The Hospital for Sick Children
Technology Assessment at SickKids (TASK)

FULL REPORT

UPDATED

A MICROCOSTING AND COST-CONSEQUENCE ANALYSIS OF GENOMIC TESTING STRATEGIES IN AUTISM SPECTRUM DISORDER

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List of Abbreviations

aCGH	Array-based comparative genomic hybridization
ACMG	American College of Medical Genetics and Genomics
ASD	Autism spectrum disorder
CAD	Canadian dollar
CMA	Chromosomal microarray analysis
CGES	Clinical genome and exome sequencing
CIHI	Canadian Institute for Health Information
CNV	Copy number variant
DD	Developmental delay
DSA	Deterministic sensitivity analysis
FISH	Fluorescence in situ hybridization
GE^3LS	Genomics and its ethical, economic, environmental, legal, and social aspects
HTA	Health technology assessment
ID	Intellectual disability
MCA	Multiple congenital anomaly
MIS	Management Information Systems
MOHLTC	Ontario Ministry of Health and Long Term Care
PSA	Probabilistic sensitivity analysis
qPCR	Real-time polymerase chain reaction
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variant
TCAG	The Centre for Applied Genomics
WES	Whole exome sequencing
WGS	Whole genome sequencing

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Executive Summary

Background

Chromosomal microarray analysis (CMA) is currently the first-tier clinical genetic test for individuals with autism spectrum disorder (ASD). Clinical genome and exome sequencing (CGES) technologies are promising tools for demonstrating genetic causality, due to their higher diagnostic yield compared with CMA for cases presenting with positive phenotypes for autism spectrum disorder. It is not yet clear whether genomic technologies can add value for money invested or how best to translate these technologies from research to clinical care. An economic evaluation of CGES technologies requires a comprehensive and accurate estimation of all costs involved in the sequencing workflow.

Objectives

The primary objective of this study is to estimate costs associated with CMA, whole exome sequencing (WES) and whole genome sequencing (WGS) tests for a targeted patient population consisting of children with ASD from an institutional payer perspective over 5 years. The secondary objective is to compare the incremental costs and diagnostic yields of CMA, WES and WGS in hypothetical clinical testing scenarios in an exploratory cost-consequence analysis.

Methods

Using a bottom-up microcosting approach, the opportunity cost per sample excluding mark-ups, fees and charges for CMA, for WES on the Illumina HiSeq® 2500 platform and for WGS on the Illumina HiSeq® 2500 and HiSeq X™ platforms for patients with ASD were estimated from an institutional payer perspective based on the diagnostic laboratory practices at The Hospital for Sick Children (SickKids), Canada. The cost per sample was determined for each year of a five-year program. Total program costs to service the ASD patient population were also estimated over five years. A probabilistic sensitivity analysis (PSA) was conducted to incorporate parameter uncertainty in the model. Three one-way deterministic sensitivity analyses (DSA) were conducted to examine the effects of changing the inputs for the overhead cost, the total volume of CGES tests in the institution, and the number of primary variants found by CGES tests, while other inputs remained the same. To calculate incremental diagnostic yields for clinical testing scenarios, diagnostic yields were sought from recently published studies reporting diagnostic yields for CMA, WES or WGS in ASD. Due to uncertainty in diagnostic yield

estimates, a one-way sensitivity analysis was conducted where the diagnostic yield for each test was varied for each scenario

Results

The cost per ASD sample in Year 1 was $1655 (95% CI: 1611, 1699) for WES, $2851 (95% CI: 2750, 2956) for WGS on the HiSeq X™ platform and $5519 (95% CI: 5244, 5785) for WGS on the HiSeq® 2500 platform, compared to $744 (95% CI 714, 773) for CMA. Reagent supply costs accounted for the largest proportion of costs for each type of CGES. The total institutional program cost to offer CMA for ASD diagnosis over five years was $1.05 million (95% CI: 1.01, 1.09) compared to $2.31 million (95% CI: 2.25, 2.37) for WES, $7.78 million (95% CI: 7.39, 8.15) for WGS HiSeq® 2500 and $3.98 million (95% CI: 3.84, 4.13) for WGS HiSeq X™ based on 300 ASD cases per year. The ratio of incremental cost to incremental diagnostic yield ranged from $25 459 for CMA+WES vs. CMA to $195 056 for WGS HiSeq® 2500 vs. CMA +WES. There is a substantial variation in the ratio depending on the diagnostic yield. For the CMA+WES vs. CMA scenario, the ratio varied from $10 745 to $71 948. If the WGS diagnostic yield was 42.4%, the cost per additional patient with a positive finding decreased substantially. If WGS replaced CMA, the ratio decreased to $6 367 for HiSeq X™ and $14 428 for HiSeq® 2500. For WGS vs. CMA+WES, the incremental cost per additional patient with positive finding was $1 702 for the HiSeq X™ and $11 733 for HiSeq® 2500.

Conclusions

This study is the first to estimate the cost of clinical exome and genome sequencing using a bottom-up microcosting approach in a clinical paradigm. The WGS using older technology (HiSeq® 2500) was the most expensive test, costing almost three times as much as WES and seven times as much as CMA. The new technology using the HiSeq X™ platform reduced the cost of WGS test by 48%. Labour costs were reduced for HiSeq X™ due to improved automation and streamlining of sample processing. Overall, supplies, followed by equipment and labour, constituted the largest proportion of total cost for all three tests. A cost-consequence analysis revealed a cost of over $25000 per additional patient with a pathologic variant if CMA were to be replaced by CMA+WES or by WGS. Additional research is required to assess the impact of CGES on the pathway of care for children with ASD and to measure ultimate improvements in health outcomes as a result of testing. This study provides comprehensive cost data for use in future economic evaluations of clinical genome and exome sequencing in ASD and allows for a costing model that can be easily adapted to other pediatric patient populations.

1 Introduction

1.1 Background

Clinical genetic testing is routinely offered to patients with developmental delay (DD) or multiple congenital anomalies (MCA) as well as to children with a clinical diagnosis of autism spectrum disorder (ASD) to identity genetic variants known to be associated with specific diagnoses. Genetic tests may also be undertaken for children with mixed co-morbidities for whom a definitive diagnosis has been elusive (1). Genetic testing for ASD in particular has been increasingly integrated into clinical practice because of the need to establish a diagnosis early and refer children for treatment (2). These tests are often done alongside or following traditional detailed clinical diagnostic assessment to provide additional insight into the cause of the disorder and identify recurrence risk in families (3). Chromosomal microarray analysis (CMA) is currently the first-tier clinical genetic test for individuals with suspected ASD (1, 3). CMA, which uses either array-based comparative genomic hybridization (aCGH) or single nucleotide polymorphism (SNP) array technologies, can detect submicroscopic copy number variations (CNVs) across the genome.

While chromosomal microarray has been widely used in genetic testing in ASD, it has failed to identify genetic etiology for the majority of autism cases (4). The CMA diagnostic yield is about 7% to 20% in patients with developmental disorders as a whole and lower for specific conditions such as ASD (1, 5-8). The advancement of massively parallel high-throughput clinical genome and exome sequencing (CGES) technologies has made possible the detection of a broad range of genetic variation. CGES is being used for discovery of candidate genes in DD, intellectual disability (ID) and ASD (9-13) and increasingly in the diagnosis of these conditions. CGES typically refers to both whole exome sequencing (WES) and whole genome sequencing (WGS). Whole exome sequencing targets the protein-coding portion of the genome, which represents about 1% of the genome and can detect single nucleotide variants (SNV), including *de novo* mutations, and some CNVs (14, 15). Whole genome sequencing covers every single base in the genome and can detect small and large *de novo* and inherited variations in coding and noncoding regions of DNA, including CNVs and SNVs (14-16).

To date, studies in both research and clinical settings have focused primarily on WES, as WGS is more resource-intensive and costly (17) and is farther behind WES in translation to clinical practice. The

diagnostic yield of WES across the developmental disorders such DD, ID, ASD and speech delay is in the range of 8% to 33% (18-22). There are fewer studies that report the diagnostic yield of WGS. The most recent WGS diagnostic yield estimates are 42% for ASD (10) and 42% for ID (9) and 34% for congenital malformations and neurodevelopmental delay in a research setting (23). Both WES and WGS can generate findings unrelated to the purpose of the test, commonly called secondary or incidental findings, but that may predict risk for other conditions and have a significant impact on a patient's health (24).

The Ontario Ministry of Health and Long-Term Care (MOHLTC) funds health care services for residents of Ontario, Canada delivered through the Ontario Health Insurance Program. The MOHLTC approved the reimbursement of CMA for Ontario residents with a variety of developmental disorders in 2010. The MOHLTC does not currently reimburse diagnostic laboratories that perform clinical WES, but pays for the test on a case-by-case basis for approved physician requests for clinical WES, typically done through laboratories in the United States (25). The use of WGS has not yet been approved for reimbursement by the Ontario government.

Due to its higher hypothesized diagnostic yield, potential for closer medical management of primary findings, and perceived ability to eliminate the need for multiple genetic tests, the demand for CGES is increasing (26). CGES may be useful in cases where traditional genetic tests are negative or inconclusive (9, 18). While using a sequence of genetic tests, such as CMA followed by CGES, in addition to clinical assessment, may be more effective in reaching a diagnosis, it may also result in significant added costs and a longer time to diagnosis. The potential for secondary findings from CGES may also provide benefits to patients and families but is also expected to contribute to additional medical management and health care system costs.

It is not yet clear whether CGES can add value for money invested and how best to translate these technologies from research to clinical care (27, 28). Health technology assessment (HTA) is concerned with the evaluation of emerging health care technologies including diagnostic tests. Typically referred to as GE^{3}LS (genomics and its ethical, economic, environmental, legal, and social aspects), HTA of genomic sequencing technologies is essential to generate high quality evidence to support policies that are equitable and that maximize health benefits to the population. An economic evaluation is a core part of

HTA and compares the costs and consequences of new technologies to standard care to quantify the additional costs associated with the technology per unit of added benefit thus providing insight into whether these technologies add value for money and are appropriate to adopt (28, 29).

Recent reviews highlight the paucity of economic evaluation studies that compare WES and WGS technologies with standard of care (14, 30, 31). There is a gap in the literature with respect to an accurate measurement of opportunity costs associated with the entire sequencing workflow (14). These costs include initial set-up, acquisition and maintenance costs of the sequencing equipment, bioinformatics analysis and storage, data interpretation and reporting, labour costs for each step of the workflow, and overhead. WGS and to a lesser extent WES, generate a large amount of data that require a substantial storage capacity, as well as bioinformatics capability to identify clinically meaningful variants and personnel resources required to interpret these variants (14, 32). While the laboratory costs of sequencing have decreased dramatically in recent years (33, 34), there is not as yet a reliable and comprehensive estimate of actual test costs. Full economic evaluation of CGES technologies that assess the incremental costs of CGES in terms of benefits to patients require accurate estimations of all costs involved in the workflow.

1.2 Study objectives

The primary objective of this study is to estimate the precise costs associated with CMA, WES and WGS tests using a microcosting approach for a targeted patient population consisting of children with ASD. In the microcosting approach, the volume of use and unit price of each resource use component is estimated (35) and the entire workflow process of a genetic test is tracked. The secondary objective of the study is to compare the incremental costs and diagnostic yields of CMA, WES and WGS in hypothetical clinical testing scenarios for children with ASD in a cost-consequence analysis.

2 Methods

2.1 Study design and clinical translation context

Using a bottom-up microcosting approach, the opportunity cost per sample excluding mark-ups, fees and charges for CMA, WES and WGS tests for patients with ASD were estimated from an institutional payer perspective based on the diagnostic laboratory practices at The Hospital for Sick Children

(SickKids), Toronto, Canada. Costs for all tests were estimated for clinical application. The cost per sample was determined for each year of a five-year program. Total program costs to service an ASD patient population were also estimated over five years.

Both WES and WGS continue to be funded primarily through research grants as basic science discovery research to expand knowledge of causal variants and to strengthen the understanding of genotype-phenotype relationships alongside the early stages of translation into clinical practice. Currently, clinical CMA is performed at the Cytogenetics laboratory, operated by the SickKids Department of Paediatric Laboratory Medicine. Clinical WES was introduced onsite by the Department of Paediatric Laboratory Medicine's Genome Diagnostics laboratory as part of a two-year research project funded by the SickKids Centre for Genetic Medicine. In 2015, the department began offering clinical WES to all medical specialities within the hospital. Whole genome sequencing is performed for SickKids patients as part of a five-year Genome Clinic Research Project, funded by the SickKids Centre for Genetic Medicine. The Genome Clinic was launched in 2013 and has been enrolling approximately 100 children each year in an effort to evaluate the diagnostic utility of WGS. Until recently, WGS has been performed off site by a private provider. Using expert opinion, the WES cost components were modified to approximate WGS testing as if it were performed and fully clinically available at the hospital. In December 2015, SickKids obtained new high capacity sequencers (Illumina HiSeq X™) housed at The Centre for Applied Genomics as part of a multi-institutional genomics research program.

A target population approach focusing on costs encountered as part of the referral and diagnostic pathway for children with ASD was selected. This is in contrast to a centralized clinic approach in which genetic test costing would be undertaken for a heterogeneous group of children with mixed diagnoses and complex etiologies. The target population approach more closely simulates the institutional costs for children with ASD referred for genetic testing as part of the ASD diagnostic pathway.

2.2 Microcost item identification

The major cost categories across all three tests are labour, small and large equipment, supplies and follow-up testing. For WES and WGS, bioinformatics is an additional cost category, reflecting the large computing component of CGES. Bioinformatics is not included in the cost of CMA since it is a negligible cost. A list of major categories and sub-categories for each technology is presented in Table 1. Each of

the sub-categories were further broken down into individual microcost items according to laboratory operating procedures for CMA, WES and WGS and these are described in detail below and in Appendices 1-4. The resource use and unit price data for each input were provided by the laboratory staff, industry or extracted from published or grey literature such as Canadian Institute for Health Information's *Standards for Management Information Systems in Canadian Health Service Organizations* ("MIS Standards")(36). Where possible and appropriate, a range encompassing all plausible values of an input's resource use or unit price was provided in addition to a point estimate. Sample costs for each input were calculated by multiplying resource use by unit price. For labour, time in minutes for each task was multiplied by wage rates. Price data were reported in 2015 Canadian dollars (CAD) and reflected estimates collected in 2013 to 2016. Prices collected prior to 2015 were adjusted for inflation using Statistics Canada's health and personal care consumer price index. Appendices 1-4 list all inputs for each test and include each input's estimated resource use, estimated unit price, range and data sources.

Table 1 Categories of resource use for CMA, WES and WGS tests.

Major Category	**Minor Category**	
	CMA	*WES/WGS*
Labour	Specimen preparation	Specimen preparation
	DNA extraction	Library preparation
	Microarray sample processing	Sequencing
	Analysis	Bioinformatics
	Clinical interpretation	Bioinformatics management & maintenance
	Reporting	Clinical interpretation
		Reporting
Supplies	Sample handling	Sample handling
	Scanner consumables	Preparation kits
		Consumables
		Reagents
Follow-up testing	qPCR/FISH	qPCR /Sanger sequencing
Bioinformatics	Not applicable	Bioinformatics file storage
		Bioinformatics computation use
Small Equipment	Not applicable	Small equipment
Large Equipment	Microarray platform	Sequencing equipment
	Equipment contract	Equipment contract

Abbreviations: CMA, Chromosomal microarray analysis; WES, Whole exome sequencing; WGS, Whole genome sequencing; qPCR, Real-time polymerase chain reaction; FISH, Fluorescence in situ hybridization

2.3 Assumptions

The assumptions of the microcosting model are summarized in Table 2. A time horizon of five years was selected based on the estimated useful lifetime of the small and large equipment. Exceptions were thermocyclers and pipette sets, used in WES and WGS, which are replaced every two and a half years. Future costs were discounted using a discount rate of 3% with the assumption that costs were incurred at the end of the year. Small and large equipment items were depreciated using a straight-line depreciation method. An opportunity cost of 3% was added to the cost of large equipment, such as array or sequencing machines and their maintenance contracts. The opportunity cost refers to the next best use of funds invested in equipment and is approximated by the return on undepreciated value of equipment at each time point (37). Resource use and unit prices were assumed to remain the same from year to year.

Bioinformatics in the WES and WGS models included multiple sub-categories. The labour cost associated with bioinformatics analyst's time to perform sample logistics and data processing was estimated. Storage of sequenced data and computation tasks were also costed. Computation tasks utilized 72 compute nodes housed at SickKids, each with 40 compute cores and 256 GB of RAM. Equipment and labour costs associated with purchasing and maintaining computing nodes were estimated but bioinformatics software costs were not included. Periodic validation, quality control and pipeline updating and testing were not included.

Overhead costs comprise administrative and infrastructure costs. They were added to labour, large and small equipment and bioinformatics costs. A query to MOHLTC's Ontario Case Costing Initiative returned an estimate of overhead costs for major interventions in different hospitals in Ontario. The average Ontario overhead cost in 2010/11 was 23% with a range of 10 to 37%. An internal request to SickKids' Case Costing and Decision Support yielded a SickKids-specific overhead estimate for patient wards of 28 to 30%. Based on these two sources, the reference overhead cost case was assumed to be 23%, with a range of 10 to 30%.

The labour steps for each test were compared to the laboratory labour components in the 2010-11 and 2012-13 MIS Standards. The MIS Standards provide a standardized framework for collecting and reporting financial and statistical data on day-to-day operations of health service organizations (36). The

MIS Standards provide average time required for standard laboratory activities, although time per activity may vary between institutions. The labour resource use, calculated as time in minutes per each step, was obtained from MIS Standards or directly from the SickKids labs.

The price per HiSeq X™ instrument assumed an initial purchase of a minimum of five sequencers. Costs were calculated for sample processing on a single sequencer. The equipment resource use per sample depends on the total number of tests done in the institution for all patients. As overall test volume increases, the equipment resource use and equipment cost per ASD patient decreases. For CMA, the average total number of tests done per year in the institution for all indications was 3948, based on the 2013/14 fiscal year (DJ Stavropoulos pers. comm. 2015). Based on the approximate volume of clinical whole exomes indicated at SickKids, it was further assumed that the annual number of WES and WGS tests for all indications could vary from 500 to 1000 per sequencer and was assumed to be 500 (100% of all tests) in the reference case. Based on the prevalence of ASD (38), it was assumed that 300 genetic tests would be run per year for children with a clinical diagnosis of ASD.

The following cost items were patient population specific: total test volume in the institution, number of primary variants, number of secondary variants, interpretation time for primary and secondary variants and reporting time for primary and secondary variants. It was assumed that all other cost items did not depend on the patient population. Training and start-up costs were not included in the model. These costs are incurred prior to offering the service and can be substantial, depending on the institution. The costs of pre-test and post-counselling, variant discovery research and development, validation testing (not including follow-up testing) and additional bioinformatics analyses for multiple examinations of primary variant findings were also excluded.

Table 2. Microcosting analysis assumptions.

Test	Description
All Tests	• Costs are applied at the end of each year • Volumes of resource use and prices per unit do not change over 5 years • Large equipment's useful lifetime is 5 years • Small equipment's useful lifetime is 5 years except thermocyclers and pipette sets which are replaced every 2.5 years • Large and small equipment cost are amortized over 5 years • 3% opportunity cost is applied to depreciation of large equipment only • 23% overhead cost is assumed, ranging from 10 to 30% • 3% discount rate is applied to all items • 300 tests for patients with ASD are conducted each year at the institution
CMA	• Overhead cost is applied to labour and small and large equipment • Follow-up testing includes qPCR and FISH • 3948 tests are conducted each year
WES/WGS	• Costs associated with special validation or special follow-up testing and additional bioinformatics analyses for multiple examinations of primary variant findings are not included, except where indicated • Library preparation and sequencing time is fixed. Efficiencies from running multiple samples simultaneously can be assigned to the per sample cost estimate • Follow-up testing includes Sanger Sequencing for WES and Sanger Sequencing/qPCR for WGS • Overhead cost is applied to labour, small and large equipment, and bioinformatics • The total capacity in the institution for patients with all indications is a maximum of 1000 cases per year per sequencer • 2 primary variants are found per ASD patient on average, ranging from 0 to 4 • 3-5% of ASD tests find secondary variants • Interpretation time per ASD test variant is 30 minutes • High performance computing cluster maintenance time is 1 hour per year per node • The maximum number of tests are run each time during batch runs (i.e., a slide that can run 3 cases per test is not used to run a single case)

Abbreviations: ASD, autism spectrum disorder; CMA, Chromosomal microarray analysis; WES, Whole exome sequencing; WGS, Whole genome sequencing; qPCR, Real-time polymerase chain reaction; FISH, Fluorescence in situ hybridization

2.4 Microcost item valuation

2.4.1 Chromosomal microarray analysis

2.4.1.1 Labour

The labour inputs in the CMA microcosting model were provided by the Cytogenetics Laboratory director and are listed in Appendix 1. The inputs can be classified into the following categories: specimen preparation, DNA extraction, microarray sample processing, analysis and clinical interpretation and report writing. Most of the CMA labour inputs corresponded to the MIS Standards' inputs. The labour time per sample for each such input was acquired from MIS Standards. The resource use based on the MIS Standards was verified with the information provided by the lab. To validate the estimate, the total time to conduct CMA on one sample obtained from the MIS Standards was compared with the total time measured by the lab director. For labour inputs without the corresponding MIS Standards, resource use was estimated by the lab. Only point estimates of resource use were provided; ranges were not assigned.

Each labour input was linked to a hospital employee. Labour time in minutes for each input was multiplied by salary and benefits per minute to obtain labour cost. Employees include nurses, lab technicians, lab technologists, microarray specialists, resource technologists and cytogeneticists. Salaries of each employee were obtained by either an informal survey of lab staff, reported salaries from SickKids or Government of Ontario Public Sector Salary Disclosure. Benefits of 18% were added to each annual salary based on SickKids policy. Because of the confidential nature of this information, reporting of unit prices for labour items has been suppressed. The ranges for salaries were based on the SickKids salary scale, lab staff survey or expert opinion within the lab. When it was not possible to obtain a salary range from these sources, the salary was assumed to vary by 20% from its point estimate.

2.4.1.2 Equipment

The large equipment cost estimates were based on the microarray platform currently used by the Cytogenetics Laboratory, Affymetrix (Santa Clara, USA) GeneChip® 3000Dx platform. The platform includes two Fluidics stations and one hybridization oven. An additional large equipment item was one-year maintenance service contract, constituting 10% of the platform price (Appendix 1). There were no small equipment items. Two bundles of equipment were needed to process 2000 tests per year. Since the lab processes twice that amount annually on average, the equipment resource use for an ASD

sample was calculated by allocating the proportion of use for all patients with all indications in the institution using the following formula: 2/all tests per year. The platform price was provided by the manufacturer. The manufacturer did not provide a price range. In order to account for price and currency fluctuations, unit price of the platform and unit price of its maintenance contract were assumed to vary by 10% from their point estimates.

2.4.1.3 Supplies

Supplies included cost of shipping of a sample to the lab and cost of scanner consumables (Appendix 1). Scanner consumables included microarray slide and reagents and were treated as a single item. One-time shipping and handling charge and one unit of scanner consumables were required per one sample. Ranges for the resource use of these items were not provided. The unit price of shipping and handling was obtained from FedEx. The unit price of scanner consumables was provided by an Affymetrix representative. Price ranges for both of these items were not available. In order to account for price and currency fluctuations, unit prices of shipping and handling and scanner consumables were assumed to vary by 10% from their point estimates.

2.4.1.4 Follow-up testing

Based on the personal communication with the lab director, the follow-up testing with Fluorescence in situ hybridization (FISH) and real-time polymerase chain reaction (qPCR) for the proband and two parents were assumed to occur in 10% and 5% of cases, respectively. The point estimates of unit price per trio (proband and two parents) for FISH and qPCR were based on internal SickKids molecular genetics costing. Unit prices of FISH and qPCR were assumed to vary by 10% from their point estimates. An exception is the lower bound of the FISH price, which was based on the 2013 British Columbia laboratory reimbursement fee (39).

2.4.2 Whole exome sequencing

2.4.2.1 Labour

The labour inputs in the WES microcosting model were provided by The Centre for Applied Genomics' (TCAG) laboratory manager and are listed in Appendix 2. TCAG is an in-house SickKids core genomics facility that conducts WES and WGS for research purposes. Although TCAG is a research facility, the costs of clinical and research exome and genome testing are comparable. An exception are reagents, the price of which might be slightly different in a clinical application (40).

None of the WES inputs corresponded to the MIS Standards except specimen preparation. For labour inputs not in the MIS Standards, resource use (time in minutes) was estimated by the lab. Labour categories included specimen preparation, library preparation, sequencing, bioinformatics, bioinformatics maintenance, clinical interpretation and report writing. Specimen preparation was assumed to have the same labour steps as in CMA. Total minutes for each task in the library preparation and sequencing categories were fixed, regardless of the number of samples processed per run. The resource use per sample for each task was calculated by dividing the total time per task by the number of samples processed per run. It was assumed that eight samples could be processed.

Labour estimates were specified for the analysis of sequenced data performed at TCAG and maintenance of the high performance computing cluster at the SickKids' Centre for Computational Medicine. For the former component, labour time was based on the TCAG bioinformatics manager's estimates as follows. For the two steps – variant calling and annotation – the total minutes per month required for each of these two tasks were assumed to be fixed. It was further assumed that the instrument's capacity was 75-100 whole exomes per month, with variant calling requiring one FTE unit of labour for variant calling and annotation requiring 0.25 FTE units of labour to process this number of exomes. The resource use per sample for variant calling and annotation was calculated by dividing the labour time by the average instrument's capacity per month, 87.5 exomes (average of 75-100 exomes).

For the bioinformatics maintenance component, five labour steps were defined: alignment, removal of duplicates, recalibration, SNV/indel variant calling and annotation. The calculation time and the number of nodes required for each step in the bioinformatics pipeline were also obtained from the TCAG bioinformatics manager. One hour of labour was assumed to be required to support one node per year (41). The bioinformatics maintenance labour resource use in minutes was estimated by multiplying the calculation time by the time needed to support the required number of nodes. Ranges for labour volume were provided.

Clinical interpretation and report writing depended on the number of primary variants prioritized and found to be clinically relevant to the disease of interest. For ASD this number was set to vary from zero to four variants found with an average of two variants per case (reference case). Clinical interpretation required 30 minutes per variant and report writing required 15 minutes per variant. If no variants were

found, then each task would take 15 minutes each. Based on expert opinion, it was assumed that addressing secondary variants required 20 to 40 minutes with an average of 30 minutes for clinical interpretation and report writing. It was further assumed that secondary variants were found in 4% of cases. Time needed for clinical interpretation and report writing for secondary variants was calculated by multiplying the total time by the proportion of cases that have them.

Hospital and lab employees who are involved in WES testing include nurses, lab technicians, lab technologists, bioinformatics analysts and high performance computing staff. Salaries of each staff member were obtained by either an informal survey of lab staff, reported salaries from SickKids or Government of Ontario Public Sector Salary Disclosure. Benefits of 18% were added to each annual salary based on SickKids policy. Because of the confidential nature of this information, reporting of unit prices for labour items has been suppressed. For most of the inputs, the salary range was based on the SickKids salary scale, lab staff survey or expert opinion within the lab. When it was not possible to obtain a salary range from these sources, salaries was assumed to vary by 20% from their point estimates.

2.4.2.2 Equipment

The large equipment cost estimates were based on the Illumina (San Diego, USA) HiSeq® 2500 sequencing platform with about 84x coverage. The estimates include the cost of the platform, its maintenance contract and Bioanalyzer and TapeStation instruments made by Agilent Technologies Inc. (Santa Clara, USA).The price of the sequencer and its maintenance contract were provided by the TCAG lab manager. The maintenance contract was approximately 10-25% of the cost of the sequencer per year. The price of a Bioanalyzer and TapeStation was provided by the manufacturer and TCAG lab manager. Small equipment consisted of the tube microcentrifuge, plate microcentrifuge, thermomixer, vortex, pipette sets, magnet particle concentrator, and thermocycler. Small equipment prices were estimated by TCAG lab manager. The sample costs for ASD patients for large and small equipment was determined by allocating the proportion of use for all patients with all indications in the institution. Since thermocyclers and pipette sets are replaced every 2.5 years, their resource use was calculated using the following formula: 2/all tests per year for all indications. The price ranges for large equipment and for some of the small equipment were based on the expert opinion of the TCAG lab manager. For items without price ranges, unit prices were assumed to vary by 10% from their point estimates.

2.4.2.3 Supplies

Supplies included costs of shipping of a sample to TCAG laboratory, Agilent SureSelect exome kits, other library preparation consumables and reagents (Appendix 2). The price of sequencing reagents was based on high throughput flow cell sequencing technology of eight samples per lane. Each item was packaged as one unit per sample and priced accordingly. Ranges for the resource use of these items were not provided. The price of shipping and handling was obtained from FedEx. The unit prices of other items, but not the ranges, were provided by the TCAG lab manager. In order to account for price and currency fluctuations, the unit prices of shipping and handling, Agilent SureSelect exome kits, library preparation consumable and reagents were assumed to vary by 10% from their point estimates.

2.4.2.4 Follow-up testing

Sanger sequencing is the only follow-up test routinely used for WES, since small copy number variants (CNV) are not commonly identified with WES and only single nucleotide polymorphisms (SNP) can be detected and validated. Sanger sequencing is done in about 50% of cases (40). One follow-up test is run in the proband and two in parents. In total, three tests are run. The price of the test per sample was obtained from Blons *et al.* (37), where the cost of Sanger sequencing was estimated using microcosting and time-motion methods based on molecular testing in cancer performed in ten French laboratories. The cost estimate included labour, depreciated equipment and consumables and was reported as a range.

2.4.2.5 Bioinformatics

Costs were calculated for bioinformatics data file storage and computational use. The resource use for data file storage depended on file size and length of storage time and was calculated in gigabytes per year. The storage resource use and unit price were provided by the TCAG bioinformatics manager. Ranges were assigned to storage resource use items. To be consistent with price ranges for other micro-items, storage unit prices were assumed to vary by 10% from their point estimates.

The bioinformatics computational use included the five pipeline steps specified above. The resource use (CPU per hour) for each step was calculated by multiplying the number of computing jobs by the number of CPUs (cores) and time (in minutes) required to complete the job. In order to account for additional processing time needed to keep the high performance computing facility operating below full capacity, 25% was added to the total resource use. The estimates were obtained from the TCAG

bioinformatics manager. The resource use ranges were based on 0% to 50% processing time usage. Prices in CAD per CPU per hour were based on the quote by the Scalar Decisions Inc., a company that set up the SickKids' Centre for Computational Medicine and High Performance Computing facility. The quote was $9560 CAD per node, assuming each node has 128 GB RAM and 20 cores. Price was depreciated using straight-line depreciation method over five years. To be consistent with price ranges for other microcost items, computing unit prices were assumed to vary by 10% from their point estimates.

2.4.3 Whole genome sequencing

For increased generalizability to different institutions, two WGS sequencing platforms were considered, HiSeq® 2500 and a more recent HiSeq X™. The latter technology can sequence 16 samples per run, compared to 4 to 8 samples for Illumina HiSeq® 2500 to achieve a 30-40X read depth. The Illumina HiSeq X™ requires greater initial investment, but has lower reagent prices. Appendices 3 and 4 contain resource use and price data for HiSeq® 2500 and HiSeq X™, respectively.

2.4.3.1 Labour

As with WES, WGS total minutes for each input in the library preparation and sequencing categories were fixed, regardless of the number of samples processed per run. There were fewer inputs in the library preparation category for WGS compared to WES, since it was not necessary to extract the exome from the genome. The total minutes per run for each of the remaining inputs in the library preparation category, as well for each input in the specimen preparation and sequencing category were the same for WES and WGS (Appendices 3 and 4). The labour time per sample for each input in these categories was calculated by dividing the total time per task by the number of samples processed per run. It was assumed that 8 to 16 samples could be processed during the library preparation tasks using HiSeq® 2500. Due to automation, HiSeq X™ can process 48 samples during the library preparation tasks. For sequencing tasks, the older WGS technology (HiSeq® 2500) allows for about 4 to 8 samples to be sequenced per run, while the newer WGS technology (HiSeq X™) can sequence 16 samples per run.

Labour resource use and prices were estimated for the analysis of sequenced data performed at TCAG and the maintenance of the high performance computing cluster at the SickKids' Centre for Computational Medicine. The capacity of the HiSeq® 2500 platform is 20-25 genomes per month. Processing this number of genomes required one FTE unit of labour for variant calling and 0.25 FTE units

of labour for annotation. For HiSeq X™ with its specialized HiSeq Analysis Software (HAS), base calling, variant calling and alignment are streamlined and do not require the same input from an analyst as the 2500 platform. The capacity of one HiSeq X™ instrument is 150 genomes per month. Based on expert opinion, 1.5 FTE unit of labour is required to process a maximum of 150 genomes per month. This labour time was assumed to include sample logistics management (i.e. starting computing jobs, tracking samples, transferring data) as well as data processing (i.e. periodic updates to the annotation pipeline). Since the HiSeq X™ machines at SickKids are not currently operating at full capacity, labour time was assumed to vary by 10% to account for uncertainty. The resource use per sample for bioinformatics was calculated by dividing the labour time by each instrument's capacity per month.

The HiSeq® 2500 bioinformatics maintenance component includes the five pipeline steps specified for WES plus post-recalibration merge. HiSeq X™ pipelines steps are: Alignment/Remove Duplicates/Re-alignment, SNV/indel variant calling, CNV/SV calling; statistics; and annotation. The calculation time and the number of nodes required for each step in the bioinformatics pipeline were obtained from the TCAG bioinformatics manager and analyst. One hour of labour was assumed to be required to support one node per year [40]. The bioinformatics maintenance labour resource use in minutes was estimated by multiplying the calculation time by the time needed to support the required number of nodes. Ranges for labour volume use were provided. The number of nodes and calculation time were higher for WGS than for WES.

As with WES, zero to four primary variants could be found, with an average of two variants. Clinical interpretation required 30 minutes per variant and report writing required 15 minutes per variant, in addition to 15 minutes required for each task regardless of how many variants were found. Based on expert opinion, it was assumed that addressing secondary variants required 20 to 40 minutes with an average of 30 minutes for clinical interpretation and report writing. It was further assumed that secondary variants were found in 4% of cases. Time needed for clinical interpretation and report writing for secondary variants was calculated by multiplying the total time by the proportion of cases that have them.

Hospital and lab employees involved in WGS testing include nurses, lab technicians, lab technologists, bioinformatics analysts and high performance computing staff. As with WES, salaries of each staff

member were obtained by either an informal survey of lab staff, reported salaries from SickKids or Government of Ontario Public Sector Salary Disclosure. Because of the confidential nature of this information, reporting of unit prices for labour items has been suppressed. Benefits of 18% were added to each annual salary based on SickKids policy. For most of the inputs, the salary range was based on the SickKids salary scale, lab staff survey or expert opinion within the lab. When it was not possible to obtain a salary range from these sources, salary was assumed to vary by 20% from its point estimate.

2.4.3.2 Equipment

The resource use for small and large equipment was identical for WGS and WES, regardless of the sequencing platform. The sample costs for ASD patients for large and small equipment was determined by allocating the proportion of use for all patients with all indications in the institution. The price estimates for small equipment were the same as well. The price of HiSeq® 2500 sequencing platform was the same for both WGS and WES. The HiSeq X™ platform required greater initial investment. The HiSeq X™ estimates include the cost of the platform, its maintenance contract and Bioanalyzer and TapeStation instruments. The price for one HiSeq X™ instrument was based on the assumption that five sequencers were purchased at SickKids (Appendix 4). In order to account for price and currency fluctuations, unit prices of small and large equipment were assumed to vary by 10% from their point estimates.

2.4.3.3 Supplies

Supplies included costs of shipping a sample to TCAG laboratory, Illumina Nano DNA library preparation reagents, other library preparation consumables and reagents and HiSeq® 2500 and HiSeq X™ sequencing reagents (Appendices 3 and 4). The price of HiSeq® 2500 reagents per sample was about three times as high as the price of HiSeq X™ reagents. Ranges for resource use were not assigned, as it was assumed that one unit of supplies was required per sample. In order to account for price and currency fluctuations, unit prices of shipping and handling, library preparation and sequencing reagents were assumed vary by 10% from their point estimates.

2.4.3.4 Follow-up testing

For WGS, follow-up testing includes Sanger sequencing, FISH and qPCR tests. About 50% of patients and their parents undergo Sanger sequencing and about 10% of patients and their parents undergo either FISH or qPCR follow-up testing (40). Since FISH testing is done infrequently, it was assumed that only

qPCR is done in 10% of cases. The price estimate and range for qPCR was the same for WGS and CMA. The price estimate and its range for Sanger sequencing were the same for WGS and WES.

2.4.3.5 Bioinformatics

Costs were calculated for the bioinformatics data file storage and computational use. The resource use for the data file storage depended on file size and length of storage time and was calculated in gigabytes per year. For HiSeq® 2500, the bioinformatics computation use included the five pipeline steps specified for WES plus post-recalibration merge. The resource use (CPU per hour) for each step was calculated by multiplying the number of computing jobs by the number of CPUs (cores) and time (in minutes) required to complete the job. The resource use estimates, along with ranges, were obtained from the TCAG bioinformatics manager. Unit prices for storage and computational use were the same for WES and WGS, with a 10% range.

For HiSeq X™, the cost was estimated for storage use and for each of the pipeline steps specified in Section 2.4.3.1. Unit prices for storage were same for across the two WGS platforms. With an exception of annotation which is done using an older technology (HiSeq® 2500), prices for computational use were based on the recent TCAG's purchase of 72 compute nodes (40 cores, 256 GB RAM) for processing WGS samples on HiSeq X™. The price of each node was 26,804 CAD over five years, including warranty. To be consistent with price ranges for other microcost items, computing unit prices were assumed to vary by 10% from their point estimates.

The resource use for bioinformatics file storage and computation was greater for WGS compared to WES, since WGS stored files are larger and WGS requires a greater number of jobs and more time to complete a job. HiSeq X™ is more costly in terms of computation use compared to HiSeq® 2500, due to a higher cost of compute nodes.

2.5 Microcosting analysis

Costs per sample were calculated and aggregated by category and by year over the 5-year time horizon separately for CMA, WES and WGS. Total program costs over five years were also estimated for each test technology.

2.5.1 Sensitivity analysis

An assessment of uncertainty is an essential part of an economic analysis (37, 42, 43). Using expert opinion, ranges covering all plausible values of input's resource use or unit price were obtained. Standard one-way sensitivity analysis was not practical due to a large number of parameters. To assess parameter uncertainty, a probabilistic sensitivity analysis (PSA) was conducted. In PSA, ranges provided by experts were used to define probability distributions for each input's resource use and unit price. Monte Carlo simulations were conducted to vary these parameters simultaneously. Deterministic sensitivity analysis (DSA) was also conducted for selected parameters that were highly uncertain or expected to vary substantially between institutions. These included the overhead cost, the number of tests performed per year for all indications, and the number of primary variants found. The DSAs permitted an examination of how changing the values of highly uncertain inputs one at a time affected the results.

2.5.2 Probabilistic sensitivity analysis

Probability distributions were defined for inputs which were either proportions or for which upper and lower bound were provided in addition to a point estimate (Tables 3-6). The source for some estimates was often the same expert. Since no evidence existed for any specific form of correlation, all input distributions were assumed to be independent. To propagate variance in the model, 10000 values were drawn from each input's distribution. Point estimates of inputs with fixed values, i.e. inputs for which ranges were not provided, were repeated 10000 times. Similarly, for parameters varied in the deterministic sensitivity analysis, i.e. overhead cost, number of tests and number of variants, their reference level values were repeated 10000 times.

2.5.2.1 Labour

Probability distributions were defined for labour resource use inputs for which plausible ranges were specified (Tables 3-6, Appendices 1-4). All labour unit prices for each of the three tests were assigned plausible ranges; therefore, probability distributions were specified for each input's unit price. Each input included in PSA had a point estimate, lower bound and upper bound provided by experts. Each input parameter was described by truncated normal distribution where a point estimate corresponded to the mean of the normal distribution and lower and upper bounds corresponded to 99.7% confidence interval (i.e. upper and lower bounds were assumed to lie within three standards deviations from the mean):

$$X \sim N(\mu, \sigma^2),$$

where X is a resource use or unit price input, bounded at zero, $0 < X < \infty$, μ corresponds to the point estimate of X, $\sigma = \frac{u-l}{6}$, u is the upper bound and l is the lower bound of a range. The 99.7% confidence level was chosen to convey a level of confidence in choosing the upper and lower bounds for an input. The normal distribution was truncated at zero, since resource use and prices cannot be negative.

For library preparation and sequencing categories of WES and WGS tests, the number of samples were simulated using truncated normal distribution; the total number minutes per task was assumed to be constant. Similarly, total number of minutes per month required for variant calling and annotation was assumed to be constant, while the number of tests done per month was modelled using truncated normal distribution. Labour steps involving secondary variants were modeled using two steps: total number of minutes required for clinical interpretation and report writing was described using truncated normal distribution and proportion of patients for whom secondary variants were found was described by beta distribution (see Section 2.5.2.3 for definition of beta distribution).

2.5.2.2 Equipment and supplies

Resource use volume of large and small equipment depended on the total number of tests performed in the institution and was varied in the DSA. Resource use of supplies was fixed at one per sample. Unit prices for equipment and supplies were assigned ranges. Unit prices of equipment and supplies were described by the truncated normal distribution with point estimate corresponding to the mean of truncated normal distribution and lower and upper bounds corresponding to 99.7% confidence interval.

2.5.2.3 Follow-up testing

The resource use for FISH, qPCR and Sanger sequencing were quantified as the proportion of cases in which follow-up testing was done (Appendices 1-4). At the individual case level, the follow-up testing can be described by binomial distribution. In order to represent uncertainty in the proportion of follow-up tests, the beta distribution, a conjugate to the binomial distribution, was used (42):

$$X \sim Beta(\alpha, \beta),$$

where X is a resource use parameter for follow-up testing, α is the number of follow-up tests and β is the total number of tests less the number of follow-up tests. Since the proportion of follow-up testing was provided by an expert, that proportion was applied to the total number of tests to obtain the number of follow-up tests. Unit prices for each follow-up test were assigned ranges. Unit prices of follow-up tests were described by the truncated normal distribution with point estimate corresponding to the mean of the normal distribution and lower and upper bounds corresponding to 99.7% confidence interval.

2.5.2.4 Bioinformatics

Ranges were provided for bioinformatics resource use and unit price. Resource use inputs and prices were described by the truncated normal distribution with point estimate corresponding to the mean of the normal distribution and lower and upper bounds corresponding to 99.7% confidence interval.

2.5.3 Deterministic sensitivity analysis

Three one-way DSAs were conducted to examine the effects of changing the inputs for i) the overhead cost; ii) the total volume of tests in the institution; and iii) the number of primary variants, while other inputs remained the same. For all three testing technologies, the reference overhead cost was set at 23%. In the deterministic sensitivity analysis, the overhead cost was varied from 10 to 30%. For WES and WGS tests, the reference case number of all tests for patients in the institution per sequencer was set to 500. As the new sequencing technologies are implemented, the volume of referrals for testing is expected to increase. In order to examine how the cost per ASD patient for equipment changes with an increasing number of tests across the institution, the number of WES or WGS tests for all indications was varied from 500 to 1000. A third DSA was conducted to vary the number of primary variants found in ASD cases. For WES and WGS, the clinical interpretation and report writing time depends on the number of variants found. On average, two variants are found per ASD case. The number of variants was varied from zero to four.

Table 3. CMA parameter estimates and distributions used in the probabilistic sensitivity analysis.

Cost Items	Volume of use per sample		Unit price	
	Estimate	Distribution	Estimate	Distribution
LABOUR				
Specimen preparation (units: minutes)				
Pediatric venipuncture	7.6	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Packaging with testing documentation	1.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Service recipient primary registration	1.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Printing and sorting of specimen labels	0.4	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Creation of recipient folder	5.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Packaging with testing documentation	1.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Service recipient limited registration	1.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
DNA extraction (units: minutes)				
Extraction using an automated kit	2.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Manual nucleic acid quantitation	5.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Freezing of cells/tissue without cryopreservation	9.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Nucleic acid quantitation using spectrophotometer with sample retention technology	1.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Microarray sample processing (units: minutes)				
Assay preparation - manual worksheet prep	2.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Fluorochrome labelling without dye swap	4.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Dilution of specimens	2.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
DNA Fragmentation by Restriction Enzyme Digestion	2.3	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Ligation	1.5	Fixed	Conf.	Trun. Normal μ,σ=Conf.
PCR amplification	2.3	Fixed	Conf.	Trun. Normal μ,σ=Conf.
PCR purification by magnetic beads	12.4	Fixed	Conf.	Trun. Normal μ,σ=Conf.
DNA Fragmentation by Restriction Enzyme Digestion	2.3	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Fluorochrome labelling without dye swap	1.1	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Microarray slide hybridization	4.1	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Microarray slide washing and drying, automated	8.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Microarray slide scanning	10.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Analysis (units: minutes)				
Data preparation	8.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Data analysis	12.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.

Report writing (units: minutes)				
Collation and write up, simple	2.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Collation and write up, intermediate	10.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Collation and write up, complex	50.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Technical checking/reporting of molecular genetic interpretation	5.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Clinical interpretation and professional signoff (units: minutes)				
Clinical interpretation and professional signoff, straightforward	8.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Clinical interpretation and professional signoff, moderate	8.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Clinical interpretation and professional signoff, complex	3.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
LARGE EQUIPMENT				
Affymetrix 1 GeneChip 3000Dx, 2 Fluidics stations, 1 hybridization oven	2/all tests	Fixed	252934	Trun. Normal μ=252934, σ=8431
1-year service contract	1/all tests	Fixed	25000	Trun. Normal μ=25000,σ=833
SUPPLIES				
Shipping and handling	1.0	Fixed	52.5	Trun. Normal μ=52.5,σ=1.8
Microarray slide and reagents per patient	1.0	Fixed	Conf.	μ,σ=Conf.
FOLLOW-UP TESTING				
Proportion of patients who undergo FISH followup (proband and two parents)	0.1	Beta α=395,β=3553	667.7	Trun. Normal μ=667.7,σ=24.2
Proportion of patients who undergo qPCR followup (proband and two parents)	0.05	Beta α=197,β=3751	684.8	Trun. Normal μ=684.8,σ=22.8

Abbreviations: CMA, Chromosomal microarray analysis; PCR, Polymerase chain reaction; FISH, Conf., Confidential; Trun. Normal, Truncated normal; FISH, Fluorescence in situ hybridization; qPCR, Real-time polymerase chain reaction. 'All tests' indicates the total volume of tests performed in the institution for all indications.

Table 4. WES parameter estimates and distributions used in the probabilistic sensitivity analysis.

Cost Items	Volume of use per sample		Unit price	
	Estimate	Distribution	Estimate	Distribution
LABOUR				
Specimen preparation (units: minutes)				
Pediatric venipuncture	7.6	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Packaging with testing documentation	1.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Service recipient primary registration	1.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Printing and sorting of specimen labels	0.4	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Creation of recipient folder	5.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Packaging with testing documentation	1.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Service recipient limited registration	1.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Library preparation (units: minutes)				
DNA quantification	2.5	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Pre-prep reagents	2.5	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Shearing	2.5	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Purification	5.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
End repair	5.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
A-tailing	5.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Adapter ligation	5.6	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Pre-hybridization PCR	5.6	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Pre-hybridization quality control	7.5	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Lyofilization	2.5	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Hybridization	3.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Hybridization washes	18.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Post-hybridization PCR	5.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Post-hybridization quality control	15.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Sequencing (units: minutes)				
HiSeq wash	3.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Sequencing prep	3.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
HiSeq post-run wash	5.6	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Run quality control	1.9	Fixed	Conf.	Trun. Normal μ,σ=Conf.
cBot	3.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.

Bioinformatics uUnits: minutes)				
Variant calling (total time per month/samples per month)	96	Total time fixed (8400 minutes); Samples per month: Trun. Normal μ=87.5.5,σ=4.2	Conf.	Trun. Normal μ,σ=Conf.
Annotation (total time per month/samples per month)	24	Total time fixed (2100 minutes); Samples per month: Trun. Normal μ=87.5, σ=4.2	Conf.	Trun. Normal μ,σ=Conf.
Bioinformatics maintenance (units: minutes)				
Alignment	0.01	Trun. Normal μ=0.01,σ=0.0007	Conf.	Trun. Normal μ,σ=Conf.
Remove Duplicates	0.0034	Trun. Normal μ=0.0034,σ=0.0003	Conf.	Trun. Normal μ,σ=Conf.
Recalibration	0.017	Trun. Normal μ=0.017,σ=0.001	Conf.	Trun. Normal μ,σ=Conf.
SNV/indel variant calling	0.009	Trun. Normal μ=0.009,σ=0.0006	Conf.	Trun. Normal μ,σ=Conf.
Annotation (ANNOVAR)	0.009	Trun. Normal μ=0.009,σ=0.0006	Conf.	Trun. Normal μ,σ=Conf.
Clinical interpretation (units: minutes)				
Classification of primary variants	60	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Classification of secondary variants (total interpretation time × proportion of cases)	1.2	Total time: Trun. Normal μ=30,σ=3.3; Proportion of cases: Beta α=12,β=288	Conf.	Trun. Normal μ,σ=Conf.
Report writing (units: minutes)			Conf.	Trun. Normal μ,σ=Conf.
Addressing primary variants	45	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Addressing secondary variants (total report writing time × proportion of cases)	1.2	Total time: Trun. Normal μ=30, σ=3.3; Proportion of cases: Beta α=12,β=288	Conf.	Trun. Normal μ,σ=Conf.
LARGE EQUIPMENT				
Illumina HiSeq 2500	1/all tests	Fixed	750000	Trun. Normal μ=750000, σ=16667
1-year service contract	1/all tests	Fixed	75000	Trun. Normal μ=75000, σ=5417
Agilent BioAnalyzer/Tape station	1/all tests	Fixed	38500	Trun. Normal μ=38500, σ=1500
SMALL EQUIPMENT				
Tube microcentrifuge	1/all tests	Fixed	2276	Trun. Normal μ=2276,σ=84.3
Plate microcentrifuge	1/all tests	Fixed	5059	Trun. Normal μ=5059,σ=168.6
Thermomixer	1/all tests	Fixed	5059	Trun. Normal μ=5059,σ=168.6
Vortex	1/all tests	Fixed	455	Trun. Normal μ=455,σ=16.9
Pipette sets	2/all tests	Fixed	1619	Trun. Normal μ=1619,σ=101.2
Magnet particle concentrator for tubes	1/all tests	Fixed	708	Trun. Normal μ=708, σ=23.6
Thermocyclers	2/all tests	Fixed	3035	Trun. Normal μ=3035,σ=101.2

SUPPLIES				
Shipping & Handling	1	Fixed	52.5	Trun. Normal μ=52.5,σ=1.8
SureSelect Baits	1	Fixed	242.2	Trun. Normal μ=242.2,σ=8.1
SureSelect Library prep	1	Fixed	58.5	Trun. Normal μ=58.5,σ=1.9
Other library prep consumables	1	Fixed	50.0	Trun. Normal μ=50.0,σ=1.7
Reagents (8 samples per lane)	1	Fixed	274.4	Trun. Normal μ=274.4,σ=9.1
FOLLOW-UP TESTING (proportion of patients)				
Sanger sequencing	0.5	Beta α=150,β=150	38.5	Trun. Normal μ=38.5,σ=0.8
BIONFORMATICS				
Bioinformatics file storage (units: GB per year)				
trimmed fastq	6.8	Trun. Normal μ=6.8,σ=0.75	0.40	Trun. Normal μ=0.40,σ=0.013
temporary BAM files	2.5	Fixed	0.40	Trun. Normal μ=0.40,σ=0.013
final rem-dup, recalibrated, locally re-aligned BAM file	4.5	Trun. Normal μ=4.5,σ=0.50	0.40	Trun. Normal μ=0.40,σ=0.013
Bioinformatics computation use (units: CPU time per hour)				
Alignment	90.0	Trun. Normal μ=90,σ=6	0.011	Trun. Normal μ=0.011,σ=0.00037
Remove Duplicates	1.3	Trun. Normal μ=1.3,σ=0.08	0.011	Trun. Normal μ=0.011,σ=0.00037
Recalibration	5.0	Trun. Normal μ=5,σ=0.33	0.011	Trun. Normal μ=0.011,σ=0.00037
SNV/indel variant calling	6.3	Trun. Normal μ=6.3,σ=0.42	0.011	Trun. Normal μ=0.011,σ=0.00037
Annotation (ANNOVAR)	20.0	Trun. Normal μ=20,σ=1.33	0.011	Trun. Normal μ=0.011,σ=0.00037

Abbreviations: WES, Whole exome sequencing; SNV, Single nucleotide variant; PCR, Polymerase chain reaction; Conf., Confidential; Trun. Normal, Truncated normal. 'All tests' indicates the total volume of tests performed in the institution for all indications.

Table 5. WGS (Illumina HiSeq® 2500) parameter estimates and distributions used in the probabilistic sensitivity analysis.

Cost Items	Volume of use per sample		Unit price	
	Estimate	Distribution	Estimate	Distribution
LABOUR				
Specimen preparation (units: minutes)				
Pediatric venipuncture	7.6	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Packaging with testing documentation	1.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Service recipient primary registration	1.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Printing and sorting of specimen labels	0.4	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Creation of recipient folder	5.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Packaging with testing documentation	1.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Service recipient limited registration	1.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Library preparation (units: minutes) total time/number of samples per batch				
DNA quantification	1.7	Total number of minutes fixed Number of samples per batch: Trun. Normal μ=12,σ=1.3	Conf.	Trun. Normal μ,σ=Conf.
Pre-prep reagents	1.7		Conf.	Trun. Normal μ,σ=Conf.
Shearing	1.7		Conf.	Trun. Normal μ,σ=Conf.
Purification	3.3		Conf.	Trun. Normal μ,σ=Conf.
End repair	3.3		Conf.	Trun. Normal μ,σ=Conf.
A-tailing	3.3		Conf.	Trun. Normal μ,σ=Conf.
Adapter ligation	3.8		Conf.	Trun. Normal μ,σ=Conf.
Sequencing (units: minutes)				
HiSeq wash	5.0	Total number of minutes fixed Number of samples per batch: Trun. Normal μ=6,σ=1.3	Conf.	Trun. Normal μ,σ=Conf.
Sequencing prep	5.0		Conf.	Trun. Normal μ,σ=Conf.
HiSeq post-run wash	7.5		Conf.	Trun. Normal μ,σ=Conf.
Run quality control	2.5		Conf.	Trun. Normal μ,σ=Conf.
cBot	5.0		Conf.	Trun. Normal μ,σ=Conf.
Bioinformatics (Units: minutes),				
Variant calling (total time per month/samples per month)	373.3	Total time fixed (8400 min); Samples per month: Trun. Normal μ=22.5,σ=0.83	Conf.	Trun. Normal μ,σ=Conf.
Annotation (total time per month/samples per month)	93.3	Total time fixed (2100 min); Samples per month: Trun. Normal μ=22.5,σ=0.83	Conf.	Trun. Normal μ,σ=Conf.

Bioinformatics maintenance (units: minutes)				
Alignment	0.57	Trun. Normal μ=0.57,σ=0.047	Conf.	Trun. Normal μ,σ=Conf.
Remove Duplicates	0.10	Trun. Normal μ=0.10,σ=0.008	Conf.	Trun. Normal μ,σ=Conf.
Recalibration	0.58	Trun. Normal μ=0.58,σ=0.048	Conf.	Trun. Normal μ,σ=Conf.
Post-recalibration merge	0.29	Trun. Normal μ=0.29,σ=0.024	Conf.	
SNV/indel variant calling	0.88	Trun. Normal μ=0.88,σ=0.073	Conf.	Trun. Normal μ,σ=Conf.
Annotation (ANNOVAR)	0.021	Trun. Normal μ=0.021,σ=0.002	Conf.	Trun. Normal μ,σ=Conf.
Clinical interpretation (units: minutes)				
Classification of primary variants	75	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Classification of secondary variants (total interpretation time × proportion of cases)	1.2	Total time: Trun. Normal μ=30,σ=3.3; Proportion of cases: Beta α=12,β=288	Conf.	Trun. Normal μ,σ=Conf.
Report writing (units: minutes)				Trun. Normal μ,σ=Conf.
Addressing primary variants	45	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Addressing secondary variants (total report writing time × proportion of cases)	1.2	Total time: Trun. Normal μ=30,σ=3.3; Proportion of cases: Beta α=12,β=288	Conf.	Trun. Normal μ,σ=Conf.
LARGE EQUIPMENT				
Illumina HiSeq 2500	1/all tests	Fixed	750000	Trun. Normal μ=750000, σ=16667
1-year service contract	1/all tests	Fixed	75000	Trun. Normal μ=75000, σ=5417
Agilent BioAnalyzer/Tape station	1/all tests	Fixed	38500	Trun. Normal μ=38500, σ=1500
SMALL EQUIPMENT				
Tube microcentrifuge	1/all tests	Fixed	2276	Trun. Normal μ=2276,σ=84.3
Plate microcentrifuge	1/all tests	Fixed	5059	Trun. Normal μ=5059,σ=168.6
Thermomixer	1/all tests	Fixed	5059	Trun. Normal μ=5059,σ=168.6
Vortex	1/all tests	Fixed	455	Trun. Normal μ=455,σ=16.9
Pipette sets	2/all tests	Fixed	1619	Trun. Normal μ=1619,σ=101.2
Magnet particle concentrator for tubes	1/all tests	Fixed	708	Trun. Normal μ=708, σ=23.6
Thermocyclers	2/all tests	Fixed	3035	Trun. Normal μ=3035,σ=101.2
SUPPLIES				
Shipping & Handling	1	Fixed	52.5	Trun. Normal μ=52.5,σ=1.8
Illumina Nano DNA library prep	1	Fixed	30.0	Trun. Normal μ=30.0,σ=1.0
Other library prep consumables	1	Fixed	50.0	Trun. Normal μ=50,σ=1.7
Sequencing reagents	1	Fixed	4055	Trun. Normal μ=4055,σ=135.2
FOLLOW-UP TESTING (proportion of patients)				
Sanger sequencing	0.5	Beta α=150,β=150	38.5	Trun. Normal μ=38.5,σ=0.84

qPCR followup	0.1	Beta α=30, β=270	684.8	Trun. Normal μ=684.8,σ=22.8
BIONFORMATICS				
Bioinformatics file storage (units: GB per year)				
trimmed fastq	75.0	Trun. Normal μ=75.0,σ=8.3	0.40	Trun. Normal μ=0.40,σ=0.013
temporary BAM files	12.5	Fixed	0.40	Trun. Normal μ=0.40,σ=0.013
final rem-dup, recalibrated, locally re-aligned BAM file	150.0	Trun. Normal μ=150.0,σ=16.7	0.40	Trun. Normal μ=0.40,σ=0.013
Bioinformatics computation use (units: CPU time per hour)				
Alignment	750.0	Trun. Normal μ=750.0,σ=50.0	0.011	Trun. Normal μ=0.011,σ=0.00037
Remove Duplicates	17.5	Trun. Normal μ=17.5,σ=1.2	0.011	Trun. Normal μ=0.011,σ=0.00037
Recalibration	752.5	Trun. Normal μ=752.5,σ=50.2	0.011	Trun. Normal μ=0.011,σ=0.00037
Post-recalibration merge	4.4	Trun. Normal μ=4.4,σ=0.3	0.011	Trun. Normal μ=0.011,σ=0.00037
SNV/indel variant calling	1200	Trun. Normal μ=1200,σ=80.0	0.011	Trun. Normal μ=0.011,σ=0.00037
Annotation (ANNOVAR)	60.0	Trun. Normal μ=60.0,σ=4.0	0.011	Trun. Normal μ=0.011,σ=0.00037

Abbreviations: WGS, Whole genome sequencing; qPCR, Real-time polymerase chain reaction; SNV, Single nucleotide variant; Conf., Confidential; Trun. Normal, Truncated normal. 'All tests' indicates the total volume of tests performed in the institution for all indications.

Table 6. WGS (Illumina HiSeq X™) parameter estimates and distributions used in the probabilistic sensitivity analysis.

Cost Items	Quantity of Use per Sample		Unit Price	
	Estimate	Distribution	Estimate	Distribution
LABOUR				
Specimen Preparation (Units: minutes)				
Pediatric venipuncture	7.6	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Packaging with testing documentation	1.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Service recipient primary registration	1.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Printing and sorting of specimen labels	0.4	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Creation of recipient folder	5.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Packaging with testing documentation	1.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Service recipient limited registration	1.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Library preparation (Units: minutes) total time/number of samples per batch				
DNA quantification	0.4	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Pre-prep reagents	0.4	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Shearing	0.4	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Purification	0.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
End repair	0.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
A-tailing	0.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Adapter ligation	0.9	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Sequencing (Units: minutes)				
HiSeq wash	1.9	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Sequencing prep	1.9	Fixed	Conf.	Trun. Normal μ,σ=Conf.
HiSeq post-run wash	2.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Run quality control	0.9	Fixed	Conf.	Trun. Normal μ,σ=Conf.
cBot	1.9	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Bioinformatics (Units: minutes)* , total time/number of samples per month				
Data processing	84.0	Trun. Normal μ=84, σ=2.8	Conf.	Trun. Normal μ,σ=Conf.
Bioinformatics Maintenance (Units: minutes)*				
Alignment/Remove Duplicates/Re-alignment, HiSeq Analysis Software (HAS)	0.022	Trun. Normal μ=0.022,σ=0.0018	Conf.	Trun. Normal μ,σ=Conf.

SNV/indel variant calling, HiSeq Analysis Software (HAS)	0.005	Trun. Normal μ=0.005,σ=0.0004	Conf.	Trun. Normal μ,σ=Conf.
CNV/SV calling, HiSeq Analysis Software (HAS)	0.004	Trun. Normal μ=0.004,σ=0.0003	Conf.	Trun. Normal μ,σ=Conf.
Statistic, HiSeq Analysis Software (HAS)	0.003	Trun. Normal μ=0.003,σ=0.0003		
Annotation (ANNOVAR)	0.021	Trun. Normal μ=0.021,σ=0.0017	Conf.	Trun. Normal μ,σ=Conf.
Clinical Interpretation (Units: minutes)				
Classification of primary variants	75	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Classification of secondary variants (total interpretation time × proportion of cases)	1.2	Total time: Trun. Normal μ=30,σ=3.3; Proportion of cases: Beta α=12,β=288	Conf.	Trun. Normal μ,σ=Conf.
Report Writing (Units: minutes)				Trun. Normal μ,σ=Conf.
Addressing primary variants	45	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Addressing secondary variants (total report writing time × proportion of cases)	1.2	Total time: Trun. Normal μ=30,σ=3.3; Proportion of cases: Beta α=12,β=288	Conf.	Trun. Normal μ,σ=Conf.
LARGE EQUIPMENT				
Illumina HiSeq X	1/all tests	Fixed	1150000	Trun. Normal μ=1150000, σ=38333
1-year service contract	1/all tests	Fixed	119025	Trun. Normal μ=119025, σ=3968
Agilent BioAnalyzer/Tape station	1/all tests	Fixed	38500	Trun. Normal μ=38500, σ=1500
SMALL EQUIPMENT				
Tube microcentrifuge	1/all tests	Fixed	2276	Trun. Normal μ=2276,σ=84.3
Plate microcentrifuge	1/all tests	Fixed	5059	Trun. Normal μ=5059,σ=168.6
Thermomixer	1/all tests	Fixed	5059	Trun. Normal μ=5059,σ=168.6
Vortex	1/all tests	Fixed	455	Trun. Normal μ=455,σ=16.9
Pipette sets	2/all tests	Fixed	1619	Trun. Normal μ=1619,σ=101.2
Magnet particle concentrator for tubes	1/all tests	Fixed	708	Trun. Normal μ=708, σ=23.6
Thermocyclers	2/all tests	Fixed	3035	Trun. Normal μ=3035,σ=101.2
SUPPLIES				
Shipping & Handling	1	Fixed	52.5	Trun. Normal μ=52.5,σ=1.8
Illumina Nano DNA library prep	1	Fixed	30.0	Trun. Normal μ=30.0,σ=1.0
Other library prep consumables	1	Fixed	50	Trun. Normal μ=50,σ=1.7
Sequencing reagents	1	Fixed	1290	Trun. Normal μ=1290,σ=43.0
FOLLOW-UP TESTING (proportion of patients)				
Sanger sequencing	0.5	Beta α=150,β=150	38.5	Trun. Normal μ=38.5,σ=0.84

qPCR followup	0.1	Beta α=30, β=270	684.8	Trun. Normal μ=684.8,σ=22.8
BIONFORMATICS*				
Bioinformatics File Storage (Units: GB per year)				
fastq	90.0	Trun. Normal μ=90.0,σ=10.0	0.40	Trun. Normal μ=0.40,σ=0.013
final rem-dup, recalibrated, locally re-aligned BAM file	60.0	Trun. Normal μ=60.0,σ=6.67	0.40	Trun. Normal μ=0.40,σ=0.013
Bioinformatics Computation Use (Units: CPU time per hour)				
Alignment/Remove Duplicates/Re-alignment – HiSeq Analysis Software (HAS)	160	Trun. Normal μ=160.0,σ=10.7	0.612	Trun. Normal μ=0.612,σ=0.0204
SNV/indel variant calling – HiSeq Analysis Software (HAS)	35.0	Trun. Normal μ=35.0,σ=2.33	0.612	Trun. Normal μ=0.612,σ=0.0204
CNV/SV calling – HiSeq Analysis Software (HAS)	30.0	Trun. Normal μ=30.0,σ=2.00	0.612	Trun. Normal μ=0.612,σ=0.0204
Statistics – HiSeq Analysis Software (HAS)	25.0	Trun. Normal μ=25.0,σ=1.67	0.612	Trun. Normal μ=0.612,σ=0.0204
Annotation (ANNOVAR)	60.0	Trun. Normal μ=60.0,σ=4.00	0.011	Trun. Normal μ=0.011,σ=0.00037

Abbreviations: WGS, Whole genome sequencing; qPCR, Real-time polymerase chain reaction; SNV, Single nucleotide variant; Conf., Confidential; Trun. Normal, Truncated normal. 'All tests' indicates the total volume of tests performed in the institution for all indications.

2.6 Cost-consequence analysis

A cost-consequence analysis was undertaken to determine the incremental costs per unit increase in diagnostic yield for CGES compared to standard care. Incremental costs and diagnostic yields were calculated for three scenarios deemed to reflect potential clinical practices: (1) substitution of CMA alone with a combination of CMA and WES (CMA + WES vs. CMA); (2) substitution of CMA with WGS (WGS vs. CMA); and (3) substitution of a combination of CMA and WES with WGS (WGS vs. CMA + WES). The rationale for combining CMA and WES is to detect both CNVs and SNVs. Chromosomal microarray can reliably identify CNVs, while WES alone is limited in the CNVs it can detect (19). Whole genome sequencing can identify both large and small variants (10). Therefore, combination of CMA and WES can be viewed as a substitute for WGS. Since WES can be viewed as a complement to CMA, clinical scenarios did not include a direct comparison of WES with CMA. These scenarios reflect how one type of technology or combination of testing technologies might fully substitute another technology. These scenarios do not consider serial testing, in which only patients who test negative on a first test, e.g. CMA, might proceed to CGES. As data on diagnostic yields for various configurations of serial testing are limited, serial testing was not considered in the cost-consequence analysis. In these scenarios, only costs of genomic diagnostic genetic tests were considered; other clinical assessments or genetic tests such as karyotyping, Fragile X or other single gene tests were not included.

To calculate incremental diagnostic yields associated with clinical scenarios, a review of published studies that reported diagnostic yields for CMA, WES or WGS for patients with a variety of developmental disorders including ASD was undertaken. Only studies done in the last five years were examined. The definition of diagnostic yield was typically the percentage of patients tested who were positive for one or more primary variants. Although the precise definition of diagnostic yield differed from study to study, in a majority of studies, variants of clinical significance were prioritized as primary variants. For CMA, this means that the diagnostic yield included variants of known or possible significance and not variants of unknown significance. Similarly for CGES, the clinical diagnostic yield included variants that were pathogenic or likely pathogenic and related to phenotype. The target population of this study are children with ASD. Therefore, only diagnostic yield estimates for patients who received an ASD diagnosis and who were from a pediatric population, were considered in the cost-consequence analysis.

3 Results

3.1 Test costs per patient with autism spectrum disorder

The results of CMA, WES, WGS (Illimina HiSeq® 2500) and WGS (HiSeq X™) microcosting models are shown in Tables 7, 8, 9 and 10, respectively. The total estimated costs per sample for each year of the five year program are shown, as well as costs for major cost categories. The percentile confidence intervals were calculated using 10000 Monte Carlo replications. Figure 1 shows the distribution of the cost per ASD sample by cost category. The results were based on reference values for overhead costs (23%), the number of total tests done per year for all indications (CMA: 3948, WES/WGS: 500) and the number of primary variants found (WES/WGS: 2).

The total cost of CMA was estimated to be $744 (95% CI: 714, 773) per ASD sample in Year 1 of the program. The largest cost component was supplies, accounting for 58% of total cost (Figure 1). The second largest cost item was labour, accounting for 19% of total cost. The total annual cost of WES was estimated to be $1655 (95% CI: 1611, 1699) per ASD sample in Year 1 of the program. Supplies and large equipment were the most expensive items at 40% and 23% of total costs, respectively (Figure 1). WGS conducted on the HiSeq® 2500 platform was estimated to cost $5519 (95% CI: 5244, 5785) per ASD sample in Year 1, with supplies constituting 74% of total cost (Figure 1). WGS conducted on the HiSeq X™ platform was estimated to cost $2851 (95% CI: 2750, 2956) per ASD sample in Year 1. The difference in total costs between the HiSeq® 2500 and the HiSeq X™ platforms was largely attributable to the higher cost of supplies and labour for the HiSeq® 2500 platform. For the newer WGS technology, supplies accounted for 48% of total cost, large equipment for 20.5% and labour for 9% (Figure 1). The WGS cost of computing and labour were higher than for WES due to greater bioinformatics and clinical interpretation demands.

Table 7. Estimated annual cost per ASD sample for CMA.

Cost Category	Year 1 (95% CI)	Year 2 (95% CI)	Year 3 (95%CI)	Year 4 (95% CI)	Year 5 (95% CI)
Labour	141.6 (132.2, 151)	137.4 (128.4, 146.6)	133.4 (124.7, 142.4)	129.6 (121, 138.2)	125.8 (117.5, 134.2)
Large Equipment	30 (28.2, 31.9)	28.4 (26.6, 30.2)	26.8 (25.2, 28.5)	25.3 (23.8, 26.9)	23.9 (22.4, 25.4)
Supplies	434.6 (409.1, 459.3)	421.9 (397.2, 445.9)	409.6 (385.6, 432.9)	397.7 (374.4, 420.3)	386.1 (363.5, 408.1)
Follow-up	98 (89, 107.4)	95.2 (86.4, 104.2)	92.4 (83.9, 101.2)	89.7 (81.5, 98.3)	87.1 (79.1, 95.4)
Overhead	39.5 (37.3, 41.7)	38.1 (36, 40.3)	36.9 (34.8, 38.9)	35.6 (33.6, 37.6)	34.4 (32.5, 36.4)
Total	743.7 (714.1, 773)	721.1 (692.4, 749.6)	699.2 (671.3, 726.9)	677.9 (650.9, 704.8)	657.3 (631, 683.4)

Estimates are given in 2015 Canadian dollars (CAD). Confidence intervals (CI) are based on 10000 Monte Carlo replications. The results were based on reference levels for overhead costs of 23% and 3948 CMA tests done for all indications per year.

Abbreviations: ASD, Autism spectrum disorder; CMA, Chromosomal microarray analysis.

Table 8. Estimated annual cost per ASD sample for WES.

Cost Category	Year 1 (95% CI)	Year 2 (95% CI)	Year 3 (95%CI)	Year 4 (95% CI)	Year 5 (95% CI)
Labour	318.4 (294.6, 342.6)	309.1 (286, 332.6)	300.1 (277.7, 323)	291.4 (269.6, 313.5)	282.9 (261.7, 304.4)
Large Equipment	385.6 (369.9, 400.9)	364.6 (349.8, 379)	344.5 (330.5, 358.1)	325.3 (312, 338.1)	306.8 (294.4, 319)
Small Equipment	8.9 (8.6, 9.2)	8.6 (8.3, 8.9)	8.4 (8.1, 8.6)	8.1 (7.9, 8.4)	7.9 (7.6, 8.1)
Supplies	657.7 (633.3, 681.7)	638.6 (614.8, 661.8)	620 (596.9, 642.5)	601.9 (579.5, 623.8)	584.4 (562.7, 605.6)
Follow-up	112 (101.1, 123.1)	108.7 (98.1, 119.5)	105.6 (95.3, 116)	102.5 (92.5, 112.7)	99.5 (89.8, 109.4)
Bioinformatics	6.7 (6, 7.5)	6.5 (5.8, 7.2)	6.3 (5.6, 7)	6.1 (5.5, 6.8)	6 (5.3, 6.6)
Overhead	165.5 (158.9, 172.1)	158.4 (152.1, 164.8)	151.6 (145.6, 157.7)	145.1 (139.2, 151)	138.8 (133.2, 144.5)
Total	1654.8 (1611, 1698.5)	1594.6 (1552.3, 1636.8)	1536.5 (1495.6, 1577.4)	1480.4 (1440.9, 1520.1)	1426.3 (1388.2, 1464.7)

Estimates are given in 2015 Canadian dollars (CAD). Confidence intervals (CI) are based on 10000 Monte Carlo replications. The results were based on reference levels for overhead costs of 23%, 500 total tests done for all indications per year, and two primary variants found per test.
Abbreviations: ASD, Autism spectrum disorder; WES, Whole exome sequencing.

Table 9**.** Estimated annual cost per ASD sample for WGS, Illumina HiSeq® 2500 platform.

Cost Category	Year 1 (95% CI)	Year 2 (95% CI)	Year 3 (95%CI)	Year 4 (95% CI)	Year 5 (95% CI)
Labour	518.4 (469.4, 568.7)	503.3 (455.7, 552.2)	488.7 (442.5, 536.1)	474.4 (429.6, 520.5)	460.6 (417.1, 505.3)
Large Equipment	385.6 (370, 401.3)	364.6 (349.9, 379.4)	344.5 (330.6, 358.5)	325.3 (312.1, 338.5)	306.8 (294.5, 319.3)
Small Equipment	8.9 (8.6, 9.2)	8.6 (8.3, 8.9)	8.4 (8.1, 8.6)	8.1 (7.9, 8.4)	7.9 (7.6, 8.2)
Supplies	4066.3 (3803.2, 4324.7)	3947.9 (3692.4, 4198.7)	3832.9 (3584.9, 4076.4)	3721.2 (3480.4, 3957.7)	3612.9 (3379.1, 3842.4)
Follow-up	178.6 (158.2, 200.5)	173.4 (153.6, 194.7)	168.3 (149.1, 189)	163.4 (144.8, 183.5)	158.7 (140.5, 178.2)
Bioinformatics	123.2 (108.1, 138.7)	119.7 (104.9, 134.6)	116.2 (101.9, 130.7)	112.8 (98.9, 126.9)	109.5 (96, 123.2)
Overhead	238.3 (225.8, 251)	229.1 (217.1, 241.4)	220.3 (208.6, 232.2)	211.7 (200.4, 223.3)	203.5 (192.6, 214.7)
Total	5519.3 (5243.7, 5785.4)	5346.6 (5078.8, 5605)	5179.2 (4919.2, 5430.2)	5017 (4764.8, 5260.9)	4859.9 (4614.9, 5096.8)

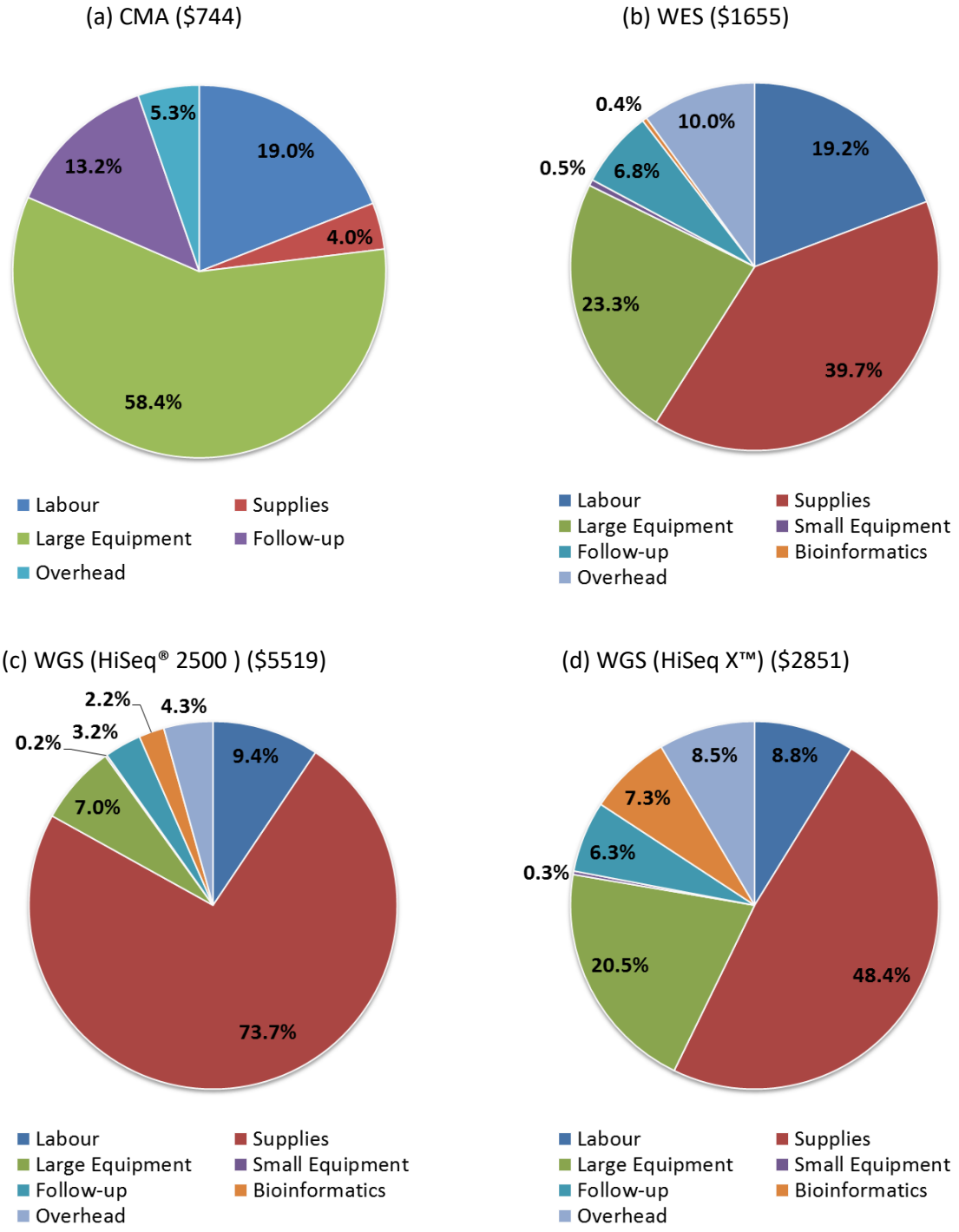
Estimates are given in 2015 Canadian dollars (CAD). Confidence intervals (CI) are based on 10000 Monte Carlo replications. The results were based on reference levels for overhead costs of 23%, 500 total tests done for all indications per year, and two primary variants found per test.
Abbreviations: ASD, Autism spectrum disorder; WGS, Whole genome sequencing.

Table 10. Estimated annual cost per ASD sample for WGS, Illumina HiSeq X™ platform.

Cost Category	Year 1 (95% CI)	Year 2 (95% CI)	Year 3 (95%CI)	Year 4 (95% CI)	Year 5 (95% CI)
Labour	250.5 (225.9, 274.5)	243.2 (219.3, 266.5)	236.1 (212.9, 258.7)	229.2 (206.7, 251.2)	222.5 (200.7, 243.9)
Large Equipment	583.8 (550, 617.3)	552 (520, 583.7)	521.6 (491.4, 551.5)	492.5 (463.9, 520.7)	464.6 (437.7, 491.3)
Small Equipment	8.9 (8.6, 9.2)	8.6 (8.3, 8.9)	8.4 (8.1, 8.7)	8.1 (7.9, 8.4)	7.9 (7.6, 8.2)
Supplies	1380.1 (1297.6, 1464.6)	1339.9 (1259.8, 1421.9)	1300.9 (1223.1, 1380.5)	1263 (1187.5, 1340.3)	1226.2 (1152.9, 1301.3)
Follow-up	178.8 (158.4, 200.9)	173.6 (153.8, 195.1)	168.5 (149.3, 189.4)	163.6 (145, 183.9)	158.9 (140.8, 178.5)
Bioinformatics	207.5 (189.9, 225)	201.4 (184.4, 218.4)	195.6 (179, 212)	189.9 (173.8, 205.9)	184.3 (168.7, 199.9)
Overhead	241.7 (231.2, 252.1)	231.2 (221.2, 241.3)	221.2 (211.6, 230.8)	211.5 (202.4, 220.8)	202.3 (193.5, 211.1)
Total	2851.2 (2750, 2955.5)	2750 (2652, 2851.2)	2652.3 (2557.2, 2750.4)	2557.9 (2466.1, 2653)	2466.7 (2377.8, 2558.7)

Estimates are given in 2015 Canadian dollars (CAD). Confidence intervals (CI) are based on 10000 Monte Carlo replications. The results were based on reference levels for overhead costs of 23%, 500 total tests done for all indications per year, and two primary variants found per test.
Abbreviations: ASD, Autism spectrum disorder; WGS, Whole genome sequencing.

Figure 1. Proportion of total annual cost per ASD test by cost category for CMA, WES, WGS (HiSeq® 2500 /HiSeq X™), Year 1.

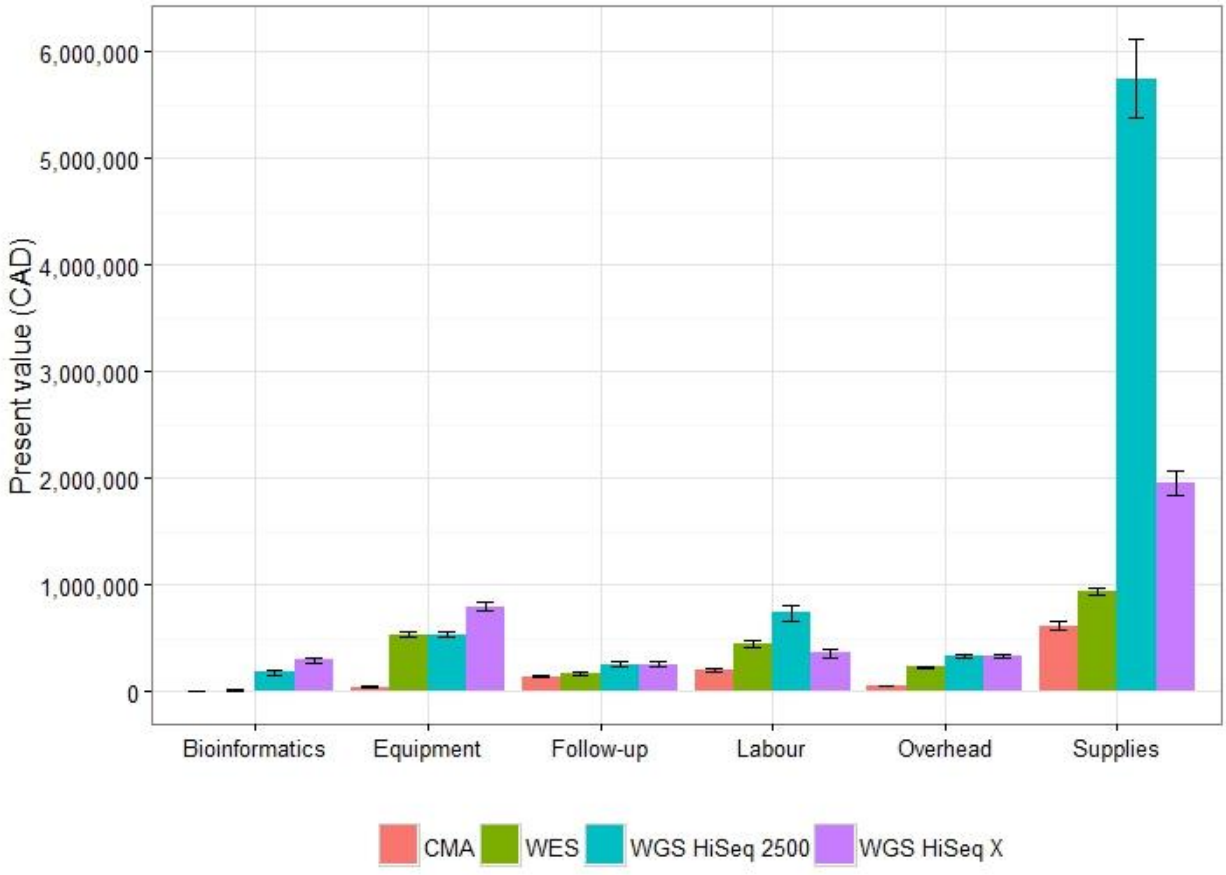


Estimates are given in 2015 Canadian dollars (CAD).
Abbreviations: ASD, Autism spectrum disorder; CMA, Chromosomal microarray analysis; WES, Whole exome sequencing; WGS, Whole genome sequencing

3.2 Program costs for autism spectrum disorder

The estimated total institutional program cost for CMA tests over the five-year period (present value) based on 300 ASD cases per year was $1.05 million (95% CI: 1.01, 1.09). The program costs of WES and WGS tests for ASD over the five-year period were also based on 300 cases per year. Estimated WES program costs were $2.31 million (95% CI: 2.25, 2.37). Estimated WGS program costs were $7.78 million (95% CI: 7.39, 8.15) for the HiSeq® 2500 platform and $3.98 million (95% CI: 3.84, 4.13) for the HiSeq X™ platform. Figure 2 shows the present value of program costs for each cost component and for each test. The program cost of supplies was the largest among the cost components for all three tests.

Figure 2. Present value of program costs over five years for CMA, WES, WGS (HiSeq® 2500 /HiSeq X™).



Estimates are given in 2015 Canadian dollars (CAD). Program costs are based on 300 ASD cases annually. Confidence bands are based on 10000 Monte Carlo replications. Abbreviations: ASD, Autism spectrum disorder; CMA, Chromosomal microarray analysis; WES, Whole exome sequencing; WGS, Whole genome sequencing

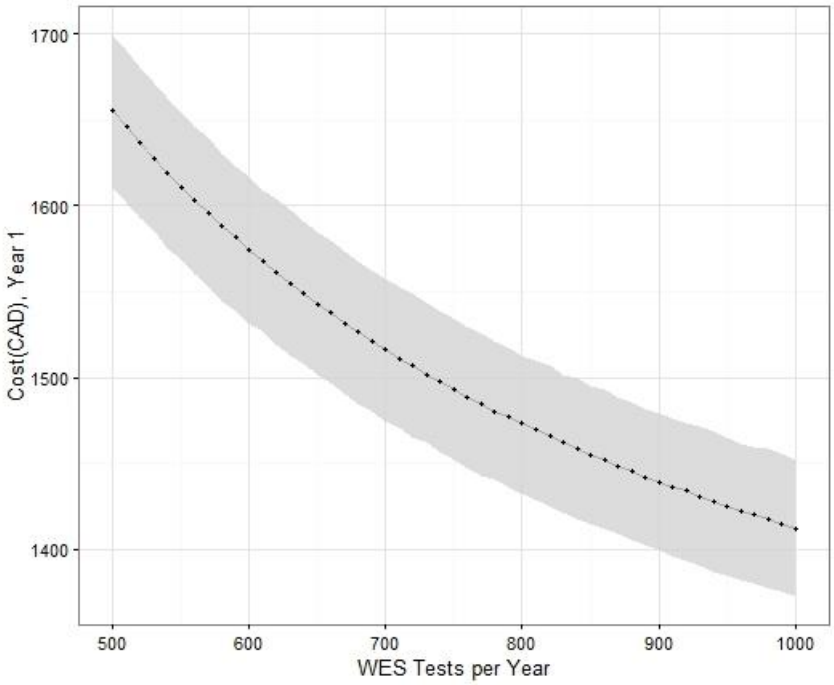
3.3 Deterministic sensitivity analysis

Figure 3 shows the effect of increasing the number of annual WES tests for all indications on ASD sample and program costs in Year 1. Due to economies of scale, the sample and program costs of WES decreased by 15% when the number of WES tests for all indications increased from 500 to 1000. Figures 4 and 5 show the effect of increasing the number of annual WGS tests for all indications on ASD sample and program costs in Year 1 for the HiSeq® 2500 and HiSeq X™ platforms, respectively. Increasing the number of tests for all indications from 500 to 1000, reduced the sample and program costs of WGS done on HiSeq® 2500 platform by 4%. The sample and program cost of WGS done on the HiSeq X™ platform declined by 13%. The relatively small cost reduction for WGS conducted on the HiSeq® 2500 platform was due to its equipment cost constituting a smaller part of total cost compared to the cost of supplies.

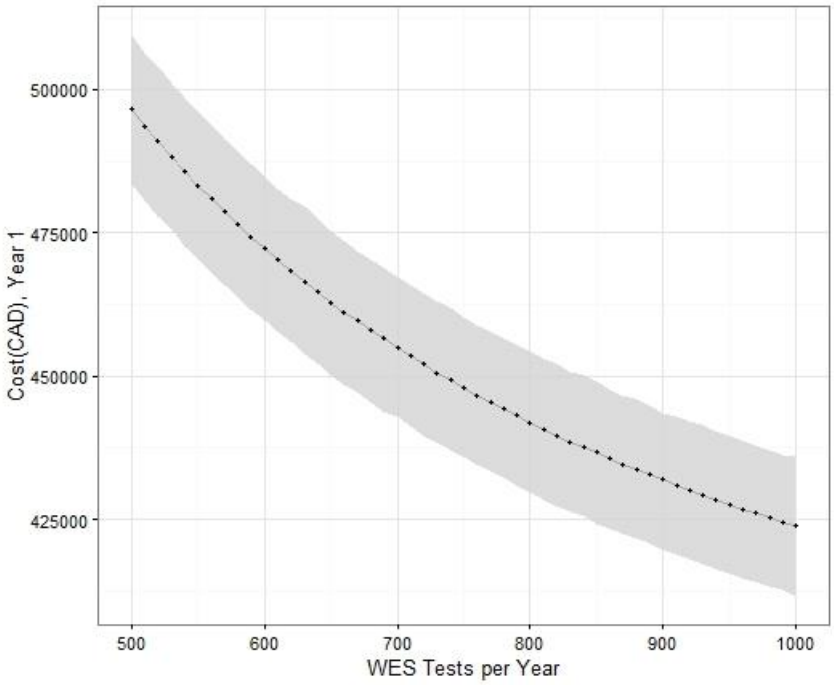
Tables 11 and 12 are summaries of deterministic sensitivity analyses that varied the overhead cost and the number of variants. The results were fairly robust to changes in overhead assumptions. Increasing the overhead cost to 30% led to a modest 1.7% increase in sample cost for CMA, 3.0% increase for WES, 1.3% for WGS (HiSeq® 2500) and 2.5% for WGS (HiSeq X™). Decreasing the overhead cost to 10% lead to a 2.9% decrease in sample cost for CMA , 5.7% for WES, 2.5% for WGS (HiSeq® 2500) and 4.8% for WGS (HiSeq X™). Compared to the base case value of two primary variants found, when the number of primary variants found was reduced to zero, the cost per sample of the WES test was reduced by 8.0% and the cost per sample for the WGS test was reduced by 2.9% for the HiSeq® 2500 platform and 5.6% for the HiSeq X™ platform. The cost increase when four variants were found instead of two was 9.6% for the WES, 2.9% for WGS (HiSeq® 2500) and 5.5% for WGS (HiSeq X™).

Figure 3. Deterministic sensitivity analysis of the effect of increasing the number of WES tests per year for all indications from 500 to 1000 on sample and program costs in Year 1.

(a) Estimated ASD sample cost in Year 1



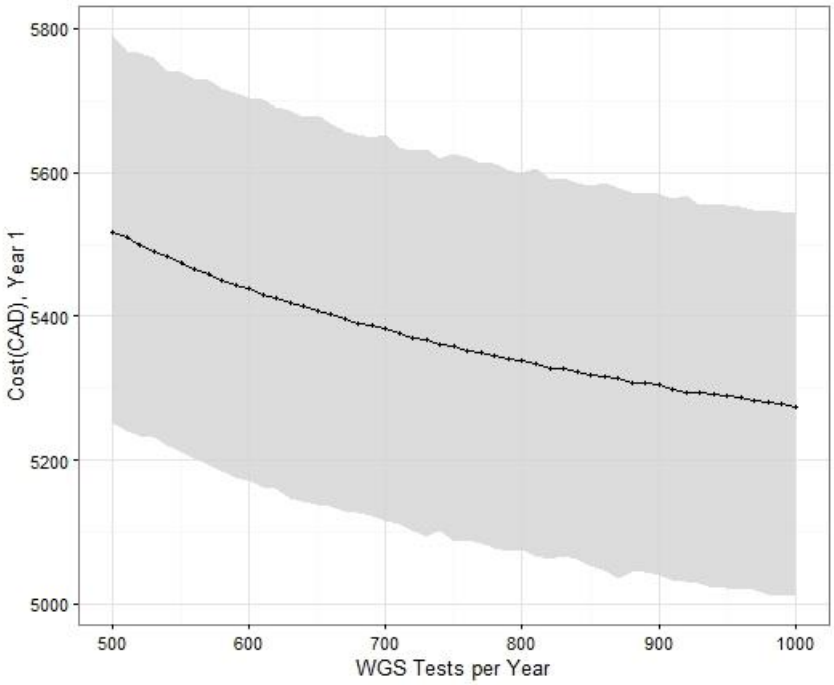
(b) Estimated ASD program cost in Year 1 (keeping the ASD tests constant at 300 per year)



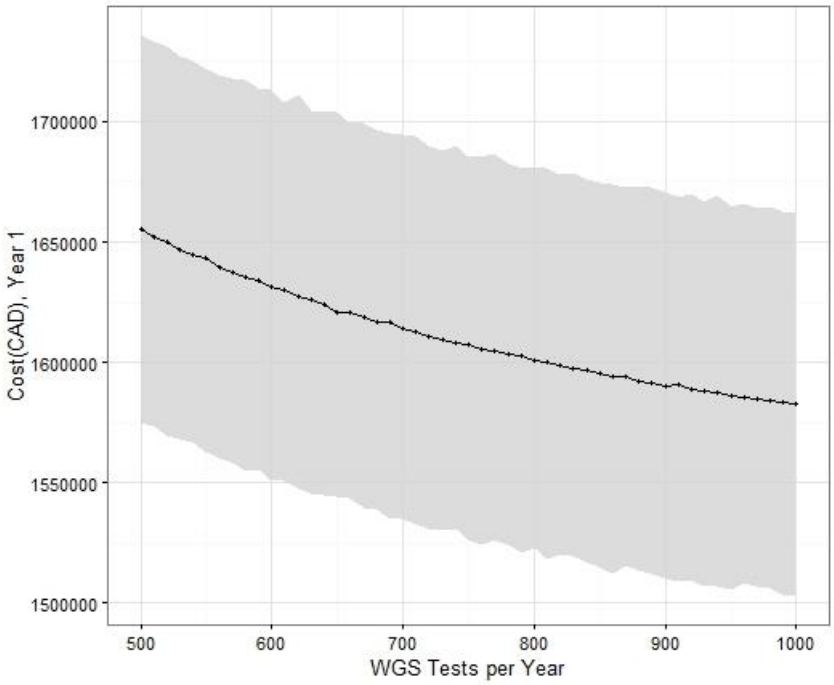
Costs are reported in 2015 CAD. Confidence bands are based on 10000 Monte Carlo replications.
Abbreviations: ASD, Autism spectrum disorder; WES, Whole exome sequencing

Figure 4. Deterministic sensitivity analysis of the effect of increasing the number of WGS (HiSeq® 2500) tests per year for all indications from 500 to 1000 on sample and program costs in Year 1.

(a) Estimated ASD sample cost in Year 1



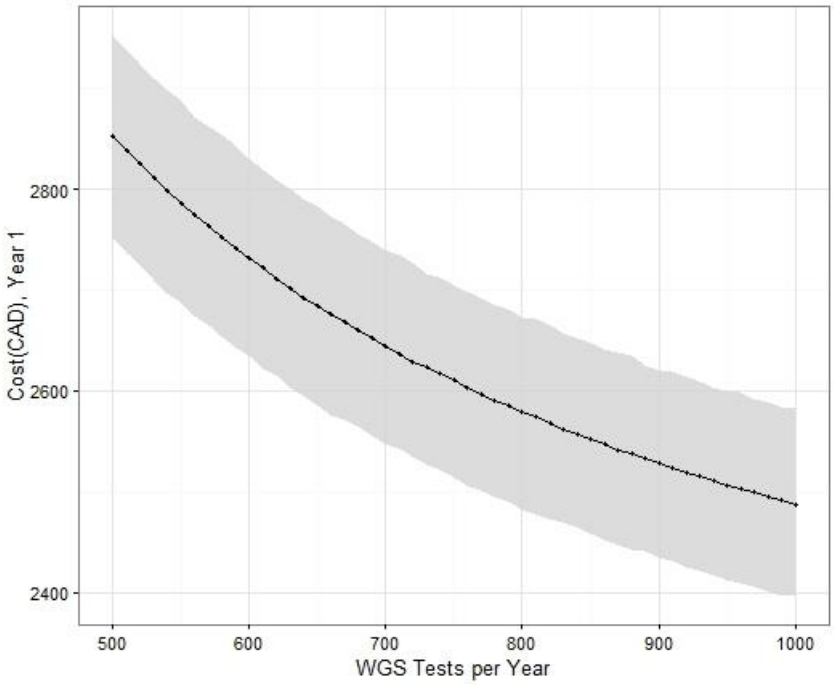
(b) Estimated ASD program cost in Year 1 (keeping the ASD tests constant at 300 per year)



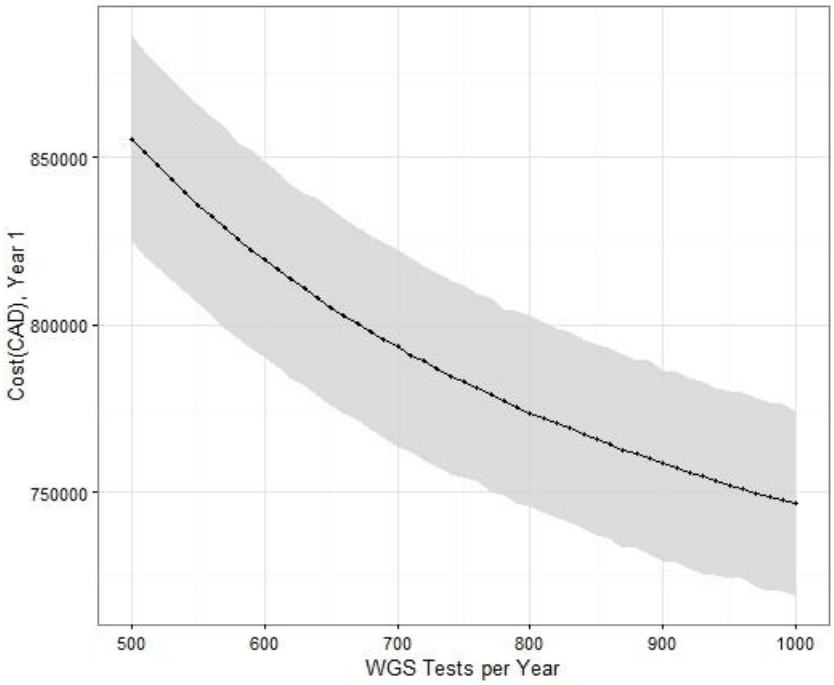
Costs are reported in 2015 CAD. Confidence bands are based on 10000 Monte Carlo replications.
Abbreviations: ASD, Autism spectrum disorder; WGS, Whole genome sequencing

Figure 5. Deterministic sensitivity analysis of the effect of increasing the number of WGS (HiSeq X™) tests per year for all indications from 500 to 1000 on sample and program costs in Year 1.

(a) Estimated ASD sample cost in Year 1



(b) Estimated ASD program cost in Year 1 (keeping the ASD tests constant at 300 per year)



Costs are reported in 2015 CAD. Confidence bands are based on 10000 Monte Carlo replications.
Abbreviations: ASD, Autism spectrum disorder; WGS, Whole genome sequencing

Table 11. Deterministic sensitivity analysis of estimated total cost per ASD sample for CMA, WES and WGS, varying the overhead cost proportion.

Overhead cost	Year 1 (95% CI)	Year 2 (95% CI)	Year 3 (95%CI)	Year 4 (95% CI)	Year 5 (95% CI)
CMA					
10%	721.3 (692.2, 750.1)	699.4 (671.3, 727.4)	678.3 (650.8, 705.4)	657.7 (631.1, 684.1)	637.8 (612, 663.4)
30%	755.7 (725.8, 785.1)	732.7 (703.7, 761.3)	710.4 (682.3, 738.1)	688.8 (661.4, 715.7)	667.8 (641.3, 694)
WES					
10%	1561.3 (1520.3, 1602.1)	1505.1 (1465.4, 1544.6)	1450.8 (1412.5, 1489)	1398.4 (1361.4, 1435.3)	1347.9 (1312, 1383.7)
30%	1705.2 (1659.7, 1750.3)	1642.8 (1598.9, 1686.4)	1582.6 (1540.3, 1624.9)	1524.6 (1483.6, 1565.5)	1468.5 (1429, 1508.1)
WGS, HiSeq® 2500					
10%	5383 (5117, 5645.7)	5215.5 (4957.4, 5470.3)	5053.2 (4802.7, 5300.7)	4895.8 (4652.6, 5136.4)	4743.4 (4507.3, 4977)
30%	5591.9 (5314.9, 5859)	5416.3 (5147.8, 5675.7)	5246.2 (4985.5, 5498.2)	5081.5 (4828.3, 5326)	4921.8 (4676.1, 5159.2)
WGS, HiSeq X™					
10%	2716.1 (2617.5, 2812.5)	2620.8 (2525.1, 2714)	2528.6 (2436.1, 2619.2)	2439.6 (2350.2, 2527.5)	2353.7 (2267, 2438.9)
30%	2924.8 (2821.4, 3031)	2820.4 (2720.4, 2922.8)	2719.6 (2623.2, 2818.5)	2622.2 (2528.9, 2718.1)	2528.3 (2437.9, 2621.3)

Estimates are given in 2015 Canadian dollars (CAD). Confidence intervals (CI) are based on 10000 Monte Carlo replications.

Abbreviations: ASD, Autism spectrum disorder; CMA, Chromosomal microarray analysis; WES, Whole exome sequencing; WGS, Whole genome sequencing

Table 12**.** Deterministic sensitivity analysis of estimated total cost per ASD sample for WES and WGS, varying the number of primary variants found.

No. of primary variants	Year 1 (95% CI)	Year 2 (95% CI)	Year 3 (95%CI)	Year 4 (95% CI)	Year 5 (95% CI)
WES					
0	1522.8 (1486.2, 1559.2)	1466.5 (1431.2, 1501.6)	1412.1 (1378.1, 1446)	1359.6 (1326.9, 1392.3)	1309 (1277.4, 1340.7)
4	1813.6 (1755.7, 1870.7)	1748.8 (1692.6, 1804)	1686.2 (1631.8, 1739.8)	1625.7 (1572.9, 1677.6)	1567.4 (1516.2, 1617.5)
WGS, HiSeq® 2500					
0	5362.6 (5099.6, 5627.1)	5194.4 (4938.9, 5450.8)	5031.5 (4783.3, 5280.8)	4873.6 (4632.6, 5115.5)	4720.7 (4486.7, 4955.3)
4	5678.8 (5404.8, 5952.5)	5501.4 (5235.3, 5767.2)	5329.5 (5071.1, 5587.6)	5163 (4912.2, 5413.6)	5001.6 (4758.2, 5244.8)
WGS, HiSeq X™					
0	2694.1 (2595.9, 2792)	2597.5 (2502.2, 2692)	2504.2 (2412.1, 2595.6)	2414.1 (2325, 2502.8)	2327.1 (2241, 2412.7)
4	3011 (2904.7, 3119.5)	2905.1 (2802, 3010.1)	2802.8 (2703.2, 2904.2)	2704 (2607.7, 2802.1)	2608.6 (2515.3, 2703.2)

Estimates are given in 2015 Canadian dollars (CAD). Confidence intervals (CI) are based on 10000 Monte Carlo replications.

Abbreviations: ASD, Autism spectrum disorder; CMA, Chromosomal microarray analysis; WES, Whole exome sequencing; WGS, Whole genome sequencing

3.4 Cost-consequence analysis

The review of the literature for papers reporting diagnostic yield in patients with ASD is summarized in Table 13. Of the twenty studies found, only studies that reported diagnostic yield for the ASD population were used in the cost-consequence analysis. Since the focus of this study is a clinical application of WES and WGS, only diagnostic yield for clinical variants was considered (i.e. pathogenic or likely pathogenic variants). For CMA, three such studies were identified. In the first study, Shen *et al.et al.* (8) recruited 933 patients aged 13 months to 22 years with a diagnosis of autistic disorder or PDD-NOS and performed CMA on 848 of them with a diagnostic yield of 7.0%. In the second study, McGrew *et al.* (7) estimated the diagnostic yield for CMA in a primarily pediatric practice for patients with confirmed diagnosis of autism to be 9.4%. Tammimies *et al.* (22) conducted CMA on 258 children diagnosed with ASD and estimated a diagnostic yield for CMA alone to be 9.3%. Of the three studies, Tammimies *et al.* study was most recent, published in 2015, therefore a diagnostic yield of 9.3% was adopted for CMA in the cost-consequence analysis. Tammimies *et al.* also conducted CMA and WES on 95 children diagnosed with ASD and reported a diagnostic yield of 15.8% for a combination of CMA and WES.

Currently, there are no studies that estimate clinical WGS diagnostic yield for children with autism. Yuen *et al.* (10) performed WGS on 85 quartet families with two ASD-affected siblings and reported a diagnostic yield of 42.4%. This yield includes variants of uncertain clinical significance and is not directly comparable to the diagnostic yield reported in the Tammimies *et al.* Based on Jiang *et al.* (16), it was assumed that WGS can detect 10% more single nucleotide variants missed by WES in clinical WGS application. Based on expert opinion, the hypothetical clinical WGS diagnostic yield can be calculated by adding 10% more variants to the diagnostic yield of a combination of CMA and WES, resulting yield of 17.38% (44). However, this calculation does not take into account non-coding variants, as well CNVs detected by WGS in addition to those detected by CMA. Therefore, 42.4% was still utilized in the study as a best case for the WGS diagnostic yield.

The incremental costs and incremental diagnostic yields for the three clinical scenarios for patients seen in Year 1 of the testing program are shown in Table 14. A ratio of incremental cost to incremental diagnostic yield was also calculated to determine the additional cost for every additional new pathologic variant finding above and beyond the standard comparator. For the first scenario, CMA+WES vs. CMA, the incremental cost was $1655 and the incremental diagnostic yield was 0.065. The incremental cost

per additional patient with a positive finding was $25459. For the second scenario, WGS (HiSeq® 2500) vs. CMA, the incremental cost to diagnostic yield ratio was $58959. The incremental cost per additional patient with a positive finding was reduced by more than half if WGS is performed on the HiSeq X™ platform, $26020. For the third scenario, WGS vs. CMA +WES, the incremental cost was $3121 for WGS done on the HiSeq® 2500 platform and $453 for WGS done on the HiSeq X™ platform. The incremental yield was estimated to be 0.016. Thus the incremental cost was $195056 for the HiSeq® 2500 platform and $28300 for the HiSeq X™ platform for every additional patient with a positive finding above and beyond the comparator.

If the diagnostic yield of WGS was 42.4%, the cost per additional patient with positive finding would decrease substantially. Comparing WGS with CMA, the incremental diagnostic yield was 0.331 and the incremental cost to incremental yield ratio decreased to $6367 for the HiSeq X™ platform and $14428 for the HiSeq® 2500 platform. For the third scenario, WGS vs. CMA+WES, the incremental diagnostic yield was 0.266. The incremental cost per additional patient with positive finding was estimated to be $11733 for the HiSeq® 2500 platform and $1702 for the HiSeq X™ platform.

Table 13. Summary of selected CMA, WGS, WES diagnostic yield studies in patients with neurodevelopmental disorders.

Citation	Sample Size	Indication	Age group	Inclusions/Exclusions	Definition of diagnostic yield	Diagnostic yield (%)
Stavropoulos *et al.* (2016)(23), WGS, Canada	100	Various, including DD	Pediatric	Inclusion: all patients who met standard clinical criteria for CMA	Diagnostic yield was reported as the proportion of individuals with variants related to the primary indication providing a molecular diagnosis. Variants of clinical significance were prioritized (pathogenic)	34 (95 CI: 25-44)
Yuen *et al.* 2015(10), WGS, Canada	170 85 quartet families	ASD	Pediatric	Exclusion: either of affected siblings had chromosomal abnormalities or fragile X mutation.	Diagnostic yield was reported as the proportion of quartet families where either of affected siblings had variants that fell into the following categories: Class I: Genes known to be involved in ASD; Class II: Genes that have been functionally implicated in ASD; Class III: Novel ASD-risk genes identified by a large-scale exome-sequencing study and meta-analysis from the Autism Sequencing Consortium; Class IV: Remaining mutations, classified as being associated with genes that are involved in known autosomal dominant neurodevelopmental disorders.	42.4
Taylor *et al.* 2015(45), WGS, U.K.	217	Various, including DD	Not specified	Inclusion: patients with Mendelian and immunological disorders with strong suspected genetic component and in whom previous genetic testing failed to identify any pathogenic variants	Diagnostic yield was reported as the proportion of patients with variants with high level of evidence of pathogenicity, classes A-C: Class A: Mutation found in a new gene for the phenotype, with additional genetic evidence (in unrelated cases) and/or functional data supporting causality; Class B: Mutation found in a gene known for a different phenotype, with additional genetic evidence and/or functional data supporting causality; Class C: Mutation found in a gene known for this phenotype.	21.0
Gilissen *et al.* 2014 (9), WGS, Netherlands	50	Severe ID (IQ < 50)	52% <10 years; 16% 10-20 years; 32% >20 years	Inclusion: patients who underwent genetic testing and in whom no molecular diagnosis was established	Diagnostic yield was reported as the proportion of patients for whom conclusive diagnosis was achieved. Variants were classified as mutations in known ID gene and disruptive or predicted to be pathogenic and mutations in candidate ID and disruptive or predicted to be pathogenic, as well as showing a functional link.	42.0
Soden *et al.* 2014 (46),	119	DD, ID, cerebral	Pediatric	Inclusion: Families with one or more	Diagnostic yield was referred to as the proportion of families with a molecular diagnosis. Rare variants were	45.0

WGS/WES, U.S.		palsy and ASD		children suspected of having a monogenetic disease, but without a definitive diagnosis.	evaluated for pathogenicity using ACMG guidelines. Potentially pathogenic variants identified in candidate disease genes were not included in molecular diagnosis, unless validated.	
Jacob *et al.* 2013(47), WGS, U.S.	25	Various	23 pediatric and 2 adult	Not specified	Diagnostic yield was referred to as the proportion of patients with definitive diagnosis. ACMG guidelines were used to classify pathogenicity of variants.	27.0
Tammimies *et al.* 2015(22), WES/CMA, Canada	258	ASD	Mean age ± SD = 4.5 years ± 2.8 years	Inclusion: children referred to developmental pediatric clinic with ASD diagnosis	Diagnostic yield was referred to as the proportion of patients with clinically significant results. Prioritized variants were classified as clinically significant (pathogenic or likely pathogenic) according to the ACMG guidelines.	CMA: 9.3 (95% CI: 6.1-13.5) WES: 8.4 (95% CI: 3.7-15.9) CMA+WES: 15.8 (95% CI: 9.1-24.7)
DDD Study 2015 (48) WES/CMA, U.K.	1133	Severe develop. disorders (inc. ID, DD)	Pediatric with a median age of 5.5 years.	Inclusion: patients with severe undiagnosed neurodevelopmental disorders and/or congenital abnormalities	Diagnostic yield was the proportion of patients with probable pathogenic variants in robustly implicated developmental disorder genes or with pathogenic deletions or duplications.	31.0
Srivastava *et al.* 2014(49), WES, U.S.	78	Neuro-develop. Disorders (DD, ID, cerebral palsy and ASD)	Pediatric patients with mean age of 8.6+/- 5.8 years	Inclusion: patients with a variety of neurodevelopmental disorders, with diagnostically unrevealing prior genetic and metabolic testing	Diagnostic yield was reported as the proportion of patients for whom molecular diagnosis was made (patients with pathogenic or likely pathogenic variants). Pathogenic variant was defined as a variant in a gene associated with the patient's phenotype that has been previously reported as a disease-associated mutation. Likely pathogenic variant was defined as a novel variant that is likely deleterious in a gene previously linked to the patient's phenotype.	41.0
Atwal *et al.* 2014 (50), WES, U.S.	35	Various, including DD and CMA	Not specified	Inclusion: patients seen in medical genetics clinic and by medical geneticists.	Diagnostic yield was reported as the proportion of patients for whom causal gene mutation was identified (i.e. pathogenic and disease causing variants).	22.8
Yang *et al.* 2014(19), WES, U.S.	2000	Neurologic al plus other	45.0%: <5 years of age; 42.2% 5 to 17 years of age;	Inclusion: Patients were referred from physician for clinical WES. The request for	Diagnostic yield was reported as the proportion of patients with a molecular diagnosis. WES case was classified as molecularly diagnosed if pathogenic or likely pathogenic variants were detected in Mendelian	Neurological: All ages: 27.2 (95% CI:23.5-31.2) <5 years: 30.4

		organ systems	12.2% adults; 0.6% fetal samples	WES was based on physician's discretion with no inclusion/ exclusion criteria by the lab	disease genes that overlapped with described phenotypes of the patients, and for recessive disorders if the variants were on both alleles of the same gene. The pathogenicity of variants was assessed using ACMG guidelines.	(95% CI:24.3-37.3) 5-18 years: 26.1 (95% CI:21.1-31.9)
Lee *et al.* 2014 (21), WES, U.S.	814	Various, including DD	64% children	Inclusion: Patients were referred for WES from clinic or referring physicians. Most cases were had substantial inconclusive prior genetic investigation	Diagnostic yield was reported as the proportion of patients for whom a conclusive molecular diagnosis was made (cases with identified causative variant in a well-established clinical gene; primarily pathogenic and likely pathogenic variants). The pathogenicity of variants was determined using ACMG guidelines.	DD+ASD (Trio): All: 21 (95% CI: 12-35) <5 years: 25 (95% CI: 11-47) 5-18 years: 17 (95% CI: 6-38)
Yang *et al.* 2013 (18), WES, U.S.	250	Neurological and neuro-logical plus other organ systems	50% < 5 years; 38% 5-18 years; 11% adults; 2% fetal samples from terminated pregnancies	Inclusion: patients were referred for WES by the patient's physician	Diagnostic yield was reported as the proportion of patients for whom molecular diagnosis was made based on the diagnostic criteria. Confirmed variants were required to have occurred in genes in which mutations had been previously reported to cause disease with a presentation consistent with that observed in the patient. Rare variants were classified using the ACMG guidelines.	Neurological disorders: 33 (95% CI: 23 46)
de Ligt *et al.* 2012 (20), WES, Netherlands	100	Severe ID (IQ < 50)	37% < 10 years; 41% 10-20 years; 22% > 20 years	Inclusion: patients with unexplained severe ID with no diagnosis using genetic testing and metabolic screening	Diagnostic yield was reported as the proportion of patients for whom molecular diagnosis was made. A case was classified as molecularly diagnosed if (1) pathogenic variants in known ID genes (published literature) were detected or (2) pathogenic variants in candidate ID genes (identified using in-house database) were detected and the mutated gene showed a functional link to ID. Pathogenicity of variants was evaluated based on exiting guidelines.	16.0
Henderson *et al.* 2014 (6), CMA, U.S.	1780	DD, ID, seizures, ASD	Median age of with abnormal CMA=4.7 years	Not specified	Diagnostic yield was reported as the proportion of patients with abnormal CMA results. Cases with variants of uncertain significance were not included.	12.7
Roberts *et al.* 2014 (51)CMA, U.S.	215	ASD and learning disability	Mean age ± SD = 10 years ± 9.7 years; age range = 5	Inclusion: ASD or learning disability patients referred for genetic services	Diagnostic yield was reported as the proportion of patients with variants that fall into either of the following categories. Cases with abnormal CMA findings were categorized into (1) diagnostic CNV if the variant was previously reported to be associated with	ASD: 20% (Inc. variants of unknown significance) [9% diagnostic variants]

			months to 52 years	Exclusion: recognized syndrome such as Down syndrome, fragile X syndrome, or single gene disorders	ASD or learning disability and (2) non-diagnostic variant or variant of unknown significance.	
McGrew *et al.* 2012 (7), CMA, U.S.	85	ASD	Pediatric	Inclusion: Patients with diagnosis of autism.	Authors reported the following: (1) proportion of with abnormal CMA results, which included clinically significant variants, likely clinically significant variants or variants of unknown significance based on lab interpretation and literature review; (2) proportion of patients with abnormal CMA result classified as clinically significant or likely clinically significant.	Abnormal (clinically/likely clinically significant): 9%
Coulter *et al.* 2011 (5), CMA, U.S.	1792	DD, ID, ASD, MCA	Pediatric	Exclusion: Patients with known or suspected diagnosis of Down syndrome.	Authors reported diagnostic yield for patients with abnormal variants or variants of possible significance. CMA variants were classified as (1) abnormal, (2) variants of possible significance, (3) variants of unknown significance, (4) reported copy number variants (normal/benign) (see guidelines).	13.1
Shen *et al.* 2010 (8), CMA, U.S.	933	ASD	Age at diagnosis ranged from 13 months to 22 years.	Inclusion: patients with autism diagnosis.	Diagnostic yield was reported as the proportion of patients with variants classified as abnormal (variants associated with known genomic disorders or variants of possible significance). Variants of unknown significance were not included in the calculation of diagnostic yield.	7.0 (95% CI: 5.5 - 8.5)
Miller *et al.* 2010 (1), CMA, Various (systematic reviews)	21698	DD, ID, ASD, MCA	Not specified	Inclusion: patients with unexplained developmental delay, ID, ASD or MCA.	Diagnostic yield was derived from each study and reported as the proportion of patients with abnormal variants. Variants of unknown significance were not included in the reported diagnostic yield. CNVs are interpreted as (1) abnormal (e.g. well-established syndromes, de novo variants and large deletions); (2) variants of unknown significance; (3) likely benign.	12.2
Schaefer *et al.* 2010 (52), CMA, U.S.	68	ASD	Not specified, possibly pediatric	Inclusion: Patients with ASD referred for CMA.	Proportion of patients with abnormal (or clinically significant) copy number variants (14 of 68 patients).	22.0

Abbreviations: DD, Developmental delay; ASD, Autism spectrum disorder; ID, Intellectual disability; MCA, Multiple congenital anomalies; CNV, Copy number variant; SNV, Single nucleotide variant; CMA, Chromosomal microarray analysis; WGS, Whole genome sequencing; WES, Whole exome sequencing; ACMG, American College of Medical Genetics and Genomics

Table 14. Estimated total annual incremental cost per ASD sample, estimated incremental diagnostic yield and estimated incremental cost per additional patient with a positive finding, Year 1.

Scenario	Incremental sample cost (CAD) (95% CI)	Incremental diagnostic yield (diagnosis rate)	Incremental ratio (CAD/diagnosis rate)
1. CMA+WES vs. CMA	1654.8 (1611, 1698.5)	0.065	25458.5
2. WGS vs. CMA			
2.1 WGS (HiSeq® 2500) vs. CMA	4775.7 (4499.2, 5042.6)	0.081	58959.3
2.2 WGS (HiSeq X™) vs. CMA	2107.6 (2002.9, 2215.2)	0.081	26019.8
3. WGS vs. CMA+WES			
3.1. WGS (HiSeq® 2500) vs. CMA+WES	3120.9 (2841.6, 3392.1)	0.016	195056.2
3.2. WGS (HiSeq X™) vs. CMA+WES	452.8 (339.2, 570.2)	0.016	28300.0

Estimates are given in 2015 Canadian dollars (CAD). Confidence intervals (CI) for incremental cost are based on 10000 Monte Carlo replications.
Abbreviations: ASD, Autism spectrum disorder; CMA, Chromosomal microarray analysis; WES, Whole exome sequencing; WGS, Whole genome sequencing

4 Discussion

In this study, the sample and program costs of CMA, WES and WGS genetic tests for children with ASD were estimated. WGS using older technology (HiSeq® 2500) was the most expensive test, costing almost three times as much as WES and seven times as much as CMA. The new technology using the HiSeq X™ platform reduced the cost of WGS test by 48%. Labour costs were reduced for HiSeq X™ due to improved automation and streamlining of sample processing. Overall, supplies, followed by equipment and labour, constituted the largest proportion of the total cost for all three tests. WGS displayed the highest supply costs due to the greater consumption of costly reagents required for sequencing in WGS compared to WES. Bioinformatics costs were substantially higher for WGS than for WES, due to greater computing demands in WGS. Equipment, supplies and bioinformatics were the largest contributors to cost differences between WES/WGS and CMA. The costs of WES and WGS were also high relative to CMA in part due to the requirement to perform validation testing in the proband (e.g. Sanger sequencing) on all positives and equivocal findings to rule out false positives. As the technology further evolves and improves, the need for validation testing should be reduced.

The precise positioning of CMA, WES and WGS in the diagnostic pathway for ASD and other pediatric conditions is not yet known. CMA is useful for detecting microdeletions and duplications which cannot be detected by WES, although these can be detected by WGS (30). The bioinformatics pipelines for both WES and WGS are still in development and WGS is currently mainly perceived as a research application. Variant discovery and linkage to phenotypes is proceeding at an astonishing rate however, creating pressure to introduce WGS into clinical practice (53). As variant discovery and phenotype linkage continues, it will overlap with the early stages of clinical implementation, necessitating frequent updates to microcosting and diagnostic yield estimates.

In addition to CGES, the pipeline of CMA continues to evolve and improve. Which test or combination of tests might ultimately replace older technology remains an open question. In the present analysis, alternative scenarios are presented as complete substitutions, e.g. combination testing with CMA plus WES for all patients replacing CMA alone, or WGS replacing CMA. This approach would be very costly, as the cost-consequence analysis revealed an *incremental* cost of over $25000 for every additional patient with a pathologic variant beyond expected CMA results if CMA were to be wholly replaced by CMA+WES or by WGS with our current knowledge of diagnostic yield. In reality the testing pathway is likely to be more complex, where, for example, only syndromic patients with a negative first line test (CMA) go on to receive a second line test such as WES. Another more cost-effective option may be to target newer sequencing technologies to high risk infant siblings of children already diagnosed with ASD, in whom a higher diagnostic yield is expected (54). The precise sequence and type of serial testing will vary with the patient population, the anticipated diagnostic yields as well as the cost of testing. It is also likely to vary, at least in the short-term, between clinical practitioners. Practice variation in genetic test ordering between clinicians makes it difficult to determine the potential for savings through the avoidance of older generation genetic tests. It is hoped that as CGES becomes more established in clinical practice, test ordering protocols that prevent the ordering of superfluous tests will be implemented. It must also be recognized that introduction of CGES may lead to more cascade genetic testing in family members, further increasing costs. As the variant discovery research continues, rigorous criteria for family member testing must also be developed, so that testing is limited to detection and validation of phenotypically deleterious variants.

Other published studies have looked at the cost of CMA and WES. The estimated cost per sample of the CMA test was comparable to estimates reported in the literature. Trakadis and Shevell (2010) (55) reported the cost of microarray to be approximately $682 CAD (2010) for children with global development delay based on the local experience at the CHU Hospital Sainte-Justine in Montreal. The authors also reported the Signature Genomics (Spokane, WA, USA) microarray fee of 1650 CAD (2010) and the GeneDx (Perry Parkway, Gaithersburg, MD, USA) microarray fee of 1595 CAD (2010). Woodworth *et al.* (2007) (56) estimated the cost of CMA for diagnosis of idiopathic learning disability using data from four participating genetic centres in United Kingdom to be 442 £ (2006) (924 CAD, 2006), using the average 2006 exchange rate of 2.09 between £ and CAD (57)). Regier *et al.* 2010 (58) reported a cost of microarray testing of 710 CAD (2007/2008) from the Cytogenetics Laboratory at the British Columbia Children's Hospital for a decision analytic model of diagnostic testing for genetic causes of intellectual disability in children.

As these tests are still early in the clinical translation pathway, studies that provide estimates of WES or WGS costs are limited (14, 30). Towne *et al.* (2013) (59) reported an approximate trio-WES cost of 3700 USD per family in a conference abstract and Wright *et al.* (2013) (60) noted that WGS costs approximately 6000 £ ($9 660 CAD, 2013) and WES costs approximately 200-500 £ (322-805 CAD, 2013). Neither study provided a breakdown of costs that were included in these estimates. Monroe *et al.* (2016) (61) examined the use of WES in patients with intellectual disability and estimated the cost of trio-WES to be 3972 in 2014 US dollars (4409 CAD, 2014). The estimate included the costs of patient registration and blood draw, DNA isolation, sample preparation, exome enrichment, sequencing on an Illumina HiSeq® 2500, interpretation, reporting of results, data storage and infrastructure. Monroe *et al.* also calculated the costs that could potentially be saved by replacing the standard genetic and metabolic testing with WES as a first diagnostic approach. On average, WES was found to save 3547 USD (3937 CAD) per patient who receives a diagnosis and 1727 USD (1917 CAD) for patients who do not receive a diagnosis using WES.

While examining isolated test costs as well as institutional program costs are necessary prerequisites to full economic evaluations, studies that examine costs to a health region or jurisdiction are also necessary, especially if the workflow is segmented. For example, regional centralization for certain steps, such as the sequencing, computing and data storage may increase efficiency and reduce costs to

the health care system compared to relying on individual institutional providers (34). Indeed, as demand for CGES grows, health regions may form partnerships to offer a CGES service to their regional population. While introducing a CGES service may involve substantial start-up costs, savings could be realized through large scale purchasing contracts, although this may entail overhead and administrative costs as well as transaction fees.

This study focused on developing a comprehensive and accurate test cost, with full recognition that the greatest source of increased costs to the healthcare system may lie not in the tests themselves, but in the referrals that ensue as a result of positive findings. Currently, national organizations in the US, Canada and the UK have developed or are in the process of developing guidelines to recommend which primary medically actionable variants should be reported, and the extent to which incidental or findings of unknown clinical significance should be reported (24, 34). Interestingly, the brief literature review performed for this study to determine diagnostic yields for genetic testing in ASD revealed a range of classification systems and definitions of primary variants (table 13). While an "abnormal" finding was often specified as a primary variant, this was not always clearly defined. In addition to agreement on variant classification, it's clear that lists of reportable findings in guidelines will require frequent updating. These lists are expected to grow as our understanding of the genetic basis of disease and risk of disease grows (53). Where the line is drawn with regard to reporting requirements will have a profound effect on queues for specialist consultations and health system costs (62, 63). It is important therefore that guidelines recognize the impacts of reporting requirements on the health care system, as well as on patients and their families.

The study has several strengths. All stages and costs involved in the workflow of CMA, WGS and WES were accounted for using the microcosting approach generating the first fully comprehensive per sample and program cost estimates of CGES. The provision of estimates for two different WGS platforms increased the generalizability of the findings and its value for decision-makers. Uncertainty associated with parameter estimates was captured in the probabilistic sensitivity analysis using Monte Carlo simulations. Parameters that were highly uncertain or expected to vary substantially between institutions were varied in the deterministic sensitivity analysis demonstrating robustness of the results to changes in assumptions regarding overhead costs and the number of variants found. Predicting costs and volumes of use before a technology has been clinically established presents with certain challenges.

This study showed how the economies of scale can be realized to reduce sample costs as the number of total CGES tests increase, in advance of full implementation. The study also showed where cost savings can be realized. For all three tests, a decrease in the cost of supplies would result in a substantial decrease in the total sample and program costs. Although the estimates in this report are for an ASD patient population, the microcosting model was deliberately constructed to be flexible and easily adapted to other patient populations by simply varying the number of primary variants and the volume of testing in the institution.

There are several limitations to the study. WES has only very recently been implemented in clinical use and WGS is currently a purely research application. The WGS costs were calculated as expected costs in a clinical setting based on WES microcosting and expert opinion, rather than by costing the research application or by applying charges from an external service provider. Thus the actual costs of WGS once clinical testing is introduced may diverge from the predicted estimates. The cost estimates did not include training of technical and lab personnel, or implementation costs. These could be considerable, especially in early generations of a technology experiencing rapid evolution. The cost estimates were based on only one institution. Since CGES is done in very few hospitals in Canada and since the focus of the study is a bottom-up microcosting approach, this precluded using a panel of experts to estimate parameters. The same expert was often used for different resource use and price estimates. However, there was no evidence for any specific form of correlation between responses and independence was assumed. Briggs *et al.* (2002) (64) suggested that the gamma distribution should be used for resource use parameters and the normal distribution should be used for unit cost (price) parameters. In this study, there was not enough information to use the gamma distribution and as a result, the normal distribution was used for both resource use and price parameters.

For most of the price parameters, a range of 10% was not based on an expert opinion, but instead chosen to reflect potential price and currency fluctuations. Nevertheless, this range was within the variation for other parameters reported by experts. A five-year time horizon was chosen based on a projected shelf-life for the sequencing equipment, and because procurement decisions for large equipment can be based on a 5-year budget plan. In reality, the life cycle for sequencers may be shorter due to rapid evolution of the sequencing technology. A shorter life cycle would result in higher costs due to a shorter period of amortization.

Another limitation is the fact a diagnostic yield for clinical WGS has not yet been estimated and a hypothetical yield was used in this study. Therefore, caution must be exercised when interpreting the incremental ratios. A full economic evaluation needs to be undertaken where the test costs and yields are preferably obtained from the same ASD population.

This study is the first to estimate the cost of whole exome and whole genome sequencing using a bottom-up microcosting approach. Additional research is required to assess the impact of CGES on the pathway of care for children with ASD and to measure ultimate improvements in health outcomes as a result of testing. The cost estimates generated in this study can be used in future health technology assessments that investigate the cost-effectiveness of CGES in the developmental delay and autism population. It is essential that programs of health services and policy research that perform such studies are executed in tandem with translation of CGES into clinical practices to generate evidence to inform institutional and provincial health policy decision-makers (65).

5 Conclusion

There is a lack of research on the cost-effectiveness of clinical genome and exome sequencing. An economic evaluation of genomic sequencing technologies requires a comprehensive and accurate estimation of all costs involved in the sequencing workflow. For cases presenting with positive phenotypes for developmental delay or autism spectrum disorder, clinical genome and exome sequencing are promising tools for demonstrating genetic causality, due to higher diagnostic yield compared with the standard of care, chromosomal microarray. In this study, the costs of CGES per ASD sample were $1655 (95% CI: 1611, 1699) for WES, $2851 (95% CI: 2750, 2956) for WGS on Illumina HiSeq X™ platform and $5519 (95% CI: 5244, 5785) for WGS on the Illumina HiSeq® 2500 platform, compared to $744 (95% CI 714, 773) for CMA. HiSeq® 2500 Reagent supply costs accounted for the largest proportion of costs for each type of CGES. Using recent diagnostic yield literature, a cost-consequence analysis revealed an incremental cost of over $25000 over and above current CMA test costs for every additional patient with a pathologic variant not found on CMA if CMA were to be wholly replaced by CMA+WES or by WGS. This suggests that based on current costs and diagnostic yields, substitution of CMA would not be cost-effective in ASD. Rather, WES or WGS may be reserved as second line testing for negative or equivocal patients, or used in target populations with high rates of suspected ASD, such as infant siblings of confirmed cases. As the costs of testing continues to decrease while

diagnostic yields of CGES in ASD increase, the willingness of decision-makers to pay for each additional pathologic variant found will influence whether CGES represents good value for money. This study provides comprehensive cost for use in future economic evaluations of clinical genome and exome sequencing in ASD, and allows for a costing model that can be easily adapted to other pediatric patient populations.

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