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Original Article

Diagnostic Yield of Intellectual Disability Gene Panels

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ABSTRACT

Background: Recent technological advances have improved the understanding and identification of the genetic basis of intellectual disability (ID) and global developmental delay (GDD). Next-generation sequencing panels of ID genes are now available for clinical testing; however, their overall yield in clinical practice has not yet been investigated.
Aim: We determined the diagnostic yield of ID gene panels in a clinical setting and explored whether any clinical features are associated with an increased diagnostic yield.
Methods: We performed a systematic retrospective chart review of all patients with ID/GDD who underwent an ID gene panel between April 2014 and July 2017 at our institution. Chi-square analysis assessed whether any specific clinical features were significantly associated with a positive diagnostic yield.
Results: Forty-eight subjects (18 females, 30 males; median age: 7.5 years) were included. Consanguinity was present in 17%, autism in 38%, seizures in 42%, nonspecific dysmorphic features in 67%, and abnormalities on neurological examination in 56%; furthermore, 29% of the cohort was nonverbal and 4% was nonambulatory. Four different gene panels were used. The diagnostic yield was 21% (10/48) overall, and 38% with the more recent trio-based panel. Eight of 10 patients had *de novo* pathogenic dominant mutations, one had an inherited pathogenic autosomal dominant mutation, and one had compound heterozygous pathogenic recessive mutations. No clinical feature was significantly associated with an increased diagnostic yield.
Conclusions: Our study suggests that ID gene panels have a high yield and are a valuable diagnostic tool in the evaluation of children with ID/GDD.

Introduction

Intellectual disability (ID) affects 1% of the population and is defined by deficits in intellectual functioning and adaptive behavior with onset before age 18 years.[1] A diagnosis of global developmental delay (GDD) is given to children under age five years with a significant functional delay in two or more developmental domains, including motor, speech or language, cognition, social, and activities of daily living.[1]

The causes of ID are wide ranging and include acquired, environmental, and genetic factors.[2] In the past decade, important advances in genetic technologies, such as chromosomal microarrays and next-generation sequencing, have allowed the deciphering of the heterogeneous genetic basis of ID.[3] Up to 40% patients with ID have a monogenic form.[4] To date, pathogenic variants in over 700 genes have been identified to be responsible for the autosomal dominant, recessive, and X-linked forms of ID.[3] *De novo* mutations, i.e., those present in the proband but absent in the parents, have been demonstrated to represent a common and important cause of ID.[3] The American Academy of Pediatrics recommends chromosomal microarray and fragile X testing as first-line tests for children with ID, and consideration of brain imaging in patients with

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microcephaly, macrocephaly, or abnormal neurological examination.[5] Similarly, the American Academy of Neurology guidelines recommend brain imaging, chromosome microarray analysis, and fragile X testing as part of the standard diagnostic evaluation of a child with GDD or ID.[6] In children in whom initial history and physical examination do not uncover an underlying cause of their ID/GDD, the diagnostic yield of chromosomal microarray is 15% to 20%[7,8] and that of fragile X testing is approximately 2%.[8,9] The diagnostic yield of trio-based whole-exome sequencing (WES), where the entire coding regions of the genome are queried in the proband and parents, is between 13% and 35% in cohorts of children with severe ID.[3,10,11] Although WES is clinically available, it is not widely offered because of high costs and/or financial coverage barriers from governmental agencies and health insurance companies. Next-generation sequencing panels of ID genes have the advantage of being less expensive (US $1500 to $3500 versus approximately $4000 for clinical WES[12]) and being focused on targeted genes, thus resulting in fewer incidental findings.[13] Moreover, most companies offer evaluation for copy number variants (i.e., exonic deletions or duplications) affecting genes included in the panels. Although targeted ID panels are increasingly being used, their overall diagnostic yield in clinical practice has not yet been investigated. In this study, our primary objective was to determine the diagnostic yield of ID gene panels used in a clinical setting in children with ID/GDD. We also sought to determine whether any clinical features were associated with a greater diagnostic yield.

Materials and Methods

This study was approved by our institutional research ethics board. All individuals with ID/GDD who underwent genetic testing using an ID gene panel at the McGill University Health Centre (MUHC) between April 2014 and July 2017 were included in this retrospective study. These individuals were identified through the hospital laboratory database that tracks all individuals who underwent an ID gene panel. GDD was defined as a delay in two or more domains (gross motor, fine motor, language, and social), and ID was defined as an intelligence quotient less than 70 on standardized neuropsychologic testing.

At our institution, ID gene panels are performed in children with ID/GDD when initial investigations, which usually consist of chromosomal microarray, fragile X testing, neuroimaging, and variable metabolic testing, have not revealed an underlying cause. The need and extent of metabolic testing is physician-dependent and not systematically performed in all patients unless there is clinical suspicion of an underlying metabolic abnormality. Rarely, adult patients with ID are assessed at our institution and may have an ID panel done. All patients were evaluated either by a pediatric neurologist or a medical geneticist. We retrospectively reviewed the medical charts of all patients and systematically collected the following information: age, gender, family history of ID (parent or sibling with ID) or consanguinity, presence of seizures, autism, microcephaly, severity of ID/GDD (whether patients were verbal and ambulatory), dysmorphic features, and abnormal findings on neurological examination. We reviewed the results of all investigations including brain imaging, chromosomal microarray, karyotype, fragile X testing, any single-gene molecular analysis, and metabolic evaluation. ID panels were performed at Fulgent (Temple City, CA, USA), GeneDx (Gaithersburg, MD, USA), MNG Laboratories (Atlanta, GA, USA), or the University of Chicago (Chicago, IL, USA). The use of the specific panels varied throughout the years (see Table 1). A list of the genes included in each panel is available in Supplemental Materials.

Chi-square analysis and Bonferroni correction for multiple comparisons were used to determine whether the presence of specific clinical features was associated with a significant increase in the rate of diagnostic yield. A P value ≤ 0.05 was used for establishing statistical significance. All statistical analyses were performed using MedCalc.

Results

Cohort characteristics

In total, 50 patients underwent an ID gene panel between April 2014 and July 2017. Two patients were excluded because they did not have ID or GDD, and thus a total of 48 individuals were included in our study. The demographic data and clinical features of our cohort are summarized in Table 2. Subjects included 18 females and 30 males, with a mean age of 8.6 years (± 3.86, median 7.5, range 3 to 45) at the time of ID gene panel testing. In total, there were five subjects older than 18 years included in our study. In terms of clinical features, 29% of the cohort (14/48) was nonverbal, 4% (2/48) was nonambulatory, 42% (20/48) had seizures, and 38% (18/48) had autism. In addition, 38% (18/48) of the cohort had microcephaly (prenatal in 3, postnatal in 12, and not specified in 3), 56% (27/48) had an abnormal result of neurological examination (which included hypotonia in nine, spasticity in seven, and abnormal gait in four; oromotor apraxia in three; dyspraxia in one, elbow contractures in one; and mild bilateral ptosis in one), and 67% (32/48) had nonspecific dysmorphic features. In terms of previous investigations, 96% (46/48) of the cohort had undergone chromosome microarray, 92% (44/48) brain imaging (43 with magnetic resonance imaging, one with computed tomography), 67% (32/48) fragile X testing, 19% (9/48) karyotype analysis, 27% (13/48) methylation testing for Angelman/Prader-Willi syndrome, 92% (44/48) various metabolic testing, and 15% (7/48), or 39% (7/18 females), sequencing of *MECP2* for Rett syndrome.

ID gene panel results

The distribution of the types of ID gene panels performed in the 48 patients was as follows: 27 (56%) patients had a 391-495 gene panel at Fulgent (18 with deletion and duplication analysis), four (8%) had a 348-361 gene panel at MNG Laboratories, one (2%) had a 143 gene panel at the University of Chicago, and 16 (33%) had a trio-based 2000-2323 gene panel at GeneDx (Table 1).

Overall, targeted next-generation sequencing using ID gene panels identified the underlying genetic diagnosis in 10 of 48 patients, representing a diagnostic yield of 21%. Causal variants were identified in the following genes: *TBL1XR1* (OMIM*608628), *SYNGAP1* (OMIM*603384), *PIGL* (OMIM*605947), *MAF* (OMIM*177075), *AHDC1* (OMIM*1615790), *HRAS* (OMIM*190020), *CDKN1C* (OMIM*6008556), *HIVEP2* (OMIM*143054), *BRAF* (OMIM*164757), and *DDX3X* (OMIM*300160). Eight patients had causal *de novo* pathogenic variants in dominant ID genes (seven autosomal and one X-linked), one patient had a causal autosomal dominant mutation inherited from a symptomatic mother, and one patient had compound heterozygous mutations in an autosomal recessive ID gene. Details of the mutations and the associated clinical features of the patients are listed in Table 3.

Variants of uncertain significance (VUS) were reported in 85% subjects who had ID gene panels. The mean number of VUS per subject was 3.6, with a range of zero to 10 variants.

To examine whether any specific clinical characteristic was associated with a positive diagnostic yield, we performed a chi-square test of independence and Bonferroni correction for

TABLE 1.
Diagnostic Yield of ID Gene Panels

Company	Number of Genes in Panel*	With Del/Dup Analysis	Individual Tested	Cost per Test (USD)	Number of Patients Tested	Time of Testing			Number of Patients With Identified Causal Variant	Diagnostic Yield of Gene Panel (%)
						4/2014 to 6/2015	7/2015 to 6/2016	7/2016 to 7/2017		
GeneDx	2308	Yes	Proband & parent trio	~2700	16	3	-	13	6	38
Fulgent	495	No	Proband	~1100	9	-	7	2	1	11
		Yes	Proband	~1100-1500	18	-	9	9	2	11
MNG Laboratories	361	Yes	Proband	~1900	4	-	4	-	1	25
U. Chicago	143	Yes	Proband	~2700	1	1	-	-	0	0
Total					48	4	20	24	10	21

Abbreviations:
Del/dup = Deletion and duplication analysis
U = University
USD = United States dollar
* Genes listed in supplementary materials

multiple comparisons. No significant relationship was found for any of the clinical characteristics or initial tests.

Discussion

In this study, we determined that the diagnostic yield of next-generation sequencing ID panels using a clinical setting in subjects with variable degrees of ID/GDD is 21% overall, and up to 38% in the most recent trio-based panels containing the largest number of genes (greater than 2000). This is the first study to assess the yield of clinically available ID gene panels in a group of unselected patients with unexplained ID/GDD. Of note, all our patients had been previously investigated with unrevealing chromosomal microarray, fragile X testing, and brain imaging. Our observed yield with ID gene panels is higher than that of chromosomal microarray (15% to 20%[7,8]) and fragile X testing (0.5% to 2%[8,9]), which are part of the recommended standard investigations in the diagnostic evaluation of children with ID/GDD, according to the American Academy of Neurology and American Academy of Pediatrics.[5,6] Determination of the underlying genetic cause of GDD/ID in a child is of great importance for families and treating physicians, as it allows more specific genetic counseling, improves prognostication and anticipation of co-morbidities, limits further unnecessary testing, and decreases parental anxiety.[6]

The diagnostic yield in our study is comparable with that observed in other studies investigating the use of targeted sequencing of ID genes in custom-designed panels. A study by Grozeva et al. screened 253 ID genes in 986 individuals with moderate to severe ID and obtained a diagnostic yield of 11%.[14] Martinez et al. performed trio-based screening of 1256 genes in a cohort of 92 patients and obtained an yield of 32%.[15] Similarly, Redin et al. sequenced 217 targeted ID genes in 106 patients and their parents and obtained a diagnostic yield of 25%.[16] In comparison, trio-based WES or whole-genome sequencing in patients with ID identified causal pathogenic mutations in 13% to 42% of ID cohorts.[9,17,18] Gieldon et al. performed partial exome sequencing of 4813 OMIM listed genes in 106 patients with varying degrees of ID and reported an yield of 34%, equivalent to that of whole-exome testing.[19] Of note, the yield in our study from the trio-based gene panels, which was also the most recent panel and included the largest number of genes (2000-2323), was 38% (6/16). It is likely that the yield of genetic testing will increase with the discovery of novel ID genes and the confirmation of candidate ID genes.[3]

In our cohort, eight of the 10 patients in the diagnostic group had a pathogenic *de novo* mutation in a dominantly inherited ID gene, illustrating the important role of *de novo* mutations in ID.[20,21] We did not identify the underlying genetic cause in any of the eight patients with consanguineous parents, highlighting the important genetic heterogeneity, and suggesting the presence of many yet undiscovered recessively inherited ID genes. Mutations in recessive ID genes have been found to underlie a high number of cases in populations with higher consanguinity rates.[22,23] A WES study in 404 consanguineous families with ID revealed likely causal variants in known or candidate ID genes in 52% of families.[22]

TABLE 2.
Demographic and Clinical Features of the GDD/ID Cohort

Clinical characteristics	All Subjects	Subjects With Negative Yield on Gene Panel	Subjects With Positive Yield on Gene Panel
	n = 48 (%)	n = 38 (%)	n = 10 (%)
M:F	30:18	23:15	7:3
Mean age (years)	8.6, SD 3.9	8.9, SD 3.9	6.7, SD 3.2
Median age (years)	7.5 (range 3–45)	7.0 (range 4–45)	8.0 (range 3–42)
Presence of family history of ID (parents or siblings)	4 (8)	4 (11)	0
Presence of consanguinity	8 (17)	8 (21)	0
Presence of autism	18 (38)	15 (39)	3 (30)
Presence of seizures	20 (42)	16 (42)	4 (40)
Presence of nonspecific dysmorphic features	32 (67)	22 (58)	10 (100)
Presence of microcephaly	18 (38)	16 (42)	2 (20)
Nonverbal	14 (29)	9 (24)	5 (50)
Nonambulatory	2 (4)	2 (5)	0
Abnormal neurological exam	27 (56)	19 (50)	8 (80)

Abbreviations:
F = Female
M = Male
All comparisons between groups were nonsignificant ($P > 0.05$).

TABLE 3.
Clinical Characteristics in Subjects With Positive Yield on ID Gene Panels

Subject	Gene	Variant	Inheritance	Gender	Age (years)	Family History of ID	Consanguinity	ASD	Seizures	Dysmorphisms	Microcephaly	Nonverbal	Nonambulatory	Abn Neuro Exam
1	*TBL1XR1* (NM_024665.5)	c.724A>C; p.Thr242Pro	AD, *de novo*	M	3	-	-	-	-	+	+	+	-	+
2	*SYNGAP1* (NM_006772.2)	c.1717C>T; p.Arg573Trp	AD, *de novo*	M	9	-	-	-	+	+	+	-	-	+
3	*PIGL* (NM_004278.3)	c.60G>A; p.Trp20* c.262C>T; p.Arg88Cys	AR, maternal AR, paternal	M	8	-	-	+	+	+	+	+	-	+
4	*MAF* (NM_005360.4)	c.206C>G; p.Pro69Arg	AD, maternal	M	19	-	-	+	+	+	-	+	-	+
5	*AHDC1* (NM_001029882.1)	c.2693dupT; p.Ala899Gfs*4	AD, *de novo*	M	4	-	-	-	-	+	-	-	-	+
6	*HRAS* (NM_005343.2)	c.448C>T; p.Gln150*	AD, *de novo*	F	20	-	-	-	-	+	-	-	-	-
7	*CDKN1C* (NM_005992.1)	Deletion of exons 2-3; p.Phe264_Arg305delins48	AD, *de novo*	F	42	-	-	-	-	+	-	-	-	+
8	*HIVEP2* (NM_006734.3)	c.1894G>A; p.Asp632Asn	AD, *de novo*	M	4	-	-	+	+	+	-	+	-	+
9	*BRAF* (NM_004333.4)	c.735A>C; p.Leu245Phe	AD, *de novo*	M	3	-	-	-	-	+	-	-	-	+
10	*DDX3X* (NM_001193416.1)	c.1026-6T>G	X-linked D, *de novo*	F	9	-	-	-	-	+	-	+	-	-

Abbreviations:
Abn neuro exam = Abnormal neurological examination
AD = Autosomal dominant
AR = Autosomal recessive
ASD = Autism spectrum disorder
F = Female
M = Male
ID = Intellectual disability

We were unable to establish whether any clinical features were associated with a greater diagnostic yield, likely in part due to the small size of our cohort. Although not statistically significant, a greater proportion of individuals with a positive diagnostic yield were nonverbal (5/10, 50%, versus 14/38, 24%), had nonspecific dysmorphic features (10/10, 100%, versus 22/38, 58%), and had abnormal results of neurological examinations (8/10, 80%, versus 19/38, 50%). It is also possible that the yield is similar across varying severity of ID/GDD, such as in microarray testing, where the degree of ID was found to not predict the yield of the test.[7]

In our center, pediatric neurologists and genetics now preferentially order trio-based panels because these offer the largest number of genes tested, have a higher yield, and report a smaller number of variants of unclear significance. Furthermore, sending the parental samples at the same time as the proband's avoids having to recontact the parents for segregation testing of variants, resulting in a more rapid variant interpretation and final report.

Our retrospective study has a number of limitations. Different ID panels with wide range of gene numbers were used, and this is a reflection of the rapidly evolving available testing and the discovery of novel ID genes. The number of patients included in this study may have been too small to allow the identification of possible clinical features associated with a greater yield on testing. Given this study's retrospective nature, individuals did not undergo identical investigations such as metabolic evaluation or neuropsychologic testing, and some clinical details were missing.

In summary, our study revealed that the diagnostic yield of ID panels in a clinical setting was 21% overall, and up to 38% with the largest recent trio-based panels. This yield is comparable with the reported yield of WES, strongly supporting the use of ID gene panels in the diagnostic evaluation of children with ID/GDD. We recommend the use of the trio ID gene panels rather than nontrio panels, not only because of the greater yield and number of genes tested but also because of the ease of variant interpretation and smaller number of reported VUS that need to be discussed with patients and families. However, with the constant discovery of novel ID genes, along with the advances in sequencing technologies, decreasing costs, and availability of tests, the choice of optimal testing will regularly need to be reassessed.

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Supplementary data

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