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

FULL REPORT

A MICROCOSTING AND COST-CONSEQUENCE ANALYSIS OF GENOMIC TESTING STRATEGIES (INCLUDING TRIOS) IN AUTISM SPECTRUM DISORDER: AN UPDATE

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TASK Technology Assessment at SickKids

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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
CGES	Clinical genome and exome sequencing
CIHI	Canadian Institute for Health Information
CMA	Chromosomal microarray analysis
CNV	Copy number variant
DD	Developmental delay
DPLM	Department of pediatric laboratory medicine
DSA	Deterministic sensitivity analysis
FISH	Fluorescence in situ hybridization
GATK	Genome Analysis Toolkit
GE^3LS	Genomics and its ethical, economic, environmental, legal, and social aspects
HTA	Health technology assessment
ID	Intellectual disability
MCA	Multiple congenital anomalies
MIS	Management Information Systems
MOHLTC	Ontario Ministry of Health and Long-Term Care
PA	Probabilistic 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 ASD. It is not yet clear precisely how the value of CGES technologies can be maximized in a diagnostic pathway 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 report update is to estimate costs associated with CMA, whole exome sequencing (WES) and whole genome sequencing (WGS) (proband and trio) 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 and NextSeq® 550 platforms) and for WGS (probands and trios) on the Illumina HiSeq X™ platform for pediatric patients with ASD were estimated. This was done 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 pediatric population were also estimated over five years. A probabilistic analysis (PA) 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 (probands and trios) in ASD. A scenario analysis was also conducted to address a hypothetical, best case scenario of diagnostic yield for WGS-proband. This was varied in each test scenario.

Results

The cost per ASD sample in Year 1 was $1960 (95% CI: 1899, 2020) for WES (HiSeq® 2500), $1981 (95% CI: 1909, 2054) for WES (NextSeq® 550), $3350 (95% CI: 3234, 3467) for WGS-proband (HiSeq X™) and $6556 (95% CI: 6278, 6832) for WGS-trio (HiSeq X™) compared to $825 (95% CI: 789, 859) for CMA. Reagent supply costs accounted for the largest proportion of costs for each type of test. The total institutional program cost to offer CMA for ASD diagnosis over five years was $1.16 million (95% CI: 1.11, 1.21) compared to $2.73 million (95% CI: 2.65, 2.82) for WES (HiSeq®2500), $2.79 million (95% CI: 2.69, 2.89) for WES (NextSeq® 550), $4.68 million (95% CI: 4.52, 4.85) for WGS-proband (HiSeq X™) and $27.78 million (95% CI: 26.59, 28.95) for WGS-trio (HiSeq X™) based on 300 ASD cases per year. The ratio of incremental sample cost to incremental diagnostic yield ranged from $30,154 for CMA plus WES (HiSeq®2500) vs. CMA to $105,349 for WGS-trio (HiSeq X™) vs. CMA plus WES (HiSeq®2500). There is a substantial variation in the ratio depending on the diagnostic yield. For the WGS vs. CMA plus WES scenario, the ratio varied from $34,506 to $105,349. If the WGS diagnostic yield was 42.4%, the cost per additional patient with a positive finding decreased substantially. If WGS-proband replaced CMA, the ratio decreased to $7,630. For WGS-proband vs. CMA plus WES, the incremental sample cost per additional patient with a positive finding was $2,127 for WES- HiSeq® 2500 and $2,049 for WES-NextSeq®550.

Conclusions

This study estimated the cost of trio genome sequencing, in addition to the evaluation of proband through both WES and WGS, using a bottom-up microcosting approach in a clinical paradigm. In contrast, previous study investigated probands only in genome analysis. WGS-trio (HiSeqX™) was the most expensive test, costing almost two times as much as WGS-proband (HiSeq X™), over three times as much as WES on both platforms and almost eight times as much as CMA. The new technology using the NextSeq® 550 platform reduced the cost of WES test only by 1%. Labour and large equipment costs were reduced for the newer platform while the reagent cost increased. Overall, supplies constituted the largest proportion of total cost for all three tests. A cost-consequence analysis revealed a cost of over $30,000 per additional patient with a positive finding if CMA were to be replaced by CMA plus WES or by WGS proband or trio. 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 CMA 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, 5-9]. 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 [10-14] 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 [15, 16]. 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 [15-17].

Traditionally, WGS has been conducted with probands (patients) only, with follow-up testing extended to include the two biological parents in addition to the probands. However, the use of trio testing is on

the rise for both WES and WGS. Trio includes the proband and two biological parents for the purposes of testing. This sequencing method enhances the speed and likelihood of accurate diagnosis by decreasing the number of candidate variants [18]. It reduces the need for follow-up tests, such as Sanger sequencing and results in shorter filtration and prioritization time and therefore costs. Furthermore, trio sequencing provides clinical sensitivity associated with the interpretation of novel genes in addition to the increased diagnostic utility. Diagnostic rate can also be improved and the chance of missing a *de novo* mutation is reduced by tailoring medical reviews and cross-checking by geneticists/ genetic counsellors [19].

To date, studies in both research and clinical settings have focused primarily on WES, as WGS is more costly [20] and is farther behind WES in translation to clinical practice. In addition, trio sequencing is more costly compared to proband only sequencing analysis. The diagnostic yield of WES across the developmental disorders such DD, ID, ASD and speech delay is in the range of 8% to 33% [21-26]. There are fewer studies that report the diagnostic yield of WGS. The most recent WGS diagnostic yield estimates are 42% for ASD [11] and 42% for ID [10] and 34% for congenital malformations and neurodevelopmental delay in a research setting [27]. More recently, CGES was conducted on pediatric patients with one or more DD/ID-related phenotypes and included trio sequencing in the analysis [28]. 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 [29].

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 in the exceptional access program for approved physician requests for clinical WES, typically done through laboratories in the United States [30]. In 2017 the MOHLTC approved the use of clinical genome-wide sequencing in the exceptional access program for patients suspected to have a rare monogenic disease and for whom the results would impact clinical decision-making and care for the individual and/or family [31]. The use of clinical WGS has not yet been approved

for reimbursement by the Ontario government. It is however offered at the Centre for Applied Genomics (TCAG) at SickKids on the Illumina HiSeq X™ platform for research purposes only.

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 [32]. CGES may be useful in cases where traditional genetic tests are negative or inconclusive [10, 21]. 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.

The optimal positioning of CGES in a diagnostic pathway that maximizes value for money invested and how best to translate these technologies from research to clinical care [33, 34] is not yet clear. Health technology assessment (HTA) is concerned with the evaluation of emerging health care technologies including diagnostic tests. Typically referred to as GE^3LS (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 [34, 35].

In the last few years, economic evaluations of WES have become more common. Recently, a qualitative review summarized economic evaluations of four individual studies that analyzed WES (proband and trio) with clinical areas that included epilepsy and ID [36]. There has been some evidence emerging in the literature with respect to an accurate measurement of opportunity costs associated with the entire sequencing workflow [15]. 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. Two Australian studies had evaluated the cost-effectiveness of clinical exome sequencing in comparison to standard of care for monogenic disorders

[37, 38]. However, any evidence on comparative analysis involving WGS is yet to arise. 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 [15, 39]. While the laboratory costs of sequencing have decreased dramatically in recent years [40, 41],, there is paucity of studies that comprehensively estimate 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

Cost estimation and incremental analysis of CGES in comparison to CMA had previously been conducted. The first report of this kind was published in 2016 [42]. The current report updates and supersedes the 2016 report and includes the addition of trio WGS. A summary of the changes between the current report update and the 2016 report are listed in Appendix 1.

The primary objective of this report update is to estimate the precise cost per proband for CMA, WES and WGS and the cost per trio for WGS 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 [43] and the entire workflow process of a genetic test is tracked. The secondary objective 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

CMA, WES and WGS (proband and trio) have different work flow processes, with exome and genome sequencing exhibiting some similarities. Appendices 2-5 illustrate this technical pathway from sample processing/ specimen preparation to clinical interpretation & report writing, with their respective components.

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 for each component in the work flow process. This was done 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, where available. 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. In Ontario, WGS is primarily used for research purposes whereas WES is in transition from research into clinical practice. Currently, clinical CMA is performed at the Cytogenetics laboratory, operated by the Department of Pediatric Laboratory Medicine (DPLM) at SickKids. Clinical WES was introduced onsite by DPLM'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 SickKids patients for select patient cohorts, such as the Genome Clinic Research Project (2013-present), Cardiac Genome study (2016 - present), Complex Care study (2016- present) and NICU study (2016-2018). Until recently, WGS has been performed off site by a private provider and is presently available at TCAG. Data to date for genomic analysis has largely been based on probands. In the near future however, it is anticipated that additional resources will be dedicated to analyze trios.

A target population approach focusing on costs encountered as part of the referral and diagnostic pathway for children with ASD was selected. This approach more closely simulates the institutional costs for children with ASD referred for genetic testing as part of the ASD diagnostic pathway. 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, such as congenital anomalies (CA) and developmental delay (DD). This approach was undertaken in a separate analysis and is detailed in the update of the supplemental report (Report No. 2018-01) [42].

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 in Tables 3-7 and in Appendices 6-10. 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 (CIHI) *Standards for Management Information Systems in Canadian Health Service Organizations* ("MIS Standards")[44]. 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 estimates from different reporting years were used for costing of the individual items (2012 to 2016). If there were updates in unit prices, the most recently available figures were taken (2018). Otherwise, the prices as reported in the previous version of the report were assumed to stay the same based on the consultation with lab managers. For labour prices, unless 2018 prices were reported, previously available salaries were adjusted with an yearly increase of 1.5% with the exception of lab director salary which was adjusted with a total increase of 1.5% from 2015 to 2018. All prices were reported in Canadian dollars (CAD).

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
	Report writing	Filtration and triage
		Clinical interpretation
		Report writing
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 included	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 [45]. Resource use and unit prices were assumed to remain the same from year to year. The following cost items were patient population specific: total test volume in the institution, number of primary variants, number of secondary variants, filtration time for primary and

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.

The labour steps for each test were compared to the laboratory labour components in the 2016 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 [44]. 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 relevant laboratories of The Hospital for Sick Children.

For whole exomes, two sequencing platforms were compared, namely HiSeq® 2500 and the new addition, NextSeq® 550. Presently, there are two NextSeq® 550 in use at the DPLM. HiSeq X™ was used for the whole genome analyses of proband and trio. The price per HiSeq X™ instrument assumed an initial purchase of a minimum of five sequencers. Costs for each sequencer were calculated based on 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 of which 3143 tests were attributable to developmental delay, based on the 2013/14 fiscal year. These figures did not change in recent years. For ASD, the number of tests conducted was 300 per year (DJ Stavropoulos pers. comm. 2018). 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 [46], it was assumed that 300 genetic tests would be run per year for children with a clinical diagnosis of ASD. For trio, it was assumed that the WGS tests could vary from 1500 to 3000 per sequencer and was assumed to be 1500 (100% of all tests) in the reference case. Based on the prevalence of ASD stated above, it was assumed that for trio, it would be 900 genetic tests that would be run per year for children with ASD diagnosis.

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 20 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 as Genome Analysis Toolkit (GATK) is an open software with no associated licencing fee. Periodic validation, quality control and pipeline updating and testing were not included. Similarly, sharing of storage space was also not routinely captured and therefore was not included in the analysis.

Overhead costs comprise administrative and infrastructure costs. They were added to labour, large and small equipment and bioinformatics costs. It was not applied to supplies (including supplies used for follow up testing) as they are bought at retail price that includes markup. This in turn is already higher than the true opportunity cost and if overhead was applied, it would result in an overestimation of the costs. A query to MOHLTC's Ontario Case Costing Initiative returned an estimate of overhead costs for acute inpatients of top 50 CMG groupers, top 50 diagnoses and top 50 procedures for all age groups and for all case types in all hospitals in Ontario. The average Ontario overhead cost in 2016/2017 was 22.3% with a range of 15.8 to 35.1%. Hospital specific overhead costs for SickKids revealed the estimate to be 31.6%. Based on this information, the reference overhead cost case was assumed to be 22.3%, with a range of 15.8 to 31.6%.

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. Assumptions: Microcosting analyses.

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 • 22.3% overhead cost is assumed, ranging from 10 to 30% • 3% discount rate is applied to all items • 300 tests for patients (probands) with ASD and 900 tests (300 x 3) for trios (probands and parents) with ASD are conducted each year at the institution
CMA	• Overhead cost is applied to labour and large equipment • Follow-up testing includes qPCR and FISH • 3948 tests are conducted each year of which 3143.26 tests are attributable to developmental delay and 300 are attributable to ASD • Small equipment costs are negligible
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, with the exception of trio for which the maximum cases is assumed to be 3000 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 • Filtration time to analyze ~300-400 variants in order to flag variants of interest is 60 minutes for WES and WGS-proband (55 minutes for primary variants and 5 minutes for secondary variants). For WGS-trio, it is 30 minutes (25 minutes for primary variants and 5 minutes for secondary variants) • Interpretation time per ASD test variant (primary) is 30 minutes. For secondary variants, it is 20-40 minutes (both proband and trio) • Report writing time is 15 minutes for primary variant and 20-40 minutes for secondary variant (both proband and trio) • High performance computing cluster maintenance time is 1 hour/ year/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) • Each compute node has a warranty of 5 years (3 years with purchase and 2 years of extra purchase of warranty)

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 6. The inputs can be classified into the following categories: specimen preparation, DNA extraction, microarray sample processing, analysis and clinical interpretation and report writing. Half of the CMA labour inputs corresponded to the MIS Standards' inputs. The labour time per sample for each 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 salaries were assumed to vary by 20% from their point estimates.

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 22% of the platform price (Appendix 6). 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. Relative to the large volume of CMA samples run, small equipment costs were negligible and were excluded.

2.4.1.3 Supplies

Supplies included cost of shipping of a sample to the lab and cost of scanner consumables (Appendix 6). 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 sample. Ranges for the resource use of these items were not provided. The unit price of shipping and handling of blood samples was obtained from FedEx. The unit price of scanner consumables was provided by an Affymetrix representative at a volume discount of 19.3%. 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. FISH was varied by 5% and 15% whereas qPCR was varied by 3% and 10% from their corresponding point estimates. 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.

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 Appendices 7 and 8. TCAG is an in-house SickKids core genomics facility that conducts WES and WGS for research purposes and only conducts data analysis. Clinical interpretation and report writing were modelled as two separate steps. 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 [47].
The only set of WES inputs that corresponded to the MIS Standards are the inputs for 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, filtering and triage, 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 number of sequencing steps for HiSeq® 2500 platform was five whereas NextSeq® 550 only required two steps. 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 bioinformatics analysis of sequenced data performed at TCAG and for the maintenance of 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. Exome output per month for HiSeq® 2500 was 70-95 and for Next Seq® 550, it was 64-96. For both platforms, variant calling requires one FTE unit of labour and annotation requires 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 exome output per month, 83 exomes and 80 exomes for each of the platforms respectively.

For the bioinformatics maintenance component, following labour steps were defined: alignment (BWA), mark duplicates (PICARD), recalibration (GATK), indel realignment (GATK), SNV/indel variant calling (GATK HaplotypeCaller), annotation (ANNOVAR). 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 (20 cores) per year [48]. 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.

In the filtering and triage step, approximately 300-400 variants were examined to flag variants of interest for both primary and secondary variants. Time required for filtration of primary variants was 55 minutes and for secondary variants the requirement was 5 minutes. 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 clinical interpretation and report writing of secondary variants required 20 to 40 minutes with an average of 30 minutes. It was further assumed that secondary variants were found in 3% - 5% of cases, with the reference case analysis being 4%. 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 were assumed to vary by 20% from their point estimates.

2.4.2.2 Equipment

The large equipment costs were estimated for each of HiSeq® 2500 and Next Seq® 550 sequencing platforms Illumina (San Diego, USA). For both equipment platforms, 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 prices of the HiSeq®2500 and NextSeq®550 sequencers and the maintenance contracts were provided by the TCAG lab manager and the director of DPLM, respectively. More recently, the NextSeq® 550 platform and the corresponding maintenance contract were

purchased at a discounted price. The maintenance contracts were approximately 9-10% of the cost of the sequencers per year. The price of a Bioanalyzer and TapeStation was provided by the manufacturer and TCAG lab manager and was the same for both platforms. 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 and was the same for both sequencers. 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 blood sample to TCAG laboratory or DPLM, depending on the sequencer, Agilent SureSelect exome kits, other library preparation consumables and reagents (Appendices 7 and 8). The price of sequencing reagents was based on high throughput flow cell sequencing technology of eight samples per lane and was the only item that was different (with different prices) between the two sequencing platforms. Each item was packaged as one unit per sample and priced accordingly. 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 and the associate director of DPLM, respectively. 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, as there were no ranges provided.

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 [47]. One follow-up test is run in the proband and two in parents, for a total of three tests. The price of the test per sample was obtained from the associate director of DPLM.

2.4.2.5 Bioinformatics

The costs calculated were for bioinformatics data file storage and computational use, which were indifferent between the two sequencers. Software costs were not included as GATK is an open software with no licensing fee. The resource use for data file storage depended on file size and length of storage time and was calculated in gigabytes per year. For both HiSeq® 2500 and NextSeq® 550, the bioinformatics computation use included the pipeline steps specified in Section 2.4.2.1. 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 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. The quote was $26,804 CAD per node (includes extra warranty for an additional 2 years for a total of 5 years), assuming each node has 256 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

The present analysis includes estimates for both proband and trio WGS. In contrast to the previous version of the report (2016-02.2), only HiSeq X™ was considered as the HiSeq® 2500 platform is no longer produced and supported by Illumina Inc. HiSeq X™ can sequence 16 samples per run to achieve a 30-45X read depth. The Illumina HiSeq X™ requires greater initial investment, but has lower reagent prices. Appendices 9 and 10 contain resource use and price data for HiSeq X™, for proband and trio testing, respectively.

2.4.3.1 Labour

Total minutes for each input in the specimen preparation, library preparation and sequencing categories were determined. These values were tripled for trios since the number of samples processed per run is three times that of a proband. 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-HiSeq®2500 and WGS-proband (Appendices 7 and 9). 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. Due to automation, HiSeq X™ can process 48 samples during the library preparation tasks and 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 output range for one HiSeq X™ instrument is 64-96 genomes per month. The resource use per sample for variant calling and annotation was calculated by dividing the labour time by the average of 72 genomes per month. Based on expert opinion, 1.25 FTE unit of labour is required to process this range of genome output 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). The resource use per sample for bioinformatics was calculated by dividing the labour time by the average output per month.

Bioinformatics maintenance components for HiSeq X™ pipelines steps are: alignment (BWA), mark duplicates (PICARD), recalibration (GATK), indel realignment (GATK), SNV/indel variant calling (GATK HaplotypeCaller), annotation (ANNOVAR), CNV detection (custom), CNV annotation (custom), SV detection (MANTA) and SV annotation (custom). The calculation time and the number of nodes required for each step in the bioinformatics pipeline were obtained from the TCAG bioinformatics manager. 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 differed between WGS and WES.

In the filtering and triage step, approximately 300-400 variants were examined to flag variants of interest for both primary and secondary variants. Time required for filtration of primary variants for proband was 55 minutes and for secondary variants the requirement was 5 minutes. Filtration time for primary variants of trios were 25 minutes while the time required for secondary variants remained the same as probands.

As with WES, zero to four primary variants could be found, with an average of two variants, for both probands and trios. Similarly, clinical interpretation of probands and trios 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 3% - 5% of cases, with the reference case analysis being 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. 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 were assumed to vary by 20% from its point estimates.

2.4.3.2 Equipment

The resource use for small and large equipment was identical for WGS and WES, regardless of the sequencing platform or whether it was a proband or a trio being analyzed. The sample costs for ASD patients for small and large 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 incrementally more expensive by a factor of 3.72 in comparison to the NextSeq® 550 platform for WES. NextSeq® 550 platform was bought with a

discount. Both platforms also required maintenance contracts and Bioanalyzers and TapeStations. For WGS, the HiSeq X™ platform was used for sequencing both probands and trios. It required greater initial investment. As with the other two platforms, the HiSeq X™ estimates also included 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 9 and 10). In order to account for price and currency fluctuations, unit prices of small equipment were assumed to vary by 10% from their point estimates. Unit prices of platforms and maintenance contracts of large equipment were given ranges by experts or were varied by 10% for HiSeq® 2500, NextSeq® 550 and HiSeq X™. Since a range was quoted for the price of Bioanalyzer/TapeStation, the mean was taken as the point estimate with the lower and higher range of the quote used for accounting of fluctuations. This was done on all models of CGES.

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 X™ sequencing reagents (Appendices 9 and 10). Ranges for resource use were not assigned, as it was assumed that one unit of supplies was required per sample. This resource use was tripled to account for the estimation of trios. 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 probands and their parents undergo Sanger sequencing and about 10% of probands and their parents undergo either FISH or qPCR follow-up testing [47]. Since FISH testing is done infrequently, it was assumed that only qPCR is done in 10% of cases. For trios, 10% are subjected to Sanger sequencing in order to be able to detect any *de novo* mutations, while qPCR is performed in 30% of the families. 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

The costs calculated were for bioinformatics data file storage and computational use. Software costs were not included as GATK is an open software with no licensing fee. The resource use for the data file storage depended on file size and length of storage time and was calculated in gigabytes per year. The

resource use (CPU per hour) for each step was calculated by multiplying the number of computing jobs by the number of CPUs (cores) the time (in hours) required to complete the job and by 25% overhead for the wait period of saturated nodes to complete. This overhead was varied as 0% and 50% for lower and upper bounds, respectively. 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 the computation use for the pipeline steps specified in Section 2.4.3.1. As with the WES platforms, prices for computational use were based on TCAG's purchase of 72 compute nodes (20 cores, 40 threads, 256 GB RAM) for processing WGS samples (probands and trios) on HiSeq X™. The price of each node was $26,804 CAD over five years, including warranty. Resource use calculation was the same for WGS as it was for WES.

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. Within WGS, file storage was three times greater for trios than for probands.

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 (HiSeq® 2500 and NextSeq® 550) and WGS (HiSeq X™) for both proband and trio. Total program costs over five years were also estimated for each platform. The models were built on Microsoft Excel. Both PA and DSAs were run as 10,000 Monte Carlo simulations on R program for statistical computing and graphics.

2.5.1 Probabilistic analysis

For each input's resource use and unit price, a range and probability distribution was established in consultation with experts. 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 (Appendices 6-10). 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, 10,000 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 10,000 times.

Most input parameters were 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. This method applied to equipment, supplies and bioinformatics. With the exception of the proportion of patients for whom secondary variants were found (follow-up testing), all other labour steps were also modelled using truncated normal distribution, as stated above.

The resource use for FISH, qPCR and Sanger sequencing were quantified as the proportion of cases in which follow-up testing was done (Appendices 6-10). 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 [49]:

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

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.

2.5.2 Sensitivity analysis

An assessment of uncertainty is an essential part of an economic analysis [45, 49, 50]. Deterministic sensitivity analysis (DSA) was conducted for selected parameters that were highly uncertain or expected

to vary substantially between institutions. For the parameters that were varied, reference level values were repeated 10,000 times. The DSAs permitted an examination of how changing the values of highly uncertain inputs one at a time affected the results.

2.5.2.1 Deterministic sensitivity analysis (DSA)

Three one-way DSAs were conducted to examine the effects of changing the inputs while other input parameters remained the same: i) the overhead cost; ii) the total volume of tests in the institution; and iii) the number of primary variants. For all three testing technologies on four platforms, the reference overhead cost was set at 22.3%. In the DSA, the overhead cost was varied from 10 to 30%. For WES and WGS-proband tests, the reference case number of all tests for patients in the institution per sequencer was set to 500. For the WGS-trio test on the other hand, this number was set at 1500. 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 for WES and WGS-proband and for WGS-trio, it was varied from 1500 to 3000. 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 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	3.0	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	398152	Trun. Normal μ=398152,σ=13,271
1-year service contract	1/all tests	Fixed	89277.20	Trun. Normal μ=89277.20,σ=2976
SUPPLIES				
Shipping and handling	1.0	Fixed	37.61	Trun. Normal μ=37.61,σ=1.25
Microarray slide and reagents per patient	1.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
FOLLOW-UP TESTING				
Proportion of patients who undergo FISH follow-up (proband and two parents)	0.1	Beta α=395,β=3553	680.00	Trun. Normal μ=680.00,σ=22.7
Proportion of patients who undergo qPCR follow-up (proband and two parents)	0.05	Beta α=197,β=3751	223.90	Trun. Normal μ=223.90,σ=7.46

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

Table 4. WES (HiSeq® 2500) parameter estimates and distributions used in the probabilistic 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 (units: minutes)				
Variant calling (total time per month/samples per month)	101.2	Total time fixed (8400 minutes); Samples per month: Trun. Normal μ=82.5,σ=4.2	Conf.	Trun. Normal μ,σ=Conf.
Annotation (total time per month/samples per month)	25.3	Total time fixed (2100 minutes); Samples per month: Trun. Normal μ=82.5, σ=4.2	Conf.	Trun. Normal μ,σ=Conf.
Bioinformatics maintenance (units: minutes)				
Alignment	0.0034	Trun. Normal μ=0.0034,σ=0.00023	Conf.	Trun. Normal μ,σ=Conf.
Mark Duplicates	0.00034	Trun. Normal μ=0.00034,σ=0.0000285	Conf.	Trun. Normal μ,σ=Conf.
Recalibration – step 1	0.00051	Trun. Normal μ=0.00051,σ=0.0000285	Conf.	Trun. Normal μ,σ=Conf.
Recalibration – step 2	0.00685	Trun. Normal μ=0.00685,σ=0.001141	Conf.	Trun. Normal μ,σ=Conf.
Post-recalibration merge	0.000171	Trun. Normal μ=0.000171,σ=0.0000285	Conf.	Trun. Normal μ,σ=Conf.
Indel realignment	0.00685	Trun. Normal μ=0.00685,σ=0.001141	Conf.	Trun. Normal μ,σ=Conf.
SNV/indel variant calling	0.00456	Trun. Normal μ=0.00456,σ=0.0001522	Conf.	Trun. Normal μ,σ=Conf.
Annotation	0.000685	Trun. Normal μ=0.000685,σ=0.0001141	Conf.	Trun. Normal μ,σ=Conf.
Filtering & Triage (units: minutes)				
Filtration of primary variants	55.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Filtration of secondary variants	5.0	Fixed	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)				
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	2250	Trun. Normal μ=2250,σ=83.3
Plate microcentrifuge	1/all tests	Fixed	5000	Trun. Normal μ=5000,σ=166.7
Thermomixer	1/all tests	Fixed	5000	Trun. Normal μ=5000,σ=166.7
Vortex	1/all tests	Fixed	450	Trun. Normal μ=450,σ=16.7
Pipette sets	2/all tests	Fixed	1600	Trun. Normal μ=1600,σ=101.2
Magnet particle concentrator for tubes	1/all tests	Fixed	700	Trun. Normal μ=700, σ=23.3
Thermocyclers	2/all tests	Fixed	3000	Trun. Normal μ=3000,σ=100
SUPPLIES				
Shipping & Handling	1	Fixed	37.61	Trun. Normal μ=37.61,σ= 1.25
SureSelect Baits	1	Fixed	195	Trun. Normal μ=195,σ=6.50
SureSelect Library prep	1	Fixed	22.50	Trun. Normal μ=22.50,σ=0.75
Other library prep consumables	1	Fixed	70	Trun. Normal μ=70,σ=2.33
Reagents (8 samples per lane)	1	Fixed	337.50	Trun. Normal μ=337.50,σ=11.25
FOLLOW-UP TESTING (proportion of patients)				
Sanger sequencing	0.5	Beta α=150,β=150	53.33	Trun. Normal μ=53.33,σ=1.78
BIONFORMATICS				
Bioinformatics file storage (units: GB per year)				
trimmed fastq	6.75	Trun. Normal μ=6.75,σ=0.75	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	11.875	Trun. Normal μ=11.875,σ=0.79	0.612	Trun. Normal μ=0.612,σ=0.02
Mark Duplicates	2.5	Trun. Normal μ=2.5,σ=0.167	0.612	Trun. Normal μ=0.612,σ=0.02
Recalibration (step 1)	1.875	Trun. Normal μ= 1.875, σ= 0.125	0.612	μ=0.612,σ=0.02
Recalibration (step 2)	14.375	Trun. Normal μ= 14.375,σ= 0.958	0.612	Trun. Normal μ= 0.612,σ=0.02
Post-recalibration merge	1.25	Trun. Normal μ=1.25,σ=0.083	0.612	Trun. Normal μ=0.612,σ=0.02
Indel Realignment	28.75	Trun. Normal μ=28.75,σ=1.916	0.612	Trun. Normal μ=0.612,σ=0.02
SNV/indel variant calling	9.583	Trun. Normal μ=9.583,σ=0.639	0.612	Trun. Normal μ=0.612,σ=0.02
Annotation	5.0	Trun. Normal μ=5.0,σ=0.333	0.612	Trun. Normal μ=0.612,σ=0.02

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. WES (Next Seq® 550) parameter estimates and distributions used in the probabilistic 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)				
Sequencing prep	3.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Run quality control	1.9	Fixed	Conf.	Trun. Normal μ,σ=Conf.

Bioinformatics (units: minutes)				
Variant calling (total time per month/samples per month)	105	Total time fixed (8400 minutes); Samples per month: Trun. Normal μ=80,σ=5.33	Conf.	Trun. Normal μ,σ=Conf.
Annotation (total time per month/samples per month)	26.25	Total time fixed (2100 minutes); Samples per month: Trun. Normal μ=80,σ=5.33	Conf.	Trun. Normal μ,σ=Conf.
Bioinformatics maintenance (units: minutes)				
Alignment	0.00342	Trun. Normal μ=0.00342,σ=0.00023	Conf.	Trun. Normal μ,σ=Conf.
Mark Duplicates	0.00034	Trun. Normal μ=0.00034,σ=0.0000285	Conf.	Trun. Normal μ,σ=Conf.
Recalibration – step 1	0.00051	Trun. Normal μ=0.00051,σ=0.0000285	Conf.	Trun. Normal μ,σ=Conf.
Recalibration – step 2	0.00685	Trun. Normal μ=0.00685,σ=0.001141	Conf.	Trun. Normal μ,σ=Conf.
Post-recalibration merge	0.000171	Trun. Normal μ=0.000171,σ=0.0000285	Conf.	Trun. Normal μ,σ=Conf.
Indel realignment	0.00685	Trun. Normal μ=0.00685,σ=0.001141	Conf.	Trun. Normal μ,σ=Conf.
SNV/indel variant calling	0.00456	Trun. Normal μ=0.00456,σ=0.0001522	Conf.	Trun. Normal μ,σ=Conf.
Annotation	0.000685	Trun. Normal μ=0.000685,σ=0.0001141	Conf.	Trun. Normal μ,σ=Conf.
Filtering & Triage (units: minutes)				
Filtration of primary variants	55.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Filtration of secondary variants	5.0	Fixed	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)				
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 NextSeq® 550	1/all tests	Fixed	201250	Trun. Normal μ=201250, σ=6708.33
1-year service contract	1/all tests	Fixed	17940	Trun. Normal μ=17940,σ=598
Agilent BioAnalyzer/Tape station	1/all tests	Fixed	38500	Trun. Normal μ=38500,σ=1500

SMALL EQUIPMENT				
Tube microcentrifuge	1/all tests	Fixed	2250	Trun. Normal μ=2250,σ=83.3
Plate microcentrifuge	1/all tests	Fixed	5000	Trun. Normal μ=5000,σ=166.7
Thermomixer	1/all tests	Fixed	5000	Trun. Normal μ=5000,σ=166.7
Vortex	1/all tests	Fixed	450	Trun. Normal μ=450,σ=16.7
Pipette sets	2/all tests	Fixed	1600	Trun. Normal μ=1600,σ=101.2
Magnet particle concentrator for tubes	1/all tests	Fixed	700	Trun. Normal μ=700,σ=23.3
Thermocyclers	2/all tests	Fixed	3000	Trun. Normal μ=3000,σ=100
SUPPLIES				
Shipping & Handling	1	Fixed	37.61	Trun. Normal μ=37.61,σ= 1.25
SureSelect Baits	1	Fixed	195	Trun. Normal μ=195,σ=6.50
SureSelect Library prep	1	Fixed	22.50	Trun. Normal μ=22.50,σ=0.75
Other library prep consumables	1	Fixed	70	Trun. Normal μ=70,σ=2.33
Reagents (8 samples per lane)	1	Fixed	707.83	Trun. Normal μ=707.83,σ=23.59
FOLLOW-UP TESTING (proportion of patients)				
Sanger sequencing	0.5	Beta α=150,β=150	53.33	Trun. Normal μ=53.33,σ=1.78
BIONFORMATICS				
Bioinformatics file storage (units: GB per year)				
trimmed fastq	6.75	Trun. Normal μ=6.75,σ=0.75	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	11.875	Trun. Normal μ=11.875,σ=0.79	0.612	Trun. Normal μ=0.612,σ=0.02
Mark Duplicates	2.5	Trun. Normal μ=2.5,σ=0.167	0.612	Trun. Normal μ=0.612,σ=0.02
Recalibration (step 1)	1.875	Trun. Normal μ= 1.875,σ= 0.125	0.612	μ=0.612,σ=0.02
Recalibration (step 2)	14.375	Trun. Normal μ= 14.375,σ= 0.958	0.612	Trun. Normal μ= 0.612,σ=0.02
Post-recalibration merge	1.25	Trun. Normal μ=1.25,σ=0.083	0.612	Trun. Normal μ=0.612,σ=0.02
Indel Realignment	28.75	Trun. Normal μ=28.75,σ=1.916	0.612	Trun. Normal μ=0.612,σ=0.02
SNV/indel variant calling	9.583	Trun. Normal μ=9.583,σ=0.639	0.612	Trun. Normal μ=0.612,σ=0.02
Annotation	5.0	Trun. Normal μ=5.0,σ=0.333	0.612	Trun. Normal μ=0.612,σ=0.02

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 6. WGS-proband (Illumina HiSeq X™) parameter estimates and distributions used in the probabilistic 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)				
Variant calling (total time per month/samples per month)	116.67	Total time fixed (8400 minutes); Samples per month: Trun. Normal μ=80,σ=5.33	Conf.	Trun. Normal μ,σ=Conf.
Annotation (total time per month/samples per month)	29.17	Total time fixed (2100 minutes); Samples per month: Trun. Normal μ=80,σ=5.33	Conf.	Trun. Normal μ,σ=Conf.

Bioinformatics Maintenance (Units: minutes)				
Alignment	0.0822	Trun. Normal μ=0.0822,σ=0.00685	Conf.	Trun. Normal μ,σ=Conf.
Mark Duplicates	0.00171	Trun. Normal μ=0.00171,σ=0.000143	Conf.	Trun. Normal μ,σ=Conf.
Recalibration	0.03	Trun. Normal μ=0.03,σ=0.0023	Conf.	Trun. Normal μ,σ=Conf.
Post-recalibration merge	0.00034	Trun. Normal μ=0.00034,σ=0.0000285	Conf.	Trun. Normal μ,σ=Conf.
Indel realignment	0.02	Trun. Normal μ=0.02,σ=0.00171	Conf.	Trun. Normal μ,σ=Conf.
SNV/indel variant calling	0.04	Trun. Normal μ=0.04,σ=0.00342	Conf.	Trun. Normal μ,σ=Conf.
Annotation	0.00205	Trun. Normal μ=0.00205,σ=0.000171	Conf.	Trun. Normal μ,σ=Conf.
CNV detection	0.00514	Trun. Normal μ=0.01,σ=0.000428	Conf.	Trun. Normal μ,σ=Conf.
CNV annotation	0.00003	Trun. Normal μ=0.00003,σ=0.00000238	Conf.	Trun. Normal μ,σ=Conf.
SV detection	0.01	Trun. Normal μ=0.01,σ=0.00057	Conf.	Trun. Normal μ,σ=Conf.
SV Annotation	0.000029	Trun. Normal μ=0.000029,σ=0.00000238	Conf.	Trun. Normal μ,σ=Conf.
Filtering & Triage (units: minutes)				
Filtration of primary variants	55.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Filtration of secondary variants	5.0	Fixed	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	2250	Trun. Normal μ=2250,σ=83.3
Plate microcentrifuge	1/all tests	Fixed	5000	Trun. Normal μ=5000,σ=166.7
Thermomixer	1/all tests	Fixed	5000	Trun. Normal μ=5000,σ=166.7

Vortex	1/all tests	Fixed	450	Trun. Normal μ=450,σ=16.7
Pipette sets	2/all tests	Fixed	1600	Trun. Normal μ=1600,σ=101.2
Magnet particle concentrator for tubes	1/all tests	Fixed	700	Trun. Normal μ=700,σ=23.3
Thermocyclers	2/all tests	Fixed	3000	Trun. Normal μ=3000,σ=101.2
SUPPLIES				
Shipping & Handling	1	Fixed	37.61	Trun. Normal μ=37.61,σ=1.25
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=1.67
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	53.33	Trun. Normal μ=53.33,σ=1.78
qPCR followup	0.1	Beta α=30,β=270	223.90	Trun. Normal μ=223.90,σ=7.46
BIONFORMATICS				
Bioinformatics File Storage (Units: GB per year)				
Trimmed 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	285	Trun. Normal μ=285,σ=19	0.612	Trun. Normal μ=0.612,σ=0.0204
Mark Duplicates	12.5	Trun. Normal μ=12.5,σ=0.83	0.612	Trun. Normal μ=0.612,σ=0.0204
Recalibration	57.5	Trun. Normal μ=57.5,σ=3.83	0.612	Trun. Normal μ=0.612,σ=0.0204
Post-recalibration merge	2.5	Trun. Normal μ=2.5,σ=0.167	0.612	Trun. Normal μ=0.612,σ=0.0204
Indel Realignment	86.25	Trun. Normal μ=86.25,σ=5.75	0.612	Trun. Normal μ=0.612,σ=0.0204
SNV/indel variant calling	86.25	Trun. Normal μ=86.25,σ=5.75	0.612	Trun. Normal μ=0.612,σ=0.0204
Annotation	15	Trun. Normal μ=15,σ=1	0.612	Trun. Normal μ=0.612,σ=0.0204
CNV Detection	37.5	Trun. Normal μ=37.5,σ=2.5	0.612	Trun. Normal μ=0.612,σ=0.0204
CNV Annotation	0.104	Trun. Normal μ=0.104,σ=0.00694	0.612	Trun. Normal μ=0.612,σ=0.0204
SV Detection	25	Trun. Normal μ=25,σ=1.67	0.612	Trun. Normal μ=0.612,σ=0.0204
SV Annotation	0.104	Trun. Normal μ=0.104,σ=0.00694	0.612	Trun. Normal μ=0.612,σ=0.0204

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 7. WGS-trio (Illumina HiSeq X™) parameter estimates and distributions used in the probabilistic analysis.

Cost Items	Quantity of Use per Sample		Unit Price	
	Estimate	Distribution	Estimate	Distribution
LABOUR				
Specimen Preparation (Units: minutes)				
Pediatric venipuncture	22.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Packaging with testing documentation	3.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Service recipient primary registration	5.4	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Printing and sorting of specimen labels	1.2	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Creation of recipient folder	15.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Packaging with testing documentation	3.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Service recipient limited registration	5.4	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Library preparation (Units: minutes)				
total time/number of samples per batch				
DNA quantification	1.3	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Pre-prep reagents	1.3	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Shearing	1.3	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Purification	2.5	Fixed	Conf.	Trun. Normal μ,σ=Conf.
End repair	2.5	Fixed	Conf.	Trun. Normal μ,σ=Conf.
A-tailing	2.5	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Adapter ligation	2.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Sequencing (Units: minutes)				
HiSeq wash	5.6	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Sequencing prep	5.6	Fixed	Conf.	Trun. Normal μ,σ=Conf.
HiSeq post-run wash	8.4	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Run quality control	2.8	Fixed	Conf.	Trun. Normal μ,σ=Conf.
cBot	5.6	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Bioinformatics (Units: minutes)				
Variant calling (total time per month/samples per month)	116.67	Total time fixed (8400 minutes); Samples per month: Trun. Normal μ=80,σ=5.33	Conf.	Trun. Normal μ,σ=Conf.
Annotation (total time per month/samples per month)	29.17	Total time fixed (2100 minutes); Samples per month: Trun. Normal μ=80,σ=5.33	Conf.	Trun. Normal μ,σ=Conf.

Bioinformatics Maintenance (Units: minutes)				
Alignment	0.25	Trun. Normal μ=0.25,σ=0.02054	Conf.	Trun. Normal μ,σ=Conf.
Mark Duplicates	0.01	Trun. Normal μ=0.01,σ=0.000428	Conf.	Trun. Normal μ,σ=Conf.
Recalibration	0.08	Trun. Normal μ=0.08,σ=0.00685	Conf.	Trun. Normal μ,σ=Conf.
Post-recalibration merge	0.0010	Trun. Normal μ=0.0010,σ=0.0000856	Conf.	Trun. Normal μ,σ=Conf.
Indel realignment	0.06	Trun. Normal μ=0.06,σ=0.00514	Conf.	Trun. Normal μ,σ=Conf.
SNV/indel variant calling	0.12	Trun. Normal μ=0.12,σ=0.01027	Conf.	Trun. Normal μ,σ=Conf.
Annotation	0.01	Trun. Normal μ=0.01,σ=0.000513	Conf.	Trun. Normal μ,σ=Conf.
CNV detection	0.02	Trun. Normal μ=0.02,σ=0.00128	Conf.	Trun. Normal μ,σ=Conf.
CNV annotation	0.0000856	Trun. Normal μ=0.0000856,σ=0.00000713	Conf.	Trun. Normal μ,σ=Conf.
SV detection	0.02	Trun. Normal μ=0.02,σ=0.00171	Conf.	Trun. Normal μ,σ=Conf.
SV Annotation	0.0000856	Trun. Normal μ=0.0000856,σ=0.00000713	Conf.	Trun. Normal μ,σ=Conf.
Filtering & Triage (units: minutes)				
Filtration of primary variants	25.0	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Filtration of secondary variants	5.0	Fixed	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 $\times$ 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)				
Addressing primary variants	45	Fixed	Conf.	Trun. Normal μ,σ=Conf.
Addressing secondary variants (total report writing time $\times$ 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	2250	Trun. Normal μ=2250,σ=83.3
Plate microcentrifuge	1/all tests	Fixed	5000	Trun. Normal μ=5000,σ=166.7
Thermomixer	1/all tests	Fixed	5000	Trun. Normal μ=5000,σ=166.7

Vortex	1/all tests	Fixed	450	Trun. Normal μ=450,σ=16.7
Pipette sets	2/all tests	Fixed	1600	Trun. Normal μ=1600,σ=101.2
Magnet particle concentrator for tubes	1/all tests	Fixed	700	Trun. Normal μ=700,σ=23.3
Thermocyclers	2/all tests	Fixed	3000	Trun. Normal μ=3000,σ=101.2
SUPPLIES				
Shipping & Handling	3	Fixed	37.61	Trun. Normal μ=37.61,σ=1.25
Illumina Nano DNA library prep	3	Fixed	30.0	Trun. Normal μ=30.0,σ=1.0
Other library prep consumables	3	Fixed	50	Trun. Normal μ=50,σ=1.67
Sequencing reagents	3	Fixed	1290	Trun. Normal μ=1290,σ=43.0
FOLLOW-UP TESTING (proportion of patients)				
Sanger sequencing	0.1	Beta α=150,β=150	53.33	Trun. Normal μ=53.33,σ=1.78
qPCR followup	0.3	Beta α=30,β=270	223.90	Trun. Normal μ=223.90,σ=7.46
BIONFORMATICS				
Bioinformatics File Storage (Units: GB per year)				
Trimmed fastq	270.0	Trun. Normal μ=270.0,σ=30.0	0.40	Trun. Normal μ=0.40,σ=0.013
final rem-dup, recalibrated, locally re-aligned BAM file	180.0	Trun. Normal μ=180.0,σ=60.0	0.40	Trun. Normal μ=0.40,σ=0.013
Bioinformatics Computation Use (Units: CPU time per hour)				
Alignment	855	Trun. Normal μ=855,σ=57	0.612	Trun. Normal μ=0.612,σ=0.0204
Mark Duplicates	37.5	Trun. Normal μ=37.5,σ=2.5	0.612	Trun. Normal μ=0.612,σ=0.0204
Recalibration	172.5	Trun. Normal μ=172.5,σ=11.5	0.612	Trun. Normal μ=0.612,σ=0.0204
Post-recalibration merge	7.5	Trun. Normal μ=7.5,σ=0.5	0.612	Trun. Normal μ=0.612,σ=0.0204
Indel Realignment	258.75	Trun. Normal μ=258.75,σ=17.25	0.612	Trun. Normal μ=0.612,σ=0.0204
SNV/indel variant calling	258.75	Trun. Normal μ=258.75,σ=17.25	0.612	Trun. Normal μ=0.612,σ=0.0204
Annotation	45	Trun. Normal μ=45,σ=3	0.612	Trun. Normal μ=0.612,σ=0.0204
CNV Detection	112.5	Trun. Normal μ=112.5,σ=7.5	0.612	Trun. Normal μ=0.612,σ=0.0204
CNV Annotation	0.3125	Trun. Normal μ=0.3125,σ=0.02083	0.612	Trun. Normal μ=0.612,σ=0.0204
SV Detection	75	Trun. Normal μ=75,σ=5	0.612	Trun. Normal μ=0.612,σ=0.0204
SV Annotation	0.3125	Trun. Normal μ=0.3125,σ=0.02083	0.612	Trun. Normal μ=0.612,σ=0.0204

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 five scenarios deemed to reflect potential clinical practices: (1) substitution of CMA alone with a combination of CMA and WES (CMA plus WES vs. CMA); (2) substitution of CMA with WGS-proband (WGS-proband vs. CMA); (3) substitution of CMA with WGS-trio (WGS-trio vs. CMA); (4) substitution of a combination of CMA and WES with WGS-proband (WGS-proband vs. CMA plus WES); and (5) substitution of a combination of CMA and WES with WGS-trio (WGS-trio vs. CMA plus 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 [22]. Whole genome sequencing can identify both large and small variants [11]. 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 (Illimina HiSeq® 2500), WES ((Illimina NextSeq® 550), WGS-proband (HiSeq X™) and WGS-trio (HiSeq X™) microcosting models are shown in Tables 8, 9, 10, 11 and 12 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 10,000 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 (22.3%), the number of total tests done per year for all indications (CMA: 3948, WES/WGS-proband: 500, WGS-trio: 1500) and the number of primary variants found (WES/WGS: 2).

The total cost of CMA was estimated to be $824.50 (95% CI: 789.00, 858.90) per ASD sample in Year 1 of the program. The largest cost component was supplies, accounting for 60.8% of total cost (Figure 1). The second largest cost item was labour, accounting for 18.4% of total cost. The total annual cost of WES conducted on the HiSeq® 2500 platform was estimated to be $1960.00 (95% CI: 1898.90, 2020.20) per ASD sample in Year 1 of the program. Supplies and labour were the most expensive items at 32.8% and 25.8% of total costs, respectively (Figure 1). WES conducted on the NextSeq® 550 platform was estimated to cost $1980.60 (95% CI: 1908.60, 2053.60) per ASD sample in Year 1, with supplies constituting 50.6% of total cost. Labour cost was almost the same as with HiSeq® 2500 (25.2%). Large equipment was more expensive for HiSeq® 2500, accounting for 19.7% of the cost where as it accounted for only 5.8% of the total cost for NextSeq® 550 platform (Figure 1). WGS-proband conducted on the HiSeq X™ platform resulted in a per ASD sample cost of $3350.30 (95% CI: 3233.70, 3467.40) in Year 1. WGS-trio cost on the same platform was $6556.00 (95% CI: 6277.50, 6832.00). The difference in total costs between the proband and the trio was largely attributable to the differences in the cost of supplies (40.8% vs. 62.5%) and large equipment (17.4% vs. 3.0%), respectively. Processing of three samples resulted in $4099.90 as supplies cost, which was about thrice as much as the cost for the proband of $1367.50. The cost of large equipment followed the same trend. The cost of small equipment on the

other hand was one third for trio of that of the cost for the proband. Other differences between the cost categories included follow-up (5.3% for proband vs. 1.5% for trio). Bioinformatics component had a greater contribution in the cost per trio sample estimation (12.5% for proband vs. 19.2% for trio) whereas labour contributed more in the proband cost estimation (13.9% for proband vs. 7.2% for trio). The difference between the proband and the trio cost estimation for each of these two cost categories was 6.7% (Figure 1).

Table 8. 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	151.3 (139.3, 163.5)	146.9 (135.3, 158.8)	142.7 (131.3, 154.2)	138.5 (127.5, 149.7)	134.5 (123.8, 145.3)
Large Equipment	50.1 (47.1, 53.1)	47.4 (44.6, 50.2)	44.8 (42.1, 47.4)	42.3 (39.8, 44.8)	39.9 (37.5, 42.2)
Supplies	501.2 (470.3, 531.1)	486.6 (456.6, 515.6)	472.4 (443.3, 500.6)	458.7 (430.4, 486)	445.3 (417.8, 471.8)
Follow-up	76.9 (69.1, 84.8)	74.6 (67.1, 82.3)	72.5 (65.2, 79.9)	70.4 (63.3, 77.6)	68.3 (61.4, 75.3)
Overhead	44.9 (42.1, 47.7)	43.3 (40.6, 46.1)	41.8 (39.2, 44.4)	40.3 (37.8, 42.9)	38.9 (36.4, 41.4)
Total	824.5 (789, 858.9)	798.9 (764.5, 832.4)	774.1 (740.8, 806.7)	750.1 (717.8, 781.7)	726.9 (695.6, 757.5)

Estimates are given in 2018 Canadian dollars (CAD). Confidence intervals (CI) are based on 10,000 Monte Carlo replications. The results were based on reference levels for overhead costs of 22.3% and 3948 CMA tests done for all indications per year.

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

Table 9. Estimated annual cost per ASD sample for WES, 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	506.3 (465.1, 546.7)	491.5 (451.5, 530.7)	477.2 (438.4, 515.3)	463.3 (425.6, 500.3)	449.8 (413.2, 485.7)
Large Equipment	385.5 (370.0, 400.9)	364.5 (349.9, 379.0)	344.4 (330.6, 358.1)	325.2 (312.1, 338.1)	306.8 (294.4, 319.0)
Small Equipment	8.80 (8.5, 9.1)	8.50 (8.2, 8.8)	8.30 (8.0, 8.5)	8.00 (7.8, 8.3)	7.80 (7.5, 8.1)
Supplies	643.2 (617.9, 668.2)	624.4 (599.9, 648.8)	606.3 (582.5, 629.9)	588.6 (565.5, 611.5)	571.5 (549.0, 593.7)
Follow-up	155.4 (138.9, 173.0)	150.9 (134.9, 167.9)	146.5 (131.0, 163.0)	142.2 (127.2, 158.3)	138.1 (123.4, 153.7)
Bioinformatics	49.1 (45.8, 52.3)	47.6 (44.5, 50.8)	46.2 (43.2, 49.3)	44.9 (41.9, 47.9)	43.6 (40.7, 46.5)
Overhead	211.8 (201.9, 221.5)	203.4 (193.9, 212.8)	195.4 (186.2, 204.5)	187.6 (178.7, 196.4)	180.2 (171.5, 188.7)
Total	1960.0 (1898.9, 2020.2)	1890.9 (1831.8, 1949.4)	1824.3 (1767.0, 1880.9)	1759.9 (1704.4, 1814.8)	1697.7 (1644.0, 1750.8)

Estimates are given in 2018 Canadian dollars (CAD). Confidence intervals (CI) are based on 10,000 Monte Carlo replications. The results were based on reference levels for overhead costs of 22.3%, 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 10. Estimated annual cost per ASD sample for WES, Illumina NextSeq ® 550 platform.

Cost Category	Year 1 (95% CI)	Year 2 (95% CI)	Year 3 (95% CI)	Year 4 (95% CI)	Year 5 (95% CI)
Labour	499.8 (457.8, 544.2)	485.2 (444.4, 528.4)	471.1 (431.5, 513.0)	457.4 (418.9, 498.0)	444.0 (406.7, 483.5)
Large Equipment	115.1 (109.0, 121.2)	108.8 (103.1, 114.6)	102.8 (97.4, 108.3)	97.1 (92.0, 102.2)	91.6 (86.8, 96.4)
Small Equipment	8.8 (8.5, 9.1)	8.5 (8.2, 8.8)	8.3 (8.0, 8.5)	8.0 (7.8, 8.3)	7.8 (7.5, 8.1)
Supplies	1002.7 (955.9, 1048.4)	973.5 (928.0, 1017.8)	945.1 (901.0, 988.2)	917.6 (874.8, 959.4)	890.9 (849.3, 931.5)
Follow-up	155.3 (138.7, 172.4)	150.8 (134.6, 167.4)	146.4 (130.7, 162.5)	142.1 (126.9, 157.8)	138.0 (123.2, 153.2)
Bioinformatics	49.0 (45.9, 52.3)	47.6 (44.6, 50.8)	46.2 (43.3, 49.3)	44.9 (42.0, 47.9)	43.6 (40.8, 46.5)
Overhead	150.0 (140.5, 160.1)	145.0 (135.8, 154.8)	140.1 (131.2, 149.6)	135.4 (126.8, 144.6)	130.9 (122.5, 139.8)
Total	1980.6 (1908.6, 2053.6)	1919.4 (1849.5, 1990.2)	1860.0 (1792.2, 1928.8)	1802.5 (1736.6, 1869.2)	1746.7 (1682.7, 1811.5)

Estimates are given in 2018 Canadian dollars (CAD). Confidence intervals (CI) are based on 10,000 Monte Carlo replications. The results were based on reference levels for overhead costs of 22.3%, 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 11. Estimated annual cost per ASD sample (proband) 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	464.7 (417.2, 515.3)	451.2 (405.0, 500.3)	438.1 (393.2, 485.8)	425.3 (381.8, 471.6)	412.9 (370.6, 457.9)
Large Equipment	583.6 (549.8, 617.0)	551.8 (519.9, 583.4)	521.4 (491.2, 551.2)	492.3 (463.8, 520.4)	464.4 (437.5, 491.0)
Small Equipment	8.80 (8.5, 9.1)	8.50 (8.2, 8.8)	8.30 (8.0, 8.5)	8.00 (7.8, 8.3)	7.80 (7.5, 8.1)
Supplies	1367.5 (1284.5, 1448.9)	1327.7 (1247.1, 1406.7)	1289.0 (1210.7, 1365.8)	1251.5 (1175.5, 1326.0)	1215.0 (1141.2, 1287.4)
Follow-up	177.0 (159.0, 195.4)	171.8 (154.4, 189.7)	166.8 (149.9, 184.2)	162.0 (145.5, 178.8)	157.2 (141.3, 173.6)
Bioinformatics	419.4 (390.6, 449.1)	407.2 (379.2, 436.0)	395.3 (368.2, 423.3)	383.8 (357.4, 411.0)	372.6 (347.0, 399.0)
Overhead	329.3 (314.5, 344.1)	316.4 (302.1, 330.8)	304.0 (290.2, 317.9)	292.0 (278.8, 305.4)	280.5 (267.7, 293.4)
Total	3350.3 (3233.7, 3467.4)	3234.6 (3121.7, 3348.1)	3122.8 (3013.7, 3232.7)	3014.8 (2909.2, 3121.3)	2910.5 (2808.1, 3013.6)

Estimates are given in 2018 Canadian dollars (CAD). Confidence intervals (CI) are based on 10,000 Monte Carlo replications. The results were based on reference levels for overhead costs of 22.3%, 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 12. Estimated annual cost per ASD sample (trio) for WGS, Illumina HiSeq X™ platform.

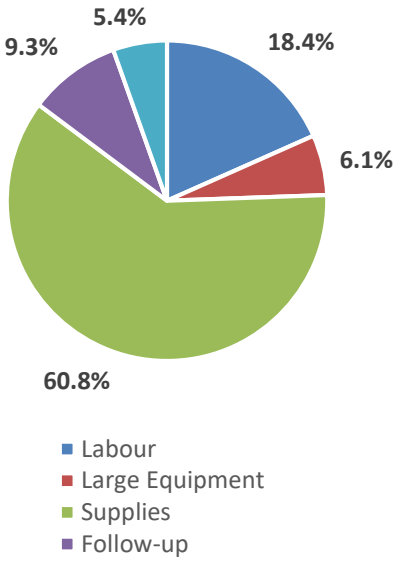
Cost Category	Year 1 (95% CI)	Year 2 (95% CI)	Year 3 (95% CI)	Year 4 (95% CI)	Year 5 (95% CI)
Labour	473.7 (430.6, 520.5)	459.9 (418.1, 505.3)	446.5 (405.9, 490.6)	433.5 (394.1, 476.3)	420.9 (382.6, 462.5)
Large Equipment	194.6 (183.4, 206.1)	184.0 (173.4, 194.9)	173.9 (163.8, 184.1)	164.2 (154.7, 173.8)	154.9 (145.9, 164.0)
Small Equipment	2.90 (2.8, 3.0)	2.80 (2.7, 2.9)	2.80 (2.7, 2.8)	2.70 (2.6, 2.8)	2.60 (2.5, 2.7)
Supplies	4099.9 (3847.7, 4348.8)	3980.5 (3735.6, 4222.1)	3864.6 (3626.8, 4099.2)	3752.0 (3521.2, 3979.8)	3642.7 (3418.6, 3863.9)
Follow-up	96.2 (87.8, 104.8)	93.4 (85.2, 101.8)	90.7 (82.7, 98.8)	88.1 (80.3, 95.9)	85.5 (78.0, 93.1)
Bioinformatics	1258.3 (1172.8, 1346.7)	1221.6 (1138.7, 1307.4)	1186.0 (1105.5, 1269.4)	1151.5 (1073.3, 1232.4)	1118.0 (1042.0, 1196.5)
Overhead	430.3 (408.5, 452.4)	416.7 (395.5, 438.0)	403.5 (382.9, 424.2)	390.7 (370.7, 410.8)	378.3 (358.9, 397.8)
Total	6556.0 (6277.5, 6832.0)	6359.0 (6088.5, 6627.1)	6167.9 (5905.2, 6428.3)	5982.6 (5727.7, 6235.4)	5802.8 (5555.4, 6048.2)

Estimates are given in 2018 Canadian dollars (CAD). Confidence intervals (CI) are based on 10,000 Monte Carlo replications. The results were based on reference levels for overhead costs of 22.3%, 1500 total tests done for all indications per year, and two primary variants found per test.

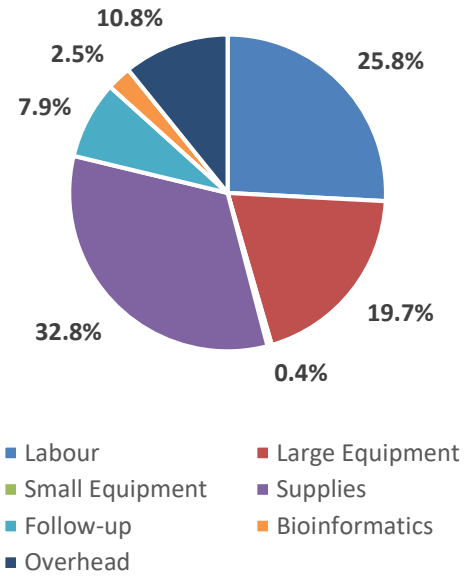
Abbreviations: ASD, Autism spectrum disorder; WGS, Whole genome sequencing.

Figure 1. Proportion of total annual cost per ASD test by cost category for CMA (a), WES (HiSeq ® 2500) (b), WES (NetSeq® 550) (c), WGS - proband (HiSeq® HiSeq X™) (d), WGS - trio (HiSeq X™) (e), Year 1.

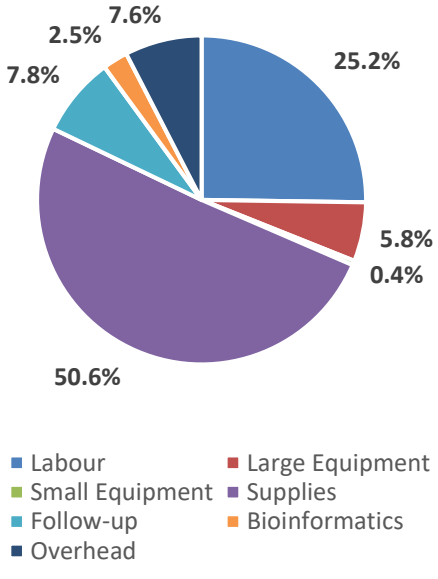
(a) CMA ($824.50)



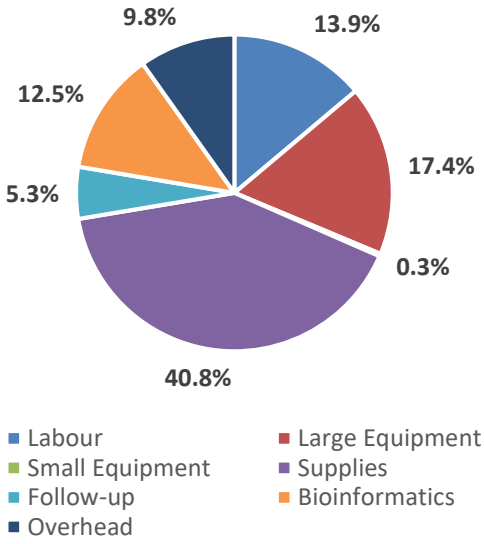
(b) WES- HiSeq ® 2500 ($1960.00)



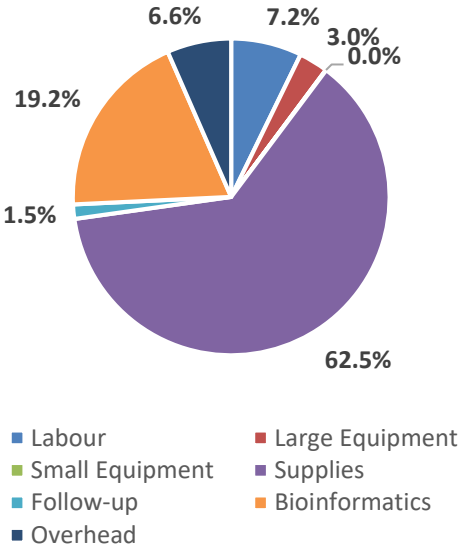
(c) WES (NetSeq® 550) ($1980.60)



(d) WGS (HiSeq X™ proband) ($3350.30)



(e) WGS (HiSeq X™ trio) ($6556.00)



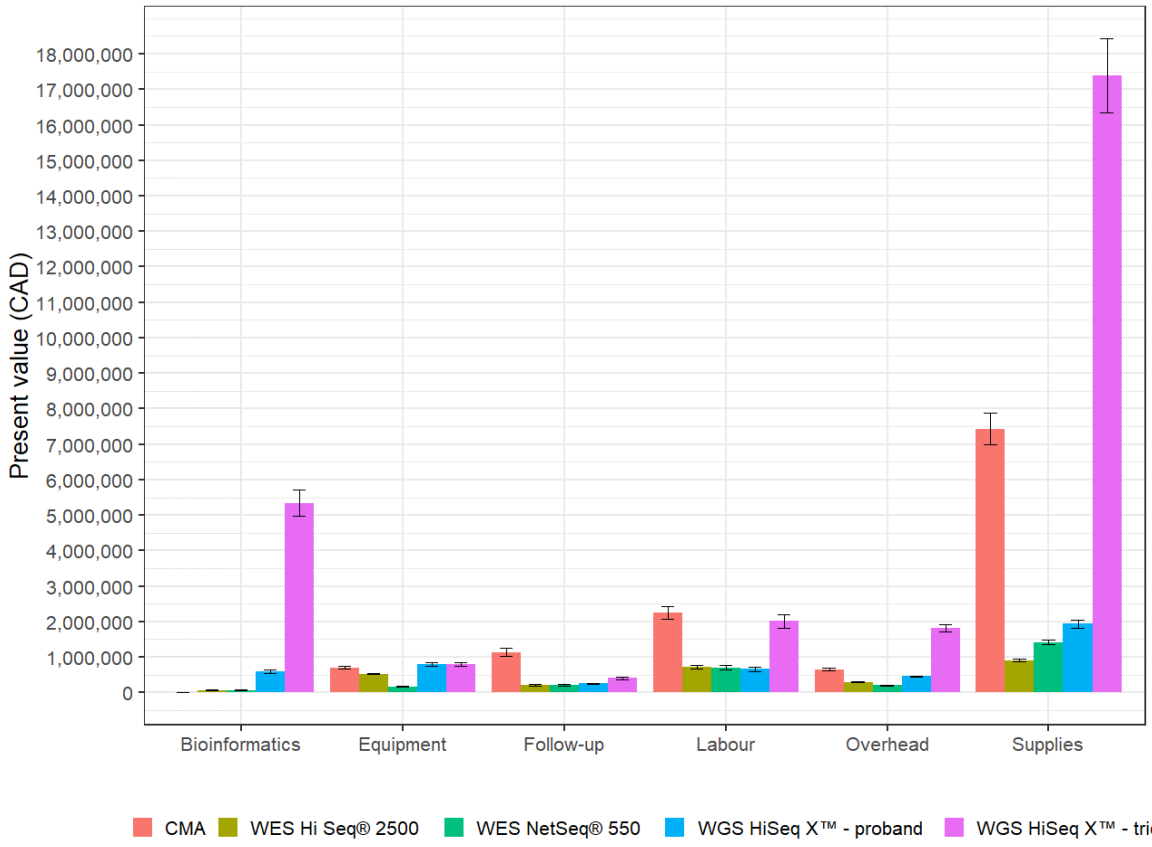
Estimates are given in 2018 Canadian dollars (CAD).

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 $1.16 million (95% CI: 1.11, 1.21). The program costs of WES and WGS tests for ASD over the five-year period were also based on 300 cases per year. The estimated WES program cost on HiSeq® 2500 platform was $2.73 million (95% CI: 2.65, 2.82). On the NextSeq® 550 platform, the estimate was $2.79 million (95% CI: 2.69, 2.89). WGS-proband program cost on the HiSeq X™ platform was $4.68 million (95% CI: 4.52, 4.85) and $27.78 million (95% CI: 26.59, 28.95) for the WGS-trio on the same platform. Figure 2 shows the present value of program costs for each cost component and for each test. Equipment component includes the cost of both small and large equipment. The program cost of supplies was the largest among the cost components for all five tests.

Figure 2. Present value of program costs over five years for CMA, WES (HiSeq® 2500/NextSeq® 550), WGS – proband and trio (HiSeq X™).



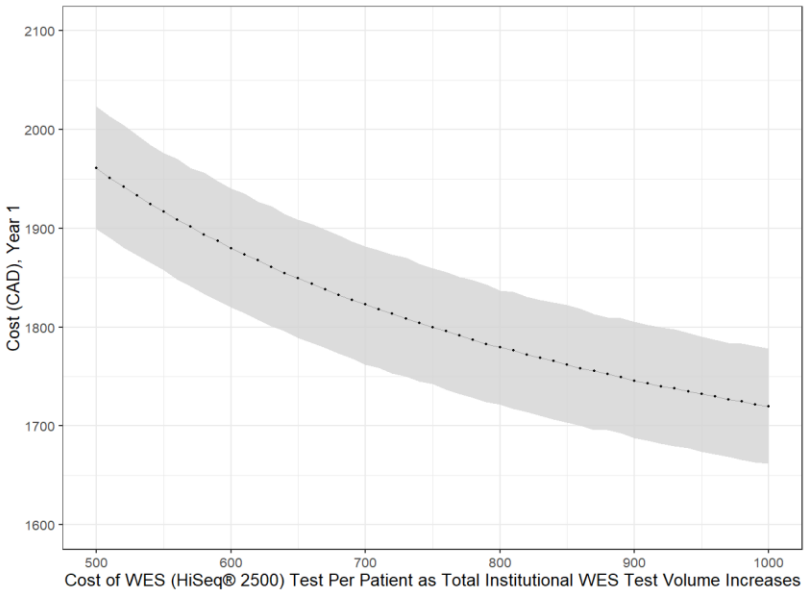
Estimates are given in 2018 Canadian dollars (CAD). Program costs are based on 300 ASD cases annually for CMA, WES/WGS proband tests and 900 ASD cases annually for WGS-trio tests. Confidence bands are based on 10,000 Monte Carlo replications.
Abbreviations: ASD, Autism spectrum disorder; CMA, Chromosomal microarray analysis; WES, Whole exome sequencing; WGS, Whole genome sequencing.

3.3 Deterministic sensitivity analysis (DSA)

Figures 3 and 4 show the effect of increasing the number of annual WES tests for all indications on ASD sample costs in Year 1 conducted on HiSeq® 2500 and NextSeq® 550 platforms, respectively. Due to economies of scale, the sample costs of WES decreased by 13.3% when the number of WES tests for all indications increased from 500 to 1000 on HiSeq® 2500. Cost efficiency on NextSeq® 550 was minimal of 3.5%. This may be attributed to the increased cost of sequencing reagents needed for the platform and the relatively low price of the platform. Figures 5 and 6 show the effect of increasing the number of annual WGS tests for all indications on ASD sample costs in Year 1 for probands and trios on the HiSeq X™ platform, respectively. Increasing the number of tests for all indications from 500 to 1000 reduced the sample costs of WGS done for probands by 12%. The sample costs of WGS done for trios on the HiSeq X™ platform declined by 1.6% when the number of tests for all indications were increased from 1500 to 3000. This three factor increase in the number of institutional tests was due to the test of trio, which by definition comprises of a proband and two parents. The relatively minimal cost reduction for trios was attributed to its equipment and follow-up costs constituting a smaller part of total cost compared to the three factor increase in the cost of supplies and computation.

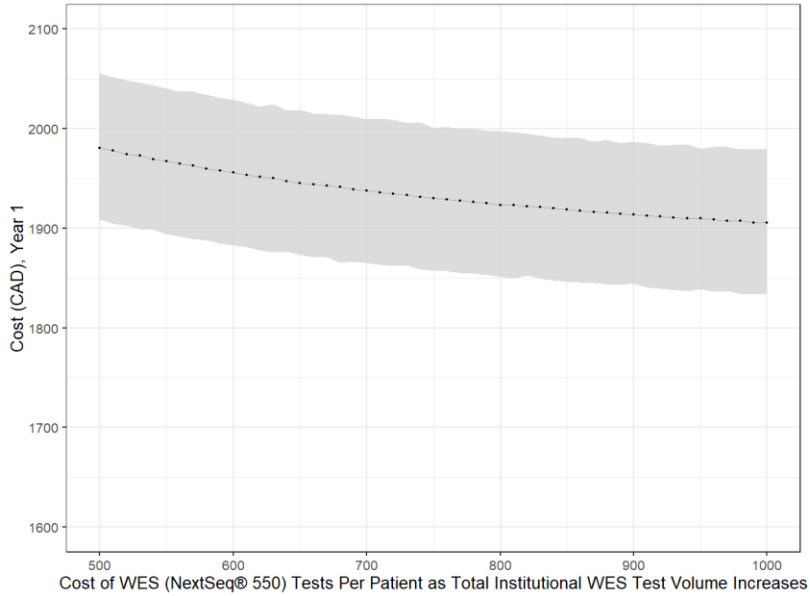
Tables 13 and 14 are summaries of DSAs that varied the overhead cost and the number of variants. The results were fairly robust to changes in overhead assumptions, with a range of 1.9% decrease to 6.3% increase. Increasing the overhead cost to 30% led to a modest 1.9% increase in sample cost for CMA, 3.7% increase for WES on HiSeq® 2500, 2.6% increase for WES on NextSeq® 550, 3.4% for WGS-proband (HiSeqX™) and 2.3% for WGS-trio (HiSeq X™). Decreasing the overhead cost to 10% led to a 3.1% decrease in sample cost for CMA, 6.3% for WES on HiSeq® 2500, 4.4% decrease for WES on NextSeq® 550, 5.7% for WGS-proband (HiSeqX™) and 3.8% for WGS-trio (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 on HiSeq® 2500 was reduced by 7.5%, and on NextSeq® 550 it was reduced by 7.4%. The cost per sample for the WGS-proband test was reduced by 5.1% and for the WGS-trio, it was reduced by 2.6%, both on the HiSeq X™ platform. The cost increase when four variants were found instead of two was 8.4% for the WES on HiSeq® 2500, 8.3% on NextSeq® 550, 4.9% for WGS-proband (HiSeq X™) and 2.5% for WGS-trio (HiSeq X™).

Figure 3. Deterministic sensitivity analysis of the effect of increasing the number of WES (HiSeq® 2500) tests per year for all indications from 500 to 1000 on sample costs in Year 1.



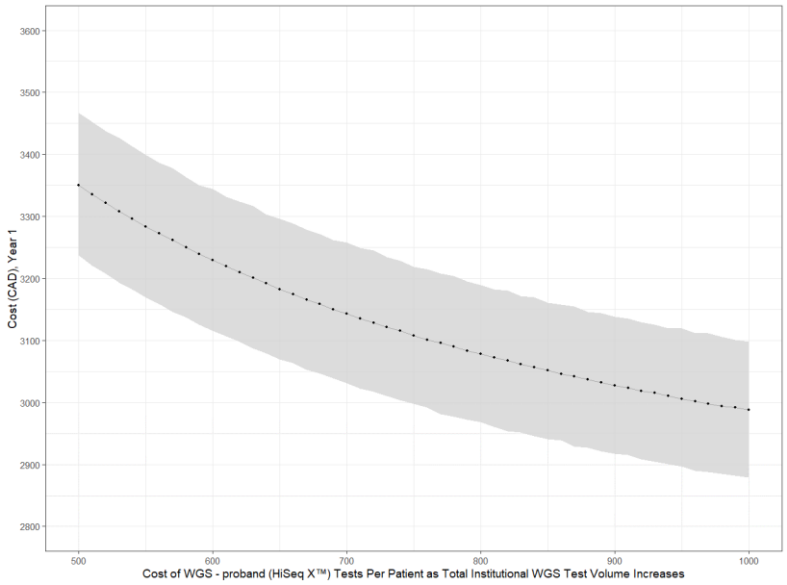
Costs are reported in 2018 CAD. Confidence bands are based on 10,000 Monte Carlo replications.
Abbreviations: ASD, Autism spectrum disorder; WES, Whole exome sequencing.

Figure 4. Deterministic sensitivity analysis of the effect of increasing the number of WES (NextSeq® 550) tests per year for all indications from 500 to 1000 on sample costs in Year 1.



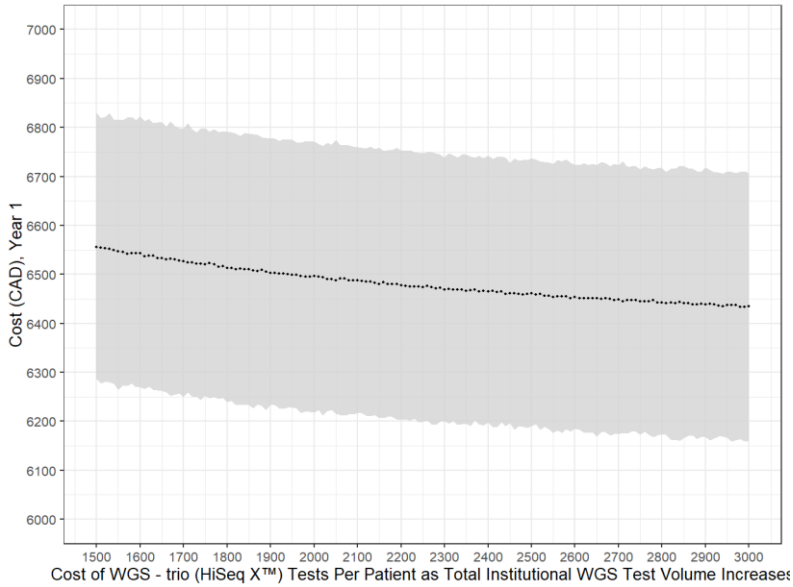
Costs are reported in 2018 CAD. Confidence bands are based on 10,000 Monte Carlo replications.
Abbreviations: ASD, Autism spectrum disorder; WES, Whole exome sequencing.

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



Costs are reported in 2018 CAD. Confidence bands are based on 10,000 Monte Carlo replications.
Abbreviations: ASD, Autism spectrum disorder; WGS, Whole genome sequencing

Figure 6. Deterministic sensitivity analysis of the effect of increasing the number of WGS-trio (HiSeq X™) tests per year for all indications from 1500 trios to 3000 trios on trio sample costs in Year 1.



Costs are reported in 2018 CAD. Confidence bands are based on 10,000 Monte Carlo replications.
Abbreviations: ASD, Autism spectrum disorder; WGS, Whole genome sequencing.

Table 13. Deterministic sensitivity analysis of estimated total cost per ASD sample for CMA, WES and WGS, varying 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%	799.7 (765.2, 833.6)	775.0 (741.6, 807.9)	751.1 (718.6, 783.0)	727.9 (696.4, 758.9)	705.4 (674.8, 735.5)
30%	840.0 (804.3, 875.0)	813.9 (779.2, 847.9)	788.6 (754.9, 821.6)	764.0 (731.3, 796.0)	740.3 (708.5, 771.3)
WES, HiSeq®2500					
10%	1843.2 (1786.7, 1899.3)	1778.7 (1723.9, 1833.3)	1716.5 (1663.3, 1769.3)	1656.4 (1604.9, 1707.4)	1598.3 (1548.4, 1647.7)
30%	2033.1 (1969.3, 2096.2)	1961.2 (1899.3, 2022.3)	1891.7 (1831.9, 1951.1)	1824.7 (1766.7, 1882.2)	1759.9 (1703.8, 1815.7)
WES, NextSeq® 550					
10%	1897.9 (1829.4, 1967.0)	1839.4 (1772.9, 1906.4)	1782.7 (1718.2, 1847.8)	1727.8 (1665.2, 1791.0)	1674.5 (1613.7, 1735.9)
30%	2032.4 (1957.8, 2108.1)	1969.4 (1897.0, 2042.8)	1908.4 (1838.0, 1979.6)	1849.2 (1781.0, 1918.2)	1791.9 (1725.7, 1859.0)
WGS, HiSeq X™ - proband					
10%	3168.6 (3057.1, 3280.7)	3060.1 (2951.9, 3168.5)	2955.2 (2850.6, 3060.2)	2853.8 (2752.5, 2955.2)	2755.8 (2657.6, 2854.0)
30%	3463.9 (3343.7, 3584.9)	3343.8 (3227.6, 3460.9)	3227.8 (3115.5, 3341.1)	3115.7 (3007.0, 3225.1)	3007.3 (2902.2, 3113.6)
WGS, HiSeq X™ - trio					
10%	6318.7 (6046.6, 6590.3)	6129.2 (5865.3, 6393.1)	5945.4 (5689.3, 6201.7)	5767.1 (5518.3, 6015.9)	5594.2 (5352.7, 5835.7)
30%	6704.6 (6423.1, 6981.8)	6502.9 (6229.5, 6772.3)	6307.3 (6041.7, 6569.1)	6117.5 (5859.6, 6372.0)	5933.5 (5683.0, 6180.6)

Estimates are given in 2018 Canadian dollars (CAD). Confidence intervals (CI) are based on 10,000 Monte Carlo replications.

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

Table 14. Deterministic sensitivity analysis of estimated total cost per ASD sample for WES and WGS, varying the number of primary variants.

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, HiSeq® 2500						
	0	1823.4 (1772.3, 1875.0)	1758.3 (1708.9, 1808.2)	1695.5 (1647.8, 1743.7)	1634.9 (1588.7, 1681.4)	1576.3 (1531.5, 1621.4)
	4	2123.9 (2046.3, 2199.2)	2050.1 (1974.8, 2122.9)	1978.8 (1905.8, 2049.4)	1909.9 (1839.2, 1978.2)	1843.4 (1774.6, 1909.5)
WES, NextSeq® 550						
	0	1843.9 (1779.6, 1910.5)	1786.6 (1724.2, 1851.2)	1731.1 (1670.6, 1793.8)	1677.4 (1618.7, 1738.2)	1625.2 (1568.3, 1684.3)
	4	2144.7 (2058.9, 2230.5)	2078.6 (1995.5, 2162.0)	2014.6 (1933.9, 2095.7)	1952.6 (1874.1, 2031.3)	1892.5 (1816.3, 1968.9)
WGS, HiSeq X™ (proband)						
	0	3186.3 (3076.9, 3297.1)	3075.4 (2969.7, 3182.5)	2968.3 (2866.2, 3071.6)	2864.8 (2766.1, 2964.2)	2764.8 (2669.3, 2861.2)
	4	3514.2 (3387.9, 3641.2)	3393.8 (3271.5, 3517.2)	3277.4 (3159.0, 3396.9)	3164.9 (3050.3, 3280.7)	3056.1 (2945.3, 3168.7)
WGS, HiSeq X™ (trio)						
	0	6391.9 (6114.9, 6666.4)	6199.7 (5930.7, 6466.4)	6013.3 (5752.0, 6272.4)	5832.4 (5578.7, 6084.2)	5657.0 (5410.6, 5901.3)
	4	6720.1 (6439.3, 6998.7)	6518.3 (6245.9, 6788.8)	6322.6 (6058.2, 6585.1)	6132.8 (5875.9, 6387.6)	5948.7 (5699.1, 6195.8)

Estimates are given in 2018 Canadian dollars (CAD). Confidence intervals (CI) are based on 10,000 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 15. Of the twenty-three 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.* [25] 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. This value was taken for the combined analysis of CMA+WES as WES alone does not capture CNVs fully.

Currently, there are no studies that estimate clinical WGS diagnostic yield for children with autism. Yuen *et al.* [11] 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 by Tammimies *et al.* Publication by Jiang *et al.* [17], 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 in a yield of 17.38% [51]. This value was included for the cost-consequence analysis of WGS-proband. However, since this calculation does not take into account non-coding variants, as well as CNVs detected by WGS in addition to those detected by CMA, 42.4% reported by Yuen *et al.* (10) was utilized in the study as a best case scenario analysis of the WGS diagnostic yield in probands. In addition, expert opinion indicated an increase in the diagnostic yield by approximately 2% for trio in comparison to proband in genome sequencing. Hence, 19.38% was adapted as the diagnostic yield value in the cost-consequence analysis of trio.

The incremental costs and incremental diagnostic yields for the four clinical scenarios for patients seen in Year 1 of the testing program are shown in Table 16. A ratio of incremental cost to incremental diagnostic yield was also calculated to determine the additional cost for every additional patient with a positive finding above and beyond the standard comparator. For the first scenario, CMA plus WES (HiSeq ® 2500) vs. CMA, the incremental cost was $1960.00 and the incremental diagnostic yield was 0.065. The incremental cost per additional patient with a positive finding was $30,153.85. While the diagnostic yield remained the same, the incremental cost was $20 more with the NextSeq® 550 platform. The incremental cost per additional patient with a positive finding for CMA plus WES (NextSeq® 550) vs. CMA was $30,470.77. For the second scenario of WGS-proband (HiSeq X™) vs. CMA, the incremental cost was $2525.80 with the incremental diagnostic yield of 0.081. When WGS-trio (HiSeq X™) was compared to CMA, the incremental cost was 2.27 times higher compared to the proband scenario with an incremental diagnostic yield of 0.101. For the proband analysis, incremental cost to diagnostic yield ratio was found to be $31,259.90. The incremental cost per additional patient with a positive finding increased by a factor of 1.8 in the trio analysis to $56,860.12. For the third scenario of WGS-proband vs. CMA plus WES, the incremental cost was $565.80 for WES conducted on the HiSeq® 2500 platform and $545.20 for WES done on the NextSeq® 550 platform. The incremental yield was estimated to be 0.0158. Thus the incremental cost was $35,810.13 for the HiSeq® 2500 platform and $34,506.33 for the NextSeq® 550 platform for every additional patient with a positive finding above and beyond the comparator. In the fourth scenario of WGS-trio vs. CMA plus WES, incremental cost was comparable when WES was conducted on the HiSeq® 2500 platform vs. the NextSeq® 550. With the same diagnostic yield of 0.0358, the incremental costs for every additional patient with a positive finding were $105,349.16 and $104,773.74, respectively.

If the diagnostic yield of WGS was 42.4%, the cost per additional patient with a positive finding would decrease substantially. Comparing WGS-proband with CMA, the incremental diagnostic yield was 0.331 and the incremental cost to incremental yield ratio decreased to $7630.82 on the HiSeq X™ platform. For the second scenario, WGS-proband vs. CMA plus WES, the incremental diagnostic yield was 0.266 for both HiSeq® 2500 and NextSeq® 550. The incremental cost per additional patient with a positive finding were estimated to be $2127.07 and $2049.62 on each of the platforms for exome sequencing, respectively.

Table 15. 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 (%)
Bowling *et al.* (2017) WES and WGS, USA	309 trios (284 families)	DD/ID	Mean age of 11 years	Inclusion: mild to severe ID with condition not accounted for by known causes; Autistic features with DD/ID phenotypes	Diagnostic yield was reported as the proportion of individuals with pathogenic or likely pathogenic variants, similar to the criteria by ACMG recommendations	Trio: 29.1%; Duo: 19.0%; Singleton: 15.0%
Rossi *et al.* (2017) [26], WES, USA	163	ASD, Autistic features	Mean age ± SD = 9.0 years ± 6.7 years	Exclusion: Secondary or incidental findings unrelated to the current clinical indication of the probands	Diagnostic yield was reported as the proportion of individuals with positive or likely positive findings in characterized genes	25.8%
Stavropoulos *et al.* (2016)[27], 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)
DDD Study (2015) [52], 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
Tammimies *et al.* (2015) [25], 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)

Taylor *et al.* (2015) [53], 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
Yuen *et al.* (2015) [11], 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
Atwal *et al.* (2014) [54], 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
Gilissen *et al.* (2014) [10], 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
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

Lee *et al.* (2014) [24], 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)
Roberts *et al.* (2014) [55], CMA, U.S.	215	ASD and learning disability	Mean age ± SD = 10 years ± 9.7 years; age range = 5 months to 52 years	Inclusion: ASD or learning disability patients referred for genetic services Exclusion: recognized syndrome such as Down syndrome, fragile X syndrome, or single gene disorders	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 or learning disability and (2) non-diagnostic variant or variant of unknown significance	ASD: 20% (Inc. variants of unknown significance) [9% diagnostic variants]
Soden *et al.* (2014) [56], WGS/WES, U.S.	119	DD, ID, cerebral palsy and ASD	Pediatric	Inclusion: Families with one or more children suspected of having a monogenetic disease, but without a definitive diagnosis	Diagnostic yield was referred to as the proportion of families with a molecular diagnosis. Rare variants were evaluated for pathogenicity using ACMG guidelines. Potentially pathogenic variants identified in candidate disease genes were not included in molecular diagnosis, unless validated	45.0
Srivastava *et al.* (2014) [57], 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
Yang *et al.* (2014) [22], WES, U.S.	2000	Neurological plus other	45.0%: <5 years of age; 42.2% 5 to 17	Inclusion: Patients were referred from physician for clinical WES. The	Diagnostic yield was reported as the proportion of patients with a molecular diagnosis. WES case was classified as molecularly diagnosed if pathogenic or	Neurological: All ages: 27.2

		organ systems	years of age; 12.2% adults; 0.6% fetal samples	request for WES was based on physician's discretion with no inclusion/ exclusion criteria by the lab	likely pathogenic variants were detected in Mendelian 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:23.5-31.2) <5 years: 30.4 (95% CI:24.3-37.3) 5-18 years: 26.1 (95% CI:21.1-31.9)
Jacob *et al.* (2013) [58], 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
Yang *et al.* (2013) [21], 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) [23], 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
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
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) [59], 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
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)

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; WES, Whole exome sequencing; WGS, Whole genome sequencing; ACMG, American College of Medical Genetics and Genomics

Table 16. 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			
1.1 CMA+WES (HiSeq 2500®) vs. CMA	1960.0 (1898.9, 2020.2)	0.065	$30,153.85
1.2 CMA+WES (NextSeq® 550) vs. CMA	1980.6 (1908.6, 2053.6)	0.065	$30,470.77
2. WGS vs. CMA			
2.1 WGS-proband (HiSeq X™) vs. CMA	2525.8 (2403.8, 2647.7)	0.081	$31,259.90
2.2 WGS-trio (HiSeq X™) vs. CMA	5731.5 (5450.9, 6007.8)	0.101	$56,860.12
3. WGS-proband vs. CMA+WES			
3.1 WGS-proband (HiSeq X™) vs. CMA+WES (HiSeq 2500®)	565.8 (429.8, 704.3)	0.0158	$35,810.13
3.2 WGS-proband (HiSeq X™) vs. CMA+WES (NextSeq® 550)	545.2 (404.7, 688.5)	0.0158	$34,506.33
4. WGS-trio vs. CMA+WES			
4.1 WGS-trio (HiSeq X™) vs. CMA+WES (HiSeq 2500®)	3771.5 (3489.0, 4055.6)	0.0358	$105,349.16
4.2 WGS-trio (HiSeq X™) vs. CMA+WES (NextSeq® 550)	3750.9 (3460.1, 4040.5)	0.0358	$104,773.74

Estimates are given in 2018 Canadian dollars (CAD). Confidence intervals (CI) for incremental cost are based on 10,000 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-trio (HiSeqX™) was the most expensive test, costing almost two times as much as WGS-proband (HiSeq X™), over three times as much as WES on both platforms and almost eight times as much as CMA. Per person cost of a trio however was found to be cheaper than a proband test by a factor of 1.53. The costs of WES for two different platforms, NextSeq® 550 and HiSeq® 2500, were nearly the same.

Labour and large equipment costs were reduced for the newer NextSeq® 550 platform while the reagent costs increased. Trio sequencing of WGS reduced the equipment and follow up costs while amplifying the bioinformatics, labour and supplies costs. Overall, supplies constituted the largest proportion of the total cost for all three tests. Of these, WGS-trio displayed the highest supply costs due to the greater consumption of costly reagents required for sequencing trios compared to probands on WGS or WES. Bioinformatics was also highest for WGS-trio due to the substantially higher computing demands. Labour was highest for both CMA and WGS-trio while it was similar between WES on both platforms and WGS probands. Equipment, supplies, labour 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 and trio (e.g. Sanger sequencing) on all positives and equivocal findings to rule out false positives. The need for validation testing was reduced by 40% for trios on WGS compared to probands on WGS and WES, which contributed to the reduction in overall cost of trio sequencing.

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 [60]. WES is transitioning into clinical practice while 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 [61]. 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, i.e. combination testing with CMA plus WES for all patients replacing CMA alone, WGS replacing CMA or WGS replacing CMA plus WES. This approach would be very costly, as the cost-consequence analysis revealed an *incremental* cost of over $30,000 for every additional patient with a positive finding beyond expected CMA results if CMA were to be wholly replaced by CMA+WES or by WGS (proband or trio) with our current knowledge of diagnostic yield. If WGS-proband replaced CMA plus WES, it is less expensive in comparison to CMA plus WES being replaced by WGS-trio. The Incremental ratio of this option is greater than $100,000 per additional patient with a positive finding. In reality, the testing pathway is likely to be more complex, where, for example, only syndromic patients with a negative first tier test (e.g. CMA) go on to receive a second tier 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 [62]. 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. Trio and duo sequencing options, if implemented in clinical practice, would influence the value gained and consequently the associated 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) [63] 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) [64] 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 [65]). Regier *et al.* 2010 [66] 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 [15, 60]. Towne *et al.* (2013) [67] reported an approximate trio-WES cost of $3700 USD per family in a conference abstract and Wright *et al.* (2013) [68] 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) [69] 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. Stark *et al.* (2017) analyzed the cost-effectiveness of singleton WES by comparing it to standard of care in infants with monogenic disorders. If WES was performed as a last resort, after thorough investigation by existing standard of care, the incremental cost per standard diagnosis was found to be $8,112 AUD ($7605 CAD, 2015) if on the other hand, WES was a first line test, the cost savings was reported to be $2,182 AUD ($2045 CAD, 2015). In the first scenario, all appointments, pathology tests, imaging, genetic testing and other costs related to standard of care were included. In the second scenario, only the first tier genetic test cost with respect to standard of care was included in the incremental analysis. Tan *et al*. (2017) [38] conducted similar research in children with suspected monogenic conditions who were non-diagnostic following microarray testing. Costs captured in their analysis included the initial visit to tertiary services for diagnostic purposes, the first clinical genetics assessment, enrollment and WES reporting. Other costs included specialist appointments, case conferences and transportation. Compared to standard diagnostic care without WES, care with WES

generated an incremental cost per additional diagnosis of $5760 AUD ($5400 CAD, 2015). In the alternate scenario where WES was performed after the first clinical genetics consultation, there was a savings of $5461 AUD ($5120 CAD, 2015) per additional diagnosis. This cost savings increased to $9020 AUD ($8457 CAD, 2015) if the WES was conducted at the initial tertiary presentation. In contrast to the study by Stark *et al.* which investigated infants, the Tan *et al.* study analyzed patients who were older than two years of age.

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 [41]. 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 [29, 41]. Interestingly, the brief literature review performed for the present report 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 [61]. Where the line is drawn with regard to reporting requirements will have a profound effect on queues for specialist consultations and health system costs [70, 71]. 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, WES and WGS were accounted for using the microcosting approach generating fully comprehensive per sample and program cost estimates of CGES. The provision of estimates for both proband and trio on the WGS platform provides information for decision makers on the value that trio analysis can add in comparison to proband analysis if WGS were to be implemented as standard clinical practice. Uncertainty associated with parameter estimates was captured in the probabilistic analysis using Monte Carlo simulations. Parameters that were highly uncertain or expected to vary substantially between institutions were varied in DSA 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 economies of scale can be realized to reduce sample costs as the volume of total CGES tests increases, in advance of full implementation. The level of this economic efficiency differed between platforms and between proband and trio sequencing. 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 changing the resource use items, 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. Furthermore, clinical interpretation and report writing were modelled to try and adapt it to a clinical setting but 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. In the case of trio analysis of WGS, the reduced need for Sanger sequencing decreased the turnaround time for families by 2-3 weeks, which was not incorporated in the economic models. Other downstream consequences, if modelled, would provide further insight into the possible benefits and/or disadvantages of trio analysis in comparison to proband testing. WES and CMA as combination tests are two parallel tests to be ordered. WGS was modelled as a single test to be compared against WES plus CMA. Although it is approximately equal in diagnostic yield to WES plus CMA, there may be potential efficiencies in ordering tests that would be evident if modelled. Software costs associated with bioinformatics were not included in the analysis for both WES and WGS as GATK is an open software. This is true in the research setting, however, in clinical practice these costs maybe incurred. Briggs *et al.* (2002) [72] 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. This is reflected in the need to update the 2016 estimates with the current report. A shorter life cycle would result in higher costs due to a shorter period of amortization.

Another limitation is the fact that 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. Furthermore, this study only costed routine, standard resource use items relevant to the testing workflow pathway. One-time expenses, staff training or changes to existing practices such as introducing a change in software were not included.

This microcosting study estimated the cost of WES and WGS using a bottom-up microcosting approach for probands on all tests and trios on WGS only. 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 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 [73].

5 Conclusion

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, CMA. In this study, the costs of CGES per ASD sample were $1960.00 (95% CI: 1898.90, 2020.20) for WES on HiSeq® 2500, $1980.60 (95% CI: 1908.60, 2053.60) on NextSeq® 550, $3350.30 (95% CI: 3233.70, 3467.40) for WGS-proband on Illumina HiSeq X™ platform and $6556.00 (95% CI: 6277.50, 6832.00) for WGS-trios compared to $824.50 (95% CI 789.00, 858.90) for CMA. Reagent supply costs accounted for the largest proportion of costs for each type of CGES. Of the three platforms, these costs were highest for NextSeq® 550. Between the proband and trio analysis on HiSeq X™ platform, supply costs for trios were 22% incrementally higher compared to the proband costs.

Using recent diagnostic yield literature, a cost-consequence analysis was conducted. This revealed an incremental cost of over $30,000 over and above current CMA test costs for every additional patient with a positive finding not found on CMA if CMA were to be wholly replaced by CMA+WES or by WGS proband or trio. Furthermore, incremental ratio (cost per additional patient with a positive finding) of WGS-trio was greater than $100,000. This suggests that based on current costs and diagnostic yields of ASD, WES or WGS could be reserved as second tier testing for negative or equivocal patients, or used in target populations with high rates of suspected ASD, such as infant siblings of confirmed cases. With

respect to WGS, the 'per sample' (per person) cost of a trio test is slightly more than half of the 'per sample' cost of proband only. However, the incremental change in diagnostic yield is minimal in a trio analysis for the doubling of the costs involved in the testing process. Although there are economies of scales achieved in various sub-categories of costs, it is, at this time an expensive option to consider.

In future, if the costs of testing continue to decrease, which may also be achieved through discounts, and if diagnostic yields of CGES in ASD continue to increase, the willingness of decision-makers to pay for each additional positive finding will influence whether CGES represents good value for money. 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.

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