Chapter 1 - Introduction. The Neurology of Autism, edited by Mary Coleman

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The Neurology of Autism

Edited by Mary Coleman

Oxford University Press
2005

Chapter 1
INTRODUCTION

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It was 1866 in Londontown. The doctors, coming into their own beside the surgeons, had begun to classify diseases of the brain and create the specialty of neurology. However there were many children who were hard to classify because they had something terribly wrong in brain function; they had great difficulty learning and were called idiots. Idiocy was an epithet, barely a disease entity, a neglected one at that. But all that began to change when Dr. John Langdon Down (1866) published a paper pointing out that children who were classified as idiots and had enlarged tongues protruding form their mouths did not necessarily all have the same form of idiocy. They might have had the same major symptom (mental retardation), the same age when it became obvious that something was wrong (usually after 18 months of age) and they shared a striking feature (appearance of macroglossia), but he announced that they did not all have the same disease. Dr. Langdon Down wrote that one group had mongolism, a name he chose because of the appearance of the children's eyelids, and the other group had cretinism, a disease known today as infant hypothyroidism. This paper ushered in a new era of medical interest in these children, which also led to more humane care.

Almost a century and a half later, in the twenty-first century, over 2000 different disease entities have been described where patients have mental retardation. Mental retardation is a series of neurodevelopmental syndromes due to chromosomal, genetic, infectious, endocrine and toxic etiologies; in almost all cases the disease process is underway prior to birth. Regarding genetic disease, when mutation of a single gene is both necessary and sufficient to cause disease, this is called a monogenetic trait; it is now known that over 200 of the diseases with mental retardation are classified as monogenetic diseases (Zechner et al. 2001). The disease entities of the mental retardation syndromes have been generally grouped together into two major categories: syndromic and nonsyndromic mental retardation. These categories refer to syndromes where there are multiple congenital anomalies and mental retardation (the MCA/MR syndromes) versus those syndromes without either major congenital anomalies or facial and other stigmata.

Does this story have any relevance to autism? The history of autism does not cover so many years. It was first identified by Leo Kanner in 1943 and thought to be a single psychiatric disease; he defined it as a behavioral disease entity beginning in very young children. A recent study has suggested that autism may be already underway by the time of birth in almost all the children. In a study of archived neonatal blood of children who later were diagnosed as autistic, Nelson and colleagues (2001) found very elevated levels of certain neurotrophins and certain neuropeptides in 99% of the children with autism compared to controls. They were brain-derived neurotrophic factor (BDNF), neurotrophin 4/5 (NT4/5), vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP). The study by Nelson and colleagues found similar elevations of the neurotrophins and neuropeptides in 97% of the children with later mental retardation, including those with Down syndrome. *It is of great interest that no measure distinguished the children with autism from those with mental retardation, although it did distinguish them from children with later cerebral palsy and from normal controls.*

This study suggests that the autistic syndromes, like the mental retardation syndromes, begin prior to birth in the overwhelming number of cases. It adds one more piece of evidence that autism is a member of the family of neurodevelopmental syndromes with maturational disturbances, a behavioral stepsister of the mental retardation syndromes.

In fact, the autistic syndromes share many of the characteristics of the mental retardation syndromes. Both groups of syndromes impair the brain in almost all cases during the gestational neurodevelopmental time frame; they are both present at birth but are not usually clinically apparent. They both have family histories with inheritance patterns that sweep across the landscape of genetics – from classical Mendelian to maternal inheritance patterns to trinucleotide repeat disorders to large numbers of sporadic cases. Twin, family, linkage data and case histories reveal that the inheritance pattern in autism is very complex (for review, see Folstein & Rosen-Sheidley 2001). Although both groups of syndromes have a percentage of children who develop epilepsy, the autistic syndromes actually include a higher prevalence of children with seizure disorders than a population with severe mental retardation (Gillberg & Coleman 2000a). Children with autism do not have the same neuropsychological profile as youngsters classified as retarded; nevertheless, IQ is the single best predictor of outcome in both syndromes (Gillberg & Coleman 2000b). And, most importantly, the two syndromes overlap in the majority of children who are classified as autistic (Jacobson & Janicki 1983; Bryson et al. 1988); in most studies IQ is below 70 in 70% of individuals who are defined as having autism.

THE TIMING OF AUTISM

If autistic traits develop because of neurodevelopmental missteps, when during gestation does that occur? Neuroembryology is a very complex phenomenon even though neurodevelopment of the brain seems to occur gradually and seamlessly. Rice & Barone (2000) and many others have noted evidence of critical periods of vulnerability in the developing nervous system (these periods are topics of important future research). In the case of autism, data obtained from several groups of children with autistic characteristics show evidence that the disease process can be initiated in all three trimesters (Rodier et al. 1996; Coleman 1994; Yamashita et al. 2003). (There is some postnatal evidence, too [Minshew 1996]). As evidence from these trimesters is reviewed, one useful way to look at this neurodevelopmental problem might be to classify the autistic syndromes with the same general system as the mental retardation ones, that is, as syndromic or nonsyndromic. However, in both mental retardation and autism, the concept of stigmatized versus nonstigmatized children is in reality a bit too stark. Certainly in autism a continuum from minor physical stigmata to major ones can be seen. In syndromes with large numbers of patients, it often is apparent that the variable degree of phenotypic manifestation between individuals could be represented as a continuum from the full disease to even minor or undetectable expression. The concept of syndromic autism versus nonsyndromic autism will be presented here as a structural framework in which to tentatively place the disease entities.

The first trimester, primarily a time when the body and face are formed, would be the time of syndromic autism, the period when MCA/MR syndromes are initiated. It has been demonstrated that

some of the MCA/MR syndromes which include an autistic subgroup, such as the Möbius sequence, thalidomide embryopathy or the CHARGE association (coloboma, heart defect, atresia of the choanae, retarded growth and mental development, genital anomalies, and ear malformations and hearing loss) may begin as early as 20 to 24 days post-fertilization. In addition, there is an autism autopsy case with shortening of the brain stem, a defect that could have occurred only during neural tube closure (Rodier et al. 1996). In the development of the brain, the first 8 weeks of gestational age are called the embryonic period. Children with autism and a grossly abnormal stigmata examination are ten times more likely to be diagnosed with a known genetic syndrome and are twice as likely to have structural abnormalities in their brains (Miles & Hillman 2000).

A large number of MCA/MR syndromes include a subgroup of children with autism (Table 1-1A). Estimates of the number of children with autism who have these syndromes vary widely from 7% to 37%, indicating either a different population mix, or a different method of selection by the investigators. Around 12% is a reasonable working estimate for population-based surveys (Kielinen et al. 2004). The relevant question in each MCA/MR syndrome is whether the syndrome is somehow related to autistic symptoms or whether the affected children have two completely separate syndromes – the MCA/MR syndrome and a second autistic syndrome. Each syndrome has to be evaluated separately, because the majority of children in most of the MCA/MR syndromes where autistic patients have been reported do *not* have autistic features.

In the MCA/MR syndromes that are very rare and have few cases of autism, a reasonable assumption could be that, in a coincidental double syndrome, an unlucky child described with autistic features perhaps did have the syndrome by chance. If the child also has established autism stigmata that differ from other children with the particular syndrome, this weighs in favor of coincidence instead of relevance; the unlucky patient likely has two separate disease entities. An example is the case of a boy with Myhre syndrome, who met DMS-IV criteria of autism, and had several unusual findings for his syndrome (Titomanlio et al. 2001). In addition to peculiar skin histology not usually described in the Myhre syndrome, he also had hypertelorism and partial cutaneous syndactyly of the second and third toes. These are stigmata singled out in studies of minor physical anomalies in children with nonsyndromic autism. This weighs in favor of two separate disease entities in this boy.

However, other MCA/MR syndromes, such as the disease process of the tuberous sclerosis complex, may be more prone to creating autistic symptoms in their population. Epidemiological studies suggest that 43%-86% of individuals with the tuberous sclerosis complex had a pervasive developmental disorder similar to autism (Harrison & Bolton 1997), and this disease alone may account for 1% up to 4% of children with autism in some series (Smalley 1998). It is possible that the autistic symptoms in some of these children may spring from the underlying disease process, such as the number, location and size of brain tubers of the tuberous sclerosis complex (Humphrey et al. 2004), which also can be a source of epileptic foci (Chugani et al. 1998). Because they are much more thoroughly studied, the MCA/MR syndromes can be helpful in highlighting information that may underlie autistic symptoms. However, it must always be kept in mind that each of these syndromes has many other nonautistic symptoms and it is only by putting together the information from many different disease entities that some form of standardized picture might come to light.

Several syndromes have been described where autistic features are part of the initial description of the syndrome. Examples are the HEADD syndrome (hypotonia, epilepsy, autism and developmental delay) and the Orstvik 1997 syndrome (macrocephaly, epilepsy, autism, stigmata and mental retardation) (Fillano et al. 2002; Orstavik et al. 1997; van Karnebeek et al. 2002). There is evidence of mitochondrial dysfunction and mitochondrial DNA (mtDNA) deletions in the children with the HEADD syndrome. It is too early to be sure, but apparently almost all of the patients diagnosed with these new syndromes would be considered autistic. Even so, each child with a double syndrome of both autism and any MCA/MR syndrome needs to be evaluated as an individual; this has both genetic counseling and treatment implications.

While on the topic of MCA/MR syndromes, it should be noted that a child with autism does not have to have major anomalies or major stigmata to have a disease entity beginning in the first trimester. Some minor stigmata can be traced to the first trimester (Rodier et al. 1997b). Sometimes the syndrome is missed because the stigmata and skin lesions that characterize the children's faces are not always apparent in infancy and early childhood. Examples of this delayed diagnostic phenomenon

are reported in tuberous sclerosis, Cohen syndrome and the chromosome 22q11.2 deletion syndrome. A yet other possibility is the recent revelation that a child may be clinically classified as nonsyndromic autism and yet have a mutation on the same gene affected in a MCA/MR syndrome (Turner et al. 2002); this is the same phenomenon that is seen in syndromic and nonsyndromic forms of mental retardation (Nokelainen & Flint 2002).

The second trimester is believed to be a major time when many of the neurodevelopmental errors that lead to nonsyndromic autism occur. The period from 8 to 20-24 weeks of gestational age is called the fetal period of brain development. When autism was first being studied, it was noted how beautiful and unstigmatized many of these children were. This may even have been a factor in the initial blaming of the parents who were raising such normal-looking children. But as soon as any systematic research was conducted on this question, it became clear that children with autism who looked unstigmatized were more likely to have minor physical anomalies than control children matched for age, sex and socio-economic level (Steg & Rapoport 1975; Walker 1976; Campbell et al. 1978; Rodier et al. 1997a; Links et al. 1980). The timing of formation of these minor anomalies shades between the first trimester to the beginning of the second trimester. The single most common minor anomaly in these studies found in autism was an ear anomaly, particularly low seating with posterior rotation of the ears. It was even more common in children with autism than in children with mental retardation. Other minor anomalies found in children with autism by more than one study were partial or full syndactyly of the second and third toes and a slight hypertelorism.

It is far from clear in which trimester to place the infantile forms of neurological (Table 1-1B) and psychiatric disorders (Table 1-1C) already defined in older populations. Because these beautiful children have so few stigmata, these categories temporarily go into the second trimester lists. The issue will be clearer disease by disease as the neuroanatomical and genetic basis of the primary orders are better understood.

In a prospective study of prenatal factors comparing children who developed autism with normal and mentally retarded controls, there was a statistically significant finding of second-trimester bleeding limited to the mothers of children with autism compared to retarded and normal controls (Torrey et al. 1975). There are a few reported cases of accurate timing of maternal infections in children who later developed autism. In children with autism and congenital rubella (Chess 1977) or congenital cytomegalovirus (Ivarsson et al. 1990), the maternal infections were in the second trimester. Maternal autoimmune diseases in the second trimester also may increase risk (Croen et al. 2005).

Malformations of cortical development due to abnormal neuronal and glial proliferation are seen in the neuroectodermal diseases, such as tuberous sclerosis. Evidence of neuronal migration aberrations is found in many MRIs of children with both autism and Asperger syndrome (see Chapter Two for the list of references). Table 1-2 lists some of the diseases where these neuroembryological errors are found. Both genetic and infectious etiologies can cause these aberrations. They are primarily second trimester phenomena.

Relatively few patients with autism are thought to have injuries to the central nervous system initiated during the third trimester. The period extending from 24 weeks of gestation until the time of birth is called the perinatal period by embryologists of the central nervous system. The children with autism that have been documented with third trimester insults are mostly examples of infections, such as symptomatic congenital cytomegalovirus infection (Yamashita et al. 2003).

The perinatal and postnatal periods are periods when errors established earlier may be expressed or new insults may affect brain function. After the advent of birth, the brain continues to grow, change and be vulnerable due to the persistence of some limited neurogenesis, elimination of neurons through apoptosis or programmed cell death, postnatal proliferation and pruning of synapses and activity-dependent refinement of neuronal connections (Johnston 2004).

THE NOSOLOGY OF AUTISM

So in this neurodevelopmental syndrome, the question arises as to how we are going to diagnose children with this core social disorder in the future? One encouraging development is that, in psychiatry and neurology as in all of medicine, nosology is rapidly improving so that diagnosis and treatment can be more individualized. It is now possible to augment the traditional clinicopathological method of

general diagnosis to a more comprehensive study of a disease during the lifetime of the individual through chromosomal studies, molecular genetics, brain imaging, electrophysiology and many other technologies. However, the symptoms of central nervous system disease, showing through an underlying personality structure, remain particularly difficult to interpret. This is especially so in the case of the young child with autistic symptoms.

It has been 60 years since the behavioral syndrome of autism was first described, yet problems of nosology remain. Most physicians working with these patients would agree that current testing instruments to define the symptoms of autism (Table 1-3) are clinically useful and appropriate. Autism involves multiple developmental domains; it compromises a wide range of socioemotional, language and other cognitive skills. However, many patients don't fit exactly into the designated criteria of autism or Asperger and are called atypical autism or pervasive developmental disorder not otherwise specified (PDD-NOS). Are children with specific language disorders mistakenly being put into a pool with autism? Another unsettled question refers to patients currently classified as having the very rare Childhood Disintegrative Disorder. This is a topic in itself - a family has been described of half-brothers, one with autism and one with Childhood Disintegrative Disorder (Zwaigenbaum et al. 2000).

Very important work has been done to recruit populations of children with autism as psychiatrically homogeneous as possible for research purposes. Nevertheless a number of unsolved issues remain in play. These problems are not just academic; they have implications for infant and childhood learning programs as well as pharmacological approaches.

One persistent nosological challenge is whether Asperger syndrome and autism are variations of the same disease, a question posed by Lotspeich et al. (2004) and others. There is evidence from a number of disciplines including genetics that suggests that the answer is likely to be yes (Table 1-4). The same mutations in nuclear DNA (Jamain et al. 2003) and also in mtDNA (Pons et al. 2004) have been found both in patients with autism and with Asperger syndrome. However, an increasingly difficult point is this: Are the criteria for Asperger generally being applied by clinicians too loosely? Where do you draw the line between an eccentric or odd person and a truly sick person, in other words, how broad is the phenotype? Benaron (2003), using the title "Inclusion to the point of dilution", reviewed the topic of diagnosis in autism/Asperger and raised a number of important points. One was that there are no criteria of exclusion, as is seen in so many other syndromes, resulting in increasing dilution.

Another area of debate is the presence of additional brain symptoms. Although it is well accepted that many of the children with autism would be classified on standardized testing as mentally retarded, there is a very significant percentage – up to 40% in some series – who are also prone to other psychiatric conditions, such as mood, attentional and anxiety disorders. Should there be a neurological or an epilepsy subgroup (Chapter VII)? Do mute patients belong in a separate group, since this is a very large subgroup of almost half the children in some series (Gillberg & Wahlstrom 1985)? Some of the worse clinical problems with children with autism arise from the so-called secondary symptoms – the vision and hearing impairments, the disturbing forms of self-abuse, sleep disorders that haunt the parents, the many types of food faddism, and water fascination with its danger of drowning. Are they found only in certain disease entities or throughout all autism?

Before these many questions can be decided however, the first most elementary question of nosology that must be addressed is whether autism is one disease or many diseases. A spectrum is the term used to describe continuous clinical variations spread out within the same disease entity. The utility of a spectrum is the grouping of individuals into a coherent group which all share a set of basic underlying pathological traits - such as a genotype - and thus can provide a consistent set of individuals for further research and the development of rational therapies. The implication of the term "autistic spectrum disorders" is that they are, in the end, one underlying disease. Thus, is autism one huge, sprawling, multifaceted spectrum of a disease? Or perhaps is autism a syndrome, a final common phenotype expressed by many different underlying diseases?

Is autism a spectrum or a syndrome? Study of the human genome has led to many changes in medical thinking; one of these changes is that the concept of "spectrum" and "syndrome" as used in the past has developed extended meanings. Here is one example – mutations of the *ARX* (Aristaless related homeobox) gene, important in neurodevelopment, has clinical manifestations referred to as the "spectrum of the *ARX* gene" (Kato et al. 2004). Mutations of this gene are associated with the following human phenotypes: (1) hydranencephaly with abnormal genitalia, (2) X-linked lissencenphaly with

abnormal genitalia, (3) Proud syndrome (X-linked mental retardation, agenesis of the corpus callosum and abnormal genitalia), (4) isolated agenesis of the corpus callosum (in females), (5) X-linked infantile spasms, and (6) nonsyndromic X-linked mental retardation. Other phenotypes include West syndrome, myoclonus, dystonia, ataxia and autism. So disease entities with very major brain malformations and those completely without malformations of the brain can arise from mutations of this single gene - an extreme pleiotropy of clinical expression indicating a great *allelic heterogeneity.* It ranges from the missing cortex of hydranencephaly all the way to the brain apparently without malformations of nonsyndromic mental retardation. The *ARX* gene has other fascinations as well. The location of the mutations inside the *ARX* gene and the type of its mutations tend to predict the phenotype. The *ARX* mutations in humans described to date that include a brain *without* malformations are deletion of exon 5, polyalanine expansions or duplications and missense mutations. In contrast, truncating mutations, which induce a premature termination of the protein, cause lissencephaly or hydranencephaly. This was one of the very first genes where it was noted that there tends to be a consistency of genotype-phenotype correlation by looking inside the gene itself.

Certainly on the face of it, the clinical expression of *ARX* mutations underlie what appears to mimic a syndrome (many separate disease entities) rather than a spectrum (variations of a single disease). However by some present definitions, clinically relevant mutations on one gene = one spectrum. So this kind of caveat will be kept in mind during the nosology discussion of autism/Asperger syndrome. At least there is no argument that from the point of view of educational intervention programs, it is often quite useful to consider autism, Asperger syndrome, PDD-NOS and the other phenotypic variants as part of a rather extensive clinical psychiatric spectrum, using similar individualized approaches to tackle the symptoms.

Based on twin and family studies (Bailey et al. 1995; Pickles et al. 1995) and subsequent genome scans, evidence began to develop that more than one gene might be involved in autism. This led to the conclusion that autism was a complex rather than a monogenic disorder, with the term "oligogenic disorder" used to describe the involvement of multiple loci (Risch et al. 1999; Cook 2001). The assumption was made that, like other common diseases such as Parkinson disease, multiple genetic and environmental factors would influence the risk of an individual being affected. Susceptibility or protective genes might confer or reduce the risks of developing disease. One strategy, using linkage and association studies, examined the possibility that the responsible genes are not mutations encoding aberrant gene products but apparently normal polymorphisms acting synergistically or even "independently." Investigating the genetics of separate subclinical traits or endophenotypes in relatives of children with autism seemed another constructive way to find susceptibility genes (Leboyer 2003).

After more than ten years of very hard work in these linkage and association studies by many dedicated investigators, no susceptibility gene for all of autism has yet been identified. In contrast, there is now good and growing information about Parkinson's disease. In Parkinson's disease, genetic studies have found mutations in genes from different families and several susceptibility genes; they all affect the misfolding, overexpression, insufficient disposal or aggregation of brain alpha-synuclein, which has a central role in this disease process (Olanow 2003; Golbe & Mouradian 2004). It is even beginning to be understood how damage by toxins such as MPTP to alpha-synuclein might cause clinical Parkinson-like effects similar to those that result from the gene mutations. Although the Parkinson story is not yet over and other genes and mechanisms await discovery, it is already known that the gene mutations directly or indirectly lead toward a pathogenetic cascade that goes eventually toward the brain's handling of a central protein, alpha-synuclein.

So now it can be asked if Parkinson's disease is a good pathogenetic model for autism. Will gene mutations be consistently found in autism that all eventually lead mostly toward one protein? Or perhaps would a more fruitful model be the approach used in studying the mental retardation syndromes where many, many different factors are involved - where there are chromosomal duplications, deletions, translocations, and mosaics, where there are genes with different Mendelian patterns of transmission, trinucleotide repeats, maternal inheritance patterns, and epigenetic phenomena?

One Disease Entity

The first question is as follows: Is autism, at least as originally described by Kanner, a single disease entity in the great majority of patients? One can make the argument that this is not clear-cut in view of the fact that two of his eleven patients developed a seizure disorder, making two subgroups - a seizure group and a nonseizure group – in the very first population of patients (Kanner 1943, 1971). Of course it is theoretically possible that the same gene, indicating one disease entity, can underlie a phenotype which is similar except that some patients have seizures and others are seizure-free. Another theoretical possibility is that classic autism might be caused by epigenetic silencing that can mimic genetic mutation by abolishing expression of a gene. The mosaicism and nonMendelian inheritance that are characteristic of epigenetic states can produce patterns of disease risk that resemble those of polygenic or complex traits.

But now that genetic screening has begun in large groups of children with autism, different genes have been found in different individuals and the pleiotropic nature of many genes has become apparent. In the autism literature there now exists both *locus heterogeneity* (mutations in completely different genes causing the same phenotype) and *allelic heterogeneity* (different mutations inside the same gene causing different phenotypes). An example of locus heterogeneity is seen in the tuberous sclerosis complex. In this genetically heterogeneous disorder, approximately 40% of the cases are related to mutations in the TSC1 gene on chromosome 9 (9q34) while most of the remaining cases are associated with alteration of the TSC2 gene on chromosome 16 (16p13.3). An example of allelic heterogeneity was discussed above concerning the *ARX* gene; Sherr (2003) lists the autism cases in the *ARX* spectrum.

As the number of genetic loci found by studying children with strictly defined autism has multiplied, investigators have begun to rethink their theories and have begun to wonder if the limited concordance in different genome-wide scans might possibly reflect sample heterogeneity rather than ever more numerous genes of weak effect (Buxbaum et al. 2004). Could it be that autism is more than one disease entity?

Many Disease Entities

That autism might be a syndrome with multiple etiologies had been considered by a number of authors. In 1986, Reiss et al. wrote that autism appears to be a behaviorally defined phenotype which arises from diverse causes of central nervous system damage. Bailey et al. (1993) noted that the genetic factors in autism probably would include causative genes, since autism appeared to have one of the highest heritability rates in psychiatry. By 2003, Eigsti and Shapiro were stating that autism is a heterogenous disorder and was likely to have multiple possible etiologies.

Actually, the concept that autism might be a syndrome of more than one disease had already been put forward earlier by neurological, biochemical, stigmata and family history analyses in a research study of 78 children with autism and their 78 age, sex, parent-income matched controls (Coleman 1976). Virtually everything studied in this early 1976 research project failed to show consistency. The children with autism were not found to be uniform in blood studies – in whole blood serotonin studies, 59% had elevated levels of whole blood serotonin (confirming results from as early as 1961 by Schain and Freedman) and 25% had low levels. There also was a lack of uniformity in urine studies – 22% of the children had too much uric acid in their urine (hyperuricosuria) and a different 22% had too little calcium (hypocalinuria). Neurological, stigmata and family history patterns also showed great variability. Although no individual disease entities were identified in this early study, there was a striking lack of homogeneity in the results; hence the name 'the autistic syndromes' was chosen for the study.

Since that study, at least 60 different disease entities have been identified in patients meeting standardized criteria of autism (Tables I-1, I-5, and I-6 and the epileptic syndromes in Chapter VII). Just as has been seen in the mental retardation syndromes, children with autistic features have been described with chromosomal, genetic, infectious, endocrine, toxic and space-occupying etiologies. In autism, there are groups of children with no classical neurological signs and there are others with neurological signs such as epilepsy or hypotonia or tics (see Chapter VII). The cranial circumferences

are not consistent: they may be normocephalic, macrocephalic, or microcephalic (see Chapter VI). All these major variations suggest that autism is not a single disease entity.

If autism in fact is many different diseases, there are a number of implications. One might refer to the study of gender difference in autism. If autism is many diseases, then the underlying factors leading to male predominance in the patients with a normal physical examination and a structurally normal brain by MRI (23 boys to 1 girl) and the phenotypically abnormal children (1.7 boys to 1 girl) likely have a different origin (Miles & Hillman 2000;). As more homogenous populations are identified, pathological studies, functional imaging studies, genetic studies—all research studies—can be more pinpointed and consistent. Another shift might be to move pharmacological research in the direction of tailoring treatment to individual disease entities inside the autistic syndrome.

A look at a few selected chromosomal and genetic aberrations discovered from studying children with autistic features will highlight the great variety of the autistic syndromes.

CHROMOSOMAL DISORDERS

Autism spans across the genome. Putting together all the published information on patients who meet the criteria of autism, Asperger syndrome or PDD-NOS, chromosomal aberrations or genetic mutations have been described in every chromosome (reviewed in Lauritsen et al. 1999; Gillberg & Coleman 2000c). Regarding the pattern seen in the chromosomal aberrations, the autistic syndromes are more likely to be associated with chromosomal deletions than the mental retardation syndromes, which have more cases of full trisomies. It has been estimated in the past that between 3% and 9% of a population of children with autistic traits can be found to have chromosomal aberrations (Gillberg & Wahlstrom 1985; Ritvo et al. 1990; Fombonne et al. 1997; Konstantareas & Homatidis 1999; Wassink et al. 2001). Three examples of chromosomal sites associated with autism will be discussed: regions on chromosome 15, chromosome 22 and the X chromosome.

The 15q11-q13 Cluster

The area on chromosome 15q11-q13, sometimes called the Prader-Willi/Angelman critical region (PWACR), contains a number of imprinted genes. In both the Prader-Willi syndrome and the Angelman syndrome, a large chromosome deletion that spans 4.0 to 4.5 megabases in 15q11.2-q13 has been identified in 60% to 70% of patients. A deletion in the paternally derived chromosome 15 results in Prader-Willi syndrome, while the same deletion in the maternally derived chromosome 15 underlies the Angelman syndrome. These syndromes can also be caused by uniparental disomy (when the two copies of the chromosome are derived from one of the parents); a maternal disomy results in Prader-Willi syndrome, whereas paternal disomy causes Angelman syndrome. Patients with Angelman syndrome have been reported to have autistic characteristics, but autistic symptoms usually are not described in patients with the Prader-Willi syndrome. However, even here an occasional patient with autistic features has been reported with Prader-Willi (Descheemaker et al. 2002; Veltman et al. 2004).

Patients who do not have clinically apparent Angelman or Prader-Willi syndrome but do definitely have autistic symptoms have been described in association with a number of different aberrations of the 15th chromosome that include the PWACR. Most frequently found are duplications including interstitial ones resulting in trisomic genetic load or a supernumerary marker chromosome formed by the inverted duplication of proximal chromosome 15, with either a trisomic or tetrasomic genetic load. Translocations and deletions and microdeletions also are described (for review see Gillberg and Coleman 2000c). The most consistent factor in these aberrations associated with autism is their origin in the maternal chromosome.

Most cases of children with autism and a chromosomal anomaly in the 15q11-13 region appear to have one of the forms of chromosomal duplication. Actually this area involves probably the most common supernumerary marker chromosome in humans. It should be remembered that not every patient with excessive chromosomal material encompassing 15q11-q13 is autistic (Rineer et al. 1998). If the duplication involves the PWARC, the patients are more likely to have autism or developmental delay; the asymptomatic patients tend to have duplications that fall outside this region (Bolton et al. 2001). Clinically the presentations in children meeting autistic criteria vary somewhat (Borgatti et al. 2001). Facial stigmata, if present, are usually subtle. Mental retardation is almost always present but

ranges from mild to severe. Many children have a seizure disorder, but not all. Patients with hypotonia and motor delays have been reported in the literature. Spinal deformities also have been described (Gillberg et al. 1991).

It is not yet known precisely which genes underlie the symptoms of autism in patients with 15q11-13 duplication or deletion in spite of extensive work by excellent laboratories. Of great interest are the three genes encoding $GABA_A$ receptor subunits (*GABRB3, GABRA5, GABRG3*) located there. In a study of 140 families who had a child with an autistic disorder, Cook et al. (1998) revealed linkage disequilibrium with the *GABRB3* gene encoding the beta3 subunit. A *GABRB3* polymorphism also was found to be associated with autism in a set of 80 families (Buxbaum et al. 2002). A study of a group of familial cases where the children had savant skills identified a susceptibility locus within the *GABRB3* gene (Nurmi et al. 2003), while another study using the symptoms of insistence on sameness also had increased linkage to the *GABRB3* gene (Shao et al. 2003). In a study of 226 families with one or more children affected with autism, Menold et al. (2001) reported linkage disequilibrium with the GABRG3 gene. Not all these studies have been duplicated.

Another gene (*KIAA0068*) that encodes a protein interacting with and modulating the fragile X protein is of interest. The imprinted gene *UBE3A*, involved in Angelman syndrome, is another candidate (Nurmi et al. 2001; Herzing et al. 2002). The gene *ATP10C* exhibits a similar imprinted expression to *UBE3A*, is adjacent to it and involved in ion transport (Herzing et al. 2001). And there are still a number of other imprinted genes in the PWACR area.

Probably the most interesting cases to date are those of two children with autism and 15q11-q13 inverted duplication, also called isodicentric chromosome 15, who had pronounced mitochondrial proliferation in muscle and partial respiratory chain block most parsimoniously placed at the level of complex III (Filipek et al. 2003). One of these children had no dysmorphic features; the other had dysmorphic features classified as mild. Both had global developmental delay and hypotonia, although it was episodic in one case.

The 22q11 & 22q13 Regions

The chromosomal 22q11 has been studied by investigators of both schizophrenia and autism; the chromosomal region 22q13 is also under intensive study. These regions are associated with a number of disease entities where patients have autistic features. There have been several cases of *ring chromosome 22* with autistic disorder (Assumpcao 1998; MacLean et al. 2000) which includes a deletion covering the q11-q13 region. Major malformations are absent in most cases, but it is of note that these children may have the second and third toe syndactyly which is statistically more common in children with autism (Walker 1976).

The 22q11 region is of particular interest because it is the site of the 22q11.2 deletion syndrome, often associated with the *velocardiofacial syndrome*, also known as CATCH 22 (cardiac abnormality, T cell deficit, cleft palate, and hypocalcemia). This multiple anomaly syndrome can occur after a *de novo* mutation or be transmitted as an autosomal dominant trait. Either learning difficulties or mental retardation are usually present as well as problems with speech and language including articulation. Common clinical features of this syndrome are cleft palate (velopharyngeal insufficiency), cardiac defects, and a characteristic facial appearance, although there is a high phenotypic variability. The patients have a long face, a prominent nose with a bulbous nasal tip and a narrow alar base, almond-shaped or narrow palpebral fissures, malar flattening, malformed ears, and recessed chin. These facial features are not always apparent in infancy and early childhood. For example there is a report of four children with childhood onset schizophrenia and deletions in the chromosome 22q11 region who "had subtle craniofacial and body dysmorphic characteristics that *had not been noticed previously*" (Sporn et al. 2004).

Neurobehavioral involvement has been reported by many investigators and is thought to be a major feature of the velocardiofacial syndrome (Niklasson et al. 2001), although it has been suggested that it may not be specific and rather related to the level of cognitive development (Feinstein et al. 2002). In 1996 Swillen et al. identified two individuals as having the velocardiofacial syndrome in residential homes for people with autism. A number of children with autism or PDD-NOS have since been reported to have a deletion at 22q11.2 or the velocardiofacial syndrome (Kozma 1998; Chudley et

al. 1998; Eliez et al. 2000; Niklasson et al. 2001). The velocardiofacial syndrome or the 22q11.2 deletion syndrome also has been described in patients with adult schizophrenia (Coleman and Gillberg 1996) and childhood-onset schizophrenia (Nicolson et al. 1999).

In the velocardiofacial syndrome, the volume of the brain—both grey and white matter but particularly white matter volume—is decreased (Eliez et al. 2000; Barnea-Goraly et al. 2003b). It is of interest that those patients with a maternal 22q11.2 microdeletion are reported to have a significantly greater loss of grey matter than those with a paternal microdeletion (Eliez et al. 2001a). The velocardiofacial syndrome is one of the syndromes where a diminished size of cerebellar vermal lobules VI-VII and the pons has been demonstrated (Eliez et al. 2001c). Researchers used functional imaging by fMRI to study mathematical reasoning abilities in this patient group and raised the question of aberrant activation of their brains (Eliez et al. 2001b).

Almost all cases of the velocardiofacial syndrome and two other syndromes - the DiGeorge syndrome and the conotruncal anomaly face syndrome - share a common microdeletion of chromosome 22q11.2. This is one of the most frequent interstitial deletions found in humans. A 3 year old boy with muteness, who met DSM-IV criteria for autism, was found to have a 20/22 chromosomal translocation with an interstitial deletion within the 22q11 region; his phenotype was inconsistent with the DiGeorge syndrome. A 99mTc HMPAO brain perfusion SPECT showed a hypoperfusion of the left temporoparietal cortex in this boy (Carratala et al. 1998). Although more than 30 genes have been identified in these deleted segments, neither a single gene or multiple contiguous genes have been identified as yet as responsible for these syndromes (Yamagishi 2002).

The 22q13 region also is involved in disease entities with autism. Adenylosuccinate lyase deficiency is a rare autosomal-recessive disorder of the purine *de novo* synthesis pathway with developmental delay, hypotonia and seizures. The first case report of three children indicated that they had infantile autism (Jaeken & van den Berge 1984) and a number of children since have been reported to have autistic features (Ciardo et al. 2001). Not all patients are autistic. Adenylosuccinate lyase is an essential enzyme involved in purine biosynthesis; its gene is located at 22q13.1-q13.2. Laboratory investigations in patients show the presence in urine and cerebral spinal fluid of succinylpurines, which are normally undetectable.

A terminal 22q13 deletion syndrome has been reported in a patient having the autistic syndrome and a *de novo* cryptic deletion in chromosome 22q13.3 (Goizet et al. 2000); Anderlid et al. (2002) have reported a case with a submicroscopic 22q13 deletion and autistic symptoms. Prasad et al. (2000) described three children with the 22q13 deletion syndrome, one with autism and two with PDD, and suggested that the terminal 22q13 deletion syndrome may represent a recognizable phenotype for genetic evaluation. These authors proposed that children with minor facial stigmata, normal or advanced growth, significantly delayed speech compared to motor development combined with deviant behavior be screened for deletion at 22q13. Macrocrania, small pointed chin, simple ears and high arched palate are other phenotypical features. Disruption of the ProSAP2 gene, encoding a scaffold protein involved in the postsynaptic density of excitatory synapses and preferentially expressed in the cerebral cortex and cerebellum, has been proposed as the cause of the 22q13 deletion syndrome (Bonaglia et al. 2002). Finally, the meaning is not yet clear regarding a case of Schindler disease (N-acetyl galactosaminidase deficiency) whose gene is located at 22q13→qter; Blanchon et al. (2002) have reported a patient with autistic features.

The X Chromosome

The X chromosome is of particular interest in autism. Compared to the genes on the autosomal chromosomes, it appears that the X chromosome contains a significantly higher number of genes that, when mutated, cause mental impairment (Zechner et al. 2001). Another reason for interest in this chromosome is the gender ratio favoring boys with autism, raising the possibility of increased genetic errors on the X chromosome, which in most known diseases effect males. (For the record, errors on the X chromosome also can be associated with autistic symptoms in subgroups of females; those with Rett syndrome, Turner syndrome, or Xp deletion syndrome [Thomas et al. 1999]). The X chromosome has been studied extensively because of its folate-sensitive fragile sites that have been visualized on the

chromosome. These sites are clustered at the Xq27.3-Xq28 region and include the fragile X syndrome and the FRAXE syndrome.

The fragile X syndrome is an X-linked disorder that is believed to be the single most common cause of mental retardation, with an incidence estimated at about 1:4000. It is usually caused by an expansion of the trinucleotide repeat (CGG) in the 5'-untranslated region of the FMR1 gene; however in a small number of patients, deletions and point mutations of the FMR1 gene have been identified.

There is perhaps no other developmental disorder where the pathogenesis from gene to behavioral manifestations is as well described, however imperfectly. Expansion of the number of CGG repeats beyond 230 in the *FMR1* gene usually leads to hypermethylation of this repeat. In addition, a CpG island is formed within the gene which then recruits the transcriptional silencing machinery to the gene, preventing its expression. The FMR1 gene product, FMRP, is a selective RNA binding protein that shuttles between the nucleus and cytoplasm. FMRP is important in fetal development; there is expression of the mutated gene in proliferating and migrating cells (Abitbol et al. 1993) and a fetal brain examined at 23 weeks gestation was noted to already have dendritic spine abnormalities (Jenkins et al. 1984). FMRP protein levels have a relationship with functional brain activation during working memory (Menon et al. 2000; Kwon et al. 2001), suggesting that these levels are associated with the translational machinery in the dendritic spines. Such regulation of localized protein synthesis is important to the synaptic plasticity that underlies the neural networks of learning and memory. Thus perhaps it is not unexpected that white matter in fronto-striatal and parietal sensory-motor tracts has been reported to be altered in this syndrome (Barnea-Goraly et al. 2003a).

Facial features of individuals with the fragile X syndrome include a long, narrow face, strabismus, prominent long ears, a thick nasal bridge, dental malocclusion and a prominent jaw. After puberty, macroorchidism often occurs. Excessive joint laxity, pectus excavatum, kyphoscoliosis, a single palmar crease and pes planus have been described. The behavioral phenotype of the syndrome includes pronounced gaze aversion (Garrett et al. 2004), language delay and echolalia, perseveration, stereotypies, need for sameness, hypersensitivity to sensory stimuli, preoccupations with constricted interests and social anxiety, especially with peers or unfamiliar adults.

Although this behavioral phenotype sounds very much like a child with autism, the majority do have emotional relationships with their parents and other familiar adults. They develop strong attachments and concern for others, so they do not have what is considered the core symptom of autism. However, a small percent of patients with the fragile X syndrome do meet full autism criteria; it is not many but it is higher than can be accounted for by chance (Feinstein & Reiss 1998). Some patients with fragile X appear to be mosaic as judged from their blood cells; there is a trend for cases with autism to be more prevalent in the nonmosaic full mutation cases. The rate of fragile X among patients with autism is estimated to be about 2%-4% (Wassink et al. 2001).

The FRAXE syndrome is also a trinucleotide repeat syndrome, with an expansion of the trinucleotide GCC in the gene FMR2. The children are less severely affected and less likely to be stigmatized than those with fragile X. A rare patient with high-functioning autism has been reported (Abrams et al. 1997).

GENES BEING STUDIED IN AUTISM

Genes on the X Chromosome

In addition to the *FMR1* and *FMR2* genes susceptible to excess trinucleotide repeats mentioned above, a number of other genes located on the X chromosome are under investigation in children with permanent or temporary symptoms of autism. Investigators have learned a great deal from the studies of the methyl-CpG-binding protein 2 gene, *MECP2,* located at Xq28. Normally the function of MECP2 is to turn off several genes whose promoters have been methylated, an epigenetic phenomenon.

Mutations in this gene were first found in girls with Rett syndrome (Amir et al. 1999), a disease which goes through an autistic phase (see Chapter III). There are a number of variants so the spectrum of disease is called the Rett Complex. Girls with one of the variants, the Preserved Speech Variant (PSV) of the Rett Complex, have autistic behavior and most meet DSM-IV criteria for autism. The clinical features of PSV include the typical staging of Rett syndrome, but symptoms are milder in

that the cranial circumference is often within normal limits, hand washing is more occasional, scoliosis is mild and body weight may be excessive. Pathogenic mutations of the *MECP2* gene in 55% (10 out of 18) girls with PSV have been reported; there is a family with two discordant sisters (classic Rett and PSV) bearing a late truncating mutation (Zappella et al. 2001). Investigation of phenotypical variability in Rett Complex by type of *MECP2* mutation has not shown a consistent pattern, raising the possibility of skewed X-inactivation and/or modifier genes whose products may act in an epistatic matter with MECP2 protein (De Bona et al. 2000).

A question has been raised about whether girls with autism who apparently do not have a type of Rett Complex might also have mutations in *MECP2*. It sometimes takes background knowledge in depth of both syndromes to distinguish between a Rett variant and autism in a child; Trevathan and Naidu (1988) have listed the differential criteria and Kammoun et al. (2004) helped pinpoint certain of the criteria. The Rett Complex is much more tightly diagnosed than autism; it has necessary, supportive and exclusion criteria, as well as four stages of progression. Two girls with the PSV variant of Rett syndrome were followed closely and, although they had autistic behavior during the first three stages of progression, by early adolescence they lost autistic behavior and reached an IQ close to 45 (Zappella et al. 2003). In addition to differences in the criteria, the clinical course often reveals the difference between a girl with a static form of autism and a girl with a Rett Complex variant.

Carney et al. (2003) analyzed 69 girls with autism and found two with *MECP2* mutations; neither patient exhibited classic Rett features. Similarly, Lam et al. (2002) screened 21 females with autism and mental retardation and found one with a *MECP2* mutation. Shibayama et al. (2004) also identified a mutation and variants in children with autism. Individuals with other brain disorders also have been found with *MECP2* mutations. These include severe mental retardation, a spastic syndrome in boys, schizophrenia and bipolar disorder. A previously unknown *MECP2* open reading frame defines a new protein isoform different from the previously identified protein; the new isoform is more abundant in human brain and this defective form may be the cause of the Rett Complex (Mnatzakanian et al. 2004; Kriaucionis & Bird 2004).

A recent study has shown that 5% (3 out of 63) of Rett girls are carriers of both a MECP2 mutation and a chromosome 15q11-13 rearrangement (Longo et al. 2004). This is in contrast to the only 1% of patients with autism who have a duplication of chromosome 15q11-q13. Since this study is the first of its kind, the percentage may be due to chance, but the finding itself gives important new information. All three of the girls with the 15q rearrangement were carriers of an MECP2 mutation at amino-acid R133, a hot spot for mutations in Rett Complex. Two of the girls with classic Rett had maternally inherited small deletions of 15q11-q13, while the girl with PSV had a paternally inherited small duplication of 15q11-q13.

Another interesting X chromosome gene is *ARX*, a paired-class homeobox gene located at Xp22.13. *ARX* is specifically expressed in the embryonic forebrain, and is involved in the proliferation of neural precursors and differentiation and tangential migration of interneurons. Stromme et al. (2002) have described individuals with autism and mild to moderate mental retardation who had *ARX* mutations. Individuals with autism or autistic behavior that had a duplication in this gene have been described by Turner et al. (2002). These authors also found the same duplication in a family with X-linked infantile spasms with hypsarrhythmia, a syndrome that is sometimes associated with the development of later autistic behavior. As discussed above, the phenotypes associated with *ARX* mutations range well beyond autism. And there is interest in another homeobox gene, EN2 located at 7q36, in autistic studies

Another important group of genes, the **neuroligins,** also have genes located on the X chromosome. These genes produce proteins that function as cell adhesion molecules during neurodevelopment. Among the proteins involved in the establishment of neural networks (see Chapter Two), neuroligins appear to have a central role in the formation of CNS synapses (Comoletti et al. 2004; Chih et al. 2004).

The two neuroligin genes on the X chromosome are *NLGN3*, located at Xq13, and *NLGN4*, located at Xp22.3. Deletions at Xp22.3 that include *NLGN4* have been reported in several individuals with autism (Thomas et al. 1999). Mutations in *NLGN3* and *NLGN4* have now been described in families with autism and Asperger syndrome (Jamain et al. 2003). An additional family has been reported with a 2-

base-pair deletion in *NLGN4* in individuals who have autism, PDD-NOS or nonsyndromic X-linked mental retardation among its members (Laumonnier et al. 2004).

Genes on the Autosomal Chromosomes

Family histories and sibpairs with autism have enriched the search for candidate genes. The combination of identifying mutations in genes in individual patients with autistic features combined with the search for susceptibility genes in strictly defined autism in the last ten years has resulted in a rich harvest of autosomal chromosomal sites. Potential sites exist on every chromosome. If autism is, in fact, the cognitive behavioral cousin to the cognitive mental retardation syndromes, then this richness is to be expected, since over 200 monogenetic diseases alone already have been identified for the mental retardation syndromes.

The long lists of potential autosomal gene sites for autism can be found in a number of papers. These include chapter 15 of *The Biology of the Autistic Syndromes* (3rd ed.) (Gillberg & Coleman 2000c) and two sections on candidate gene analysis and genetic mechanisms in *Autism: Neural Basis and Treatment Possibilities* (Bock & Goode 2003). Data from one genome-wide scan on Asperger syndrome is now available (Ylisaukko-oja et al. 2004). New gene mutations or likely chromosomal sites are constantly being found. While you are reading this book, it is possible that researchers have found another gene mutation or chromosomal site.

Mitochondrial DNA Studies

The mitochondria are unique among cellular organelles in that they contain their own DNA. Each mitochondrion has 2 to 10 copies of DNA in a circular molecule consisting of 16,569 base pairs. Mitochondrial DNA (mtDNA) differs from DNA in the cell nucleus in that it contains few noncoding sequences (introns), has a slightly different genetic code, and is transmitted almost exclusively from the mother. Over 95% of total brain ATP, the chemical energy of cells, is produced in the mitochondria by the process of oxidative phosphorylation. The regions of the brain that are most functionally active, such as the temporal lobe, are sites of increased mitochondrial activity. Since mitochondria play a pivotal role in cell metabolism, cellular mitochondrial density reflects metabolic activity. Skeletal muscles as well as neurons have a high mitochondrial mass, a finding that can be used to detect mitochondrial disease when the mitochondrial function is diminished.

The mutation rate of mtDNA is much higher than that of nuclear DNA. The reason for this higher mutation rate can be ascribed to the absence of a histone coat for mtDNA, a poor repair system for DNA damage, a high turnover rate of mtDNA, exposure to higher oxidative stress, and a high error rate of polymerase *gamma* which is responsible for mtDNA replication. This high mutation rate means that even control subjects may sometimes possess mutations, making for caution in interpreting a complicated literature.

Many mutations of mtDNA have now been associated with human disease. These are a diverse group of disorders that result from structural, biochemical or genetic derangement of mitochondria. Because mtDNA is inherited almost exclusively through the mother, mtDNA-related defects would be expected to exhibit the maternal inheritance pattern. This is indeed seen, but to date most cases are sporadic. This maternal inheritance pattern of mtDNA does represent a well-recognized nonMendelian genetic system. However, to make things more complicated, it is necessary to know that some of the mitochondrial diseases affecting the respiratory chain can also be due to mutations of genes in the nuclear genome, resulting in Mendelian patterns of inheritance. Thus the family history of a child with a mitochondrial disease has the possibilities of a disease that is sporadic, that has a maternal inheritance pattern or that has a classical Mendelian pattern. Other genetic characteristics – heteroplasmy, mitotic segregation and threshold effects – also are unique.

This background detail about mitochondrial diseases has been offered because evidence is mounting that a significant number of children with autism may belong to the mitochondrial subgroup. In fact the first population-based epidemiological study of mitochondrial disease in autism, completed in Portugal, has arrived at an estimate of 7.2% or more of children with autism (Oliveira et al. in press). If confirmed, this is the largest subgroup of patients with autism yet described. Furthermore,

some of the children reported with an mtDNA abnormality did not appear to be different from children with "primary autistic spectrum disorders" (Pons et al. 2004).

Speculation that children with autism might have mitochondrial diseases began after it was discovered that some children with autism had high lactate levels (Coleman & Blass 1985), raising the possibility of mitochondrial oxidative phosphorylation dysfunction (Lombard 1998). The impairment of mitochondrial energy metabolism has been documented in individuals with autism and Asperger syndrome (Minshew et al. 1993, Laszlo et al. 1994; Chugani et al. 1999). Thus it is no surprise that cases of autism and mitochondrial disease are now being reported; the reported cases have a variety of mitochondrial defects. In some children, there are mutations in mtDNA itself (Graf et al. 2000; Pons et al. 2004) as well as deletion (Fillano et al. 2002) and depletion of mtDNA (Pons et al. 2004). In other children, there is alteration of mitochondrial function likely secondary to duplication of the maternally derived chromosome 15q11-q13 region (Filipek et al. 2003).

A growing evidence indicates that certain children with autism should be evaluated for mitochondrial cytopathies. This includes those who have lactic acidosis, although it may not be always detectable, or those who have a family pattern of maternal inheritance. Other possible indications include the presence of seizures, if other known etiologies have been ruled out, or a family history of depression.

Genes in Nonsyndromic Autism

Genetic diagnosis has recently become possible in a few patients with nonsyndromic autism and Asperger syndrome. There are children now in the literature who clinically present with these disorders, yet have genetic mutations (Table 1-7). However genetic diagnosis in the majority of such children still eludes us, as of the writing of this book.

GENETIC COUNSELING

That autism is a disorder with a major genetic component is no longer in doubt (see Cook, 2001, for a summary of the evidence). Furthermore, variations such as Asperger syndrome and PDD-NOS breed true in some families (Micali et al. 2004). One group of the patients have what are called single-gene diseases; this concept of mutations in a single gene as an essential minimum for such genetic diseases is a clinically useful tool, in spite of its scientific limitations. Other members of the family of these patients sometimes have sub-clinical traits; these are called the broader autism phenotype, encompassing both psychological and biological data.

Genetic counseling for a disease entity needs to be based on valid information about the family risk of recurrence. In the disease entities listed in Tables I-1, I-4 and I-5, informed sources of such information often are available. Although many cases of autism are sporadic, there are some patients within each disease entity where family patterns can be discerned. For example, genetic patterns of autosomal recessive (adenylosuccinate lyase deficiency), autosomal dominant (tuberous sclerosis complex), X-linked (mutations in the X-linked neuroligins), trinucleotide repeats (fragile X) and mitochondrial patterns of inheritance (A3243G mtDNA mutations) can be used to counsel a family.

Unfortunately for the majority of children with autistic features, often with nonsyndromic autism, such information is not available or may not be relevant. Here the information gathered from studies of families of children with autism put together as a single disease entity can at least be a guideline. Family studies have shown that the risk of another sibling born with autism to parents who have already had a child with autism is 4.5% (Jorde et al. 1991). Another way of saying this is that the risk of reoccurrence among siblings may be at least 20 times more frequent than in the population as a whole, a much higher recurrence rate than the prevalence rate in the general population. However it should be noted that this rate is much lower than is found in single-gene diseases. In the case of autism, there is a high concordance of monozygotic twins, first reported by Rimland (1964) and later confirmed by many studies. When twins are studied using a broader spectrum of autism including related cognitive and social abnormalities, 92% of monozygotic twins were concordant versus only 10% of dizygotic twins (Bailey et al. 1995).

Neurogenetic Diseases

The sequencing of the human genome has turned out to be a necessary first step in a yet unfolding journey; the answers appear to be much more complex than mere DNA sequence. In patients with autism and their families, discovery of a genetic mutation is a most important but not necessarily a full picture of what leads to the disease process.

Inactivating mutations that result in loss of protein function can occur. The unstable trinucleotide repeat, a common type of mutation frequently found in diseases of the brain, has a different genetic mechanism, toxic gain-of-function. For example, when abnormally long polyglutamine stretches are present, cascades of sequential genes often occur, so that a mutation in the first gene in a series results in low expression of others that follow; it may be difficult to determine whether a detected underexpression of a later gene is primary or secondary. Gene sequences may be shuffled and create different proteins with alternate functions when being expressed in different organs (Mnatzakanian et al. 2004), making it harder to anticipate brain function or dysfunction from the study of a gene taken from the blood. This finding in Rett syndrome has received special attention because of its clinical implications; the concept of alternative splicing, in which a gene produces different versions of a protein, is well established.

Another genetic factor to consider in autism, especially in the mitochondrial diseases, is the amount of heteroplasmy (a mixture of wild-type and mutant mtDNA) present in living patients (DiMauro & Moraes 1993). This helps account for tremendous clinical variation inside the same family. There also is the new information that may arise from epigenetics, changes in the human genome that don't affect DNA sequence. These are modifications that help control gene activity by acting like switches; the best known epigenetic signal is DNA methylation which is generally associated with silencing of gene expression. There is growing evidence that imprinted genes are probably involved in autism, epigenetics may help disentangle future diagnoses. Finally, in regard to laboratory studies, evolutionary research regarding the brain in both genes and established neural circuits shows that sometimes earlier systems have been recruited for specific "human" functions, placing limitations on animal studies which simulate a human genetic disease. A great deal of work lies ahead.

SUMMARY

This chapter can be summarized as follows: it asserts the principle that, although we can not yet define many of them, autistic symptoms reflect a great variety of underlying disease entities, each perhaps with a somewhat different neuropathological mechanism. Autism/Asperger is a big syndrome. Now it is time, in the next chapter, to explore how so many different diseases can all lead to the same symptom complex of autism by modifying, interrupting or redirecting the neural pathways of behavior.

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Table 1–1 Some of the double syndromes where a subgroup of patients meeting autistic criteria has been described

A. Multiple congenital anomalies/mental retardation (MCA/MR) syndromes

- Angelman syndrome
- CATCH 22
- CHARGE association
- Cohen syndrome
- Cole-Hughes macrocephaly syndrome
- Cowden syndrome
- 15q11-q13 duplication syndrome
- de Lange syndrome
- Down syndrome
- Ehlers-Danlos syndrome
- Fragile X syndrome
- Goldenhar syndrome
- Hypomelanosis of Ito
- Lujan-Fryan syndrome
- Moebius sequence
- Neurofibromatosis 1
- Noonan syndrom e
- Orstavik 1997 syndrome
- Rett Complex
- Rubinstein-Taybi syndrome
- Smith-Lemli-Opitz syndrome
- Smith-Magenis syndrome
- Sotos syndrome
- Steinert's myotonic dystrophy
- Timothy syndrome
- Tuberous sclerosis complex
- Turner syndrome
- Williams syndrome

B. Neurological syndromes

- Dysmaturational syndrome with familial complex tics
- Joubert syndrome
- Leber congenital amaurosis
- Mitochondrial syndromes, including the HEADD syndrome
- Neuroaxonal dystrophy variant
- Tourette syndrome/autism
- X-linked creatine transporter defect

C. Psychiatric syndromes

- Anorexia nervosa/autism
- Infantile autistic bipolar subgroup

Table 1–2 Disease entities where both neuronal migration and autistic symptoms have been reported

MCA/MR syndrome	*Type of neuronal migration disorder*
de Lange syndrome	heterotopia
Ehlers-Danlos syndrome	heterotopia
Hypomelanosis of Ito	polymicrogyria, heterotopia
Neurofibromatosis, type 1	polymicrogyria, heterotopia
Rett syndrome	nodular heterotopia perisylvian cortical dysplasia
Smith-Lemli-Opitz syndrome	polymicrogyria, rare
Sotos syndrome	neuronal heteropia
Tuberous sclerosis complex	heterotopia

Reference: Hennekam and Barth 2003

Table 1–3 Current instruments that can be used to diagnose or assess the severity of patients with autistic symptoms

Autism Diagnostic Interview–Revised (ADI-R)
Autism Diagnostic Observation Schedule–Generic (ADOS-G)
Childhood Autism Rating Scale (CARS)
Diagnostic Interview for Social and Communication Disorders (DISCO)
Diagnostic and Statistical Manual of Mental Disorders (DSM–IV) criteria
The ICD-10 Classification of Mental and Behavioral Disorders (ICD–10) criteria

Table 1–4 Characteristics shared by autism and Asperger syndrome

Clinical:
Social impairment
Nonverbal communication difficulties
Narrow interests
Repetitive routines
Savants or splinter skills
Predominantly male
Imaging:
Variability of neural network maps
Neuropathological:
Diminished Purkinje cells in the cerebellum
Neuronal migration defects
Limbic system neuropathology
Altered minicolumn organization
Rare neuroepithelial tumors
Genetic:
Same gene mutations found in some cases (e.g., mutations in the X-linked *NLGN3* and *NLGN4* genes, and in mitochondrial DNA)

Table 1– 5 Autistic syndromes with subtle or minimal stigmatization

Miles-Hillman subgroup of nonstigmatized phenotypes
Mitochondrial disease subgroup
15q11-q13 duplication syndrome
22q13 deletion syndrome
X-linked neuroligin syndrome
Metabolic disorders (cases are scarce even within these rare diseases):
Aminoacidurias–phenylketonuria
GABA metabolism disorders–Succinic semialdehyde dehydrogenase deficiency, pyridoxine-dependency syndrome, ?GABA-transaminase deficiency
Leukodystrophies–metachromatic leukodystrophy
Mucopolysaccharidoses–Sanfilippo's syndrome
Organic acidurias–D-glyceric aciduria, (succinic semialdehyde dehydrogenase deficiency)
Peroxismal disorders–infantile Refsum disease
Purine disorders–adenylosuccinate lyase deficiency, nucleotidase-associated pervasive developmental disorder

Table 1–6 Infectious, endocrine, toxic, and space-occupying disease entities reported in children with autistic symptoms

Infectious
Congenital encephalopathies: rubella, herpes simplex, cytomegalovirus
Meningitis
Endocrine–Hypothyroidism
Perinatal toxicity–ROP (retinopathy of prematurity)
Space-occupying lesions
Arachnoid cysts
Brain tumors–neuroepithelial tumors
Toxic fetal embryopathies
Alcohol, cocaine, lead, thalidomide, valproate

Table 1–7 Examples of children with clinical nonsyndromic autism who have a genetic error

Diagnosis	*Genetic error*	*Reference*
Autism	24-bp duplication in exon 2 of *ARX* gene (Xp22.13)	Turner et al. 2002
Autism and Asperger	frameshift mutation (1-bp insertion in exon 5) in gene encoding neuroligin *NLGN4* (Xp22.3)	Jamain et al. 2003
Autism and PDD-NOS	2-bp deletion in exon 5 of the *NLGN4* gene (Xp22.3)	Laumonnier et al. 2004
Autism and Asperger	C→T transition (R451C) in gene encoding neuroligin *NLGN3* (Xq13)	Jamain et al. 2003
Autistic spectrum disorder	A3243 mtDNA mutation	Pons et al. 2003
Autism	1-bp deletion in exon 5 (c.183deIT) of the *CDKL5*/*STK9* gene (Xp22.1), resulting in a frameshift and premature truncation	Weaving et al. 2004