td>
<td>Descriptive: Count, percents</td>
<td>ASD only</td>
<td>2850</td>
<td>Pathogenic: 125 (4.4%)&lt;br&gt;Benign: 2195 (77%)&lt;br&gt;VUS: 529 (18.5%)</td>
</tr>
<tr>
<td><strong>Roberts (2014)</strong>&lt;sup&gt;42&lt;/sup&gt;</td>
<td>Descriptive (# of CNVs): Count, percents</td>
<td>All</td>
<td>215</td>
<td>Pathogenic: 32 (14.9%)&lt;br&gt;Benign: 170 (79%)&lt;br&gt;VUS: 17 (8%)</td>
</tr>
<tr>
<td><strong>Roberts (2014)</strong>&lt;sup&gt;42&lt;/sup&gt;</td>
<td>Descriptive (# of CNVs): Count, percents</td>
<td>ASD</td>
<td>65</td>
<td>Pathogenic: 6 (9.2%)&lt;br&gt;Benign: 52 (80%)&lt;br&gt;VUS: 8 (64%)</td>
</tr>
<tr>
<td><strong>Roberts (2014)</strong>&lt;sup&gt;42&lt;/sup&gt;</td>
<td>Descriptive (# of CNVs): Count, percents</td>
<td>ID/DD</td>
<td>150</td>
<td>Pathogenic: 26 (17.3%)&lt;br&gt;Benign: 118 (79%)&lt;br&gt;VUS: 9 (6%)</td>
</tr>
<tr>
<td><strong>Stobbe (2014)</strong>&lt;sup&gt;43&lt;/sup&gt;</td>
<td>Count, percent, two-tailed P value, Fisher's exact test</td>
<td>All</td>
<td>25</td>
<td>Pathogenic: 3 (12%)&lt;br&gt;Benign: NR&lt;br&gt;VUS: NR</td>
</tr>
<tr>
<td><strong>Stobbe (2014)</strong>&lt;sup&gt;43&lt;/sup&gt;</td>
<td>Count, percent, two-tailed P value, Fisher's exact test</td>
<td>Confirmed ASD</td>
<td>23</td>
<td>Pathogenic: 2 (8.7%)&lt;br&gt;Benign: NR&lt;br&gt;VUS: 9 (39%)</td>
</tr>
</tbody>
</table>

ASD=autism spectrum disorders; CNV=copy number variant, DD=developmental delay, ID=intellectual disabilities, NR=Not reported, VUS=Variant of unknown significance.
<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Analytic Method</th>
<th>Method of Adjustment Variations</th>
<th>Management Changes</th>
<th>GA/WES Tested</th>
<th>Not GA/WES Tested</th>
<th>Effect of GA/WES Tested Unadjusted: RR (95% CI), p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coulter (2011)</strong>&lt;sup&gt;39&lt;/sup&gt;</td>
<td>Descriptive</td>
<td></td>
<td>INC: Recommendations made because of CMA results: specialist referral, imaging study, diagnostic test, medication prescription. EXC: standard CMA follow-up</td>
<td>Pathologic CNV: Total: 121 Any recommendation: 65 (54%) Referral: 67 (60%) Diagnostic testing (imaging and laboratory): 45 (37%) Stop diagnostic odyssey/avoid other diagnostic testing: 110 (90%) Possible pathogenic variant: Total: 73 Any recommendation: 25 (34%) Referral: 11 (29%) Diagnostic testing: 27 (38%) Stop diagnostic odyssey/avoid other diagnostic testing: 61 (84%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Ellison (2012)</strong>&lt;sup&gt;45&lt;/sup&gt;</td>
<td>Descriptive</td>
<td></td>
<td>Actionable disorders diagnosed by CMA: Microdeletion or microduplication syndromes with organ or endocrine abnormalities that require specific follow-up; Conditions associated with increased cancer susceptibility; Duplications or deletions of dosage sensitive genes that result in genetic disease requiring follow-up.</td>
<td>Total cases tested by CMA: 46298 Any actionable disorder: 1996 (4.3%) Syndromes that require clinical action: 1733 (3.7%) Increased cancer screening: 189 (0.4%) Genetic disease management due to deletion of dosage sensitive genes: 74 (0.16%) Physician-reported actions Total cases: 81 At least 1 appropriate action taken: 76 (94%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Hayeems (2015)</strong>&lt;sup&gt;46&lt;/sup&gt;</td>
<td>Descriptive</td>
<td></td>
<td>Chart review showed any of the following prompted by CMA results: 1) recurrent surveillance, 2) specialist referral 3) imaging</td>
<td>Definitely pathologic: 114 Mean recommendations per patient: All recommendation: 2.35 Specialist referrals: 1.20</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Author (Year)</td>
<td>Method of Adjustment</td>
<td>Stratification / Regression Variables</td>
<td>Management Changes</td>
<td></td>
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<tr>
<td>Henderson (2014)</td>
<td>Descriptive</td>
<td>Impact on management any of the following actions in clinical notes that reference or deemed to be a direct consequence of the CMA results: Direct clinical action (pharmacologic treatment or contraindications, cancer-related screening, avoidance of cancer screening), specialist referrals, diagnostic (imaging or laboratory) tests.</td>
<td>Total, any indication: 187 Any impact on clinical management: 102 (54.5%) Any direct clinical action: 24 (12.8%) Specialist referral: 84 (44.9%) Imaging: 38 (20.3%) Laboratory test: 29 (15.5%) Pharmacologic treatment: 6 (3.2%) Cancer screening recommended: 11 (5.9%) Cancer screening avoided: 3 (1.6%) Contraindication: 3 (1.6%) Total, neurodevelopmental indication: 38 Any impact on clinical management: 16 (42.1%) Any direct clinical action: 3 (7.9%) Specialist referral: 12 (31.6%) Imaging: 6 (15.8%) Laboratory test: 2 (5.3%) Pharmacologic treatment: 0</td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GA/WES Tested</th>
<th>Treatment Type</th>
<th>Total Received Treatment: N (%, SD)</th>
<th>Not GA/WES Tested</th>
<th>Treatment Type</th>
<th>Total Received Treatment: N (%, SD)</th>
<th>Effect of GA/WES Unadjusted: RR (95% CI), p-value Adjusted: RR (95% CI), p-value</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

Tests: 4) laboratory tests 5) surveillance protocols 6) family member investigations.

- Definitely or likely pathologic: 186
- Mean recommendations per patient:
  - All recommendation: 2.25
  - Specialist referrals: 1.02
  - Medical imaging: 0.52
  - Laboratory tests: 0.17
  - Surveillance/screening: 0.39

Mean recommendations per patient for children with reportable variants: 2.25

Children with reportable variants: 79.6%

Children with benign variants: 62.4%

Children with reportable variants:
- 79.6% received treatment
- 20.4% did not receive treatment

Children with benign variants:
- 62.4% received treatment
- 37.6% did not receive treatment
<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Analytic Method</th>
<th>Method of Adjustment</th>
<th>Management Changes</th>
<th>GA/WES Tested</th>
<th>Treatment Type</th>
<th>Not GA/WES Tested</th>
<th>Treatment Type</th>
<th>Effect of GA/WES Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riggs (2014)</td>
<td>Descriptive</td>
<td>INC: Clinically actionable phenotype if at least one of following recommended in guidelines (Level 1) or literature (Level 2): specialist referral, diagnostic testing (includes imaging), surgical/interventional procedures, medication or lifestyle changes.</td>
<td>Pathogenic and likely pathogenic CNVs: Total: 4125 Any Level 1 or 2 recommendation: 1908 (46%)</td>
<td></td>
<td>Cancer screening recommended: 3 (7.9%) Cancer screening avoided: 0 Contraindication: 0</td>
<td></td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Saam (2008)</td>
<td>Descriptive</td>
<td>Physician-reported recommendations made because of clinically significant CMA results. INC: Specialist referral, recommendation for medical screening, stop previously recommended screening, family testing, improved access to services.</td>
<td>Total: 48 Any change in management: 34 (70.8%) At least one recommendation: 13 (27%) Referral: 7 (14.6%) Medical screening: 8 (16.7%) Stop medical screening: 1 (2.1%) Stop diagnostic odyssey, avoid additional testing: 20 (41.7%) Improved access to services: 12 (25.0%) Counseling on recurrence risk: 17 (35.4%)</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td></td>
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</tr>
<tr>
<td>Tao (2014)</td>
<td>Descriptive</td>
<td>Management change: Any of the following when prompted by CMA results: 1) recurrent surveillance, 2) specialist referral 3) diagnostic intervention, 4) medical surgical procedure, 5) pharmacologic, 6) lifestyle and other recommendations</td>
<td>Total with pathogenic variant: 37 At least 1 clinical action: 28 (75.7%) Surveillance/screening: 19 (51.4%) Specialist referral: 24 (64.9%) Diagnostic tests: 25 (67.6%) Medical/surgical procedure: 7 (18.9%) Pharmacologic treatment: 15 (40.5%) Lifestyle: 12 (32.4%)</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author (Year) Key Questions</td>
<td>Analytic Method Method of Adjustment Stratification / Regression Variables</td>
<td>Management Changes</td>
<td>GA/WES Tested Treatment Type Total Received Treatment: N (%, SD) Other</td>
<td>Not GA/WES Tested Treatment Type Total Received Treatment: N (%, SD) Other</td>
<td>Effect of GA/WES Unadjusted: RR (95% CI), p-value Adjusted: RR (95% CI), p-value</td>
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<tr>
<td>Level 1 evidence: professional association guidelines, Level 2 peer-reviewed literature: Level 3: peer-review literature/clinical judgement, Level 4: Managed symptomatically</td>
<td>Level 1 evidence: 9 (24.3%) Level 2 evidence: 10 (27.0%) Level 3 evidence: 8 (21.6%) Level 4 evidence: 1 (2.7%)</td>
<td>Total with VUS: 40 At least 1 clinical action: 1 (2.5%)</td>
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</tr>
</tbody>
</table>

CMA=chromosomal microarray; CNV=copy number variant, EXC=excluded, INC=included, ISCA=International Standards for Cytogenomic Arrays, SD=standard deviation, VUS=variant of unknown significance.
### Table C-5A. Individual Study Findings Related to Costs and Cost-effectiveness of Testing with Chromosomal Microarray or Whole Exome Sequencing Testing (CQ1)

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Key Questions</th>
<th>Analytic Method</th>
<th>Currency, Year</th>
<th>Participants</th>
<th>Cost per Patient</th>
<th>Cost per Diagnosis</th>
<th>Cost per Diagnosis 95% CI</th>
<th>Services Included</th>
<th>Additional Cost per Patient 95% CI</th>
<th>Cost per Additional Diagnosis 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Newman (2007)</strong>&lt;sup&gt;30&lt;/sup&gt;</td>
<td>2-tailed student's t-test</td>
<td>British Pounds (£), 2005</td>
<td>Participants: 36</td>
<td>Cost per patient: £1389</td>
<td>Services included: Varied by patient but includes CMA plus a variety of metabolic tests (e.g., amino acids, thyroid, mucopolysaccharide, and others), fragile X testing, FISH-specific probes, subtelomeric probes, MRI, skeletal surveys, EEG, cranial computerized tomogram, 15q methylation, homocysteine, specific syndromic testing (e.g., UBE3A (Angelman's syndrome, and others), myotonic dystrophy, chromosome breakage studies.</td>
<td>Services included: Varied by patient but includes aCGH, metabolic tests, methylation studies, skin and muscle biopsies, molecular genotyping/sequencing for specific syndromes, CT, MRI, bone age, EEG, EMG.</td>
<td>Cost per patient: £1765</td>
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<tr>
<td><strong>Trakadis (2011)</strong>&lt;sup&gt;31&lt;/sup&gt;</td>
<td>2-tailed student's t-test</td>
<td>Canadian Dollars ($), 2010</td>
<td>Participants: 33</td>
<td>Cost per patient: NR</td>
<td>Services included: Varied by patient but includes aCGH, metabolic tests, methylation studies, skin and muscle biopsies, molecular genotyping/sequencing for specific syndromes, CT, MRI, bone age, EEG, EMG.</td>
<td>Services included: Karyotype as first line test, with aCGH as second-line test if no variation detected, plus any other testing</td>
<td>Cost per patient: NR</td>
<td></td>
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</tr>
<tr>
<td><strong>Sagoo (2015)</strong>&lt;sup&gt;32&lt;/sup&gt;</td>
<td>NR</td>
<td>British Pounds (£), 2013</td>
<td>Participants: 848</td>
<td>Cost per patient: £291.05</td>
<td>Services included: aCGH as first line test, plus any other testing or consultation conducted to establish a diagnosis.</td>
<td>Services included: Karyotype as first line test, with aCGH as second-line test if no variation detected, plus any other testing</td>
<td>Cost per patient: £532.61</td>
<td></td>
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</tr>
<tr>
<td>Study (Year)</td>
<td>Study Type</td>
<td>Country/Currency</td>
<td>Participants</td>
<td>Cost per Patient</td>
<td>Cost per Diagnosis</td>
<td>Cost per Additional Diagnosis</td>
<td>Services Included</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Regier (2010)</td>
<td>Decision analysis</td>
<td>Canada</td>
<td>NA (Model)</td>
<td>Varies by results of testing</td>
<td>£3118 (95% CI, £2980 to £3254)</td>
<td>£217 (95% CI, £172 to £261)</td>
<td>Karyotype as first line test, followed by targeted FISH and/or karyotyping (unless trisomy suspected in which case karyotyping was first and aCGH only used if karyotype did not establish a diagnosis).</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Wordsworth (2011)</td>
<td>Decision analysis</td>
<td>UK</td>
<td>NA (Model)</td>
<td>Varies by results of testing and need for follow-up testing.</td>
<td>£4957 with follow-up multi-telomere FISH for normal karyotype or £2129 with follow-up multi-telomere MLPA for normal karyotype</td>
<td>-£183.90 to £98.80 depending on which follow-up testing used.</td>
<td>Karyotype as first line test, with karyotype of parents if results are of unknown clinical relevance. If karyotype normal, additional FISH or multi-telomere FISH or MLPA. Assumes diagnostic yield of 8%. Does not include follow-up testing in patients with normal karyotype and normal FISH/MLPA that would likely need additional testing to rule out genetic imbalances as diagnostic etiology.</td>
<td></td>
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</tbody>
</table>

aCGH = array comparative genomic hybridization, CMA = chromosomal microarray, CT = computerized tomography, EEG = electroencephalogram, EMG = electromyography, FISH = fluorescent in situ hybridization, MLPA = multiplex ligation-dependent probe amplification, MRI = magnetic resonance imaging, NR = not reported, WES = whole exome sequencing.
## Table C-5B. Summary of findings from five studies evaluating cost or cost-effectiveness of genetic microarray testing, data provided in currency units and years reported by studies

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>No. of Studies (No. of Participants)</th>
<th>Cost Per Patient or Diagnosis (95% CI)</th>
<th>Difference in Cost (95% CI)</th>
<th>Incremental Cost-Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Outcome (currency, year)</strong></td>
<td><strong>GA Testing</strong></td>
<td><strong>No CMA Testing</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Cost Per Patient or Diagnosis (95% CI)</strong></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td><strong>Difference in Cost (95% CI)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Incremental Cost-Effectiveness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intellectual Disability</td>
<td>2 (NA&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>Cost per diagnosis (CD, 2007)&lt;sup&gt;53&lt;/sup&gt;</td>
<td>2980&lt;sup&gt;b&lt;/sup&gt; (2727 to 3254)</td>
<td>2763 (2499 to 3052)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cost per diagnosis&lt;sup&gt;b&lt;/sup&gt; (GBP, 2006)&lt;sup&gt;54&lt;/sup&gt;</td>
<td>3118&lt;sup&gt;c&lt;/sup&gt; (NR)</td>
<td>Range 2129 to 4957&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Developmental Delay</td>
<td>1 (114)&lt;sup&gt;51&lt;/sup&gt;</td>
<td>Cost per patient (CD, 2010)</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cost per diagnosis (CD, NR 2010)</td>
<td>NR</td>
<td>NR</td>
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<td></td>
<td>Cost per patient (CD, 2010)</td>
<td>NR</td>
<td>NR</td>
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<td></td>
<td>Cost per diagnosis (CD, 2010)</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cost per patient (GBP, 2005)&lt;sup&gt;50&lt;/sup&gt;</td>
<td>1389 (NR)&lt;sup&gt;50&lt;/sup&gt;</td>
<td>1765 (NR)&lt;sup&gt;50&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cost per diagnosis (GBP, 2005)</td>
<td>291.05 (Range 190 to 1258)&lt;sup&gt;52&lt;/sup&gt;</td>
<td>532.61 (Range 390 to 1424)&lt;sup&gt;52&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intellectual disability or developmental delay or both</td>
<td>2(1636)</td>
<td>Cost per diagnosis (GBP, 2005)&lt;sup&gt;50&lt;/sup&gt;</td>
<td>Range 2399 to 4248&lt;sup&gt;(NR)&lt;sup&gt;50&lt;/sup&gt;</td>
<td>NR&lt;sup&gt;50&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cost per diagnosis (GBP, 2005)&lt;sup&gt;50&lt;/sup&gt;</td>
<td>2544.42 (NR)&lt;sup&gt;52&lt;/sup&gt;</td>
<td>4819.44 (NR)&lt;sup&gt;52&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviations: CD= Canadian Dollars; CI= confidence interval; GBP=British Pound; NA= not applicable; NR=not reported

<sup>a</sup> Both studies were conducted using decision analyses using hypothetical cohorts.

<sup>b</sup> Assumes that CMA testing increases diagnostic yield from 19.2% to 27.5%

<sup>c</sup> Assumes that CMA testing increases diagnostic yield from 8% to 18%, cost per diagnosis is 2440 with a 15% absolute increase in diagnostic yield.

<sup>d</sup> Depending on which kinds of follow-up testing after karyotype used.

<sup>e</sup> Calculated based on data provided in the study.

<sup>f</sup> When using local hospital lab for testing.

<sup>g</sup> When using commercial lab for testing.
Appendix D. Excluded Studies

List of Exclusion Codes
X1: Ineligible publication type
X2: Ineligible population
X3: Ineligible or no intervention
X4: Ineligible comparator
X5: Ineligible or no outcome
X6: EQ1 Study Published before 2010
X7: No Key Question
X8: Old Platform
X9: Ineligible setting
X10: EQ1 Ineligible country
X11: Duplicates/superseded by more recent publications


Genetic microarray and whole exome sequencing: Draft evidence report


Celestino-Soper PB, Shaw CA, Sanders SJ, et al. Use of array CGH to detect exonic copy number variations throughout the genome in autism families detects a novel deletion in


Ness GO, Lybaek H, Houge G. Usefulness of high-resolution comparative genomic hybridization (CGH) for detecting and characterizing constitutional chromosome


Warburton D, Ronemus M, Kline J, et al. The contribution of de novo and rare inherited copy number changes to congenital heart disease in an unselected sample of children with


## Appendix E. Individual Study Risk of Bias Assessments

### Table E-1A. Risk of Bias Assessment: Sample Selection and Description of Test

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Overall Risk of Bias Rating</th>
<th>Was a consecutive or random sample of patients enrolled?</th>
<th>Were inclusion/exclusion criteria appropriate?</th>
<th>Comments on Sample Selection</th>
<th>What is the level of detail the authors used to describe the test?</th>
<th>Comments on Test Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowling (2017)44</td>
<td>Low</td>
<td>Cannot determine</td>
<td>Yes</td>
<td>None</td>
<td>High, clear, all details provided</td>
<td>None</td>
</tr>
<tr>
<td>Coulter (2011)39</td>
<td>Cannot determine</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
<td>Medium, somewhat clear, most details provided</td>
<td>Resolution not reported</td>
</tr>
<tr>
<td>Ellison (2012)45</td>
<td>High</td>
<td>Yes</td>
<td>No</td>
<td>Sample includes all patients tested with CMA and does not include/exclude patients based on phenotype. Thus, the sample tested cannot be characterized based on phenotype.</td>
<td>High, clear, all details provided</td>
<td>None</td>
</tr>
<tr>
<td>Hamilton (2015)28</td>
<td>High</td>
<td>No</td>
<td>No</td>
<td>High potential for selection bias given the manner in which sample recruited.</td>
<td>Low, unclear, many details missing</td>
<td>None</td>
</tr>
<tr>
<td>Hayeems (2015)46</td>
<td>Low</td>
<td>Yes</td>
<td>Cannot determine</td>
<td>Unclear whether the exclusion of children not followed by the same tertiary pediatric hospital where genetic labs is located would result in selection bias, as presumably 'sicker' kids are followed at the tertiary center versus kids followed in the community.</td>
<td>High, clear, all details provided</td>
<td>None</td>
</tr>
<tr>
<td>Henderson (2014)40</td>
<td>Low</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
<td>High, clear, all details provided</td>
<td>Only missing genome build</td>
</tr>
<tr>
<td>Ho (2016)41</td>
<td>Low</td>
<td>Cannot determine</td>
<td>Yes</td>
<td>None</td>
<td>High, clear, most details provided</td>
<td>Uses same platform as BioMed Research Intl 77 paper excluded because of overlapping study population.</td>
</tr>
<tr>
<td>Author (Year)</td>
<td>Overall Risk of Bias Rating</td>
<td>Was a consecutive or random sample of patients enrolled?</td>
<td>Were inclusion/exclusion criteria appropriate?</td>
<td>Comments on Sample Selection</td>
<td>What is the level of detail the authors used to describe the test?</td>
<td>Comments on Test Description</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------------------</td>
<td>------------------------------------------------------</td>
<td>---------------------------------------------</td>
<td>-----------------------------</td>
<td>---------------------------------------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Newman (2007)
\(^{50}\) | Low                        | Yes                                                  | Yes                                        | None                        | Medium, somewhat clear, most details provided               | Article references a separate article for details.            |
| Regier (2010)
\(^{53}\)  | Low                        | NA                                                   | Yes                                        | Decision analysis using a hypothetical cohort | Low, unclear, many details missing                          | None                                                          |
| Riggs (2014)
\(^{47}\)  | High                       | NA                                                   | Yes                                        | No patients. Used CMA diagnosable phenotypes and cases in a database. | NA. No actual testing. Include only well described symptoms in study |
| Roberts (2014)
\(^{42}\) | Low                        | Yes                                                  | Yes                                        | None                        | Medium, somewhat clear, most details provided               | None                                                          |
| Saam (2008)
\(^{46}\)  | Cannot determine           | Yes                                                  | Yes                                        | Consecutive sample for EQ1 and EQ2, non-consecutive sample for EQ3 as study only included patients with positive CMA results. | Medium, somewhat clear, most details provided               | None                                                          |
| Sagoo (2015)
\(^{52}\) | Low                        | Yes                                                  | Yes                                        | None                        | Low, unclear, many details missing                          | None                                                          |
| Stobbe (2014)
\(^{43}\) | Low                        | Yes                                                  | Yes                                        | None                        | Medium, somewhat clear, most details provided               | Missing resolution                                            |
| Tao (2014)
\(^{69}\)  | Low                        | Yes                                                  | Yes                                        | None                        | High, clear, all details provided                           | None                                                          |
| Trakadis (2011)
\(^{51}\) | Low                        | Yes                                                  | Yes                                        | None                        | Medium, somewhat clear, most details provided               | None                                                          |
| Wordsworth (2011)
\(^{54}\) | Low                        | NA                                                   | Yes                                        | Decision analysis using a hypothetical cohort | Low, unclear, many details missing                          | None                                                          |

CMA=chromosomal microarray, EQ=efficacy question
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>7. Are the variant classification methods valid?</th>
<th>Comments on Variant Classification</th>
<th>8. Is the selection of the comparison group appropriate after considering feasibility and ethical considerations?</th>
<th>9. Does the analysis control for baseline differences between groups?</th>
<th>10. Are the measures and statistical methods used to assess outcomes appropriate?</th>
<th>11. Are the results believable taking study limitations into consideration?</th>
<th>Comments on Selection, Confounding, Measurement, or Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riggs (2014)</td>
<td>47</td>
<td>Yes</td>
<td>Likely conservative</td>
<td>NA-single arm(before/after)</td>
<td>NA-single arm(before/after)</td>
<td>Yes</td>
<td>Yes</td>
<td>Cases with well-described symptoms may be less likely to be entered in database once they are well described. May underestimate the proportion with clinical impact.</td>
</tr>
<tr>
<td>Roberts (2014)</td>
<td>42</td>
<td>Yes</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Saam (2008)</td>
<td>46</td>
<td>Cannot determine</td>
<td>Defined as alterations believed or suspected to be of clinical significance, but no additional details are provided.</td>
<td>NA-single arm(before/after)</td>
<td>NA-single arm(before/after)</td>
<td>Partially</td>
<td>Yes</td>
<td>Measurement of changes in management based on physician survey/recall.</td>
</tr>
<tr>
<td>Sagoo (2015)</td>
<td>52</td>
<td>Cannot determine</td>
<td>None</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>Stobbe (2014)</td>
<td>43</td>
<td>Yes</td>
<td>ACMG guidelines</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tao (2014)</td>
<td>49</td>
<td>Yes</td>
<td>ACMG guidelines</td>
<td>NA-single arm(before/after)</td>
<td>NA-single arm(before/after)</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>Trakadis (2011)</td>
<td>51</td>
<td>No</td>
<td>None</td>
<td>NA-single arm(before/after)</td>
<td>NA-single arm(before/after)</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>Wordsworth (2011)</td>
<td>54</td>
<td>Yes</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA=not applicable.
### Table E-2. Risk of Bias Assessment Items Specific to Cost Studies

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>12. Was the perspective (societal vs. payer) stated?</th>
<th>13. Does the analysis clearly identify the costs used and how they were valued?</th>
<th>14. Were all relevant costs included for the perspective used?</th>
<th>15. Was the analysis appropriate (i.e., appropriate discount rate, sensitivity analyses conducted?)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newman (2007)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Regier (2010)</td>
<td>Yes</td>
<td>Yes</td>
<td>Partially</td>
<td>Yes</td>
</tr>
<tr>
<td>Sagoo (2015)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Trakadis (2011)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Wordsworth (2011)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### Table E-3A. Risk of Bias Assessment for Included Systematic Review: Study Eligibility Criteria

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>1.1 Pre-defined objectives and eligibility criteria?</th>
<th>1.2 Criteria appropriate for the review question?</th>
<th>1.3 Eligibility criteria unambiguous?</th>
<th>1.4. All restrictions in criteria based on study characteristics appropriate?</th>
<th>1.5 Restrictions based on sources of information appropriate?</th>
<th>Concerns regarding study eligibility criteria.</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grant (2015)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Low</td>
<td>None</td>
</tr>
</tbody>
</table>

### Table E-3B. Risk of Bias Assessment for Included Systematic Review: Identification and Selection of Studies

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>2.1 Appropriate range of databases/electronic sources used?</th>
<th>2.2 Were methods in addition to database searching used to identify relevant reports?</th>
<th>2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?</th>
<th>2.4 Were restrictions based on date, publication format, or language appropriate?</th>
<th>2.5 Were efforts made to minimize error in selection of studies?</th>
<th>Concerns regarding methods used to select studies.</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grant (2015)</td>
<td>Yes</td>
<td>No</td>
<td>Probably yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Low</td>
<td>None</td>
</tr>
</tbody>
</table>
Table E-3C. Risk of Bias Assessment for Included Systematic Review: Data Collection and Study Appraisal

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>3.1 Were efforts made to minimize errors in data collection?</th>
<th>3.2 Were sufficient study characteristics available for both review authors and readers to be able to interpret results?</th>
<th>3.3 Were all relevant study results collected for use in the synthesis?</th>
<th>3.4 Was risk of bias formally assessed using appropriate criteria?</th>
<th>3.5 Were efforts made to minimize error in risk of bias assessment?</th>
<th>Concerns regarding methods used to select studies.</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grant (2015) 28</td>
<td>No information</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No information</td>
<td>Unclear</td>
<td>No information provided about attempts to assess individual study risk of bias.</td>
</tr>
</tbody>
</table>

Table E-3D. Risk of Bias Assessment for Included Systematic Review: Synthesis and Findings

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>4.1 Did the synthesis include all studies that it should?</th>
<th>4.2 Were all pre-defined analyses reported or departures explained?</th>
<th>4.3 Was the synthesis appropriate given the nature and similarity in the research questions, study designs, and outcomes across included studies?</th>
<th>4.4 Was between study variation minimal or addressed in the synthesis?</th>
<th>4.5 Were the findings robust?</th>
<th>4.6 Were biases in primary studies minimal or addressed in the synthesis?</th>
<th>Concerns Regarding Methods Used to Select Studies</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grant (2015) 28</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No information</td>
<td>No information</td>
<td>Low</td>
<td>Given topic, sensitivity analyses probably not needed</td>
</tr>
<tr>
<td>Author (Year)</td>
<td>Concerns Regarding Study Eligibility Criteria</td>
<td>Concerns Regarding Methods Used to Identify or Select Studies</td>
<td>Concerns Regarding Methods Used to Collect Data and Appraise Risk of Bias</td>
<td>Concerns Regarding the Synthesis of Findings</td>
<td>Did Interpretation of Findings Address all of Concerns Identified in Domains 1-4?</td>
<td>Was the Relevance of Identified Studies to the Review’s Research Question Appropriately Considered?</td>
<td>Did the Reviewers Avoid Emphasizing Results on the Basis of Their Statistical Significance?</td>
<td>Overall Risk of Bias in the Review</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------------------------</td>
<td>---------------------------------------------------------------</td>
<td>-------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Grant (2015) 28</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
<td>Low</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Unclear</td>
</tr>
</tbody>
</table>