Diagnostic Impact and Cost-effectiveness of Whole-Exome Sequencing for Ambulant Children With Suspected Monogenic Conditions

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IMPORTANT: Optimal use of whole-exome sequencing (WES) in the pediatric setting requires an understanding of who should be considered for testing and when it should be performed to maximize clinical utility and cost-effectiveness.

OBJECTIVES: To investigate the impact of WES in sequencing-naive children suspected of having a monogenic disorder and evaluate its cost-effectiveness if WES had been available at different time points in their diagnostic trajectory.

DESIGN, SETTING, AND PARTICIPANTS: This prospective study was part of the Melbourne Genomics Health Alliance demonstration project. At the ambulatory outpatient clinics of the Victorian Clinical Genetics Services at the Royal Children's Hospital, Melbourne, Australia, children older than 2 years suspected of having a monogenic disorder were prospectively recruited from May 1 through November 30, 2015, by clinical geneticists after referral from general and subspecialist pediatricians. All children had nondiagnostic microarrays and no prior single-gene or panel sequencing.

EXPOSURES: All children underwent singleton WES with targeted phenotype-driven analysis.

MAIN OUTCOMES AND MEASURES: The study examined the clinical utility of a molecular diagnosis and the cost-effectiveness of alternative diagnostic trajectories, depending on timing of WES.

RESULTS: Of 61 children originally assessed, 44 (21 [48%] male and 23 [52%] female) aged 2 to 18 years (mean age at initial presentation, 28 months; range, 0-121 months) were recruited, and a diagnosis was achieved in 23 (52%) by singleton WES. The diagnoses were unexpected in 8 of 23 (35%), and clinical management was altered in 6 of 23 (26%). The mean duration of the diagnostic odyssey was 6 years, with each child having a mean of 19 tests and 4 clinical genetics and 4 nongenetics specialist consultations, and 26 (59%) underwent a procedure while under general anesthetic for diagnostic purposes. Economic analyses of the diagnostic trajectory identified that WES performed at initial tertiary presentation resulted in an incremental cost savings of A$9020 (US$6838) per additional diagnosis (95% CI, A$4304-A$15 404 [US$3263-US$11 678]) compared with the standard diagnostic pathway. Even if WES were performed at the first genetics appointment, there would be an incremental cost savings of A$5461 (US$4140) (95% CI, A$1433-A$10 557 [US$1086-US$8004]) per additional diagnosis compared with the standard diagnostic pathway.

CONCLUSIONS AND RELEVANCE: Singleton WES in children with suspected monogenic conditions has high diagnostic yield, and cost-effectiveness is maximized by early application in the diagnostic pathway. Pediatricians should consider early referral of children with undiagnosed syndromes to clinical geneticists.
Children affected by genetic conditions often have multisystem disease, experience substantial morbidity and mortality, and have higher hospitalization rates with longer admissions compared with the general pediatric population.1-3 The evaluation for a genetic condition in a child, the so-called diagnostic odyssey, typically involves clinical assessment and multiple investigations, many of which are invasive and costly. The diagnostic trajectory can be prolonged, and many children continue to have undiagnosed conditions. Each technological advance in genetic analysis offers an opportunity to improve diagnostic yield, as exemplified by chromosomal microarray over conventional karyotyping,4 but none has the diagnostic power of genomic sequencing. Whole-exome sequencing (WES), the analysis of the protein-coding exons of genes, has the potential to revolutionize the diagnosis in children suspected of having monogenic disorders.5,6 Large-scale studies7-13 of WES in children with broad clinical presentations report diagnostic rates of 25% to 30%, applying WES after multiple genetic investigations, including single-gene or panel testing. Studies using WES as a last resort result in ascertainment bias toward diagnostically difficult cases because children with conditions diagnosed with an earlier gene test would be excluded. The application of WES to a clinically ascertained, sequencing-naive cohort is needed to understand the full impact of WES in the pediatric clinic and to assist clinicians in knowing when and for whom to consider singleton WES.

Few studies have investigated the cost-effectiveness of WES in the clinical setting, although an evaluation14 of the standard diagnostic approach hypothesized that applying next-generation sequencing at the first clinical visit could result in considerable cost savings. A study13 of syndromic children, more than half of whom had undergone single-gene or multigene panels, evaluated the cost-effectiveness of WES, but this was limited to comparing the cost of WES with the cost of prior genetic testing without including other diagnostic investigations. An evaluation15 of the retrospective use of trio WES in a cohort of 17 children with intellectual disability found that it would have been less costly than the traditional diagnostic trajectory. Although this study indicated that WES would have been less costly than other investigations, retrospective studies do not include those who receive a diagnosis through usual diagnostic care; thus, the applicability of those studies to clinical practice is limited. When undertaking a robust study of the cost-effectiveness of WES, it is important to undertake WES prospectively in a clinical cohort and to include all investigations for diagnostic purposes, such as brain magnetic resonance imaging and metabolic investigations, because it is feasible that with early WES diagnosis, these tests may no longer be necessary.

A previous prospective study16 focused on infants younger than 2 years who were suspected of having a monogenic disorder and found a diagnostic yield of 57% for WES compared with 13.5% diagnosed with standard care. In the present study, we sought to prospectively determine the diagnostic yield of WES in older, ambulant children suspected of having a monogenic condition but who were sequencing naïve, that is, having not had any single-gene or panel testing. We assessed the cost-effectiveness of the use of WES at the start of the diagnostic odyssey, first presentation to a clinical geneticist, and at the end of the standard diagnostic pathway in a clinical cohort, capturing the impact of the inclusion of WES on the cost of all investigations undertaken for diagnostic purposes in the hospital setting. Our objectives were to determine the clinical impact of WES in these children and to determine the point in the diagnostic trajectory at which WES is most cost-effective.

Methods

Study Design and Participants
We prospectively recruited ambulant children aged 2 to 18 years suspected of having a monogenic condition from the outpatient clinics of Victorian Clinical Genetics Services at the Royal Children’s Hospital, Melbourne, Australia, from May 1 through November 30, 2015. The Royal Children’s Hospital is the major tertiary referral center for children in the states of Victoria and Tasmania. All children had at least one clinical assessment by a clinical geneticist, and a panel of investigators discussed each case to determine eligibility. Our study population represents a typical cohort referred by pediatricians for genetics evaluation but remaining undiagnosed after clinical assessment. We did not include children whose diagnosis would usually be made by clinical assessment, such as achondroplasia or neurofibromatosis type 1. All children in our cohort had a nondiagnostic single-nucleotide polymorphism microarray. We included only children who had not had any prior single gene or panel sequencing test. We did not exclude those who had tests for genetic abnormalities not detectable by WES (eg, triplet repeat analysis or methylation studies). Because this was not a gene discovery project and data analysis was limited to genes currently known to cause monogenic disorders (the Mendeliome), we excluded children deemed to have novel phenotypes, in whom we were not able to derive a reasonable differential diagnosis list. At enrollment, we recorded each participant’s clinical features in PhenoTips using Human Phenotype Ontology terms.17 Each referring physician generated
a phenotype-driven list of candidate genes for prioritized analysis, which was supplemented by database searches and team discussion at recruitment.

Parents provided written informed consent after genetic counseling. This study was part of the Melbourne Genomics Health Alliance demonstration project (http://www.melbournegenomics.org.au) and approved by the Royal Melbourne Hospital Human Research Ethics Committee.18

**Exome Sequencing, Variant Detection, and Filtering**

We performed exome sequencing, variant detection, and filtering as described previously.16,19 The mean coverage obtained was 147.5 (95% CI, 109.8-233.3). Only variants in the HUGO Gene Nomenclature Committee genes associated with mendelian disease before the end of 2015 (3203 genes) were available for initial analysis. Variants were assessed using the Melbourne Genomics variant curation database, a modification of the Leiden Open Variation Database.20 Variants were prioritized on the basis of the phenotype-driven gene lists for each participant (Gene Prioritization Index) and predicted effect (Variant Prioritization Index).21 We only assessed the pathogenicity of variants relevant to the participant’s phenotype, and classification was based on the American College of Medical Genetics and Genomics standards for interpretation of sequence variants.21

Variant classifications were reviewed in a multidisciplinary team meeting. Sanger sequencing was used to confirm pathogenic and likely pathogenic variants of clinical significance. Parents also underwent Sanger sequencing to establish phase and segregation. The WES data of unsolved cases were reexamined to identify variants in newly discovered genes causative of a phenotype relevant to the child.

**Costing Approach**

We collected cost data in Australian dollars for all children22 from dates that included initial presentation to tertiary services for diagnostic purposes, first clinical genetics assessment, enrollment, and WES report. All diagnostic inpatient and outpatient episodes of care, including investigations, specialists consulted, duration of admission, and travel, were collected from medical records. We obtained costs of hospital admissions, outpatient specialist appointments, case conferences, diagnostic investigations (including operating theater and general anesthesia from the Clinical Costings Department of the Royal Children’s Hospital), and the Australian Medicare Benefits Schedule (http://www.mbsonline.gov.au). We estimated the shortest distance from home to the Royal Children’s Hospital multiplied by the cost per kilometer (A$0.20 [US$0.15] as per the Victorian Patient Assistance Scheme; https://www2.health.vic.gov.au/hospitals-and-health-services/rural-health/vptas-how-to-apply) to calculate the costs incurred by the health system for travel from home, for which the family is reimbursed by the state. We obtained costs of any flights required from the most economical carrier.

**Cost-effectiveness Analysis**

We considered 3 diagnostic counterfactual scenarios23 and the actual trajectory and compared their costs and diagnostic yields to investigate which option was most cost-effective (Figure 1). First, we considered the standard diagnostic pathway without WES, which included all investigations and clinical assessments that occurred primarily for diagnostic purposes, including microarray, without costs associated with WES. Second, we considered the standard diagnostic pathway with WES, which included all aforementioned investigations and clinical assessments with WES as the final test. The cost of singleton WES was provided by the laboratory service, and the genetic service delivery model included consultations with a clinical geneticist and genetic counselor for pretest assessment or counseling and result disclosure. We included the cost of proband and parental Sanger sequencing to validate variants detected by WES. Third, we considered WES at the first genetics appointment, which included all costs up to and including the first genetics appointment with cost of WES (test, Sanger validation, and genetic service delivery) incorporated. Fourth, we considered WES at initial tertiary presentation, which included all costs incurred leading to the initial presentation, which included all costs incurred leading to the initial presentation.
Table 1. Clinical Data for 44 Children Prospectively Recruited for WES*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21 (48)</td>
</tr>
<tr>
<td>Female</td>
<td>23 (52)</td>
</tr>
<tr>
<td><strong>Age at enrollment, y</strong></td>
<td></td>
</tr>
<tr>
<td>2-10</td>
<td>30 (68)</td>
</tr>
<tr>
<td>10-18</td>
<td>14 (32)</td>
</tr>
<tr>
<td><strong>Primary phenotype in children with diagnosed conditions</strong></td>
<td></td>
</tr>
<tr>
<td>Dysorphic with multiple congenital anomalies</td>
<td>12/21 (57)</td>
</tr>
<tr>
<td>Neurometabolic</td>
<td>6/8 (75)</td>
</tr>
<tr>
<td>Intellectual disability without congenital anomalies</td>
<td>1/7 (14)</td>
</tr>
<tr>
<td>Skeletal dysplasia</td>
<td>2/4 (50)</td>
</tr>
<tr>
<td>Dermatological</td>
<td>2/4 (50)</td>
</tr>
<tr>
<td><strong>Primary indication for WES in children with diagnosed conditions</strong></td>
<td></td>
</tr>
<tr>
<td>Unknown diagnosis</td>
<td>11/28 (39)</td>
</tr>
<tr>
<td>Genetically heterogeneous condition</td>
<td>12/14 (86)</td>
</tr>
<tr>
<td>Suspected composite phenotype</td>
<td>0/2 (0)</td>
</tr>
<tr>
<td><strong>Age at presentation to tertiary care for diagnostic purposes, mean (range), mo</strong></td>
<td></td>
</tr>
<tr>
<td>Children aged 2-10 y</td>
<td>19 (0-71)</td>
</tr>
<tr>
<td>Children aged 10-18 y</td>
<td>47 (0-121)</td>
</tr>
<tr>
<td><strong>Interval between tertiary presentation to genetics assessment, mean (range), mo</strong></td>
<td></td>
</tr>
<tr>
<td>Children aged 2-10 y</td>
<td>7 (0-65)</td>
</tr>
<tr>
<td>Children aged 10-18 y</td>
<td>25 (0-113)</td>
</tr>
<tr>
<td><strong>Interval between first genetics assessment and WES report, mean (range), mo</strong></td>
<td></td>
</tr>
<tr>
<td>Children aged 2-10 y</td>
<td>39 (2-103)</td>
</tr>
<tr>
<td>Children aged 10-18 y</td>
<td>103 (7-197)</td>
</tr>
<tr>
<td><strong>No. of specialist (excluding genetics) appointments for diagnostic purposes, mean (range)</strong></td>
<td></td>
</tr>
<tr>
<td>Children aged 2-10 y</td>
<td>5 (0-15)</td>
</tr>
<tr>
<td>Children aged 10-18 y</td>
<td>4 (1-7)</td>
</tr>
<tr>
<td><strong>Children requiring anesthesia for diagnostic purposes</strong></td>
<td></td>
</tr>
<tr>
<td>26/44 (59)</td>
<td></td>
</tr>
<tr>
<td><strong>Travel distance from home, km</strong></td>
<td></td>
</tr>
<tr>
<td>0-80</td>
<td>30 (68)</td>
</tr>
<tr>
<td>&gt;80</td>
<td>14 (32)</td>
</tr>
<tr>
<td><strong>Hospital admissions for diagnostic purposes</strong></td>
<td></td>
</tr>
<tr>
<td>10 (9 children)</td>
<td></td>
</tr>
<tr>
<td><strong>WES outcomes</strong></td>
<td></td>
</tr>
<tr>
<td>Change in management</td>
<td>6 (14)</td>
</tr>
<tr>
<td>Stopping planned investigations</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Prenatal diagnosis</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

Abbreviation: WES, whole-exome sequencing.

* Data are presented as number (percentage) of children unless otherwise indicated.

appointment at a tertiary hospital, with the cost of WES (test, Sanger validation, and genetic service delivery) replacing the initial tertiary assessment and investigations.

Sensitivity Analysis

We undertook sensitivity analysis using a higher cost of singleton WES in another Australian laboratory (A$23000) to examine robustness of the original estimates.

Statistical Analysis

We calculated mean cost per patient, mean cost per diagnosis, and incremental cost per additional diagnosis for WES used at 3 time points in the diagnostic trajectory compared with the standard diagnostic trajectory. We performed bootstrap simulations with 1000 replications to determine 95% CIs of all outcomes to examine the distribution of our estimates.24 All statistical analyses were performed in Microsoft Excel (Microsoft Corp) and SAS statistical software, version 9.4 (SAS Institute Inc).

Results

Of 61 children initially assessed, 17 were excluded (novel phenotype, n = 3; enrolled in another genomic project, n = 7; declined or consent withdrawn, n = 5; microarray diagnosis, n = 2), leaving 44 children aged 2 to 18 years who were prospectively recruited from outpatient clinics (Table 1). There were 21 male children (48%) and 23 female children (52%). Thirty (68%) lived within 80 km of the Royal Children’s Hospital, whereas 14 (32%) lived in a regional center. The largest phenotypic subgroup was dysmorphic with multiple congenital anomalies (21 of 44 [48%]). None of the children had undergone prior single-gene or panel testing. The median number of genes for prioritized analysis was 15 (range, 1-60). The main clinical indications for WES were unknown diagnosis (64%), genetically heterogeneous condition (32%), or a composite of 2 or more phenotypes (5%). The mean number of Human Phenotype Ontology terms recorded per child was 10 (range, 3-27). A Human Phenotype Ontology term related to intellectual or developmental disability was recorded in 31 of 44 children (70%); however, most had additional dysmorphisms, and only 7 had intellectual disability as their primary phenotype with nonspecific features.

The mean age at initial presentation to tertiary services for diagnostic purposes was 28 months (range, 0-121 months). The mean interval between first presentation to tertiary services and genetics assessment was 13 months (range, 0-113 months). Two children had antenatal ultrasound anomalies detected, and their first postnatal contact with tertiary services was pediatric and genetics assessment at birth. The mean duration from consent to WES report was 181 days (range, 40-283 days). Most children (37 of 44 [84%]) were referred by general or specialist pediatricians, and all had nondiagnostic chromosomal microarray. All children with developmental or intellectual disability underwent testing for fragile X syndrome. In addition to a clinical geneticist, the mean number of specialists seen by each child for diagnostic purposes was 4 (range, 1-7). Twenty-six children (59%) required general anesthesia for diagnostic purposes. Nine children were admitted to the hospital for diagnostic workup; one child required 2 admissions.

Whole-exome sequencing achieved a molecular diagnosis in 23 of 44 children (52%) (eTable in the Supplement). In 8 children (35%) with diagnosed conditions, the causative gene was clinically unexpected and not in the prioritized gene list. Most of the diagnoses were de novo heterozygous mutations (14 of 23 [61%]), followed by autosomal recessive (6 of 23 mutations), and rare autosomal dominant (10 of 23 [43%]).
Whole-exome sequencing at initial tertiary presentation resulted in incremen
tal savings per additional diagnosis of A$35054 (US$13620–US$26583).
Whole-exome sequencing applied at initial tertiary presentation or first
genetics appointment (A$13481 [US$10225]; 95% CI, A$10097–A$17957
[US$11681–US$9792]) followed by WES performed at first genetics
appointment (A$8026–US$11747). Whole-exome sequencing performed at
initial tertiary presentation had the lowest cost per patient (A$15816 [US$3933];
95% CI, A$4637–A$5811 [US$3517–US$4407]), followed by WES performed
at the first genetics appointment (A$7047 [US$5347]; 95% CI, A$4637–A$5811
[US$3517–US$4407]) and traditional pathway combined with WES (A$8293
[US$6580–US$13820] [US$5825–US$10482]), followed by WES performed at
first genetics appointment (A$7047 [US$5347]; 95% CI, A$4637–A$5811
[US$3517–US$4407]). No diagnoses were made with the pathway without WES
(by study design), but the pathway combined with WES achieved 23
diagnoses (23 of 44 [52%]). Whole-exome sequencing applied at initial
tertiary presentation or first genetics appointment would have resulted in
the same number of diagnoses but at lower cost. Whole-exome sequencing at
initial tertiary presentation had the lowest mean cost per diagnosis (A$9922
[US$7526]; 95% CI, A$7566–A$12 498 [US$5738–US$9478]). The cost per patient
of the standard diagnostic pathway without WES was A$12 912
(US$9792) (95% CI, A$10 583–A$15 490 [US$8026–US$11747]). Whole-exome
sequencing performed at initial tertiary presentation had the lowest cost per
patient (A$15 816 [US$3933]; 95% CI, A$4637–A$5811 [US$3517–US$4407]),
followed by WES performed at the first genetics appointment (A$7047
[US$5347]; 95% CI, A$4637–A$5811 [US$3517–US$4407]).

Whole-exome sequencing at initial tertiary presentation resulted in
incremental savings per additional diagnosis of A$9020 (US$6840) (95%
CI, A$15 404–A$4304 [US$11 681–US$3265]) compared with the standard
diagnostic pathway. If WES were performed after the first clinical genetics
consultation, there would still be an incremental savings per additional
diagnosis of A$5 461 (US$4143) (95% CI, A$10 577–A$14 433 [US$8009–
US$1087]) compared with the standard diagnostic pathway. Adding WES
to the standard diagnostic pathway does not offer a cost savings, but it incurs
an additional cost of A$5 760 (US$4371) (95% CI, A$4 692–A$7 799 [US$3 561–US$5 981]) per diagnosis. The
cost-effectiveness plane (Figure 2) for this cohort confirms that WES performed
at initial tertiary presentation was most cost-effective compared with the
standard diagnostic pathway. The bootstrapped analyses indicate that these results
can be extrapolated to a larger cohort.

Discussion
Our study confirms the clinical utility of WES in syndromic children
and provides strong evidence to support its use early in the
diagnostic trajectory. The children prospectively recruited for
singleton WES were suspected of having a genetically hetero-
genous condition or had features overlapping several condi-
tions. We excluded children with novel phenotypes more suited
to gene discovery projects and those with highly specific features
more appropriately investigated with single-gene tests. In
subgroup analysis, we found that the lowest diagnostic yield (14%)
was in those with intellectual disability without congenital
anomalies. Trio WES may have a higher yield in this subgroup.25,26
The higher diagnostic yield (86%) in those suspected of having a genetically heterogeneous condition compared
with those with an unknown diagnosis (39%) suggests that
the former represents a more genetically tractable group, which
is consistent with the relatively high yields of targeted panels
for specific phenotypes.27,28 A targeted panel with multiplex
ligation–dependent probe amplification instead of WES in those
with a genetically heterogeneous condition may have resulted in
a higher diagnostic yield because of better coverage and dis-
cover of indels, with different cost implications. However, WES
affords a more comprehensive approach to genetically hetero-
genous conditions, allowing reanalysis of new genes and analy-
sis of the Mendelio, and is likely to be more cost-effective than
gene-by-gene interrogation.9

We expected that the diagnostic yield in our cohort of am-
bulant children would be lower than that observed in a previous
study of infants16 because we were ascertaining older children
who remained undiagnosed beyond infancy. The observation that
the diagnostic yield was equivalent (52%) probably reflects the
similar approach in both studies, with prospective ascertainment,
through phenotype determination, defined selection criteria, and close
clinician-laboratory partnership in the use of phenotype knowledge
for variant curation. Sustainable solutions to integrate clini-
cian input into variant curation remain an important challenge
if the true power of WES is to be realized. Although phenoty-
ping is critical, 35% of children had a diagnosis caused by a gene
outside the initially prioritized gene list. This finding not only
possibly reflects lack of clinical recognition but also underscores the
utility of WES in achieving a diagnosis even when the a priori
hypothesis is imprecise.

In our study, WES demonstrated benefits beyond resolution
of the diagnostic odyssey, leading to clinically meaningful out-
comes, such as a change in management in 6 of 23 cases (26%),
stopping of planned investigations, and prenatal or preimplan-
tation genetic diagnosis in another 2 of 23 cases (9%). Manage-
ment changes included the institution of surveillance for com-
lications, stopping of surveillance for suspected diagnoses when
an alternative diagnosis was confirmed, and referral for special-
ist assessment of known or evolving complications of the
condition. This finding is similar to what we observed with the
infant cohort and reported by others.9,13,16

We documented the diagnostic trajectory of the children in
our cohort and modeled the cost-effectiveness of WES applied
at different time points. We found that the cost of the standard
diagnostic pathway was high and that earlier application of WES
maximized cost-effectiveness. This cohort is distinct from the
neonates and infants previously described16,29,30 because they
were less acutely unwell and had a higher mean age of 28 months
at first presentation. The phenotypic subgroup of intellectual
disability without congenital anomalies would be unusual in an
infant cohort. Our study also differs from others reporting clinical
utility of WES9,10,14,31,32 because we recruited children in whom
Table 2. Bootstrapped Cost of Investigations in the 44 Children and Comparison of 3 Models of Integrating WES Into the Diagnostic Trajectory

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Costs, A$ (US$)</th>
<th>Standard Diagnostic Trajectory Without WES</th>
<th>Standard Diagnostic Trajectory With WES</th>
<th>WES at First Genetics Appointment</th>
<th>WES at First Tertiary Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomical pathologic test</td>
<td>4434.72 (3368.79)</td>
<td>4434.72 (3368.79)</td>
<td>652.30 (494.92)</td>
<td>383.55 (275.84)</td>
<td></td>
</tr>
<tr>
<td>Hematologic test</td>
<td>2299.45 (1746.75)</td>
<td>2299.45 (1746.75)</td>
<td>1415.26 (1074.20)</td>
<td>203.40 (154.33)</td>
<td></td>
</tr>
<tr>
<td>Basic biochemistry analysis</td>
<td>4869.69 (3698.73)</td>
<td>4869.69 (3698.73)</td>
<td>2860.57 (2170.65)</td>
<td>1118.65 (848.77)</td>
<td></td>
</tr>
<tr>
<td>Complex biochemistry analysis</td>
<td>28 170.79 (21 365.33)</td>
<td>28 170.79 (21 365.33)</td>
<td>12438.05 (9439.40)</td>
<td>2903.20 (2030.38)</td>
<td></td>
</tr>
<tr>
<td>Serologic or immunologic test</td>
<td>5608.30 (4253.63)</td>
<td>5608.30 (4253.63)</td>
<td>4280.40 (3247.17)</td>
<td>278.29 (211.20)</td>
<td></td>
</tr>
<tr>
<td>Genetic test (SNP microarray)</td>
<td>26 268.00 (19 921.97)</td>
<td>26 268.00 (19 921.97)</td>
<td>26 268.00 (19 921.97)</td>
<td>26 268.00 (19 921.97)</td>
<td></td>
</tr>
<tr>
<td>Genetic test (other)</td>
<td>19 220.14 (14573.71)</td>
<td>19 220.14 (14573.71)</td>
<td>7121.05 (5400.62)</td>
<td>4149.30 (3146.84)</td>
<td></td>
</tr>
<tr>
<td>Imaging</td>
<td>40 572.36 (30 769.69)</td>
<td>40 572.36 (30 769.69)</td>
<td>15658.59 (11 875.85)</td>
<td>7309.10 (5544.02)</td>
<td></td>
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<tr>
<td>Electrophysiologic test</td>
<td>41 906.70 (31781.57)</td>
<td>41 906.70 (31781.57)</td>
<td>25101.90 (19 018.95)</td>
<td>16 475.70 (12 483.14)</td>
<td></td>
</tr>
<tr>
<td>Subspecialist appointment</td>
<td>101 613.22 (77 064.64)</td>
<td>101 613.22 (77 064.64)</td>
<td>29838.91 (22 627.42)</td>
<td>4424.46 (3350.92)</td>
<td></td>
</tr>
<tr>
<td>Genetics appointment</td>
<td>69 297.35 (52 555.91)</td>
<td>69 297.35 (52 555.91)</td>
<td>150.90 (114.44)</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>OT or anesthesia</td>
<td>60 502.18 (45 884.16)</td>
<td>60 502.18 (45 884.16)</td>
<td>19497.42 (14 786.23)</td>
<td>11 788.00 (8939.68)</td>
<td></td>
</tr>
<tr>
<td>Case conference</td>
<td>10 708.70 (8121.69)</td>
<td>10 708.70 (8121.69)</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
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<tr>
<td>Travel</td>
<td>2053.00 (1557.00)</td>
<td>2053.00 (1557.00)</td>
<td>547.00 (414.83)</td>
<td>110.00 (83.42)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>18 140.56 (13 757.81)</td>
<td>18 140.56 (13 757.81)</td>
<td>11617.51 (8811.14)</td>
<td>203.40 (154.27)</td>
<td></td>
</tr>
<tr>
<td>WES</td>
<td>0.00</td>
<td>88 000.00 (66 740.99)</td>
<td>88 000.00 (66 740.99)</td>
<td>88 000.00 (66 740.99)</td>
<td></td>
</tr>
<tr>
<td>Sanger confirmation</td>
<td>0.00</td>
<td>13 800.00 (10 466.35)</td>
<td>13 800.00 (10 466.35)</td>
<td>13 800.00 (10 466.35)</td>
<td></td>
</tr>
<tr>
<td>WES appointments</td>
<td>0.00</td>
<td>30 676.80 (23 278.56)</td>
<td>50 810.76 (38 556.87)</td>
<td>50 810.76 (38 556.87)</td>
<td></td>
</tr>
<tr>
<td>Total cost</td>
<td>435 665.16 (330 701.56)</td>
<td>568 141.96 (431 325.49)</td>
<td>310058.62 (235 357.12)</td>
<td>228 205.81 (173 224.86)</td>
<td></td>
</tr>
<tr>
<td>Mean cost per patient (95% CI)</td>
<td>9901.48 (7515.01)</td>
<td>12 992.12 (10 800.01)</td>
<td>12 992.12 (10 800.01)</td>
<td>12 992.12 (10 800.01)</td>
<td></td>
</tr>
<tr>
<td>Mean cost per diagnosis (95% CI)</td>
<td>24 701.82 (18 762.19)</td>
<td>14 244.32 (10 006.88)</td>
<td>14 244.32 (10 006.88)</td>
<td>14 244.32 (10 006.88)</td>
<td></td>
</tr>
<tr>
<td>Incremental cost per additional diagnosis (95% CI)</td>
<td>5759.86 (4572.80)</td>
<td>3750.71 (2948.83)</td>
<td>-5461.15 (-3147.04)</td>
<td>-5461.15 (-3147.04)</td>
<td></td>
</tr>
<tr>
<td>Sensitivity analysis assuming WES cost of A$2300</td>
<td>9901.48 (7515.01)</td>
<td>12 992.12 (10 800.01)</td>
<td>12 992.12 (10 800.01)</td>
<td>12 992.12 (10 800.01)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NA, not applicable; OT, operating theater; SNP, single-nucleotide polymorphism; WES, whole-exome sequencing.

*Genetic test (other) comprises investigations for molecular lesions not detectable by WES.
Diagnostic Impact and Cost-effectiveness of Whole-Exome Sequencing

Diagnostic Impact and Cost-effectiveness of Whole-Exome Sequencing

ARTICLE INFORMATION
Accepted for Publication: May 1, 2017.
Published Online: July 31, 2017.

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no prior single-gene or panel testing had been performed, a change in the current paradigm of applying WES after other tests are done.36,37 In one cohort of children with complex neurologic conditions, the cost of prior genetic testing comprised 43% of the total workup.32 Even without the high costs associated with previous gene testing, our economic analysis found an incremental savings per additional diagnosis of A$9020 (US$6845) (95% CI, A$15 404–A$4304 [US$11 689–US$3266]). Whole-exome sequencing performed at first genetics appointment was also cost saving, with an incremental savings per additional diagnosis of A$5461 (US$4145) (95% CI, A$10 557–A$1433 [US$8012–US$1088]) compared with the standard diagnostic pathway without WES. Adding WES after a protracted diagnostic odyssey was the most expensive scenario. The cost estimations are conservative and did not include diagnostic investigations undertaken by community health care professionals. A future study with larger numbers of prospectively recruited children at the start of their diagnostic trajectory who are randomly assigned to pathways that involve WES applied at different times would be beneficial in confirming this finding. Trio WES may have increased diagnostic yield,26,31 but its cost-effectiveness requires formal evaluation. In a retrospective cohort of children with neurodevelopmental disorders, the diagnostic yield was 40% in the ambulatory subgroup and retrospective evaluation suggested that the break-even point at which trio WES would cost no more than standard diagnostic investigations (excluding physician appointments) would be US$7640 per family (US$2996 for singleton WES).31 A retrospective study31 of 17 children with intellectual disability with previously undiagnosed conditions found that WES delivered substantial cost savings if other genetic tests and metabolic investigations were replaced.

Although we found that WES is most cost-effective early in the diagnostic trajectory, WES is not to replace a thorough clinical geneticist evaluation. Accordingly, we incorporated the costs of genetic appointments in all scenarios that involve WES. We suggest that early referral of a syndromic child by their pediatrician to a clinical geneticist be considered; however, rather than being offered to all referred children, singleton WES has highest clinical utility in selected children in whom a phenotype-driven gene list can be generated. Although trio WES may have further improved the diagnostic yield in those with nonsyndromic intellectual disability, its cost-effectiveness needs to be determined. A similar evaluation would need to be undertaken to determine the clinical utility and cost-effectiveness of whole-genome sequencing.

Limitations
As mentioned, this study has several limitations. Our patient group comprises children with genetically heterogeneous con-
Author Contributions: Drs Gaff and White contributed equally to this work. Drs Tan and White had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Tan, Dillon, Stark, Schofield, Alam, Shrestha, Oshlack, Gaff, White.

Acquisition, analysis, or interpretation of data: Tan, Dillon, Stark, Schofield, Alam, Shrestha, Chong, Phelan, Brent, Creed, Jarmolowicz, Yap, Walsh, Downie, Amor, Savarirayan, McCullivray, Yeung, Peters, Robertson, Robinson, Sadedin, Bell, Oshlack, Georgeson, Thorne, White.

Drafting of the manuscript: Tan, Dillon, Schofield, Alam, Shrestha, Chong, Amor, Robertson, Robinson, Georgeson, White.

Critical revision of the manuscript for important intellectual content: Tan, Stark, Schofield, Alam, Amor, Yeung, Macciocca, Sadedin, Bell, Oshlack, Thorne, Gaff, White.

Statistical analysis: Tan, Dillon, Schofield, Alam, Shrestha, Oshlack, Georgeson.

Obtained funding: Amor, Gaff.

Administrative, technical, or material support: Tan, Dillon, Amor, Phelan, Brent, Creed, Jarmolowicz, Yap, Downie, Yeung, Peters, Robertson, Robinson, Macciocca, Sadedin, Georgeson.

Supervision: Tan, Stark, Schofield, Oshlack, White.

Conflict of Interest Disclosures: None reported.

Funding/Support: This study was funded by the founding organizations of the Melbourne Genomics Health Alliance (Royal Melbourne Hospital, Royal Children’s Hospital, University of Melbourne, Walter and Eliza Hall Institute, Murdoch Childrens Research Institute, Australian Genome Research Facility, and Commonwealth Scientific and Industrial Research Organisation) and the State Government of Victoria (Department of Health and Human Services). The involvement of Australian Genome Research Facility was supported by sponsorship from Bioplatforms Australia and the National Collaborative Research Infrastructure Strategy program.

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and the decision to submit the manuscript for publication.

Additional Contributions: Elin Wee, clinical coverings officer at the Royal Children’s Hospital, and Leeanne Cavanough provided administrative support. They were not compensated for their work. We thank all collaborators in the Melbourne Genomics Health Alliance demonstration project.