Original Investigation

Molecular Diagnostic Yield of Chromosomal Microarray Analysis and Whole-Exome Sequencing in Children With Autism Spectrum Disorder

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IMPORTANCE The use of genome-wide tests to provide molecular diagnosis for individuals with autism spectrum disorder (ASD) requires more study.

OBJECTIVE To perform chromosomal microarray analysis (CMA) and whole-exome sequencing (WES) in a heterogeneous group of children with ASD to determine the molecular diagnostic yield of these tests in a sample typical of a developmental pediatric clinic.

DESIGN, SETTING, AND PARTICIPANTS The sample consisted of 258 consecutively ascertained unrelated children with ASD who underwent detailed assessments to define morphology scores based on the presence of major congenital abnormalities and minor physical anomalies. The children were recruited between 2008 and 2013 in Newfoundland and Labrador, Canada. The probands were stratified into 3 groups of increasing morphological severity: essential, equivocal, and complex (scores of 0-3, 4-5, and ≥6).

EXPOSURES All probands underwent CMA, with WES performed for 95 proband-parent trios.

MAIN OUTCOMES AND MEASURES The overall molecular diagnostic yield for CMA and WES in a population-based ASD sample stratified in 3 phenotypic groups.

RESULTS Of 258 probands, 24 (9.3%, 95% CI, 6.1%-13.5%) received a molecular diagnosis from CMA and 8 of 95 (8.4%, 95% CI, 3.7%-15.9%) from WES. The yields were statistically different between the morphological groups. Among the children who underwent both CMA and WES testing, the estimated proportion with an identifiable genetic etiology was 15.8% (95% CI, 9.1%-24.7%; 15/95 children). This included 2 children who received molecular diagnoses from both tests. The combined yield was significantly higher in the complex group when compared with the essential group (pairwise comparison, $P = .002$).

Positive Results	Essential Group	Equivocal Group	Complex Group	*P* Value for 3-Group Comparison
CMA, No./total No.	7/168	4/37	13/53	<.001
% (95% CI)	4.2 (1.7-8.4)	10.8 (3.0-25.4)	24.5 (13.8-38.3)	
WES, No./total No.	2/64	2/7	4/24	.02
% (95% CI)	3.1 (0.0-10.8)	28.6 (3.7-71.0)	16.7 (4.7-37.4)	
CMA and/or WES, No./total No.	4/64	2/7	9/24	.001
% (95% CI)	6.3 (1.7-15.2)	28.6 (3.7-71.0)	37.5 (18.8-59.4)	

CONCLUSIONS AND RELEVANCE Among a heterogeneous sample of children with ASD, the molecular diagnostic yields of CMA and WES were comparable, and the combined molecular diagnostic yield was higher in children with more complex morphological phenotypes in comparison with the children in the essential category. If replicated in additional populations, these findings may inform appropriate selection of molecular diagnostic testing for children affected by ASD.

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Autism spectrum disorder (ASD) represents a group of neurodevelopmental conditions of increasing prevalance.[1] The clinical presentation and outcome vary substantially in ASD.[2] In addition to variability in the core autistic features, many affected individuals have medical, cognitive, and mental health comorbidities.[3] There is also evidence that children with ASD have an excess of minor physical anomalies, which are defined as morphological deviations present in less than 5% of the population.[4,5]

The broad phenotype spectrum of ASD is also reflected in the underlying genetic etiology, which ranges from identifiable monogenic syndromes to large chromosome imbalances.[6] Chromosomal microarray analysis (CMA) is recommended as the first-tier genetic test for individuals with ASD[7] with a yield ranging from 7.0%[8] to 9.0%.[9] Whole-exome sequencing (WES) on research cohorts of individuals with ASD have highlighted sequence-level de novo mutations in the etiology of ASD.[10,11] Despite these efforts, the molecular diagnostic yield of WES in a heterogeneous ASD sample is undefined, and CMA data on these same patients may help inform clinical practice.

The phenotypic complexity of ASD remains a challenge, and stratification using different phenotypic measures could help categorize individuals with ASD into subtypes more likely to benefit from genetic testing.[12,13] Using clinical morphology categorization, Miles et al[13] showed that 20% of children with ASD were defined as "complex," based on the presence of multiple minor physical anomalies. There is also limited information about how dysmorphology stratification of children with ASD would be related to the molecular diagnostic yields of genome-wide tests. Recently it has been shown that subsets of individuals with ASD are more likely to carry disruptive de novo and rare copy-number variants (CNVs) and sequence-level mutations.[11,14,15]

Here, we report the molecular diagnostic yields for CMA and WES in a population-based sample of children with ASD who are typical of those seen in a developmental pediatric clinic, stratified by clinical phenotype.

Methods

The study sample included children from Newfoundland and Labrador, Canada, who were consecutively referred from 2008 through 2013 from both of the developmental pediatric clinics in the province that perform multidisciplinary team assessments for ASD. Each assessment was led by a developmental pediatrician and was required for the child to receive funding for applied behavioral analysis therapy. Each child received an ASD diagnosis based on criteria from the *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition, Text Revision), which was confirmed by Autism Diagnostic Observation Schedule and Autism Diagnostic Interview-Revised assessments. The parents or guardians of all children provided written informed consent, and the study was approved by Memorial University's human research ethics authority. The research numbers assigned to the specific cases in this article and its Supplement have no relationship to any identifying information from the participants and should be considered coded.

Clinical Assessments and Morphology Classification

The family history and medical records of the child (including radiology and electroencephalography reports) were reviewed. If not already performed, brain magnetic resonance imaging and IQ testing using the Wechsler Preschool and Primary Scale of Intelligence III or the Wechsler Abbreviated Scale of Intelligence were offered. Other screens for birth defects were arranged based on a standard physical examination of the child. Morphological examinations were performed by a dysmorphologist (B.A.F.) and included measurements of height, weight, and head circumference; measurements of the face, hands, and feet; and documentation of the presence of minor physical anomalies (eMethods in the Supplement).[16] The latter are slight morphological deviations present in less than 5% of the normal population.[4] Examples include single palmar creases and low-set ears.

As per Miles et al,[13] each child was assigned a minor physical anomaly score (1 point for each embryologically unrelated minor physical anomaly and for each measurement abnormality beyond 2 SD from the mean that was not present in a parent) (eMethods in the Supplement). Using the scores, the probands were first classified into 1 of 3 morphological groups based on physical examination alone: essential (minor physical anomaly score of 0-3), equivocal (minor physical anomaly score of 4 or 5), or complex (minor physical anomaly score ≥6).[13] Each child was also assigned a major congenital anomaly score (2 points for each structural brain abnormality or other major congenital anomaly outside the brain) and a total morphology score (minor physical anomaly + major congenital abnormality scores). Using the total morphology score, each child was again classified as essential, equivocal, or complex with the same cutoffs (eFigure 1 in the Supplement). The total morphology score classification was correlated with the CMA and WES analyses. The morphological categories were assigned prior to genetic testing.

Molecular Genetics

Whole blood for DNA extraction and/or establishing lymphoblastoid cell line was collected from each proband, available parents, and siblings. Each proband was tested for fragile X syndrome. All girls had *MECP2* sequencing, and any child with a head circumference 3 SD or more above the mean had *PTEN* sequencing. If any syndrome was suspected clinically, relevant targeted sequencing was ordered.[17]

Molecular genetic analyses are summarized in eFigure 1 in the Supplement. Chromosome analysis was performed using clinical microarray, high-resolution (>1 M probes) research microarray as previously described,[18-21] or both (see eMethods in the Supplement). Copy-number variants were classified according to American College of Medical Genetics and Genomics guidelines.[22] For copy-number changes that were classified as pathogenic or variant-of-unknown-significance-likely pathogenic, confirmation and parental testing was completed using quantitative polymerase chain reaction and fluorescence in situ hybridization.[23]

One hundred probands, for whom DNA samples from both parents were available, were randomly selected without knowledge of their CNV status. The probands and parents under-

Table 1. Demographics of 258 ASD Probands

	Whole Sample (N = 258)	Categories Based on Minor Physical Anomalies and Birth Defects [% of Whole Sample][a]		
		Essential (n = 168 [65.1])	Equivocal (n = 37 [14.3])	Complex (n = 53 [20.5])
Age at diagnosis, mean (SD), y[b]	4.5 (2.8)	4.1 (2.5)	4.7 (2.5)	5.5 (3.5)[c]
No. of boys/No. of girls	216/42	140/28	34/3	42/11
Male-female ratio	5.1:1	5.0:1	11.3:1	3.8:1
Examination of parents, No. (%)				
Both	169 (65.5)	115 (68.5)	24 (64.9)	30 (56.6)
One	56 (21.7)	38 (22.6)	5 (13.5)	13 (24.5)
Neither	33 (12.8)	15 (8.9)	8 (21.6)	10 (18.9)
ASD subtype, No. (%)				
Asperger syndrome	27 (10.5)	15 (8.9)	7 (18.9)	5 (9.4)
Autistic disorder	143 (55.4)	88 (52.4)	20 (54.6)	32 (66.0)
Pervasive developmental disorder–not otherwise specified	88 (34.1)	65 (38.7)	10 (27.0)	11 (24.5)
Genetic testing received, No. (%)				
Clinical microarray	150 (58.1)	84 (50)	29 (78.4)	37 (69.8)
High-resolution research microarray[d]	244 (94.6)	161 (95.8)	32 (86.5)	51 (96.2)
Whole-exome sequencing[d]	95 (36.8)	64 (38.1)	7 (18.9)	24 (45.3)

Abbreviation: ASD, autism spectrum disorder.

[a] Minor physical anomaly + major congenital abnormality score: 0-3 (essential), 4-5 (equivocal), and ≥6 (complex).

[b] Overall statistical difference between the groups was observed using analysis of variance ($F = 5.0$, $P = .008$).

[c] Post hoc comparison with the essential group $P = .006$ after Holm correction.

[d] After quality control.

went WES using the Ion Proton system after exonic amplification with the Ion AmpliSeq Exome Kit (Life Technologies). After quality control, 95 trios were analyzed further (eTable 1 in the Supplement). Rare variants with a frequency of less than 1% in population databases (National Heart, Lung, and Blood Institute exome server[24] and 1000 Genomes[25]) were prioritized to search for putative ASD-relevant variants. Further prioritization was done based on a list of genes previously implicated in ASD or other neurodevelopmental disorders[21,26] and manual curation (eMethods in the Supplement). The variants were then categorized using the guidelines from the American College of Medical Genetics and Genomics.[27] Only variants categorized as pathogenic or uncertain significance-likely pathogenic were included in calculation of the molecular diagnosis yield.

Main Outcomes

The main outcomes measured were the phenotypic differences in the ASD probands, the yield of molecular diagnosis from CMA and WES, and the differences in the molecular diagnostic yields between the morphological groups.

Statistical Analysis

Comparisons of the differences between the phenotype characteristics and yield of molecular diagnosis were performed using the Fisher exact test with post hoc pairwise comparisons. *P* values for the pairwise tests were corrected for 3 simultaneous comparisons using Holm adjustment. The 95% confidence intervals of the proportions of molecular diagnoses were calculated based on a binomial distribution. Differences in the age at diagnosis and IQ between the morphological groups were tested using 1-way analysis of variance (ANOVA) followed by *T* statistics for pairwise comparisons and Holm correction of the *P* values. Calculations of the 95% confidence interval and difference in the prevalence of detected de novo variants between the groups were done using Poisson tests. A significance level of $\alpha = .05$ was used, and all tests were 2-sided. All 3-group comparisons had estimated power greater than 0.9. Calculations for the estimated statistical power are described in the eMethods in the Supplement. All statistical analyses were performed using R software version 3.2.0.

Results

The study sample included 258 consecutively referred children for whom written informed consent was obtained (**Table 1**). From all the referred families, less than 10% declined to participate. Twenty-seven (10.5%), 143 (55.4%), and 88 (34.1%) children were classified as having Asperger syndrome, autistic disorder, or pervasive developmental disorder-not otherwise specified, respectively. Prior to CMA and WES, 12 of 258 probands (4.7%) were also diagnosed with a clinically distinct syndrome based on physical examination with or without targeted sequencing. All but 1 of these syndromes was classified as ASD-related (11/258, 4.3%), including 1 girl with a *PTEN* (OMIM 601728) mutation and 1 girl with osteogenesis imperfecta due to a homozygous *WNT1* (OMIM 164820) mutation[28] that probably also contributed to her ASD susceptibility (eTable 2 in the Supplement).[29]

Based on the categorical approach using the minor physical anomaly scores (ie, based on dysmorphology examination alone), 179 (69.4%) of the probands were classified as essential, 51 (19.8%) had equivocal dysmorphology, and 28 (10.9%) probands were complex (**Table 2**). IQ tests were administered to 127 children (49.2%), and a score was obtained

Table 2. Phenotypic Characteristics of the 258 Probands With Autism Spectrum Disorder

			Categories Based on Minor Physical Anomalies Only					
	Whole Sample (N = 258)	*P* Value for 3-Group Comparison[a]	Essential (n = 179)	*P* Value, Essential vs Equivocal[b]	Equivocal (n = 51)	*P* Value, Equivocal vs Complex[b]	Complex (n = 28)	*P* Value, Essential vs Complex[b]
IQ, mean (SD)	102 (23)	.46[c]	103 (22)	NA	97 (23)	NA	106 (25)	NA
No. of individuals tested	109[d]		74		26		8	
No. with regressive-onset ASD (%)[e]	69 (26.7)	.59	50 (27.9)	NA	14 (27.5)	NA	5 (9.9)	NA
No. with macrocephaly (%)	63 (24.4)	.16	38 (24.4)	NA	15 (21.2)	NA	10 (19.6)	NA
No. with microcephaly (%)	1 (0.4)	.31	0 (0.0)	NA	1 (2.0)	NA	0 (0.0)	NA
No. with abnormal electroencephalography, No./Total No. (%)	20/115 (17.4)	.03	9/76 (11.8)	.91	5/23 (21.7)	.85	6/15 (40.0)	.046
No. with ≥1 major congenital anomaly excluding brain (%)	47 (18.2)	<.001	21 (11.7)	.07	13 (25.5.4)	.24	13 (46.4)	<.001
No. with ≥1 structural brain abnormality, No./Total No. (%)	48/164 (29.3)	<.001	17/106 (16.0)	.001	18/39 (46.1)	.49	13/19 (68.4)	<.001

Abbreviations: ASD, autism spectrum disorder; NA, no pairwise comparison was performed as the primary test was not significant.

[a] Statistical difference between the 3 groups was tested with Fisher exact test.

[b] Post hoc pairwise comparisons using Fisher exact test was performed; *P* values after Holm correction for 3 simultaneous tests are reported.

[c] Statistical significance was computed using 1-way analysis of variance.

[d] Additional 18 individuals were tested, but no scores could be obtained.

[e] Regressive onset was defined as loss of expressive language with or without deterioration of social interaction skills prior to age 36 months.

for 109. IQ levels were comparable between the 3 groups (Table 2). No statistical difference was observed between girls and boys (eFigure 2A in the Supplement), although distribution of the scores in girls had a more biphasic representation than in boys (eFigure 2B). The 3 morphological groups, stratified by minor physical anomaly scores, differed statistically (3-group comparison using Fisher exact test) in number of children with abnormal electroencephalography results based on 44.6% (115/258) of the children ($P = .03$), structural brain abnormalities based on 63.6% (164/258) of the sample ($P < .001$), and major congenital anomalies outside the brain ($P < .001$) (Table 2), which is consistent with previous findings.[13,30] The pairwise group comparisons showed that children in the complex group differed most in these measures, with enrichment in the complex group (Table 2). Therefore, in a post hoc setting, the total morphology score, including the presence of structural brain abnormalities and major congenital anomalies outside the brain, was used for the final morphological categories (Table 1). Using the total score for categorization, the probands classified as complex increased from 10.9% to 20.5% (53/258). We did not observe any statistical difference in the ASD subtypes or mean IQ scores between the categorical groups; however, the mean age at diagnosis differed between the groups (1-way ANOVA, $P = .008$), with a later mean age at diagnosis in the complex group (Table 1, eFigure 2 in the Supplement).

CMA Diagnosis

In the course of this study, all 258 children received some form of microarray testing. One hundred fifty children (58.1%) received clinical microarray testing, of which 125 were performed using oligonucleotide arrays (for the additional 25 children, a low-resolution bacterial artificial chromosome microarray-based comparative genomic hybridization was performed). High-resolution (>1 M probes) research microarray genotyping was performed for 94.6% (244/258) of the probands (Table 1). A total of 24 molecular diagnoses in 24 probands were identified from all CMA in the 258 probands (9.3%, 95% CI, 6.1%-13.5%) (**Table 3** and eTable 3 in the Supplement). The molecular diagnostic yields were 9.8% (95% CI, 6.4%-14.3%, 24/244) in the research microarray sample and 8.0% (95% CI, 3.9%-14.2%, 10/125) in the clinical microarray sample (Table 3). Of the 125 who underwent clinical microarray testing, 114 also had high-resolution research microarray data. Three additional clinically relevant CNVs were found in these children by research microarray analysis. The number of molecular diagnoses within the defined morphological categories were analyzed and demonstrated a difference in the diagnostic yield of CMA across the 3 morphological groups (Fisher exact test, $P < .001$) (Table 3).

The complex ASD group had significantly more pathogenic CNVs (13/53, 24.5%, 95% CI, 13.8%-38.3%) compared with the essential group (7/168, 4.2%, 95% CI, 1.7%-8.4%; Holm adjusted $P < .001$). For the clinically significant variants where parental testing was possible, 57.1% (12/21) were de novo events in the proband (eTable 3 in the Supplement); 11 of 12 of these de novo events were detected in probands in the complex category. Maternal inheritance was observed for 23.8% (5/21) and paternal inheritance for 19% (4/21) of the variants.

Molecular Diagnoses From WES

After quality control of the WES data (100 trios), 95 probands were analyzed further. For these, a mean coverage depth of 108× was achieved; 90.5% of the exonic regions targeted for sequencing were covered at least 20× (eTable 1 in the Supplement). Among the 95 probands, 8 children with 9 mutations received an ASD-related molecular diagnosis (8.4%, 95% CI, 3.7%-15.9%) (eTable 4 in the Supplement); 2 of these children already had 1 molecular diagnosis from CMA (cases 3-0075-000 and 3-0095-000, eTable 3 and 4 in the Supplement). The

Table 3. Molecular Diagnostic Yield of Chromosomal Microarray Analysis and Whole-Exome Sequencing

	Whole Sample			Categories Based on Minor Physical Anomalies and Birth Defects									
				Essential		Equivocal				Complex			
	No./Total No.	Yield, % (95% CI)	*P* Value for 3-Group Comparison[a]	No./Total No.	Rate, % (95% CI)	No./Total No.	Yield, % (95% CI)	*P* Value for Essential vs Equivocal[b]	OR (95% CI)	No./Total No.	Yield, % (95% CI)	*P* Value for Essential vs Complex[b]	OR (95% CI)
Chromosomal microarray	24/258	9.3 (6.1-13.5)	<.001	7/168	4.2 (1.7-8.4)	4/37	10.8 (3.0-25.4)	.51	2.8 (0.5-11.6)	13/53	24.5 (13.8-38.3)	<.001	7.4 (2.5-23.4)
Clinical microarray	10/125	8.0 (3.9-14.2)	.002	1/67	1.5 (0.0-8.0)	2/26	7.7 (0.0-25.1)	.50	5.3 (0.3-328.5)	7/32	21.9 (13.2-52.9)	.004	17.9 (2.1-841.1)
Research microarray	24/244	9.8 (6.4-14.3)	<.001	7/161	4.4 (1.8-8.8)	4/32	12.5 (3.5-29.0)	.53	3.1 (0.6-13.3)	13/51	25.5 (14.3-39.6)	<.001	7.4 (2.6-23.6)
Whole-exome sequencing	8/95	8.4 (3.7-15.9)	.02	2/64	3.1 (0.0-10.8)	2/7	28.6 (3.7-71.0)	.41	11.5 (0.7-191.3)	4/24	16.7 (4.7-37.4)	.40	6.0 (0.7-71.7)
Combined analysis[c]	15/95	15.8 (9.1-24.7)	.001	4/64	6.3 (1.7-15.2)	2/7	28.6 (3.7-71.0)	.31	5.7 (0.4-54.2)	9/24	37.5 (18.8-59.4)	.002	8.7 (2.1-44.2)
Diagnosis by CMA only	7/95	7.4 (0.3-14.6)		2/64	3.1 (0.0-10.8)	0/7	0.0 (0.0-41.0)			5/24	20.8 (7.1-42.2)		
Diagnosis by WES only	6/95	6.3 (2.4-13.2)		2/64	3.1 (0.0-10.8)	2/7	28.6 (3.7-71.0)			2/24	8.3 (10.3-27.0)		
Diagnoses by CMA and WES	2/95	2.1 (0.0-7.4)		0/64	0.0 (0.0-5.6)	0/7	0.0 (0.0-41.0)			2/24	8.3 (10.3-27.0)		

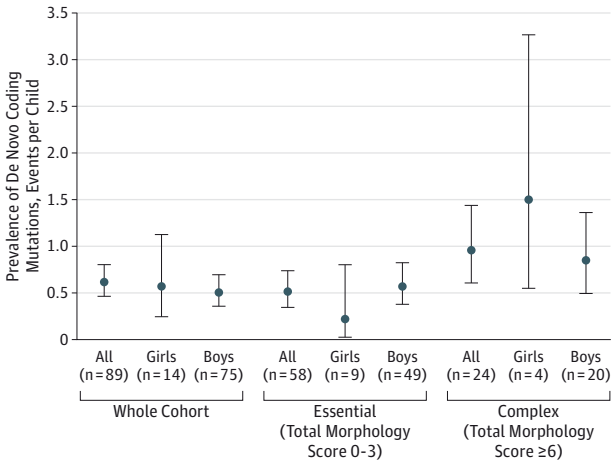
Abbreviations: CMA, chromosomal microarray analysis; OR, odds ratio; WES, whole-exome sequencing.

[a] Statistical significance between the proportions in the morphological groups was tested using Fisher exact test.

[b] Post hoc pairwise comparisons were computed using Fisher exact test and *P* values corrected using Holm adjustment based on 3 simultaneous comparisons. Pairwise *P* values are not shown for the equivocal and complex comparisons as the estimated power for Fisher exact test was <0.5.

[c] Based on the 95 children with both microarray and WES data.

Figure. Prevalence of De Novo Mutations Affecting Coding Sequence From Whole-Exome Sequencing (WES)



Prevalence estimates were calculated from de novo variants identified through WES and confirmed in blood-derived DNA from 89 children and parents (6 children had only cell line–derived DNA available). Differences in the prevalence between the different phenotypic groups (equivocal excluded) and sexes were performed using a 2-sided Poisson test. A significant increase was observed in the complex vs the essential group (P = .02), and the highest prevalance, detected in the girls in the complex group, was significantly higher than that in girls from the essential group (P = .01). The total morphology score was the sum of minor physical anomaly and major congenital abnormality score. Error bars indicate 95% confidence intervals for the mean prevalence. All putative de novo variants and pathogenic variants were confirmed using Sanger sequencing.

proportion of probands with a molecular diagnosis from WES differed between the morphological groups; the yields were 16.7% in the complex group (95% CI, 4.7%-37.4%, 4/24), 28.6% in the equivocal group (95% CI, 3.7%-71.0%, 2/7), and 3.1% in the essential group (95% CI 0.0%-10.8%, 2/64; Fisher exact test, P = 0.02) (Table 3). The mode of inheritance of the 9 mutations included 6 (66.7%) autosomal dominant (including 3 de novo mutations), 2 (22.2%) autosomal recessive, and 1 (11.1%) X-linked recessive.

Sequence-Level De Novo Variants by ASD Dysmorphology

A total of 96 de novo variants were identified and confirmed in 55 of 95 children from the WES (0-5 de novo mutations per child, eTable 5 in the Supplement). The number of de novo variants affecting the coding sequence was weakly correlated with the total morphology score based on the presence of minor physical anomalies and major congenital abnormalities (r = 0.2, P = .03) in the 89 children with blood-derived DNA available (eMethods and eFigure 3 in the Supplement). A significant increase (Poisson test, P = .02) was observed in the complex group (1.0 events per child, 95% CI, 0.6-1.5) when compared with the essential group (0.55 events per child, 95% CI, 0.4-0.8), indicating a higher burden of de novo events in syndromic children (**Figure**). The highest prevalence was detected in the girls in the complex group (1.75 events per child, 95% CI, 0.7-3.6), and this was significantly higher than that among girls from the essential group (0.33 events per child, 95% CI, 0.07-1.0; Poisson test, P = .01). No significant difference was observed between girls and boys in any comparison.

Of the 96 de novo variants, 3 were classified as mutations leading to molecular diagnosis (eTables 4 and 5 in the Supplement). These included 2 loss-of-function mutations in the ASD and intellectual disability genes *ASH1L* (c.C7189T, OMIM 607999) and *WAC* (c.576_585delGCAAGCAACA, OMIM 615049) and a de novo missense mutation in *SCN2A* (OMIM 182390).

Inherited Sequence-Level Variants

Six of the 9 mutations leading to molecular diagnosis were inherited (66.6%) (eTable 4 in the Supplement). In proband 3-0111-000, a homozygous M390R missense mutation (c.T1169G) was found in the Bardet-Biedl syndrome 1 (*BBS1*) gene (OMIM 209901). At enrollment at age 22 months, he was given a working diagnosis of an unspecified overgrowth syndrome (eTable 2 in the Supplement); however, after clinical reevaluation following WES, a diagnosis of atypical Bardet-Biedl syndrome was confirmed. A maternally inherited frameshift insertion (c.1106_1107insG) leading to premature stop codon in *TCF12* (OMIM 600480) was detected in proband 3-0459-000, who had equivocal dysmorphology, including brachycephaly and an area of left frontal cortical dysplasia. We also detected another presumed loss-of-function mutation (c.A1295G) in *FGFR2* (OMIM 176943).This proband (3-0211-000) also had equivocal dysmorphology, including dolichocephaly, deep-set eyes, and a left temporal lobe septated intraparenchymal cyst; however, there were no features of craniosynostosis.

Incidental and Medically Actionable Findings From WES

Incidental or medically actionable findings were reported for 8 of the 95 probands (8.4%) (eTable 6 in the Supplement), all of which were inherited mutations. Six (6.2%) were deemed medically actionable, ie, results for which additional baseline clinical investigations or ongoing screening are expected to improve outcome with respect to morbidity or mortality. These 6 results were communicated to the families. The incidental findings included 3 mutations occurring in *SDHB* (OMIM 185470) and *CACNA1S* (OMIM 114208), causing familial paragangliomas and malignant hyperthermia, respectively.

Combined Yield of CMA and WES

Nine of 95 probands (9.5%) who underwent WES already had a molecular diagnosis from CMA; the probands for WES were selected randomly without knowledge of their CNV status. This CMA-positive proportion is similar to that of the whole sample (9.30%) (Table 3). We therefore used this subset of probands to estimate the proportion of ASD individuals with an identifiable genetic etiology after CMA and WES, which was 15.8% (95% CI, 9.1%-24.7%, 15/95). Two children received molecular diagnoses from both tests, both in the complex group (Table 3). The combined yield in the different morphological groups was 6.3% (95% CI, 1.7%-15.2%, 4/64) in the essential, 28.6% (95% CI, 3.7%-71.0%, 2/7) in the equivocal, and 37.5% (95% CI, 18.8%-59.4%, 9/24) in the complex group (Fisher exact test, P = .001) (Table 3). The combined yield was significantly higher in the complex group when compared with the essential group (pairwise comparison, P = .002).

Discussion

In a population-based sample of children with ASD, the molecular diagnostic yield of WES (8.4%) was comparable with the yield from CMA (9.3%), which is currently recommended as the first-line genetic test for individuals with ASD.[7,31] A combined molecular diagnostic yield of 15.8% was found in those children who received both tests (Table 3). The observed yield for clinically relevant CNVs (9.3%) is slightly higher than earlier reports,[8,9] possibly due to the higher-resolution microarrays used in our study and also a better understanding of rare variants affecting new candidate genes.[32,33] In contrast to CMA, WES in ASD is still largely in the research domain.[10,11] Some clinical WES studies have provided molecular diagnostic estimates for smaller autism subgroups with additional medical conditions, and these have varied (0%-45.0%).[34-36]

In the current study, we have demonstrated differences related to morphological stratification of ASD probands based on clinical examination. Our data suggest that medical evaluation of ASD children may help identify populations more likely to achieve a molecular diagnosis with genetic testing. The morphological stratification was related to the molecular diagnostic yields, which were higher for both CMA and WES when the analysis was restricted to the subset of individuals with complex phenotypic presentations. Based on analysis of the combined diagnostic yield of CMA and WES, we estimate that more than 35% of ASD children with additional medical and dysmorphology features might be able to receive a molecular diagnosis. In contrast, only 6.0% of ASD children without syndromic features received a molecular diagnosis in our study (Table 3). If children with essential ASD who have a comorbid intellectual disability are excluded, the molecular diagnostic yield may be even lower. In our analysis, 2 of 4 children in the essential group who received molecular diagnosis by either CMA or WES had comorbid intellectual disability (eTable 7 in the Supplement). The data presented here also highlight improvements in designating molecular diagnoses for children with ASD that have occurred over the past 10 years. In 2005, Miles et al[13] published their stratification of 260 children with autistic disorder into 3 morphological groups and showed that the complex designation had 87% specificity for predicting poor outcome. In their analysis, 4.2% (11/260 children) had an identifiable genetic syndrome by clinical examination and chromosome analysis.[13] We show a more than 3.5-fold increase in the molecular diagnostic yield (15.8%) compared with the results in 2005. When the comparison was restricted to the complex groups, the diagnostic yields were 23.9% (11/46) in Miles et al[13] and 37.5% in our study (Table 3).

It seems likely that genetic testing of children with ASD will continue to increase. In a survey of parental interest in ASD genetic testing,[37] 80% of parents indicated that they would want a sibling younger than 2 years tested to identify ASD-risk mutations even if the test could not confirm or rule out the diagnosis.[38] For some children with positive genetic test results, treatment plans targeting ASD-associated medical conditions can be offered.[35,39-41] Examples include screening for cardiac defects and maturity-onset diabetes of the young in probands with 1q21.1 and 17q12 deletion syndromes, respectively, and close monitoring to avoid the development of obesity in those with 16p11.2 microdeletions. We observed that the age at diagnosis with ASD was significantly older for the complex group (Table 1), which suggests that health care professionals may need to be particularly vigilant in monitoring children with suspected or diagnosed morphological syndromes for behavioral signs of ASD.[42]

Our study illustrates the genetic and phenotypic heterogeneity of ASD (eTables 4, 5, and 7 in the Supplement).[21,43-45] The sample of 95 probands had contributions from inherited and de novo mutations found by WES in 9 ASD susceptibility genes, some of which are known to have variable expressivity and penetrance. Four of these were loss-of-function mutations in autosomal-dominant genes. For instance, mutations in *TCF12* have been observed in individuals with craniosynostosis; however, neurodevelopmental disorders ranging from mild learning disability to severe autism are known.[46-48] Genetic heterogeneity is also present within families[45]; for example, proband 3-0027-00 had a maternally inherited loss-of-function mutation in the ASD susceptibility gene, *SLITRK5,* which was absent in his brother with ASD. In accordance with earlier studies,[21,49] our data support the conclusion that ASD in girls may be genetically different than ASD in boys, as evidenced by enrichment of girls in the complex group (Table 1), slightly different distribution of IQ scores (eFigure 2 in the Supplement), and higher prevalence of de novo variants (eTable 5 in the Supplement).

Limitations of our study include a relatively small sample size as well as possible ascertainment bias related to clinical differences that may have existed between families who consented and declined (less than 10% declined to participate in the study after diagnosis in the developmental pediatric clinics). Only 63.5% of the children had brain magnetic resonance imaging, which may have skewed our final morphological classification in favor of the essential group, and only 49.2% (127/258) of the study sample underwent IQ testing. A major limitation of the study is that only 36.8% (95/258) of the children were included in the WES analysis, which could have led to an unmeasured confounding effect on the results. In addition, there are technical and interpretation limitations to the identification of variants, which were classified as molecular diagnoses. Whole-exome sequencing does not provide equal coverage for all the coding sequence regions[26,50] and lacks sensitivity and specificity for detection of structural variants.[45] Given this and the resolution limits of CMA, the inability to detect the majority of larger indels (>20 base pairs) and smaller CNVs (<20 kilobases) is also a limitation. It seems likely that whole-genome sequencing will become the primary genetic test for ASD because all classes of genetic variation might be detected in 1 experiment.[26,45,50] Genetic counseling for ASD-related genomic mutations, especially for rare sequence-level variants, is often challenging because of their variable expressivity and incomplete penetrance.[17]

Conclusions

Among a heterogeneous sample of children with ASD, the diagnostic yields of CMA and WES were comparable, and the combined diagnostic yield was higher among children with more complex morphological phenotypes in comparison with the children in the essential category. If replicated in additional populations, these findings may inform appropriate selection of molecular diagnostic testing for children affected by ASD.

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