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Original Article

Toward a Pathway-Driven Clinical-Molecular Framework for Classifying Autism Spectrum Disorders

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ABSTRACT

Background: The current classification system of neurodevelopmental disorders is based on clinical criteria; however, this method alone fails to incorporate what is now known about genomic similarities and differences between closely related clinical neurodevelopmental disorders. Here we present an alternative clinical molecular classification system of neurodevelopmental disorders based on shared molecular and cellular pathways, using syndromes with autistic features as examples.
Methods: Using the Online Mendelian Inheritance in Man database, we identified 83 syndromes that had "autism" as a feature of disease, which in combination were associated with 69 autism disease-causing genes. Using annotation terms generated from the DAVID annotation tool, we grouped each gene and its associated autism syndrome into three biological pathways: ion transport, cellular synaptic function, and transcriptional regulation.
Results: The majority of the autism syndromes we analyzed (54 of 83) enriched for processes related to transcriptional regulation and were associated with more non-neurologic symptoms and co-morbid psychiatric disease when compared with the other two pathways studied. Disorders with disrupted cellular synaptic function had significantly more motor-related symptoms when compared with the other groups of disorders.
Conclusion: Our pathway-based classification system identified unique clinical characteristics within each group that may help guide clinical diagnosis, prognosis, and treatment. These results suggest that shifting current clinical classification of autism disorders toward molecularly driven, pathway-related diagnostic groups such as this may more precisely guide clinical decision making and may be informative for future clinical trial and drug development approaches.

Introduction

The current diagnostic classification system of neurodevelopmental disorders (NDDs) is generally considered in need of revision to more effectively stratify patients.[1] The phenotypes of NDDs are broad and often overlapping, offering little insight into the molecular and cellular pathophysiology of disease that would guide targeted treatments. Although there have been great advances in determining underlying genetic variants, it has become clear that the clinical utility of these findings is limited owing to tremendous genetic heterogeneity and complex inheritance patterns.[2] Moreover, although there has been tremendous effort to understand the basic pathophysiology of NDDs such as autism spectrum disorder (ASD), the diagnosis is still made clinically based on criteria that encompass deficits in communication, social interaction, and other behavioral abnormalities, but without any consideration of underlying molecular variants[3]; this creates a disconnect between "genetic" and "clinical" diagnoses, creating disparities in diagnoses and hindering progress toward molecularly targeted therapies. Recently, there has been a move toward gene- or variant-specific classification of NDDs, driven largely by decreasing cost and increasing clinical availability of genome-wide analyses such as chromosomal microarray, whole-exome sequencing, and whole-genome sequencing.[4–10] Although currently only about 20% of all patients with autism receive a molecular diagnosis, we expect this number to increase as

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noncoding variants and gene expression changes are increasingly recognized.[11] NDDs, and in particular ASD, are genetically heterogeneous. In ASD, for instance, almost 1000 disease-associated genes have been identified so far, each only accounting for a minority of cases.[12–14] With such a small number of patients with shared pathogenic variants, it is difficult for clinicians to provide prognostic information to families, and developing individual therapeutics targeted to such small numbers of cases is often not practical or cost effective.[15]

Despite genetic heterogeneity at the DNA level, it is well documented that NDDs, and ASD more specifically, share common molecular and cellular pathways of dysregulation. In particular, processes related to transcriptional regulation, protein synthesis, and synaptic function all have been consistently implicated in ASD using a variety of genomic techniques.[13,16–22] Consequently, there has been a concerted effort to develop "mechanism-centric" approaches to classify NDDs and more specifically ASD.[22–24] However, using molecular genetic data to clinically categorize or stratify patients with ASD has not been implemented in the clinic as quickly as the evolving genetic discoveries.[1,25] Here we propose an intermediate level of classification, at the level of the cellular or molecular pathway, as an important next step in clinical diagnosis and management of ASD and perhaps more broadly NDDs. Such a classification scheme would need to be clinically relevant and molecularly specific, therefore allowing clinicians to use the expanding genetic information without overburdening non-geneticist clinicians and other providers who care for these patients.

Precedence for the utility of pathway-based classification schemes has been long established in the field of oncology, for example, in medulloblastoma, where molecular subgrouping has allowed clinicians to better stratify patient risk and guide treatment regimens.[26,27] In addition, with increasing evidence showing similar dysregulated pathways that drive tumorigenesis across different cancer types, there has been a push toward centralization of therapy with treatment regimens focused on molecular diagnosis instead of tissue phenotype.[28] A similar approach to ASD is needed, as the molecular drivers of the pathophysiology in autism disorders (like in cancer) are unlikely to conform to phenotypic boundaries.

Methods and rationale

In an effort to assess the feasibility of such a classification system using ASD and related NDDs with autistic features as major components, we manually assessed all Online Mendelian Inheritance in Man (OMIM) entries (https://www.ncbi.nlm.nih.gov/omim) with "autism" as features of disease. One-hundred fifteen OMIM entries met our initial search criteria. After manual curation, four of the OMIM entries were syndromes not associated with any known gene and another 29 were either genes not associated with a specific syndrome or were syndromes that did not have autism as a major clinical phenotype and so were excluded from further analysis. Eighty-three syndromes were included in the final analysis, of which we identified 69 disease-causing genes associated with the OMIM disorders. Enrichment analysis of these genes was performed using the DAVID Bioinformatics Resource 6.8 (https://david.ncifcrf.gov). Using the DAVID functional annotation tool, we assessed for significantly over-represented molecular or cellular pathways that may be informative clinically. We defined significance as only those annotation terms that were enriched after multiple testing correction by the Benjamini-Hochberg correction for false discovery rate with P value <0.05.[29] We manually grouped the OMIM syndromes into broad biological pathways based on the corresponding annotation term, with processes related to ion transport, cellular synaptic function, and transcriptional regulation being over-represented. We defined ion transport genes as genes changing the molecular landscape of the synapse and surrounding cytosol, for example, by coding for proteins involved in ion channel function in the case of *CACNA1C*. In contrast, genes in the cellular synaptic function subgroup act at the cellular level, for instance, by dampening long-term synaptic transmission in the case of *SHANK3*. A few syndromes met the criteria for more than one pathway and were manually curated after literature review and placed into the most appropriate category. Eighty of the 83 syndromes assessed could be classified into one of these three pathways, with transcriptional regulation being the most common pathway implicated (Tables 1-3). Of the three syndromes not enriched for the three most common pathways, two were related to amino acid metabolism (hyperprolinemia; OMIM # 239500 and phenylketonuria OMIM 261600) and one was related to axonal function (Schaaf-Yang syndrome; OMIM # 615547). For the purposes of this, we chose to focus on the three most common biological pathways and so did not include these syndromes in our final analysis. Chi-square analysis was used to compare individual pathway association with different clinical symptoms (Supplementary Table 1).

To see if our pathway analysis held up using genes implicated in idiopathic autism we downloaded genes from the SFARI (Simons Foundation Autism Research Initiative) database (https://sfari.org/resources/sfari-gene).[30,31] This database uses a systems biology approach to discover candidate autism genes and so is primarily used as a research tool for scientists. In total 65 SFARI "high confidence" and "strong candidate" autism-associated genes were downloaded and a DAVID gene ontology enrichment analysis was performed on this set of genes, as above (Supplementary Tables 2 and 3). "High confidence" genes are defined as having evidence of recurrent and consistent mutations or have been implicated from association studies that reached genome-wide significance, confirmed via independent replication. "Strong candidate" genes are similarly implicated but do not require confirmation via independent replication.[32]

Below, we discuss each functional pathway individually, highlighting trends within each, focusing on clinically relevant observations. Our results suggest that a classification system such as this, which identifies phenotypic patterns shared across different syndromes with shared molecular pathophysiology, can provide clinically valuable information that may aid in the management of patients with complex disorders.

Results and implications

Cellular synaptic function

Dysfunction at the level of the synapse is thought to contribute to the pathology of a number of NDDs including ASD.[13,16,18–21] Yet, traditionally, dysregulated ion transport at the synapse has not been distinguished from cellular synaptic dysfunction. Our classification points to a phenotypic divergence among syndromes driven by disruption in either of these two pathways and suggests that these two groups may have different prognoses and different treatments. Examples of disorders with disrupted synaptic function included tuberous sclerosis 1 (OMIM # 191100) and 2 (OMIM # 613254), and Phelan-McDermid syndrome (OMIM # 606232) (Table 1).

We found that motor-related symptoms were more common in patients with syndromes associated with disrupted cellular versus molecular synaptic transport (74% vs 29%, P value 0.01, respectively). In addition, disorders with disrupted cellular synaptic function were more likely to have non-neurological involvement as part of the disease (63% vs 57%), and less likely to be associated with

TABLE 1.
OMIM Syndromes With Autism as a Significant Feature—Dysfunctional Synaptic Function

Syndrome	OMIM Number	Gene(s) Implicated	Cytogenic Location	Dysmorphic Facial Features	Motor Symptoms	Psychiatric Disorder	Behavioral Problems	Systemic Symptoms	Intellectual Disability	Epilepsy
Spinocerebellar ataxia, autosomal recessive-20	616354	SNX14	6q14.3	X	X			X		X
chr 10q22.3-23.3 deletion syndrome	612242	NRG3, GRID1	10q22.3-q23.3	X	X	X		X	X	X
Arthrogryposis, mental retardation and seizures	615553	SLC35A3	1p21.2	X	X				X	X
chr 1q21.2 deletion syndrome	612474	SEC22B, HFE2, ITGA10	1p21.2			X				X
Costello syndrome	218040	HRAS	11p15.5	X	X			X	X	X
Phelan-McDermid syndrome	606232	SHANK3	22q13.33	X	X			X	X	X
Tuberous sclerosis 2	613254	TSC2	16p13.3		X	X	X	X	X	X
Perioxome biogenesis disorder 9B	614879	PEX7	6q23.3	X	X	X		X	X	X
chr 10q26 deletion syndrome	609625	DOCK1, C10orf90	10q25-q26	X	X	X	X	X	X	X
chr 5q12 deletion syndrome	615668	PDE4D	5q12	X	X			X		X
Joubert syndrome 3	608629	AHI1	6q23.3		X			X	X	X
Tuberous sclerosis 1	191100	TSC1	9q34.13					X	X	X
Williams-Beuren region duplication syndrome	609757	POM121, STX1A, GTF2I, NCF1	7q11.23	X	X	X	X	X		X
Brunner syndrome	300615	MAOA	Xp11.3		X	X	X			
Mental retardation, X-linked 104	300983	FRMPD4	Xp22.2				X		X	X
Nance-Horan syndrome	302350	NHS	Xp22.2-p22.1	X			X		X	
Macrocephaly/autism syndrome	605309	PTEN	10q23.31	X						
chr 2p16.3 deletion syndrome	614332	NRXN1	2p16.3	X	X	X	X		X	X
Mental retardation, autosomal dominant 44	617061	TRIO	5p15.2	X	X	X	X	X	X	X

Abbreviation:
chr = Chromosome
X denotes the clinical feature is present in that particular syndrome.

intellectual disability (68% vs 86%) and behavioral problems (47% vs 57%) when compared with patients within the ion transport group (Table 4). Although further studies are needed to clarify the cause and implication of these observations, such information would be immediately useful and may guide clinicians to enroll patients in aggressive physical therapy and rehabilitation programs earlier in their disease course and earlier in their motor development.

Transcriptional regulation

Knowledge about how subtle disruptions in transcriptional regulation within the cell lead to abnormal neurodevelopment has been a recent focus of genomic research and reflects the growing number of transcriptional regulating genes implicated in ASD.[33,34] Consistent with these observations, the majority of syndromes we assessed (54 of 83 total) were enriched for functions related to transcriptional regulation and control (Table 2). Representative examples within this subgroup included Angelman syndrome (OMIM #105830), fragile X syndrome (OMIM # 300624), and CHARGE syndrome (association of congenital anomalies-Coloboma, heart anomaly, choanal atresia, retardation, genital and ear anomalies) (OMIM #214800). Syndromes enriched for transcriptional regulation tended to have more systemic non-neurological symptoms (72%), dysmorphic features (80%), and comorbid psychiatric disease (54%) such as mood, anxiety, and psychotic disorders (Table 4). In addition, the transcriptional regulation group of disorders had the lowest incidence of comorbid epilepsy (72%) when compared with the ion transport (100%) and synaptic function (84%) groups. These observations suggest that transcriptional dysregulation may be useful as a clinical tool to identify patients with more broad phenotypes, and perhaps poorer long-term outcomes when compared with individuals with synaptic and ion channel abnormalities, who appear more likely to have more narrow neurological-only phenotypes, although further work is needed to confirm this theory.

In our cohort, autism syndromes within the translational dysregulation group tended to be associated with significant non-behavior comorbidities, such as apnea in the case of Rett syndrome, which is often the cause of death.[35,36] In Rett syndrome (OMIM # 312750) specifically, recent efforts have focused on developing therapies targeting the mutated *MECP2* gene and its downstream effectors.[37] However, few therapies have been shown to be efficacious, likely reflecting the number of broad and diverse functions of *MECP2*.[37,38] We predict that other disorders within this transcriptional regulation group may also have poor responses to very targeted molecular treatment unless employed very early in postnatal development, as the molecular consequences of early disruption of these key transcriptional regulators are unlikely to be reversible later in the disease course. As such, identifying patients with variants in this pathway may signal a need for more intense management and early referral to subspecialists. Furthermore, as disruption of transcriptional regulation is likely to disrupt several developmental cellular programs or pathways downstream of the transcriptional regulator, patients with variants in transcriptional pathways are likely to need pharmacologic therapy targeted to multiple cellular abnormalities concurrently.[39]

Ion transport

Seven of the 80 syndromes analyzed enriched for processes related to ion transport (Table 3). Representative examples within this subgroup included Timothy syndrome (OMIM #601005) and Landau-Kleffner syndrome (OMIM # 245570). Within the ion transport subgroup, all had epilepsy (seven of seven), a majority had features of intellectual disability (six of seven), and most also had behavioral problems (four of seven) (Table 1). Although it is not

TABLE 2.
OMIM Syndromes With Autism as a Significant Feature—disrupted Transcriptional Regulation

Syndrome	OMIM Number	Gene(s) Implicated	Cytogenic Location	Dysmorphic Facial Features	Motor Symptoms	Psychiatric Disorder	Behavioral Problems	Systemic Symptoms	Intellectual Disability	Epilepsy
Desanto-Shinawi syndrome	616708	WAC	10p12.1	X		X	X	X		X
chr 14q11-22 deletion syndrome	613457	FOXG1B, PRKD1, SCFD1, PAX9	14q11.2-p13.1	X	X			X	X	X
Rett syndrome, congenital variant	613454	FOXG1	14q12		X		X	X	X	X
Angelman syndrome	105830	UBE3A	15q11.2-q13		X		X	X	X	X
chr 15q25 deletion syndrome	614294	SEC11A, ZNF592	15q25.2-p25.3	X	X			X		X
chr 16p13.2 deletion syndrome	616863	USP7, GLYR1, UBN1, PPL	16p13.2		X		X	X		X
chr 17q12 deletion syndrome	614527	LHX1, HNF1B, AATF, DDX52	17q12	X		X		X	X	X
Cerebral creatine deficiency syndrome 2	612736	GAMT	19p13.3	X			X	X	X	X
Cerebellar ataxia, nonprogressive, with mental retardation	614756	CAMTA1	1p36.31-p36.23	X	X		X		X	X
White-Sutton syndrome	616364	POGZ	1q21.3	X	X		X	X		X
Helsmoortel-van der AA syndrome	615873	ADNP	20q13.13	X	X	X		X		X
Velocardiofacial syndrome	192430	TBX1	22q11.21	X		X	X	X	X	X
Adenylosuccinase deficiency	103050	ADSL	22q13.1	X	X	X	X		X	X
Glycine encephalopathy	617301	AMT	3p21.31		X		X	X	X	X
Luscan-Lumish syndrome	616831	SETD2	3p21.31	X		X	X	X		X
chr 7q11.23 deletion syndrome, distal	613729	HIP1, YWHAG	7q11.23	X		X	X	X		X
Nicolaides-Baraitser syndrome	601358	SMARCA2	9p24.3	X	X			X	X	X
Kleefstra syndrome	610253	EHMT1	9q34.3	X	X	X	X	X	X	X
Renpenning syndrome	309500	PQBP1	Xp11.23		X			X	X	X
Microphthalmia, syndromic 1	309800	NAA10	Xq28		X			X	X	X
chr 2q37 deletion syndrome	600430	HDAC4	2q37	X	X	X	X		X	X
Rett syndrome	312750	MECP2	Xq28		X		X	X	X	X
chr 3pter-p25 deletion syndrome	613792	CNTN4, SETD5, ITPR1	3pter-p25	X	X			X		X
Cornelia de Lange syndrome (types 1-4)	122470, 300590, 610759, 614701	NIPBL, RAD21, SMC3, SMC1A, HDAC8	5p13.2, 8q24.11, 10q25.2, Xp11.22, Xq13.1	X	X	X	X	X	X	
Myhre syndrome	139210	SMAD4	18q21.2	X	X			X	X	
Williams-Beuren syndrome	194050	GTF2IRD1, GTF2I	7q11.23	X	X	X		X	X	
CHARGE syndrome	214800	CHD7	8q12.2	X				X	X	
Primrose syndrome	259050	ZBTB20	3q13.31	X	X	X	X	X	X	
Mental retardation, X-linked, syndromic 13	300055	MECP2	Xq28	X	X	X		X	X	X
Xq25 duplication syndrome	300979	STAG2	Xq25	X	X	X	X		X	X
MEHMO syndrome	300148	EIF2S3	Xp22.11	X	X	X	X	X	X	X
Mental retardation, X-linked associated with fragile site	309548	AFF2	Xq28	X		X	X		X	
chr 3q29 deletion syndrome	609425	PAK2, DLG1, PAK3, DLG3	3q29	X	X	X	X		X	
Potocki-Lupski syndrome	610883	RAI1	17p11.2	X	X			X	X	
Waardenburg syndrome type 2E	611584	SOX10	22q13.1	X	X			X	X	
chr 1q21.1 deletion syndrome	612474	NBPF15, HYDIN	1q21.1	X	X	X		X	X	X
chr 1q21.1 duplication syndrome	612475	NBPF15, HYDIN	1q21.1	X	X	X		X	X	X
chr 2p16.1-p15 deletion syndrome	612513	BCL11A	2p16.1-p15	X	X	X		X	X	
chr 17p13.3, centromeric, duplication syndrome	613215	PRP8, RILP, SCARF1, PITPNA, SKIP, MYO1C, CRK, YWHAE	17p13	X	X				X	
Witteveen-Kolk syndrome	613406	SIN3A	15q24	X	X	X	X	X	X	X
chr 5q14.3 deletion syndrome	612881	MEF2C	5q14.3	X	X				X	X
chr 16p11.2 deletion syndrome	613444	SH2B1	16p11.2			X		X	X	
Mental retardation with language impairment and with or without autistic features	613670	FOXP1	3p13	X	X	X	X		X	
chr 3q13.31 deletion syndrome	615433	ZBTB20, GAP43, LSAMP	3q13.31	X	X	X			X	

(*continued on next page*)

TABLE 2. (*continued*)

Syndrome	OMIM Number	Gene(s) Implicated	Cytogenic Location	Dysmorphic Facial Features	Motor Symptoms	Psychiatric Disorder	Behavioral Problems	Systemic Symptoms	Intellectual Disability	Epilepsy
Mental retardation, autosomal dominant 23	616311	SETD5	3p25.3	X	X	X			X	
chr 15q14 deletion syndrome	616898	MEIS2	15q14	X	X			X	X	
chr 11p13 deletion syndrome, distal	616902	ELP4, PAX6	11p13							
Mental retardation, autosomal dominant 41	616944	TBL1XR1	3q26.32	X	X	X			X	X
Mental retardation, autosomal dominant 42	616973	GNB1	1p36.33	X	X	X		X	X	X
Neurodevelopmental disorder with or without anomalies of the brain, eye or heart	616975	RERE	1p36	X	X		X	X	X	
Dias-Logan syndrome	617101	BCL11A	2p16.1	X	X		X			
Dyskinesia, seizures, and intellectual developmental disorder	617171	DEAF1	11p15.5	0	X	X	X		X	X
chr19q13.11 deletion syndrome, proximal	614119	TSHZ3	19q13.11	0	X			X	X	X
Fragile X	300624	FMR1	Xq27.3	X	X	X	X		X	X

Abbreviations:
chr = Chromosome
CHARGE syndrome = Association of congenital anomalies-Coloboma, heart anomaly, choanal atresia, retardation, genital and ear anomalies
MEHMO syndrome = Mental retardation, epileptic seizures, hypogonadism and hypogenitalism, microcephaly, and obesity
X denotes the clinical feature is present in that particular syndrome.

surprising that disorders with ion channel abnormalities commonly have epilepsy as a component, interestingly there is emerging evidence that certain antiepileptic drugs may improve behavioral or mood symptoms in addition to their antiepileptic properties.[40–42] A possible insight from a pathway-centric approach to diagnosis would be that patients with variants in ion transport pathways may benefit from the behavioral modification effect of certain antiepileptic's, even if they do not have overt epilepsy clinically at the time of diagnosis. Furthermore, these results suggest that even if these patients do not have seizures when identified initially, they are likely at high risk and at the very least this can guide anticipatory counseling for patients and their parents.

SFARI analysis

Importantly, there are several databases that have attempted to list and categorize autism genetic data. For our analysis we chose to use the OMIM database to identify autism syndromes, which account for about 25% of all autism cases, given the clinical bias of this database.[11] However, as a comparison, we performed a gene ontology analysis of autism-associated genes in the SFARI database, which is primarily used as a research tool. Only 13 of 65 autism candidate genes from the SFARI database were also implicated in syndromic autism defined in our OMIM search, which was not surprising given the different ways these gene sets were curated. Interestingly, our SFARI results showed enrichment for processes related to transcriptional regulation and synaptic function, corroborating our findings from our OMIM analysis (Supplementary Tables 2 and 3). However, we did not find processes related to ion channel function in the SFARI analysis, and this may be explained in part by the emphasis on idiopathic autism in the SFARI database, which may have a lower incidence of associated epilepsy when compared with syndromic autism, which was the focus of our OMIM analysis.[43] However, future work is needed to tease out differences in idiopathic and syndromic autism pathways as this information is potentially clinically useful.

Discussion and future directions

NDDs such as ASD are currently diagnosed clinically based on criteria that encompass deficits in communication, social

TABLE 3.
OMIM Syndromes With Autism as a Significant Feature—Ion Transport Dysregulation

Syndrome	OMIM Number	Gene(s) Implicated	Cytogenic Location	Dysmorphic Facial Features	Motor Symptoms	Psychiatric Disorder	Behavioral Problems	Systemic Symptoms	Intellectual Disability	Epilepsy
Cerebral creatinine deficiency syndrome-1	300352	SLC6A8	Xq28		X		X	X	X	X
chr 4q32.1-q32.2 triplication syndrome	613603	GRIA2, GLRB	4q32.1-q32.2	X				X		X
chr 15q13.3 microdeletion syndrome	612001	OCA2, CHRFAM7A, CHRNA7	15q13.3	X		X	X		X	X
chr Xp11.23-22 duplication syndrome	300801	SYN1, RP2, CACNA1F, KCND1	Xp11.23-p11.22				X	X	X	X
chr 15q11.2 deletion syndrome	615656	NIPA1, NIPA2, CYFIP1	15q11.2	X	X	X	X		X	X
Landau-Kleffner syndrome	245570	GRIN2A	16p13.2	X		X			X	X
Timothy syndrome	601005	CACNA1C	12p13.33	X				X	X	X

Abbreviation:
Chr = Chromosome
X denotes the clinical feature is present in that particular syndrome.

TABLE 4.
Summary of clinical Features Associated With Each clinical-Molecular Group

Clinical Feature	Pathway			
	Ion Transport (n = 7)	Synaptic Function (n = 19)	Transcriptional Regulation (n = 54)	Chi-square *P* Value
Dysmorphic features	71%	68%	80%	0.58
Motor symptoms	**29%**	**74%**	**81%**	**0.01**
Psychiatric disorder	43%	47%	54%	0.80
Behavioral problems	57%	47%	50%	0.91
Systemic symptoms	57%	63%	72%	0.60
Intellectual disability	86%	68%	81%	0.71
Epilepsy	100%	84%	72%	0.06

Bold text indicates difference between groups was statistically significant.

interaction, and other behavioral difficulties.[3] However, emerging molecular and genetic evidence overwhelmingly shows that in many instances what are now considered separate clinical entities may result from dysregulation of the same few underlying biological pathways.[14,19] Moreover, incorporating the underlying molecular basis of these disorders could provide valuable prognostic and therapeutic insights that are shared among molecularly related disorders, such as more appropriate counseling or targeted therapies.[25] Molecular evidence continues to accumulate suggesting that despite a wide range of phenotypes at the clinical level often the same cellular-molecular pathways are implicated. Thus, based on genetic data, it will be critically important for clinicians to begin incorporating such information into their practice. However, the majority of patients undergoing genetic testing for autism who have variants discovered will not have variants in genes associated with a well-phenotyped entity.[15,25] Furthermore, it will be difficult for clinicians to act on this gene-level information given that there would not be significant information on the clinical phenotype, developmental trajectory, and natural history of patients with the same variant because of the rarity of the individual variants themselves. However, if patients with variants in the same biologic pathway were grouped together, such as in the schema we propose here, there is the potential for greater clinical insight given the greater number of aggregated patients. In addition, a pathway-based classification may be more clinically actionable given that it is the cellular process that the genetic variant affects that ultimately results in the clinical pathology.

Our analysis of ASD is an attempt to illustrate a concept, and our approach is limited by the assumption that disease-causing genes are associated with one pathway, which in many instances is an oversimplification. For instance, the *FMR1* gene linked to fragile X syndrome is well known to be associated with cellular processes including mRNA binding and dendritic translational regulation, and recent evidence suggests that this gene may also be involved in other processes such as ion channel regulation.[44–46] Several groups have addressed this by placing genes into multiple pathways and organizing the genetic data into overlapping pathways of dysregulation.[23,24,47] However, how this added level of complexity can be easily incorporated into clinical practice will need to be resolved.

For this small pilot study, we used autism genes associated with known clinical syndromes to best characterize associated clinical phenotypes; however, this approach limited the sample size of the analysis. Follow-up studies should incorporate coding and non-coding genes associated with idiopathic autism, which would increase the power of our study and also allow us to see if the same biological pathways hold for syndromic and idiopathic autism. Our SFARI analysis was an example of how this study could be done, but it would be interesting to expand the analysis even further to include genes from other databases such as ClinVar and AutDB. In addition, as many of the genes implicated in autism are also implicated in other NDDs, future studies may attempt to integrate other neurodevelopmental syndromes such as congenital epilepsy, mental retardation, and schizophrenia syndromes into similar biological pathways, as other groups have done.[47,48] Incorporating NDDs more broadly into our analysis would show convergent and divergent pathways between disorders and could also potentially provide insight into clinical similarities and differences between NDDs.[49–51]

Although a pathway-based framework will be an important step for the field in general, it is to be noted that there are certainly individual variants that are amenable to their own therapeutic interventions. For example, patients with variants in genes related to carnitine biosynthesis or coenzyme Q_{10} (CoQ_{10}) synthesis would benefit from trials of carnitine or CoQ_{10} supplementation, respectively, and thus may not be best suited for a pathway-based approach to diagnosis.[52,53]

In summary, here we propose a molecular-genetic stratification of ASD that may complement current clinical diagnostic approaches. This strategy could provide actionable clinical insight and potentially guide therapeutic development. Although this approach is not a permanent solution to the gap between genomic and clinical diagnoses in ASD, our results suggest that pathway-based classifications of NDDs such as ASD based on emerging genetic data may soon be clinically feasible.

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Supplementary data

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