REVIEW ARTICLE

Copy number variation and autism: New insights and clinical implications

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Genomic research can lead to discoveries of copy number variations (CNVs) which can be a susceptibility factor for autism spectrum disorder (ASD). The clinical translation is that this can improve the care of children with ASD. Chromosome microarray is now the first-tiered genetic investigation for ASD, with a detection rate exceeding conventional cytogenetics and any single gene testing. However, interpretation of the results is challenging and there is no consensus on "what" and "how much" to disclose. In this article, we will review how CNV studies have improved our understanding of ASD, the clinical applications, and related counseling issues. Future direction of autism genetic research is also discussed.
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Introduction

Autism spectrum disorders (ASDs) are neurodevelopmental conditions that share features of impaired communication, impaired social interactions, and repetitive behaviors with a narrow range of interests. According to the Centers for Disease Control and Prevention (CDC) 2012 estimates, one in 88 children has been identified to have ASD in the United States.[1] The average age of diagnosis is 4.5 years, when symptoms in the three core domains become apparent (CDC

2009). Approximately 75% of children with ASD have a lifelong disability requiring substantial social and educational support.[2] ASD represents an important health burden with its astonishing 78% increase in pediatric prevalence between 2002 and 2012 (CDC data).

According to the new Diagnostic and Statistical Manual of Mental Disorders edition 5 (DSM-V),[3] the diagnosis of "autistic disorders" is replaced by "autism spectrum disorders" because autism is defined by a common set of behaviors. The three domains in DSM-IV become two with social and communication domains merged to improve the diagnostic clarity. Importantly, this revision is more adapted to the individual's clinical heterogeneity by including clinical specifiers and associated features, including any known genetic disorders. This certainly reflects the improved understanding of the clinical presentation, the pathology, and the genetic susceptibility of ASD.

Recent research has suggested that ASD might result from atypical brain structure, differentiation, synchronization, or connections.[4–6] As mentioned earlier, it was long believed that genetics played a major role in ASD. The heritability of autism was reported to be approximately 90%.[7] Concordance rate in monozygotic twins is as high as 70%,[8] while the recurrence rate in siblings is approximately 20%.[9] Understanding of the genetic origin of ASD first came from studying various genetic syndromes, for example, fragile X syndrome (*FMR1*), Rett syndrome (*MECP2*), Angelman syndrome (*UBE3A*), tuberous sclerosis (*TSC1* and *TSC2*). Genetic studies on various neurodevelopmental/psychiatric diseases, for example, bipolar disorders, schizophrenia (SZ), intellectual disability (ID), developmental delay (DD), attention deficit-hyperactivity disorders (ADHDs), provide further evidence that these conditions share considerable locus heterogeneity. An emerging model has been that certain mutations or genetic variability would disrupt the homeostasis of normal neuronal development resulting in a range of disorders as part of a neuro-developmental continuum.[10] A recent review identified more than 100 disease genes and over 40 genomic loci among patients with ASD.[11] Many of these findings were discoveries made from the study on copy number variation (CNV) in patients with ASD or other neurodevelopmental/neuropsychiatric phenotypes.[12–14]

In the subsequent discussion, we will focus on how CNV studies have improved our understanding of the genetics of ASD and their impacts on the clinical evaluation of patients with this common and potentially devastating neuro-developmental disorder. The experience in handling the interpretive complexities of CNV studies will also be important and applicable to other emerging and increasingly complex genome-based technologies.

CNVs and human genome

CNVs are segments of DNA ranging from a kilobase to several mega-bases, present in varying number of copies in different individuals.[15] They can be gains (duplication or insertional transpositions), losses (deletion), or complex rearrangements. The function of CNVs is yet to be fully understood. However, the contribution of CNVs in genomic variation has gained more attention because they encompass more nucleotides per genome than the total number of single-nucleotide polymorphisms (SNPs).[16]

The first CNV studies were published in 2004.[17,18] CNVs can involve one or multiple genes and can affect gene function by (1) disrupting the coding region as a recessive or dominant allele, (2) disrupting the regulatory landscape, (3) generating a chimeric gene, or by (4) position effect.[19] Genomic disorders are syndromes caused by alteration of specific dosage-sensitive genome segments.[20] Often the genomic region is flanked by homologous low-copy number repeats that mediate nonallelic homologous recombination, resulting in deletion/duplication of the same genomic region, for example, the 22q11.2 deletion/duplication syndrome, the Williams—Beuren syndrome and its reciprocal duplication.[21] Conversely, CNVs can be associated with no overt phenotype, even for the large CNVs involving multiple genes.[17,18] The Database of Genomic Variants (http://projects.tcag.ca/variation/) is a useful catalog that provides a comprehensive summary of structural variation in the human genome. It annotates CNVs that involve segments of DNA that are greater than 1 kb, as well as InDels (insertions and deletions) in the 100 bp—1 kb range, identified in healthy control samples.

CNV studies in ASD

Cytogenetically visible chromosomal anomalies are found in approximately 7—8% of patients with autism.[22] Almost every chromosome is affected by numeric or structural aberrations but the most consistent findings are fragile X and duplication of maternal 15q11-13.[22] Chromosome microarray (CMA) is a molecular cytogenetic technique that overcomes the limited resolution of conventional cytogenetics and allows genome-wide scanning for both microscopic and submicroscopic chromosomal aberrations.[23] This strategy has been very successful in the discovery of ASD candidate genes, making it the best known example of CNV studies for pediatric disorders.[24]

In 2007, Sebat et al reported the first family-based CNV study by examining 118 simplex, 47 multiplex, and 99 control families, and identified *de novo* CNVs in 10% of sporadic patients, compared with 3% in multiplex families and 1% in controls.[25] Disease-associated CNVs were identified at 17 loci on 11 chromosomes, suggesting multiple genes are involved in the pathogenesis. The concept of CNVs has then emerged as a possible genetic contribution to the development of autism. Multiple large studies have been performed using different platforms to examine different cohorts[25–33] and many are impactful studies in the field of autism research [as rated by the Interagency Autism Coordinating Committee (http://iacc.hhs.gov/) of the U.S. Department of Health and Human Services]. By summarizing the findings of these studies,[25–33] we can conclude the following:

1. Multiple rare *de novo* (and some inherited) CNVs contribute to ASD susceptibility. All have an individual contribution of less than 1% in the frequency of occurrence.[26,28,29,33]
2. The proportion of *de novo* CNVs is three to five times higher in families with ASD than in controls, and it is higher in simplex than multiplex families.[25,26,28,29]

3. Some patients with ASD have two or more *de novo* CNVs and typically have more severe phenotype. Approximately 27% patients with syndromic ASD have *de novo* CNVs.[31,32]

4. Up to 40% of family-specific CNVs are inherited from an apparently non-ASD parent, suggesting incomplete penetrance.[25,26,31]

5. Although many CNVs appear to involve haploinsufficient regions, some act recessively as homozygous alleles deleting both copies of a gene in consanguineous families (e.g., *PCDH10, DIA1, NHE926*).[32]

6. Some CNVs recur at the same locus among unrelated patients, and may coincide with genomic disorders associated with ASD. Some are well-known (e.g., maternal 15q11-q13 duplication),[26–31,33] while the others are more recent findings (e.g., 16p11.2 deletion/duplication[25,26,28–30,33] and 17p11.2 duplication[28]).

7. There is a relative enrichment within CNVs in ASD studies for neuronal synaptic complex genes (e.g., *SHANK2, SHANK3, NRXN1, NLGN4*).[25–28,31–33]

8. CNV studies in ASD identified the same genes spanning across different neuropsychiatric and neuro-developmental disorders including ADHD, SZ, and ID, indicating that overlapping pathways may be involved in phenotypically distinct outcomes.[12]

It is important to note that different CNVs exhibit different penetrance for ASD depending on multiple factors including dosage sensitivity and the function of the gene(s) they affect.[25–33] For CNVs with large impact on ASD susceptibility, they will typically be *de novo* in origin, higher in the frequency of occurrence and associated with more severe symptoms, for example, maternal 15q11-q13, 16p11.2 deletion and 22q13 deletion (*SHANK3* gene). Some have moderate or mild effects and will require a "second-hit" or "multiple-hits" to take the phenotype across the ASD threshold, for example, 15q11.2 deletion,[34] 16p12.1 deletion,[35] *SHANK2* mutation.[36] Others demonstrate phenotypic variation and can be seen in non-ASD individuals.[19] CNV deletions, compared with duplications, tend to have a stronger effect size on phenotype severity across the spectrum of neurodevelopmental diseases.[10]

International collaborations and recent landmark studies

Tremendous effort is made to establish collaborations for autism genetic researches. Two important examples in this regard are the Autism Genome Project (AGP) (http://www.autismgenome.org/) and the Simons Simplex Collection (http://sfari.org/sfari-initiatives/simons-simplex-collection).

The AGP aims to identify autism susceptibility genes by drawing on very large cohorts of simplex (trios with an affected child and the parents) and multiplex (a family with at least two children affected) families from more than 40 academic/research institutions across North America and Europe.[37] The probands are fully characterized in the autism phenotype. Phase I of the project was completed in 2007, while phase II was finalized in 2010. Its landmark study on CNVs, involving 996 cases with ASD and 1287

controls, has demonstrated a higher burden of rare, genic CNVs in patients compared with controls and notably higher involvement of genes previously implicated in ID. It has also implicated new ASD genes such as *SHANK2* and the *DDX53-PTCHD1* locus and using gene set analysis, the study identified novel important ASD pathways (e.g., cellular proliferation, projection and motility, and guanosine triphosphatase/Ras signaling).[38]

In contrast, the Simons Foundation Autism Research Initiative embarked on an effort to recruit and carefully phenotype more than 2000 simplex families.[39] The majority (>80%) of the study population included at least one unaffected sibling and the probands were evaluated with a battery of diagnostic measures related to ASD and related co-morbidities. Its landmark study involves 1124 cases and 872 controls, and uses the same microarray platform as the AGP study.[38] They have found that CNVs were four times more common in the probands compared with their unaffected siblings. In addition, CNVs in affected children were larger and overlapped with more genes. These findings support that autism is mostly caused by rare *de novo* events unique to each proband.[40]

Table 1[12,38,40–52] summarizes the loci/genes most often affected by CNVs from these two most comprehensive studies.

Clinical applications of CMA in the evaluation of patients with ASD

Clinical utility

In 2010, the International Standard Cytogenomic Array (ISCA) Consortium reviewed 33 published studies of 21,698 patients referred for the investigations of DD, ID, multiple congenital anomalies, and ASD and found that CMA offers a much higher diagnostic yield of 15–20% than a conventional karyotype of 3%, excluding the diagnosis of Down syndrome and other recognizable chromosomal aberrations.[53] ISCA, therefore, proposed the use of CMA in place of karyotype as the first-tier cytogenetic diagnostic test for patients with these indications. In the same year, the Autism Consortium Clinical Genetics/DNA Diagnostics Collaboration focused specifically on 933 patients with ASD and compared different testing methods. Diagnostic yield was 2.23% using G-banding karyotype and 0.46% using fragile X testing that was less sensitive compared with CMA method, which was able to detect clinically significant abnormalities in 7% of patients. They concluded that despite the potentially complicated interpretation of novel CNVs of unknown significance, CMA should be considered as a part of the initial diagnostic evaluation of patients with ASD.[54]

With these lines of evidence showing the clinical utility, the 2010 Practice Guidelines of the American College of Medical Genetics has recommended testing for CNVs as first-line test in the initial postnatal evaluation of individuals with ASD.[55] The Canadian College of Medical Genetics has also issued a position statement recommending CMA as the first-line laboratory investigation when autism is unexplained after a thorough history and physical examination.[56] More recently, clinical CNV studies in Chinese population have become available and in two recent

Table 1 Loci/genes most commonly affected by CNV in ASD in the Autism Genome Project study by Pinto et al and Simons Foundation study by Sanders et al.[12,38,40–52]

Genomic loci	Gene(s)	ASD cases (n = 2200)	Controls (n = 2159)	p	Other overlapping phenotypes
		Deletion/duplication	Deletion/duplication		
16p11.2 (700 kb)	30 genes	10/8	1/2	0.001 *	Schizophrenia,[49] ADHD[12,50]
Xp22.11 (1 Mb)	PTCHD1	10 (with nine affecting upstream noncoding RNA)/0	0/0	0.038 *	ADHD[12]
2p16.3	NRXN1	8/1	1/0	0.011 *	Schizophrenia[12,51]
7q11.23 (1.4 Mb)	22 genes	0/4	0/0	0.06	Williams–Beuren syndrome (deletion)[41]
22q11.2 (2.5 Mb)	56 genes	2/2	0/1	0.214	Schizophrenia[12,52]
1q21.1 (1.5 Mb)	14 genes	0/4	0/3	0.723	Schizophrenia[12,43]
15q13.3 (1.5 Mb)	6 genes	4/1	0/0	0.030 *	Epilepsy ± intellectual disability, schizophrenia[42,43]
15q11-q13 (5 Mb)	12 genes	0/2	0/0	0.245	Epilepsy, schizophrenia[44]
11q13.3	SHANK2	2/0	0/0	0.245	Intellectual disability[45]
22q13.33	SHANK3	0/1	0/0	0.495	Intellectual disability[46]
Xq13.1	NLGN3	1/0	0/0	1	Intellectual disability[47]
Xp22.3	NLGN4X	0/1	0/0	1	PDD-NOS, intellectual disability[48]

The number of cases (and controls) is the total number combining the studies by Pinto et al[38] and Sanders et al.[40]
*Statistically significant. ADHD = attention deficit-hyperactivity disorder; ASD = autism spectrum disorder; CNV = copy number variation; PDD-NOS = pervasive developmental disorder not otherwise specified.

publications from Taiwan, the detection rate of clinically significant abnormalities by CMA is approximately 5%.[13,14] Obviously, the application of CMA as the first-tiered genetic testing in children with ASD needs to be cautiously evaluated in different patient populations with different clinical context.

An evidence-based approach for causality

As elucidated in the study by Shen et al,[54] at present it is still challenging to determine the causation and pathogenicity of a given *de novo* variant. Our knowledge of mutation rates and population-distribution statistics of CNVs is still rudimentary. At the same time, it is difficult to know whether an inherited variant is necessarily benign in a particular genomic environment. Some CNVs annotated as benign may, in fact, be found to be associated with subtle phenotypic signs (known as "broader autism phenotype"). Sometimes, gains and losses involving multiple genes at the same genomic locus can lead to overlapping or very different phenotypes. Other independent potential factors that are genetic, epigenetic, sex-related, environmental, or stochastic in origin may also warrant attention. All these factors need to be considered carefully in the interpretation and subsequent genetic counseling for a complex disease like ASD.[19]

The ISCA Consortium aims to promote informed and uniform CNV interpretation, which will in turn be translated into better patient care. Besides its continuous effort to optimize and standardize the array design,[57] the consortium has established a framework to assess the potential clinical relevance of CNVs systematically.[58] CNVs are classified as (1) pathogenic, (2) uncertain, likely pathogenic, (3) uncertain, (4) uncertain, likely benign, or (5) benign by considering the following questions:

- Is this genomic region associated with a clinical phenotype?
- Is this clinical phenotype associated with dosage sensitivity?
- How many lines of evidence are there to support dosage sensitivity?
- Are CNVs involving this genomic region enriched in disease population?
- Is there any compelling evidence to refute its dosage sensitivity?

Peer-reviewed literature is considered the gold standard for primary evidence and large-scale case-control studies are of particular value in assessing the clinical relevance, while locus-specific database such as the ISCA Database (www. ncbi.nlm.nih.gov/dbvar/studies/nstd37/), DECIPHER,[59] or the Autism Chromosome Rearrangement Database (ACRD) (http://projects.tcag.ca/autism/) are examples of secondary evidence. The ACRD is a collection of hand curated breakpoints and other genomic features that are related to autism, taken from publicly available literature, databases, and unpublished data. It was first described in 2004[18] and is constantly updated.

Role and responsibility of the clinicians

It is important for clinicians to have a sufficient understanding of the technology. They need to be aware of the different clinical platforms and the information they can provide.[55] In general, smaller probe size and higher density enhance the accuracy and resolution. Balanced chromosome rearrangements such as translocation or inversions cannot be identified through CMA, which can only essentially detect copy number changes. A SNP-based platform can not only detect CNVs, but also copy-

number neutral abnormalities such as uniparental disomy and areas of homozygosities. Clinicians should also understand what type of follow-up tests needs to be performed, including conventional karyotype, fluorescence in situ hybridization, and quantitative polymerase chain reaction studies. Often parental studies need to be conducted to rule out the presence of chromosomal rearrangement predisposing to recurrence. Clinicians shall also be familiar about the databases and resources currently available for referencing gene location and function, CNV listings, and up-to-date clinical information for specific CNVs. Certainly, clinicians must be aware that CNV studies are just part of the overall genetic evaluation of patients with ASD (Fig. 1).[2,60–62] Clinical geneticists shall be involved to provide dysmorphology assessment[63] and targeted neurogenetic evaluation according to latest development.[64–66]

Disclosing results of autism genomic testing

Currently, there is still no international consensus regarding the disclosure of results for genomic testing in individuals with ASD in both the clinical and research setting. With the objective to determine opinions on genomic testing disclosure, we performed a systematic review involving a comprehensive search of MEDLINE and Embase for quantitative and qualitative studies on the opinions of researchers and participants, in the context of autism genomic research (using search terms "autism", "autism spectrum disorder", "genetic", "genome", "genomic", "result", and "disclosure").[67] Publications published in English before December 2011 were included, whereas those presenting ethical arguments alone were excluded. Two quantitative studies and one qualitative study[68–70] were included. The two quantitative surveys involved only researchers ($N = 168$) or participants ($N = 158$) with response rates of 40% and 41%, respectively. The qualitative study involved both (23 researchers and 34 participants) and the response rate was not stated.

Almost all (97%) participants wished to obtain individual research results, whether favorable or not, irrespective of whether they would act upon the results. Majority of researchers (80%) agreed that clinically significant findings should be disclosed, while those of uncertain significance should not be reported (85%). "Clinical significance" depends on whether the genetic finding is robust, well-replicated, or incidental. Researchers with clinical interpretive role or capability to explain the results are more inclined to disclose the results. Integrating the opinions of both parties, the qualitative study found that reportability is related to perceived meaning to participants, evidentiary standards, and epistemological commitments regarding the role of genetics in autism and concluded that disclosure standards remained context specific and not universal. Our systematic review provides limited guidance on genomic research disclosure and the meaning of "clinical significance" remains subjective and poorly defined. Furthermore, all included studies are susceptible to response bias and selection bias, limiting their validity and generalizability.

Research with larger samples evaluating different scenarios is needed to guide the decision-making process on result disclosure and to explore the ethical and legal responsibility of researchers. These studies will provide the evidence necessary to guide clinicians and scientists in result disclosure. However, the ultimate disclosure standards will be context specific and require individualized considerations for different participants, given the complexity of the issue.

Conclusion and future directions

At the time of writing this review paper, the Phase III study of AGP was just reported in the 2012 International Meeting for Autism Research, supporting and reinforcing the major findings in the Phase II study. Three studies, using exome sequencing, have investigated more than 600 families and identified a two to four times increase in de novo mutations in the coding regions of the genome among affected individuals over their controls.[71–73] The Autism Sequencing Consortium has begun studies using whole exome/genome sequencing and these projects are expected to be completed within a reasonable time frame. Both whole exome and genome sequencing make use of massively parallel sequencing (or next-generation sequencing) technology.[2] Exome sequencing investigates all the exons, or coding regions of genes which comprise of approximately 1% of the entire genome. It has been successfully applied in the identification of causative mutations in Mendelian and non-Mendelian disorders including ASD, using case-control design or using parents-affected child trios.[74] However, in conditions caused by mutations in the noncoding regions of the genome, the genetic changes will escape detection by exome sequencing and would only be detectable by whole genome sequencing. However, whole genome sequencing is relatively expensive and the huge amount of data requires sophisticated bioinformatic analysis and computational power that is still beyond the capacities of most clinical molecular laboratories. Detailed discussion of these technologies is beyond the scope of this review and readers are referred to relevant comprehensive reviews for more information.[2,74]

Massively parallel sequencing has also brought about advances in the understanding of our epigenome. Epigenetics refers to heritable changes in gene expression that occur without a change in the primary DNA sequence. Identifying the relationship between our epigenome and neurodevelopment and the link to ASD susceptibility has become an emerging area in autism research. Global DNA methylation profiling in lymphoblastoid cell lines has identified interesting candidate genes with altered expression in brain tissues from individuals with ASD,[75] whereas whole-genome research studying postmortem brain samples from tissue banks have identified chromatin changes in affected individuals.[76]

Pluripotent stem cells and animal models are new technologies that enable researches to shed lights on promising novel strategies for interventions in the future.[77] For example, the triple-Ube3a autistic mouse model with diminished glutaminergic transmission in synaptic pathway exhibited the behavior phenotype of maternal 15q11-13 duplication and triplication syndrome.[78] The FMR1 knockout mouse model with deficiency in metabotropic
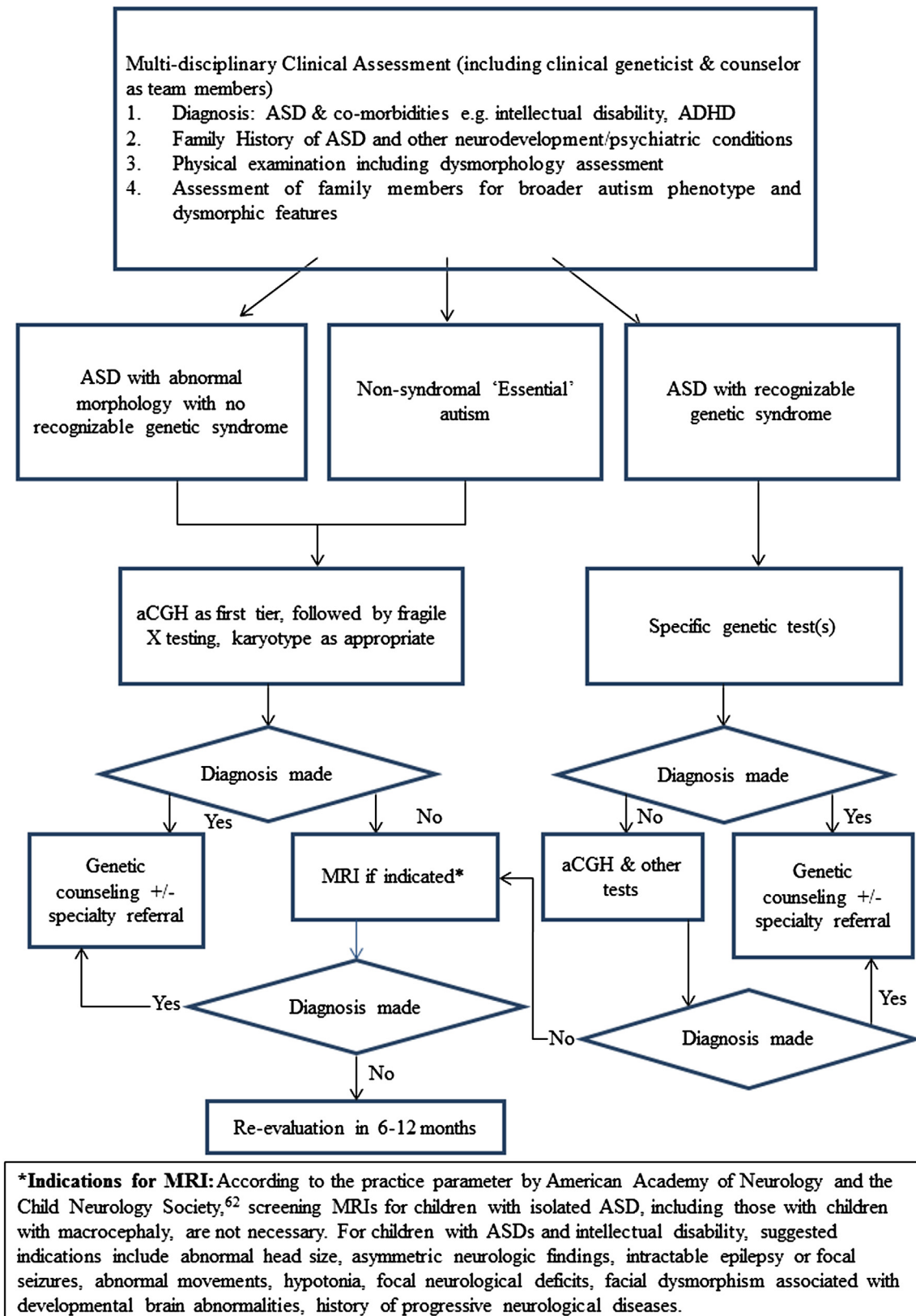
Multi-disciplinary Clinical Assessment (including clinical geneticist & counselor as team members)
1. Diagnosis: ASD & co-morbidities e.g. intellectual disability, ADHD
2. Family History of ASD and other neurodevelopment/psychiatric conditions
3. Physical examination including dysmorphology assessment
4. Assessment of family members for broader autism phenotype and dysmorphic features

ASD with abnormal morphology with no recognizable genetic syndrome

Non-syndromal 'Essential' autism

ASD with recognizable genetic syndrome

aCGH as first tier, followed by fragile X testing, karyotype as appropriate

Specific genetic test(s)

Diagnosis made

Diagnosis made

Yes · No

No · Yes

Genetic counseling +/- specialty referral

MRI if indicated*

aCGH & other tests

Genetic counseling +/- specialty referral

Yes

Diagnosis made

No

No

Yes

Diagnosis made

No

Re-evaluation in 6-12 months

*Indications for MRI: According to the practice parameter by American Academy of Neurology and the Child Neurology Society,[62] screening MRIs for children with isolated ASD, including those with children with macrocephaly, are not necessary. For children with ASDs and intellectual disability, suggested indications include abnormal head size, asymmetric neurologic findings, intractable epilepsy or focal seizures, abnormal movements, hypotonia, focal neurological deficits, facial dysmorphism associated with developmental brain abnormalities, history of progressive neurological diseases.

Figure 1 Clinical genetic evaluation of children with autism spectrum disorder (ASD).[2,60–62]

glutamate receptors showed a phenotype similar to that of fragile X syndrome.[79] These animal models played important roles in pharmaceutical exploration of treatment methods for syndromic ASD. Following promising results in animal drug trials, several medications entered into clinical trial phase. For example, the mGluR5 antagonist showed

some positive effect without clinically significant adverse effect in a pilot study in adult patients with fragile X syndrome.[80] Another example would be minocycline,[81] which was previously used in neurodegenerative disease treatment for its neuroprotective effect.[82] Encouraged with the rescue effect on the dendritic spine and synaptic structural abnormalities in the fragile X knock-out mouse,[83] Utari et al[84] put it into clinical use and reported promising result when treating 50 fragile X syndrome patients with a 2-week course of minocycline.

The incremental advance in ASD genetic research has not only improved our understanding of this complex neurodevelopmental disorder, but it has also started to have an impact on the diagnosis, classification, and intervention of ASD. It is our hope that with the data accumulated through whole genome technology, important pathways will continue to be discovered and be translated into better clinical care for children with ASD and other neurodevelopmental disorders.

Acknowledgments

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