Autism and Genetics

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Helga V. Toriello

Abstract

Autism had initially been considered to be caused by environmental influences, but it is now known that there is a strong genetic influence on the development of autism. In addition, autism is genetically heterogeneous, and may occur as a component manifestation of a genetic syndrome or occur as an isolated trait. However, the genetic evaluation of an individual is still limited by the availability of certain tests, although as technology proceeds, the approach to testing is expected to change. This chapter reviews currently recommended approaches to testing, and some speculation on what will likely be available in the future.

Introduction

Autism was first described by Leo Kanner (1896–1981) in 1943 [1] with a description of eleven children with what the author termed a "unique syndrome" which was characterized as "an inability to relate themselves in the ordinary way to people and situations from the beginning of life". The author further described this as an "extreme autistic aloneness (that) disregards, ignores, shuts out anything that comes to the child from the outside". Although Kanner suggested that these characteristics were innate, he also made the observation that family members were generally not considered to be warmhearted and had limited interest in people. It is believed that from this latter observation a theory that autism was attributable to parenting by aloof mothers (which were later termed "refrigerator mothers") took hold, and persisted into the 1970s until alternative theories suggesting that autism was biologically determined gained acceptance [2]. Then in 1998, Wakefield et al. [3] published a paper suggesting that MMR vaccinations were responsible for the development of autism. Several studies ultimately debunked this theory and a review by Gerber and Offit [4] described the various studies in this group. The current belief is that autism and related disorders are now considered to be complex traits with a significant genetic contribution to their heterogeneous and complex nature.

H.V. Toriello, Ph.D. (✉)
Department of Human Development and Pediatrics, College of Human Medicine, Michigan State University, 15 Michigan St, Room 363, Grand Rapids, MI 49503, USA
e-mail: toriello@msu.edu

I.L. Rubin et al. (eds.), *Health Care for People with Intellectual and Developmental Disabilities across the Lifespan*, DOI 10.1007/978-3-319-18096-0_60

In addition, the definition of autism and related conditions (e.g., pervasive developmental disorder, NOS) has also undergone evolution, so that the DSM-5 now considers what had been previously considered separate conditions to now be one entity termed autism spectrum disorders (ASD). This term includes Asperger syndrome, pervasive developmental disorders, and atypical autism, as well as classic autism, which were once considered to be separate conditions [5].

Autism Spectrum Disorders: Genetic Contributions

Once considered to be caused by environmental factors as noted above, ASD are now known to have a strong genetic component, with an estimated heritability of approximately 90 % [heritability is the proportion of the phenotype that is attributable to genetic factors]. Much of this estimated figure is derived from twin studies, which had found that concordance between monozygous twins was greater than the concordance between dizygous twins. For example, Smalley et al. [6] found that concordance between monozygous and dizygous twins was 64 % and 9 %, respectively; others have reported concordance rates between monozygous twins to be as high as 95 % [7]. Additional evidence that ASD have a genetic basis comes from family studies, which have found that recurrence risk to siblings for having classic autism is 5–8 % if there is only one affected child; if there are two (or more) affected children, the recurrence risk is estimated to be closer to 35 % [8]. In addition, the risk of a sibling having any form of ASD (not just classic autism) is 15–25 % [7]. The third piece of evidence for a genetic component is the finding that in as high as 40 % of affected individuals a genetic or chromosomal abnormality is discovered; this observation also provides further evidence for the extreme heterogeneity of autism. Recently Betancur [9] provided a review of over 100 genetic and chromosomal disorders which have been found to be associated with an increased risk of autism. Within this group, there are several entities which are considered nonsyndromic, indicating that even within this latter group, there is marked heterogeneity. As a result, recommendations for the genetic evaluation of the child with an ASD have been proposed, with the practice guideline provided by the American College of Medical Genetics [10] serving as the basis for further discussion (see Table 60.1).

Table 60.1 Recommended evaluation according to the American College of Medical Genetics (ACMG)

First tier evaluation – done on all patients
Three generation family history with pedigree analysis, *documentation of parental ages and pregnancy history (italicized items not part of the ACMG recommendation, but worth noting as well)*
Initial evaluation to identify known syndromes or associated conditions
Note dysmorphic features; if specific condition suspected, perform targeted testing
If clinical findings are suggestive of a metabolic or mitochondrial disorder, do appropriate testing
Chromosomal microarray
In males only, testing for fragile X
Second tier evaluation – if first tier testing is negative
MECP2 sequencing in females
If phenotype is suggestive, *MECP2* duplication testing in males
If macrocephaly is present, *PTEN* testing
Brain imaging studies if indicated
Testing which is not yet clinically available, but could become part of tier one or two in the future
Whole exome or whole genome sequencing
Testing for altered epigenetic modification patterns

Family History

A family history should include three generations, with notation of other family members who have or may have an ASD. In addition to ascertaining other family members with ASD, it is also worthwhile to note family members with psychiatric disorders. The reason for this ascertainment is that Miles [11] noted that other neuropsychiatric disorders are more common in parents of a child with an ASD. For example, alcoholism is present in 35 %, other psychiatric disorders in 35 %, and intellectual disability in 26 %; frequencies all greater than those in the general population. A study done by her group had found

that of families ascertained because of an ASD, 39 % had what was termed probable genetic alcoholism. This history is relevant in that the children in these families were less likely to have macrocephaly and more likely to have a regressive onset of their condition.

A second potentially significant finding in families of probands with an ASD is a greater frequency of autoimmune disorders, particularly in mothers. Atladóttir et al. [12] evaluated the family histories of over 3000 probands with an ASD, and found that rheumatoid arthritis (RA), type 1 diabetes (T1D), and celiac disease were significantly more common in mothers or fathers (RA and celiac disease in mothers; T1D in fathers). The reason for this association is unknown, but hypothesized to be related to a common genetic susceptibility (in the case of T1D) or the effect of maternal antibodies on fetal development (in the case of RA and celiac disease).

Parental ages (not part of the original guideline, but worthy to include in the evaluation of a child with an ASD). There is good evidence to suggest that advanced parental ages are correlated with an increased risk of having a child with an ASD. Two recently published meta-analyses of available data found that offspring born to fathers 50 years or older were 2.2 times more likely to have an ASD than were offspring born to fathers less than 30 years. Offspring born to women who were 35 years or older were 1.31 times more likely to have an ASD than were offspring who were born to women age 25–29. The proposed mechanism is increased frequency of new mutations, epigenetic modifications, or both [13, 14].

Pregnancy History

Pregnancy history (not part of the original guideline, but worthy to include in the evaluation of a child with an ASD). A number of environmental exposures during pregnancy have been found to be correlated with an increased risk of ASD, and include, but are not limited to thalidomide, valproic acid, endocrine disruptors, alcohol, and maternal diabetes and/or obesity. Thalidomide is a known teratogen associated with causing a number of congenital anomalies, most notably limb reduction defects. The term thalidomide embryopathy was applied to the constellation of anomalies produced by maternal thalidomide consumption during pregnancy. Several years after the physical findings of the embryopathy were described, it was noted that 4 % of those affected by thalidomide embryopathy also had autism; this was stated to be a 50-fold increase in frequency compared to a population frequency of 1/1000 in that population [15]. Valproic acid (valproate) is used as an anticonvulsant for seizure disorders. The fetal valproate syndrome includes spina bifida, dysmorphic features, intellectual disability, and cardiac, craniofacial, skeletal and limb anomalies in the phenotype. In children prenatally exposed to valproate, the frequency of an ASD has been found to be approximately 10 % [16]. The mechanism by which valproate exerts its teratogenic effect is thought to be via interference with folate metabolism which in turn could inhibit histone deacetylases leading to increased gene expression. De Cock et al. [17] reviewed the subject of endocrine disruptors as potential causes of ASD. Endocrine disruptors include such agents as organophosphate pesticides, dioxin-like compounds, brominated flame retardants, bisphenol A, and phytoestrogens. Although preliminary results suggest there may be an increased risk of ASD in children exposed prenatally to some or all of these substances, additional studies need to be done to confirm these findings.

The association between prenatal alcohol exposure and ASD has also been the subject of inconsistent findings. In one study, 2 of 21 children with FAS (fetal alcohol syndrome) also had a diagnosis of autism; case reports also documented this association [18]. There have also been larger studies that did not find an association between amount of maternal alcohol consumption and ASD. However, there is support from studies of mouse models which have demonstrated that alcohol affects protein expression levels in embryos, suggesting that alcohol can induce epigenetic modifications [19], which as will be discussed further on in this chapter may be the basis for the development of ASD, at least in some individuals.

Finally, Krakowiak et al. [20] found that a maternal history of diabetes, obesity or hypertension during pregnancy were collectively associated with a 1.6-fold increase in the frequency of ASD in offspring. A suggested mechanism was the increase in glucose levels in the fetus which would lead to fetal hyperinsulinemia. This in turn would lead to increased oxygen consumption and metabolism, leading then to chronic tissue hypoxia and cell damage. There are other prenatal agents that have also been investigated regarding their potential role in causing ASD, but a full discussion of all these agents is beyond the scope of this chapter. The interested reader is referred to several good reviews on the topic [16, 18, 21].

Physical Examination: Dysmorphic Syndromes

As noted above, Betancur [9] recently published a review of over 100 genetic and chromosomal disorders that are associated with an increased risk of development of an ASD. The most common among this group are fragile X, Rett and PTEN-related syndromes, as well as tuberous sclerosis. The first three are discussed later in the second tier section; herein I will review only a few of the more common conditions with which ASD is associated as a component manifestation.

Tuberous sclerosis (TS) is a heterogeneous condition which can be caused by mutation in one of two genes, hamartin and tuberin. The phenotype consists of various forms of skin lesions (including hypopigmented macules, shagreen patches, and adenoma sebaceum) and brain and/or internal organ growths, and is inherited as an autosomal dominant condition. The frequency of ASD in affected individuals is approximately 50 % whereas TS accounts for 1–2 % of all individuals with an ASD.

Angelman syndrome is caused by lack of expression of UBE3A, which is only expressed on the maternally-inherited copy of chromosome 15. This lack of expression can be caused by a mutation in the maternally-inherited gene, by a deletion of this region on the maternally-inherited chromosome, or by uniparental disomy of the paternal chromosome 15 s. The phenotype consists of dysmorphic facial features (wide mouth, prominent chin), ataxic gait, and inappropriate laughter. Over 50 % of individuals with Angelman syndrome have an ASD. This condition is an uncommon cause of ASD.

CHARGE syndrome is an acronym for the component manifestations, which are Coloboma of the iris or retina, Heart defects, Atresia choanae, Retardation of growth and/or cognition, Genital anomalies, and Ear anomalies with or without hearing loss. CHARGE syndrome is usually caused by heterozygous mutation in MLL2, and is usually the result of a new mutation in a child born to unaffected parents. In one small study, 68 % of individuals with CHARGE syndrome had an ASD.

Duchenne muscular dystrophy (DMD) is characterized by early-childhood onset of proximal muscle weakness and calf hypertrophy. This is an X-linked condition caused by mutation in the dystrophin gene. The frequency of ASD in these boys is as high as 19 %, with the ASD preceding the muscle weakness in many of the boys with DMD [22].

Joubert syndrome (JS) is a heterogeneous disorder, with at least 20 different genes responsible for the phenotype. The cardinal manifestations of JS include ocular findings (retinal dysplasia, coloboma), dysregulated breathing, and brain malformations, with a "molar tooth" sign virtually pathognomic for this condition. All affected individuals have intellectual disability to some degree, with 13–36 % described as having an ASD as well.

Physical Examination: Metabolic/Mitochondrial Disorders

As described in the ACMG practice guideline [10], metabolic or mitochondrial testing should only be undertaken in individuals who have the following symptoms:

- electrolyte or acid/base disturbances
- cyclic vomiting
- developmental regression associated with metabolic stress (e.g., illness or fever)
- alternations in muscle tone
- lactic acidosis
- seizures
- Growth failure
- Multisystem involvement
- Other (skin changes, GI dysfunction, lethargy, neurodegeneration)

Within this group, there are several conditions which are associated with an ASD. Some of the more common ones are described below [23, 24].

Phenylketonuria (PKU) is an autosomal recessive disorder caused by a deficiency of phenylalanine hydroxylase. Affected children have the combination of intellectual disability, microcephaly, decreased pigmentation, and what has been described as a musty or mousy smell. Since the advent of newborn screening, it is unusual to encounter an affected individual. Nonetheless, among untreated affected children early-onset ASD is common.

Smith-Lemli-Opitz syndrome (SLOS) is a disorder of cholesterol metabolism, attributable to homozygous or compound heterozygous mutations in the gene that encodes 7-dehydrocholesterol reductase. Affected individuals have dysmorphic features, microcephaly, genital hypoplasia, and significant syndactyly between toes 2 and 3. ASD is a common component manifestation of this condition, present in more than half of the affected individuals. It is noteworthy that Tierney et al. [25] screened 100 individuals with ASD in an attempt to identify children with SLOS; they did not find any with SLOS in their cohort of affected children, but did find that almost 20 % had significantly low levels of cholesterol, suggesting to them that disordered sterol metabolism might be one cause of ASD.

Adenylosuccinase deficiency. This condition is one of the disorders of purine metabolism, with the result that succinyl purines accumulate in the brain. In addition to an ASD phenotype, affected children have profound neurologic impairment, but are not described as being dysmorphic.

Creatine deficiency syndromes. There are three conditions in this group of disorders. Two are involved with abnormal creatine synthesis, and the third with creatine transport. The first two are caused by deficiency of either arginine: glycine amidinotransferase (AGAT) or guanidinoacetate methyltransferase (GAMT); the third is a deficiency of creatine transporter 1, encoded by SLC6A8. All three disorders are characterized by hypotonia, intellectual disability, seizures in some, and autistic-like behavior. The two creatine synthesis disorders are inherited as autosomal recessive conditions; the creatine transporter disorder is an X-linked recessive condition [26].

Cerebral folate deficiency is actually a symptom, characterized by low levels of cerebral spinal fluid levels of 5-methytetrahydrofolate (5MTHF). This condition is heterogeneous and thought to be caused by various enzyme deficiencies, as well as a component manifestation in individuals with Rett syndrome, Aicardi-Goutieres syndrome, or several mitochondrial disorders [27]. The phenotype is characterized by intellectual disability, neurologic regression and dyskinesia; seizures and ASD are also present in most affected individuals.

Mitochondrial disorders. Although some authors consider mitochondrial dysfunction a rare cause of ASD, Oliveira et al. [28] found evidence of mitochondrial dysfunction in 7 % of children diagnosed with an ASD. Although defects of Complex I were most common, other oxidative-phosphorylation defects were found as well. In a review of the subject by Haas [29], it was stressed that a history of regression, multiorgan involvement, and GI dysfunction were especially prevalent in this group. However, mitochondrial mutations have not been found in children with ASD, thus to date, sequencing of the mitochondrial genome is unlikely to reveal any causative mutations [30].

Chromosomal microarray. Several studies have reported on the utility of chromosomal microarray analysis in children with an ASD, whether or not the child has intellectual disability and/or dysmorphic features [9, 31–33]. In the past, a routine chromosome analysis using banding techniques were estimated to identify a chro-

mosome anomaly in 5 % of children with an ASD; with the advent of chromosomal microarray analysis, the detection rate has more than doubled, with most studies suggesting a detection rate of 8–12 % in this group of individuals. Therefore, unless a specific chromosomal anomaly is suspected, a microarray should be the test of choice.

Both aneuploidy syndromes and deletion or duplication syndromes have been reported in this group. For example, the frequency of an ASD in children with Down syndrome, Klinefelter (XXY) syndrome, and XYY syndrome have an ASD 10 %, 11–48 %, and 19 % of the time, respectively. Common deletion or duplication syndromes include deletion 22q11 (velocardiofacial syndrome) – ASD in 28 %; deletion 22q13 (Phelan-McDermid syndrome) – ASD in 55 %; deletion or duplication of 17p11.2 (Smith-Magenis and Potocki-Lupski syndromes, respectively) – ASD in as many as 90 %; and perhaps the most common of this group, deletion or duplication of 16p11.2 – ASD in approximately 75 %, with the 16p11.2 anomalies accounting for 1 % of all individuals with an ASD. Furthermore, Yurov et al. [34] recently described finding mosaic aneuploidy in 16 % of children with an ASD. Chromosomes involved in these aneuploidies included trisomy 9, monosomy 15, trisomy 15, monosomy 16, monosomy 18, and additional X chromosomes. The level of mosaicism was low, being no higher than 11 % in one case of an extra X, and more often between 1 and 5 %. These findings have not been reported by others, so the impact on testing guidelines is unclear.

Fragile X testing in males. Fragile X syndrome (FXS) is a relatively common cause of intellectual disability, especially in males; approximately 1–2 % of those with an ASD have FXS. FXS is caused by a trinucleotide repeat expansion in FMR1, which is the responsible gene. Affected males have the combination of speech delay, mild to moderate intellectual disability, macrocephaly, macroorchidism after puberty, and a high frequency of ASD. Approximately 60 % of those with FXS have ASD; in addition, 10–15 % of males and 5 % of females with a premutation (55–200 repeats) are also reported to have ASD, thus testing for number of repeats in FMR1 is indicated, whether or not the individual has evidence of FXS. For more detail about fragile X syndrome see the chapter elsewhere in this book.

MECP2 testing. Mutations in MECP2 are responsible for most cases of Rett syndrome (RS). RS is characterized by normal development until the age of 8–14 months; affected girls then begin to show regression and loss of previously-attained skills. Head circumference starts to cross centile lines downward, so that females with a previously normal head circumference end up with microcephaly. Affected females also tend to keep their hands in the midline, and often make a wringing motion with them. Rett syndrome is thought to account for 1 % of those with an ASD. In addition, there have been reports of individuals with MECP2 mutations that did not have Rett syndrome, thus lack of a clinical diagnosis of Rett syndrome is insufficient reason to forego testing. There is also a condition termed the MECP2 duplication syndrome. This condition affects both males and females, and is characterized by intellectual disability in almost all, hypotonia, absent speech, dysmorphic features (midface hypoplasia, depressed nasal bridge, and large ears) and ASD in 75 %. Several other manifestations may also occur, but less often than those listed above [35].

PTEN testing. There are a few conditions associated with mutations in PTEN, including Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, and others. Common to these conditions is the occurrence of hamartomas and significant macrocephaly. ASD is a relatively common component manifestation, and in those with an ASD and macrocephaly, a PTEN mutation may account for the phenotype in as many as 17 % of these cases [22].

Brain imaging. Recent imaging studies have found an unexpectedly high frequency of positive findings on MRI in children with an ASD, particularly if the affected individual also had intellectual disability. For example, Boddaert et al. [36] reported that 48 % of their population of children with non-syndromic autism had MRI abnormalities, whereas Erbetta et al. [37]

described abnormal findings in 44 % of their population of children with ASD and intellectual disability. Despite the high detection rate, Erbetta et al. [37] point out that these findings don't contribute to the diagnosis of ASD, but may be actionable in that follow up is indicated for some of the detected abnormalities.

Future tools: whole exome or whole genome sequencing. It is becoming clear that rare mutations with major impact are a significant cause of ASD. Recent studies have suggested that approximately 15–20 % of ASD is caused by mutations in genes with a major effect on the phenotype [38–40]. Most of these are new, heterozygous mutations, but there have also been reports of homozygous mutations in genes which are usually associated with a specific syndrome when the mutations are null mutations; the mutations in individuals with an ASD-only were more likely to be "milder" mutations. For example, Yu et al. [40] identified biallelic mutations in genes which usually cause PKU, a syndromic form of muscular dystrophy, or non-ketotic hyperglycinemia, among others.

Based on some of these early studies, it is now estimated that there could be several hundred genes, which when mutated, cause ASD. As the price of exome or genome sequencing comes down, it would not be surprising if this technology became part of the recommended evaluation of an individual with an ASD, particularly when parental age is increased.

Future tools: Epigenetic diagnostics. The role of epigenetic modification (that is, a change in the level of gene expression without a change in the genetic sequence) as a cause of ASD is a new and exciting area of study. For example, Wong et al. [41] found differences in methylation patterns (methylation is one form of epigenetic modification) between discordant monozygous twins, thus in part providing an explanation for the apparent paradox between high heritability and at times significant discordance levels between monozygous twins. Recent publications have described candidate genes for ASD which are known to be epigenetically modified, and thus epigenetic modification could occur in one twin and not the other. Examples of these candidate genes include the gene which encodes the oxytocin receptor OXTR which was found to have increased methylation of the promoter region in a mouse model of ASD [42]. GABRB3 codes for a protein which is a subunit of the GABA-A receptor, which is important for regulating brain development and synaptic function. Mice in whom the GABRB3 gene has been knocked out demonstrate autistic-like behavior. Therefore, underexpression of this gene via epigenetic mechanisms could also be an important cause of ASD in humans. Several other genes associated with ASD have also been found to be epigenetically modified thus this is likely to be a significant mechanism for the cause of ASD. However, the technology to assess epigenetic modification is only in its infancy, and until a means by which epigenetic modification can be inexpensively assessed becomes available, this diagnostic approach is likely a few years away.

Conclusions

Our understanding of the causes of ASD has, and will continue to evolve over time; from the "refrigerator mother" model, through vaccines as the cause, to our present day understanding of the strong genetic contribution to the etiology of ASD. Our knowledge base will continue to grow, and with it, refined methods for diagnosis, as well as the development of potential treatment options.

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