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Nuclear and Mitochondrial Genome Defects in Autisms

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In this review we will evaluate evidence that altered gene dosage and structure impacts neurodevelopment and neural connectivity through deleterious effects on synaptic structure and function, and evidence that the latter are key contributors to the risk for autism. We will review information on alterations of structure of mitochondrial DNA and abnormal mitochondrial function in autism and indications that interactions of the nuclear and mitochondrial genomes may play a role in autism pathogenesis. In a final section we will present data derived using Affymetrix[™] SNP 6.0 microarray analysis of DNA of a number of subjects and parents recruited to our autism spectrum disorders project. We include data on two sets of monozygotic twins. Collectively these data provide additional evidence of nuclear and mitochondrial genome imbalance in autism and evidence of specific candidate genes in autism. We present data on dosage changes in genes that map on the X chromosomes and the Y chromosome. Precise analyses of Y located genes are often difficult because of the high degree of homology of X- and Y-related genes. However, continued efforts to analyze the latter are important, given the consistent evidence for a 4:1 ratio of males to females affected by autism. It is also important to consider whether environmental factors play a role in generating the nuclear and mitochondrial genomic instability we have observed. The study of autism will benefit from a move to analysis of pathways and multigene clusters for identification of subtypes that share a specific genetic etiology.

Key words: autism; genome; mitochondria; dosage changes; copy number (CN); copy number variation (CNV); twins

Introduction

There are a number of recently published comprehensive reviews on genetic factors in autism. The goal of this review is not to list all chromosome abnormalities defined in autism, nor to list all regions for which evidence exists for linkage in autism or all genes found to be associated with autism. Rather, we plan to consider broader underlying genetic mechanisms, for example, genomic imbalance in autism. We will evaluate the evidence that altered gene dosage and structure impacts neurodevelop-

ment and neural connectivity through deleterious effects on synaptic structure and function, and evidence that the latter are key contributors to the risk for autism. In addition, we plan to consider information on alterations of structure of mitochondrial DNA and abnormal mitochondrial function in autism and indications that interactions of the nuclear and mitochondrial genomes may play a role in autism pathogenesis. In a final section of this review we will include data derived using Affymetrix[™] SNP 6.0 microarray analysis of DNA of a number of subjects and parents recruited to our autism spectrum disorders project. These data provide additional evidence of genomic and mitochondrial genome imbalance in autism. They also provide additional evidence of specific candidate genes in autism.

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Autism Spectrum Disorders: Definition

Autism spectrum disorders (ASD) are defined as neurobehavioral syndromes characterized by deficits in social interaction, impaired communication skills, and repetitive stereotypic behaviors that manifest by 3 years of age. Throughout the 1980s ASD were believed to be rare, with a prevalence of no more than 5 per 10,000 persons.¹ In 2003, the prevalence for autism was reported as 3.4 per 1000 (95% confidence interval [CI], 3.2–3.6) (male–female ratio, 4:1).² There is an ongoing debate as to whether the increased prevalence results from revised diagnostic criteria and increased awareness of these disorders or whether increased prevalence is due to synergistic environmental factors.

A number of authors have drawn attention to the fact that progress in defining the underlying etiology and the pathogenesis of autism may be negatively impacted by our autism definition. London³ noted that the disorder described as autism likely includes diseases with different etiologies; he used the word *autisms* to address this fact. He noted further that each of the three key manifestations of autisms—communication deficits, social impairment, and repetitive stereotypic behaviors—may be individually present in the general population, and the demarcation between pathology and normal variation is not clear cut.

London³ proposed using the etiology of the disease as the defining character. In considering the fact that autistic features occur in children raised in such orphanages where children receive little attention, London developed an interesting concept. He suggested that in children destined to develop autism the underlying brain pathology may generate an attention problem and make a child less available for stimulation.

A number of investigators have proposed that autisms may be more definitively analyzed by defining endophenotypes, i.e., determining if subgroups of patients exist that show predominance of specific autism symptoms.⁴ One

difficulty with this approach is that strict quantitative measures of impairment are often difficult to obtain in behavioral phenotypes.

Syndromic autism is defined as autism occurring in patients with physical malformations or metabolic abnormalities, specific syndromes due to gene mutations, e.g., fragile X syndrome, tuberous sclerosis, neurofibromatosis, or syndromes due to clinically detectable cytogenetic abnormalities. Further, the presence of a syndrome in an individual patient with autism may be a key factor in the pathogenesis of the disorder in that patient, but not be the only factor. Casanova⁵ emphasized that the presence of co-morbid conditions, e.g., chromosomal imbalance or mitochondrial diseases, in patients with autism symptoms, does not exclude the diagnosis of autism but provides evidence of the etiologic heterogeneity of the syndrome.

Neurobiology and Neuropathology

Pardo and Eberhart⁶ reviewed the neurobiology of autisms and proposed that these are disorders of neuronal cortical organization that occur at different levels and lead to alterations in information processing. They concurred with results from other investigators that provided evidence that synaptic and dendritic organizations of brain structures are impaired. They proposed that autism is due to disruption of pre- or postnatal neurodevelopmental trajectories. They postulated further that diverse molecular pathways and developmental processes are disrupted. These include neuronal migration, cortical organization, and formation of synaptic and dendritic connections. They noted also that environmental factors likely interact with genetic factors to impact development.

Geschwind and Levitt⁷ described autisms as developmental disconnection syndromes. They proposed a model in which higher-order association areas of the brain, which normally connect to the frontal lobe, are partially disconnected during development.

Persico and Bourgeron⁸ reviewed post-mortem neuropathologic findings, taking into

account the caveats inherent in small sample sizes and etiologic heterogeneity. They noted certain underlying alterations that occurred commonly across cases. These included increased cell proliferation, disrupted cortical and subcortical architecture and abnormal cell differentiation and synaptogenesis.

These key observations and hypotheses include the important notion that autisms, in any presentation of the syndrome, involve the development, structure, and/or function of synapses and synaptic communication. These general neurological errors and growing evidence of important roles for nuclear genomic alterations and impaired mitochondrial function serve as explanations for the varied clinical presentation and observed genetic heterogeneity of autism.

Chromosomes and Structural Genomic Changes in Autism

Clinical cytogenetic abnormalities in autism are not confined to specific chromosomes; they have been described on each of the autosomes and on sex chromosomes.⁹ Chromosomal abnormalities detected by routine clinical cytogenetics or through application of fluorescence *in situ* hybridization (FISH) occur in 7.4% of cases with autism, and these are mostly diagnosed as syndromic autism. Most common cytogenetic abnormalities found in patients who present with syndromic autism include: 15q11–q13 inverted duplication and 22q13.3 microdeletion syndrome.

Duplication of a segment of the maternally-derived chromosome 15q11–q13, either as an inverted duplication present as an extra chromosome or as an interstitial duplication, constitutes one of the most frequent chromosome abnormalities in autism. Deletions within this region lead to Angelman syndrome or Prader–Willi syndrome, and these conditions may also be associated with autism.¹⁰

Despite considerable effort by a number of investigators, no specific gene that maps within this region has been identified as most likely

to play a role in the etiology of autism. Sahoo *et al.*¹¹ described a male with Prader–Willi syndrome who met diagnostic criteria for autism and had a paternally derived microdeletion that involved 174,584 base pairs on 15q11.2. The deletion encompassed the map location of small nucleolar RNAs, snoRNA clusters HBII-438A, HBII-85, and HBII-52. The authors noted that previous studies ruled out deletion of HBII-52 snoRNA as a cause of Prader–Willi syndrome. They concluded that HBII-85sno RNA deletion is the most likely causative factor of the abnormalities detected in their patient. In studies on mice where HBII-85 was deleted, the animals manifested deficiency in motor learning, increased anxiety, and hyperphagia. Sno RNAs play a role in RNA modification and processing and in protein expression. These remain intriguing but as of yet unproven candidate genes for autism.

Follow-up studies will need to be carried out to analyze the sequence and function of HBII SnoRNA in autistic subjects.

The 22q13.3 microdeletion syndrome is characterized by global developmental delay, delayed speech, normal or accelerated growth, muscular hypotonia, and autism. Minor dysmorphic features may be present. The SHANK3 gene maps within the 22q13.3 chromosomal region^{12,13} identified a patient with autism and a deletion in intron 8 of SHANK3. The deleted intron was replaced by telomeric repeats. These investigators also identified two brothers with an unbalanced chromosomal translocation that interrupted the SHANK3 gene. These males manifested autism. Together these studies provide compelling evidence that SHANK3 is an autism candidate gene.

In addition to the major chromosomal alterations that serve as risk factors for autisms, the question then arises as to whether subtler chromosome abnormalities occur that are not detected by microscopy or FISH, and that lead to genomic imbalance and may lead to autism. Recent evidence from microarray studies indicates that structural genomic changes and copy

number variations (CNVs) occur with high frequency in the general population and with higher frequency in subjects with autism. This is discussed further below.

Sex Differences in the Frequency of Autism and Possible Roles of X and Y Chromosomes

It is important to take into account that autism occurs four times as frequently in males as in females.¹ Factors on the X chromosome must therefore be carefully examined to include neuroligins, Fragile X, and related genes and interacting genes. It will likely also be important to consider abnormalities on the Y chromosome.

Klinefelter Syndrome XXY

Van Rijn and colleagues¹⁴ reported that autistic behaviors were more frequent in the 24 Klinefelter patients (47,XXY) they studied compared to the 20 control individuals in their study. The Klinefelter individuals experienced particular difficulty with social interactions. They experienced social withdrawal, social anxiety, and shyness.

XXX Syndrome

Geerts and colleagues¹⁵ studied 38 males where XXX status (47,XXX) was diagnosed prenatally. They reported an increased frequency of language delay and motor development delay in this group and an increased risk for autism.

Turner Syndrome

Weiss and colleagues¹⁶ reported that autism-like social communication difficulties occur in one third of women with Turner syndrome and normal IQ. They carried out studies of face emotion recognition studies on women with partial deletions of the X chromosome. These studies led to identification a 5-megabase region that is apparently linked to this phenotype. Further association studies between abnormalities in face emotion recognition and

SNPs in this gene region revealed that the SNP most strongly associated with quantitative changes in this phenotype maps within a gene EFHC2. This gene, EF-hand domain containing 2, maps to chromosome Xp12.3 and encodes a product that is expressed in brain and in other tissues; it also shows 37% amino acid identity with the EFHC1-encoded product. EFHC1 plays a role in calcium current generation, neurotransmission, and neuronal pruning, areas of brain development proposed as critical, when altered, to the development of autism.

De Novo Genomic CNVs and Dosage Changes in Autism

Sebat and colleagues¹⁷ published results of studies in autism subjects and controls of de novo copy number variants. These are defined as genome alterations leading to dosage changes due to deletions or duplications of segments greater than 1 kilobase (Kb) in size. In their study they used ROMA, a form of comparative genomic hybridization on microarrays, to determine differences between autism subjects and their unaffected parents. They analyzed DNA derived from lymphoblastoid cell lines or peripheral blood. The marker density on the microarray corresponded to one probe every 35 Kb. They compared hybridization intensity of sample DNA to intensity of a reference genomic DNA. In their subsequent analysis they eliminated copy number variants present in children and their parents. They concentrated on variants detected by at least three markers in a row and with a very low frequency in the population. These parameters allowed them to identify rare de novo CNVs in their patients. They identified 14 such variants in 195 autistic subjects and in 2 of 196 unaffected individuals studied. The frequency of the changes was 10% in sporadic cases of autism and 3% in multiplex families (families with more than one child diagnosed with autism spectrum disorder (ASD)). In autism cases 12 of the 15 copy number (CN) changes detected

were deletions. In autism cases with de novo variants, 9 were male and 5 were female. In the overall autism sample the ratio of males to females was 5:1.

These investigators noted that fragile genomic syndrome was unlikely in their sample since usually a single variant per individual was detected. The average IQ was lower in the autistic subjects with de novo rare copy number variants than in the overall autistic population.

Sebat¹⁷ noted that the frequency of cytogenetically detectable changes in autism and additional variants detected in microarray analysis indicates that genomic imbalance occurs in 15% of cases of sporadic autism.

Significance of Dosage Changes

Which deletions and duplications should be considered as playing a role in the pathogenesis of a disorder? Genes involved in copy number variants that also occur in the general population with reasonably high frequency may perhaps be considered less significant. On the other hand, if one considers the theory that polymorphic changes in many genes may work together to lead to complex disease, perhaps some specific, nonunique CNVs are significant.

Intuitively, more weight is given to rare CNV that arise de novo in affected offspring of unaffected parents and are not present as polymorphic variants in that population.

In general, when one takes the genomic location of a CNV into account, protein-coding genes in those regions are considered. It is possible that in some cases abnormalities in unique sequence DNA that does not encode proteins may contribute to the pathogenesis of specific disorders, such as autism, since unique sequence DNA may nevertheless have important functions, e.g., gene regulation.

In most instances copy number changes leading to deletion are present on one member of a chromosome pair, and dosage is reduced from two copies to one copy. It is important to take

into account the fact that for many genes, e.g., genes involved in metabolism, there is strong evidence that deletion of one copy of the gene is not sufficient to cause disease (as evidenced by the fact that the heterozygous parents of children with recessive metabolic disorders have a normal phenotype). The question arises also as to whether duplication leading to extra copies of a gene is significant. In some instances copy number changes extend over an entire gene while in others the dosage alteration impacts one exon or a few exons and introns; in some instances only one intron may be impacted by deletion or duplication. Are the latter copy number changes significant in the function of the gene? Intragenic changes alter the structure of the gene and may alter the reading frame and transcription products. In some instances alternate transcripts are derived from the gene sequence and alternate splicing may be impacted by changes in an intron. Furthermore, copy number changes and alteration of sequence in an intron may impact access to transcription factors or transcription modulators, e.g., enhancers.

In determining the significance for the autism phenotype of CNVs that impact protein-coding genes, we place more emphasis on genes that are primarily expressed in the central nervous system (CNS). However, genes that are also expressed in other tissues may be important, and/or it may be that the CNS is more sensitive to the effects of altered expression levels than are other tissues.

Polymorphic CNVs

Genomic microarray studies provide evidence that copy number variations ranging from 1 kilobase to 1 megabase (Mb), occur in control individuals from different population groups. These variations include deletions, duplication and insertions.¹⁸ Regions of CNVs are unstable, so deletions in these regions occur in some individuals, while duplications occur in others. Copy number variants frequently occur in regions of the genome apparently

devoid of protein-coding genes. They may, however, occur within protein-coding genes. Additional research is necessary to determine the extent to which CNVs influence gene expression; it is possible that certain genes are more dosage-sensitive than others. There is some evidence that in polymorphic CNV, duplications are more common than deletions, and that deletion occurrences are biased away from genes. Repetitive DNA sequences serve as the substrate for CNV.

The question arises as to whether polymorphic copy number variation has little functional impact or whether it should be considered a major influence on phenotype. This influence would then derive from its effect on gene expression. In addition, copy number variation impacts genome stability.

The phenotype, including a disease phenotype, may be directed by the constellation of polymorphisms present in one individual. Subsets of those polymorphisms are inherited from each parent. In addition further structural variants may arise post zygotically. In the case of copy number variants, the precise size of the variation at a specific location varies in different individuals. The specific collective combination of variants present in an individual is different from the combination in each parent and may lead to differences in gene expression and possibly to differences in transcripts derived from a particular gene.

Stranger and colleagues¹⁹ interrogated gene expression in lymphoblastoid cell lines from 210 individuals from four different populations. Their studies demonstrated that SNP genotype and CNV captured, respectively, approximately 83.6% and 17.7% of the variation in gene expression.

De Smith and colleagues²⁰ reported gene categories that are over-represented in lists of polymorphic CNV. These included genes for sensory perception, genes for transmission of nerve impulses and brain development, and genes for acquired and innate immunity. Also high on the list of genes over-represented in

polymorphic CNV were genes defined in the Gene Ontology database as playing a role in the categories “multicellular organismal development” and “multicellular organismal processes.” Genes in these categories may contribute to the development of symptoms of autism spectrum disorders.

Autism Spectrum Disorders as Oligogenic or Multigenic Diseases

Persico and Bourgeron⁸ in their review postulated that a specific rare mutation might cause nonsyndromic autism in an individual. However, they considered it more likely that autism spectrum disorders are complex and that they are oligogenic or polygenic in nature, consequently in most cases a number of susceptibility-conferring gene variants at different loci are required for an individual to develop the disorder. They proposed further that gene–gene and gene–environment interactions are important in autism causation. They noted that common variants at specific loci might enhance the risk of autism. In some cases risk factors may include abnormal regulation of gene expression. One example presented by these authors was that in linkage and association studies in autism evidence that the SLC6A4 gene that encodes serotonin transporter is sometimes positive, but often it is negative. However, elevated serotonin levels occur in 25% of cases of autism, possibly indicating abnormal gene expression due to the effect of changes that map at other locations. These findings are consistent with a disorder influenced by the complex interaction of multiple genes.

Multiplex Autism Families and Determining Loci in One Family

The concept that even within one family multiple genetic loci may contribute to the etiology of autism gained further support from the study published by Allen-Brady and colleagues.²¹ They carried out genome-wide

SNP typing, using the 10 K Affymetrix array, on a sixth-generation Utah pedigree with 7 males affected with autism spectrum disorder. Three chromosome regions reached statistical significance in parametric linkage analysis, 3q13.2–q13.31, 3q26.31–q27.3, and 20q11.21–q13.12. A region on 7p and another on 9q met suggestive criteria for linkage. It is interesting to note that studies in Finnish autism families identified a region on 3q25–q27 linked to autism with a multipoint lod score of 4.81.²² *Neurexins* 1, at 3q26.31 (a gene important in synaptic structure and function), is an autism candidate gene in this chromosomal region. Allen-Brady and colleagues²¹ noted that, within a particular family, specific inherited risk factors might lead not only to autism but also to increased risk for other psychopathologies that may be present in siblings or, in mild form, in parents.

Candidate Gene Regions and Candidate Genes in Autism

In general, greater weight is given to chromosome regions that are shown to be involved in autism based on more than one study and particularly to candidate regions that are shown to be significant in several modes of study. Significant regions and/or genes are: those shown to be linked or associated with autism in multiplex families; gene regions deleted or duplicated in singletons in multiple studies; and genes that show sequence changes in autistic individuals.

Persico and Bougeron⁸ reviewed genetic, environmental, and epigenetic factors involved in the etiology of autism. They considered categories of proteins where changes in autism subjects were found. These include proteins involved in chromatin modeling and gene regulation, and proteins involved in cytoskeletal dynamics and signaling pathways. The latter include GTPase activating proteins, e.g., tuberin and hamartin, and guanine exchange factors, e.g., neurofibromin. Other categories of protein considered significant in autism include

neurotransmitter receptors and transporters, cell adhesion molecules present at the synapse, and second messengers such as neurobeachin.⁸ Genes in these categories are present in Table 1, where we document genes that show evidence of structural variation and copy number changes in our subjects with autism.

Autism and Defects in Synaptic Proteins

In the review of genes that follows we demonstrate that a number of genes that play a direct role in determining synaptic structure and function have been shown in different families to be associated with autisms.

In 2003, Jamain and colleagues²³ reported the occurrence of mutations in the X-linked *neurexins* genes in two pairs of brothers with autism. They noted that *neurexins* are cell adhesion molecules that play an important role in synaptogenesis. In a subsequent review of genetic factors in autism,²⁴ they proposed that this disorder is due to synaptic abnormalities. Additional support for this proposal comes from growing evidence that in autistic probands, mutations or structural abnormalities occur in genes that encode proteins active at synapses, e.g., other *neurexins* forms, *neurexins*, *contactins*, and *contactin-associated proteins*, and the synapse protein encoded by *SHANK3*. The protein encoded by *SHANK3* acts as a binding partner for *neurexins* and plays a role in the structural organization of dendrites. Within synapses *neurexins* are also associated with *neurexins* and with *contactin-associated proteins*.²⁵

Persico and Bougeron⁸ hypothesized that *neurexins* and the *SHANK3*-encoded protein participate in the assembly of postsynaptic structures and particularly in structures that are involved in pathways important for language and social communication. They proposed further that abnormal synaptic function and abnormal neural networks play a role in autism.

Neuroligins and Neurexins

Sudhof and colleagues analyzed neuroligins and neurexin for the past decade. They determined that these proteins play a key role in determining the balance between excitatory and inhibitory signals at synapses.²⁶

The Neurologin gene family is composed of at least five genes (NLGN1–5) that encode adhesion molecules expressed in postsynaptic neurons. Neuroligins interact with neurexins expressed in presynaptic neurons.²⁷ Neurexins are encoded by at least three genes (NRXN1–3), and many different transcripts are generated from these three genes through alternate splicing. Lawson-Yuen and colleagues²⁸ reviewed published evidence of NLGN3 and NLGN 4 mutations in autism, mental retardation, and pervasive developmental disorders. In addition, they reported studies on a family with deletions in exons 4, 5, and 6 in NLGN4 at Xp22.33. Family members who carried the NLGN4 deletion showed a spectrum of different neuropsychiatric illnesses. Laumonnier and colleagues²⁹ reported the occurrence of NLGN4 deletions in members of a large extended family with 13 affected males, 10 with mental retardation, 2 with autism, and 1 with pervasive developmental disability. Yan and colleagues³⁰ identified NLGN4 mutations in individuals in four different autism families.

It is important, when designing studies that test for neurologin mutations, for investigators to note that alternate splice forms of NLGN3 and 4 occur^{31,32} and alternate splicing of neuroligins generates isoforms that have different targeting and function.

Neurexin 1 signal peptide variants were identified in autistic patients.³³ In the study carried out by the Autism Genome Project Consortium,³⁴ two female siblings with typical autism were identified who each had a 300-Kb deletion on 2p16 that was not present in the parents. The presence of this *de novo* deletion was confirmed by quantitative PCR. This deletion removed coding exons from the Neurexin 1 gene.

One of the parents was most likely a gonadal mosaic for this deletion.

Sequence analysis of the Neurexin 1 gene (NRXN1) in 116 patients with autism led to identification of 5 structural variants.³⁵ The variants identified included a predicted splicing mutation. Variants identified in the autism patients were absent in 10,000 control alleles.

Levison and El-Husseini³⁶ reviewed information on the role of the neurologin–neurexin interaction on synapse maturation and functioning, and the mechanisms whereby structural defects in these proteins may lead to autism. They noted that the Neurologin 1 protein impacts presynaptic terminal maturation through its calcium-dependent interaction with beta neurexin. Studies on the crystal structure of Neurologin 1 and Neurologin 4 revealed that neuroligins dimerize through a specific domain. Following this dimerization, binding between neurologin and neurexin occurs.

There is evidence that Neurologin 1 and Neurologin 3 occur primarily in excitatory synapses.²⁶ Differences in synaptic binding of these neuroligins may depend on differential expression of the scaffolding protein PSD-95. Higher PSD-95 expression is associated with glutamate receptor expression and excitatory synapses. In inhibitory synapses higher levels of the scaffolding protein gephyrin 1 occur. In these synapses levels of neurologin 2 and GABA receptors are higher.³⁷

Contactin-Associated Proteins

One region of interest that has emerged in several recent studies is the chromosome 7q36 region and the CNTNAP2 gene that encodes the Caspr2 protein.

Caspr2, contactin-associated protein 2 is one member of the neurexin superfamily of proteins. These transmembrane proteins play a role in cell–cell interactions in the central nervous system (CNS). Poliak and colleagues³⁸ reported that the Caspr2 protein is primarily

TABLE 1. Genes in *de Novo* CN Change Regions with CNV-Overlap = 0

Sample id	Chr	CN type	CN state	Cytoband	Size (Kb)	# of markers	Start position (bp)	End position (bp)	Gene region ^a	Genes
AU24_0201	9	loss	1	p24.1	5	17	5068397	5073005		JAK2
	12	loss	1	p12.2	4	5	21187742	21191808	intron	SLC01B1
	X	gain	2	q21.31	28	7	91285599	91313739	intron	PCDH11X
AU28_0202	7	loss	1	q21.11	5	8	78167369	78172110	intron	MAGI2
	7	loss	1	q35	5	7	145873673	145878763	intron	CNTNAP2
	8	gain	3	q24.3	77	30	142406624	142483508		GPR20
	11	gain	3	p15.5	26	22	1601016	1626863	intron	HCCA2
										KRTAP5-5
										RAD9A
	11	gain	3	q13.1	125	45	66917509	67042651		PPP1CA
										TBC1D10C
										KIAA1394
									RPS6KB2	
									PTPRCAP	
									CORO1B	
									GPR152	
									CABP4	
	15	loss	1	q24.1	1364	868	70750326	72114195		7 genes ^d
	15	loss	1	q24.1	1575	583	72285241	73860506		23 genes ^d
15	gain	3	q24.3	19	13	75675899	75694590		LINGO1	
16	loss	1	q23.3	13	20	81753452	81766787	intron	CDH13	
19	gain	3	q13.32	51	26	52923460	52974307		EHD2	
									GLTSCR2	
									SEPW1	
22	gain	3	q13.31	31	42	42854883	42886112		PARVB	
X	loss	1	q23	8	4	115505412	115513193		SLC6A14	
									CXORF61	
AU39_0201	7	gain	3	q35	35	28	145619422	145654806	intron	CNTNAP2
	21	loss	1	q22.2	3	5	38829975	38832550	intron	ERG
AU44_0201	1	gain	4	p36.31	184	72	6134551	6318310		ACOT7
	3	loss	1	p12.3	19	12	81607796	81626739		GBE1
	3	loss	1	q26.31	2	8	175046386	175047941	intron	NLGN1
	3	loss	0	q26.31	32	26	176392112	176423680	intron	NAALADL2
	4	loss	1	p15.31	24	18	20644075	20668242	intron	KCNIP4
	4	loss	1	p15.2	16	6	26345224	26360962		TBC1D19
	6	loss	1	p22.2	13	17	24272644	24285902		DCDC2
	6	loss	1	q16.3	58	33	102421709	102480116		GRIK2
AU44_0201 (continued)	8	loss	1	p22	22	17	17827700	17849728		PCM1
	8	loss	1	q23.3	18	8	113409255	113427524		CSMD3
	10	loss	0	p13	7	3	14279921	14286816	intron	FRMD4A
	15	loss	1	q25.3	10	8	85300271	85309955	intron	AGBL1
	17	gain	3	p13.3	40	25	2320622	2360250		METT10D
	17	loss	1	q12	5	10	35099899	35104753		ERBB2
	17	loss	1	q24.1	7	4	59961941	59969136		CCDC45
	18	loss	1	p11.32	9	7	2726921	2735431		SMCHD1
	18	loss	0	q12.3	3	3	41498586	41501467		SLC14A2
	18	loss	1	q21.2	10	5	51219736	51229919		TCF4
	18	loss	1	q22.1	7	7	64840170	64847339	intron	CCDC102B
	19	gain	3	p13.2	278	99	12547282	12825167		XNF791
										MAN2B1
										MORG1
										C19orf56
										DHPS
									FBXW9	
									TNPO2	
									C19orf43	

Continued

TABLE 1. Continued

Sample id	Chr	CN type	CN state	Cytoband	Size (Kb)	# of markers	Start position (bp)	End position (bp)	Gene region ^a	Genes
	19	loss	1	p13.11	6	6	17042890	17048895		NY-SAR-48
	19	loss	1	q13.32	9	8	51018316	51027704		SYMPK
	19	gain	3	q13.32	101	50	52890629	52991587		GLTSCR1
										EHD2
										GLTSCR2
										SEPW1
	X	loss	0	p11.3	9	6	43892057	43900792		EFHC2
	X	loss	0	q13.2	9	5	72707782	72716662		CHIC1
	X	gain	2	q13.2	20	8	73423068	73442785		ZCCHC13
	X	gain	2	q22.3	39	13	106768913	106808383		PRPS1
	X	gain	2	q22.3	6	7	109818512	109824507		CHRD1
	X	gain	2	q25	32	9	129014137	129045726		BCORL1
										ELF4
	X	loss	0	q28	8	3	150534372	150541938		PASD1
AU46_0201 ^b	X	gain	2	q21.31	207	29	91740930	91947433		PCDH11X
AU63_0202	X	gain	2	q21.31	197	28	91750715	91947433		PCDH11X
AU80_0202	1	loss	1	q31.2	12	6	191480002	191491706		CDC73
	4	loss	1	q12	1	7	54852790	54853912	intron	PDGFA
	11	gain	4	p11.2	35	12	47370309	47405648		SLC39A13
										PSMC3
	17	gain	4	q21.32	4	4	43285583	43289137		SP6
AU80_0203	X	gain	2	q21.31	91	19	91158912	91249703		PCDH11X
AU93_0202	10	loss	1	p13	7	3	14279921	14286816	intron	FRMD4A
	10	loss	1	q22.2	10	5	74701404	74711556		TTC18
	10	loss	1	q23.1	7	3	87460016	87466650	intron	GRID1
	10	loss	1	q24.32	6	5	104066221	104072300	intron	GBF1
	10	loss	1	q25.3	5	5	116939963	116944900	intron	ATRNL1
	15	gain	4	q24.3	8	5	74413238	74421389		ISL2
AU210-0201	15	gain	3	q11.2	5307	3474	21192943	26500067		MKRN3 ^c
										MAGEL2 ^c
										NDN ^c
										C15orf2
										SNRPN ^c
										UBE3A ^c
										ATP10A
										GABRB3
										GABRA5
										GABRG3
										OCA2
AU232_0201	1	loss	1	q31.2	9	5	191480002	191488526		CDC73
	10	gain	3	q11.21	9	36	42910286	42919369		RET
AU232_0202	5	loss	0	q35.2	15	8	173295063	173310403		CPEB4
	7	loss	1	q33	6	6	134956331	134962321		NVP205

^aCN changes that are confined to an exon are indicated; the remaining CN changes involve at least one exon and in some cases an entire gene sequence.

^bIndividual AU0046-0201 shares with his father the CN change that includes part of the PCDH11X gene; this inherited change is included here since it overlaps de novo CN changes in other subjects.

^cThese five genes in the duplication region for AU0210-0201 overlap reported CNVs, but are included in this table because they overlap the inverted duplication region of chromosome 15.

^dBetween these two deletion regions are segments that contain polymorphic variations.

localized at the juxta-paranodes of myelinated axons and that it is associated with K⁺ channels. Disruption of the Caspr2 protein impacts functioning of the K⁺ channels. These investigators also noted that alternate Caspr2 encoding transcripts are present in brain. The 9-Kb transcript is predominant in the medulla, substantia nigra, and caudate nucleus. In other brain regions both 9- and 10-Kb transcripts occurred.

Based on the demonstrated localization of Caspr2 in the juxta-paranodal region of myelinated axons, a number of investigators have proposed that Caspr2, along with other neurexin-related proteins, plays a role in modulating neuron–glial interactions.

Abrahams and Geschwind³⁹ noted striking anterior cortical enrichments of expression of CNTNAP2 in human fetal brains. Their studies were designed particularly to search for specific patterns of gene expression in the frontal and superior temporal cortical regions. They studied midgestation fetuses since this is the peak period of neurogenesis and neuronal migration.

A syndrome associated with epilepsy and neuropsychiatric symptoms, including autism, that occurs in the Amish population is associated with reduced expression of Caspr2, the protein encoded by the CNTNAP2 gene.⁴⁰

Friedman and colleagues⁴¹ identified hemizygous deletions of the CNTNAP2 gene on chromosome 7q35–7q36 in two cognitively-impaired patients with epilepsy. In one patient the deletion was 11 Mb in length in the other patient the deletion was 1.5 Mb. These investigators identified a smaller deletion of 220 Kb in the CNTNAP2 gene in a patient with schizophrenia.

Alarcon and colleagues⁴² reported an association of autism and a specific SNP in CNTNAP2 in male autistic probands. The CNTNAP2 intronic SNP rs2710102 at 147,011,820 showed particularly strong association with the endophenotype that included delayed age at first word. Alarcon and colleagues⁴² indicate a

deletion in intron 1 of CNTNAP2 in an autistic subject (Fig. 2).

Arking and colleagues⁴³ identified association with autism and a specific SNP in CNTNAP2, rs7794745 at 145,926,660. They followed up this analysis with a study of parent child trios (one affected child and two unaffected parents). They observed over-transmission of the T allele to affected children.

Bakkaloglu and colleagues⁴⁴ described a rare variant of CNTNAP2 that occurred in 34 autism-affected individuals in unrelated families. This variant was not present in 400 controls studied by these investigators.

Neurotransmitter Receptors and Autism

Glutamate Receptors

In the context of synaptic function it is interesting to note that neurotransmitter receptor-encoding genes are located within regions identified by linkage and association studies as being significant in the etiology of autism. We summarize next the evidence that points to this association.

Glutamate 6 Receptor GRIK2 Gene

Genome-wide linkage studies revealed evidence for linkage of autism to chromosome 6q21. Jamain and colleagues⁴⁵ identified the glutamate receptor 6 gene that maps in this region as a possible candidate gene for autism. They carried out two follow-up association studies including an affected sib-pair analysis and a transmission disequilibrium assay (TDT) in children and parents. Results yielded significant evidence of association of specific GluR6 alleles and autism ($P = 0.0004$).

In a consanguineous Iranian family in which 6 members were affected with recessively inherited mild or severe nonsyndromic mental retardation, Motazaker and colleagues⁴⁶ identified an intragenic deletion in the GluR6 gene. This was an in-frame deletion that led to loss

of exons 7 and 8, with loss of 84 amino acids in the extracellular N terminal domain close to the ligand binding site. These investigators demonstrated that loss of these amino acids resulted in impaired ability of the receptors to form ion channels.

GRIN2A Glutamate Receptor Ionotropic NMDA 2A

Barnby and colleagues⁴⁷ carried out studies to follow up on evidence of linkage of autism to chromosome 16p11–p13. They analyzed genotypes within candidate genes, initially in 239 multiplex families and subsequently in 91 autism trios. In the study on multiplex families, specific alleles within genes ABAT (4-aminoglutamate transferase), GRIN2A (glutamate receptor ionotropic NMDA 2A), and CREBP (CRE binding proteins) were significantly associated with autism. ABAT plays a role in metabolism of the neurotransmitter gamma amino-butyric acid GABA. In GRIN2A the associated markers were found in exon 6, intron 10, and in the 3' untranslated region. In the study of trios, association of autism with specific alleles within GRIN2A was particularly noted. Haplotype analysis revealed differences between cases and controls, particularly within GRIN2A.

GRIN2A represents an interesting autism candidate gene, given its role in learning and memory. In a postmortem analysis of autistic subjects and controls, Purcell and colleagues⁴⁸ identified glutamate expression differences between the two groups.

Barnby and colleagues⁴⁷ noted that bioinformatics analysis alone might be insufficient to detect the effects of intronic variants on splicing and transcription. They postulated further that in autism and other multigenic disorders subtle changes in gene regulation and expression may be anticipated. They referred to a publication by Suzuki and colleagues,⁴⁹ who identified association of PAD14 and rheumatoid arthritis, but failed to identify missense mutations in the coding region of that gene. However, in 48 affected individuals, the researchers identified

altered PAD14 mRNA stability associated with a specific SNP haplotype.

Fragile X Mental Retardation, Autism, and Metabotropic Glutamate Receptors

Fragile X mental retardation is a form of mental retardation in which many affected individuals (usually males) manifest features of autism. Common autistic symptoms in affected males include hand flapping and poor eye contact. The disorder is due to expansion of the CCG repeats to over 200 copies within the 5' untranslated region of the FMR1 gene on chromosome Xq27.3. In premutation carriers the repeat expansion is less than 200 copies. However there is instability of the repeat size. It should be noted that this repeat expansion is distinct from the definition of CNV, since the repeat units in a CNV are greater than 1 Kb in length.¹⁵ Molecular studies of the FMR1-encoded FMRP protein⁵⁰ revealed that this protein plays an important role in regulation of protein synthesis in neurons, particularly in dendrites. There is evidence that FMRP is a negative regulator of protein synthesis that occurs in response to activation of metabotropic glutamate receptors (mGluR). In the absence of FMRP, there results excessive metabotropic AMPA glutamate receptor activity and receptor trafficking, which leads to altered synaptic activity. Support for this theory as it relates to the downstream effects of FMRP loss comes from animal models of Fragile X mental retardation, and evidence exists that treatment with AMPA receptor antagonists results in phenotypic rescue.⁵⁰

GABA Receptors

Genome dosage changes in the 15q12 region represent the most common chromosome change in autism. This region harbors three GABA receptor genes. The GABRB3 gene was found to be associated with autism in a study by Shao and colleagues⁵¹ on subjects with a specific autistic subtype, namely strong insistence on sameness.

In a study of 14 genes that each encode a GABA receptor subunit and are located on 3 different chromosomes, Ma and colleagues⁵² identified a strong association of autism with the GABRA4 on chromosome 4p. Subsequent analyses carried out by these investigators to search for epistasis revealed that GABRA4 increases autism risk through its association with GABRA1 (chromosome 5q34–q35). Their findings support the hypothesis that complex gene interactions may contribute to autism risk.

Tuberous Sclerosis, Synaptic Function, and Autism

Autism occurs in 25 to 50% of subjects with tuberous sclerosis.⁵³ Autism and mental retardation in some individuals with this disorder were in the past attributed to the occurrence of cortical tubers and seizures. Recent studies using animal models of the disorder have provided evidence that loss of TSC1 or TSC2 gene function leads to altered synaptic activity, as is hypothesized as important for autism. Von der Brélie and colleagues⁵⁴ carried out studies of hippocampal synaptic plasticity in rats heterozygous for an autosomal dominant germline mutation in the TSC2 gene. They discovered significant differences between TSC2 heterozygous mutant and control rats in potential for activity-dependent hippocampal synaptic modulation.

TSC1 and TSC2 gene products—hamartin and tuberin—together play a key role in regulation of a growth control pathway that involves Rheb (Ras homolog enriched in brain) and TOR (target of Rapamycin). TOR is a component of two different complexes, TORC1 and TORC2. There is evidence that TORC1 is primarily involved in growth control while the TORC2 complex is a regulator of actin organization and cytoskeletal function. The latter play important roles in axon guidance and synaptic assembly. Late phase, long-term potentiation is a form of synaptic plasticity that encodes long-term memory. Kovacs and colleagues⁵⁵ determined that the TORC1 activity plays a role in this process.

Studies in rat and mouse models of tuberous sclerosis by Tavaz and colleagues,⁵⁶ revealed that morphological changes occur in hippocampal neurons. These include enlargement of soma and dendritic spines and altered properties of glutamatergic synapses. They determined that regulation of actin and cofilin polymerization played a role in generation of altered morphology. These investigators concluded that perturbations of neuronal morphology and function contributed to neurological symptoms of tuberous sclerosis.

Mitochondrial Dysfunction in Autism

A number of investigators who have carried out studies of mitochondrial function or biomarkers in autism have concluded that autism spectrum disorders may be characterized by disturbed brain energy metabolism due to impaired mitochondrial function. Their evidence is primarily the observation of diagnosed autism patients who have biochemical evidence of mitochondrial defects.

Fillano and colleagues⁵⁷ described 12 children who presented clinically with hypotonia, intractable epilepsy, autism, and developmental delay (HEADD syndrome). Reduced levels of specific mitochondrial respiratory enzymes were found in seven of the eight cases who underwent muscle biopsy. In five cases increased levels of large-scale mitochondrial DNA deletions were found. Structural mitochondrial abnormalities were detected in three patients.

Filipek and colleagues⁵⁸ reported mitochondrial hyperproliferation and respiratory complex III deficiency in two autistic children who were also positive for a 15q inverted duplication. Pons and colleagues⁵⁹ described five patients with autism and mitochondrial DNA mutations. In two of these patients a mitochondrial DNA A3243G mutation occurred in the gene that encodes mitochondrial tRNA Leu gene. This mutation is often associated with the MELAS syndrome: mitochondrial encephalopathy and seizures. In two additional autism patients Pons and colleagues identified

the A3243G mutation only in mothers of the affected children. They postulated that mutant mitochondria might be more predominant in the brain tissue of the affected children. In the fifth autism case described in this paper, Pons and colleagues documented evidence of mtDNA depletion and deficiency of mitochondrial respiratory chain enzymes.

Correia and colleagues⁶⁰ examined 210 autistic subjects. They identified lactic acidemia in 36 patients (17%) and the lactate pyruvate ratio was elevated in 53 of 196 patients studied. In 7 of the 30 fully assessed patients who also underwent muscle biopsy, the investigators confirmed mitochondrial disease. On the basis of their results Correia and colleagues proposed that mitochondrial disease might be one of the most common medical conditions associated with autism.

Holzman⁶¹ analyzed maximal respiratory rates in lymphoblastoid cell lines in nine pairs of subjects. Each pair comprised one autistic subject and one unaffected close relative. He reported that the maximal respiratory rate was 40 to 50% higher in autism-affected members of the pairs. Furthermore, inhibition of activity of complex I electron transport components occurred in affected individuals. He interpreted these results as indicative of compensatory increased cellular respiratory capacity due to the occurrence of partial inhibition of ATP synthesis. Holzman expressed the opinion that while environmental factors may impact the clinical state of patients with diseases of energy metabolism, there is no evidence that they cause autism spectrum disorders.

Mitochondrial DNA Structural and Dosage Changes

Given evidence that impaired mitochondrial function plays a role in autism, it is important to consider factors that lead to this impairment. Important among these are structural defects in the mitochondrial genome that may lead to dosage changes involving specific genes.

Kajander and colleagues⁶² reviewed disorders in which mitochondrial heteroplasmy occurs and different populations of mitochondria are present, some with partial deletions of DNA and some with partial duplications of DNA. These disorders are often characterized by impaired muscle function and/or impaired central nervous system function. Kajander and colleagues reported that different inheritance patterns occurred in disorders characterized by rearranged mitochondria. In some cases sporadic inheritance or maternal transmission was observed and in these cases a single primary rearrangement was observed. In other cases, where inheritance was autosomal multiple species of mitochondria with different rearrangements occurred. Breakpoints in the DNA sequence within the rearranged mitochondria often occurred at specific repeat sequence elements. Kajander and colleagues noted that the deletions usually lie between the origin of replication sites of the two DNA strands, heavy and light. The predominant breakpoints in their study were located at sites between 7818 base pairs and 16,071 base pairs. The question arises as to whether the rearrangements are due to abnormal recombination or to replication slippage. These investigators reported that use of long range PCR for analysis of mitochondrial DNA revealed that low levels of deleted and rearranged mitochondrial sequences may be detected even in healthy individuals. They presented evidence that the variant pool of mitochondria is increased under pathological conditions and under conditions of oxidative stress. These variant mitochondria lead to cellular dysfunction.

Samuels and colleagues⁶³ reported that two 13 base pair direct repeats in mitochondrial DNA predispose to common deletion formation. The most common deletion is 4977 base pairs in size; one end of the deletion is usually located between 8 and 9 Kb with the other positioned at approximately 13 Kb. In patients with syndromes due to deletions of mitochondrial DNA multiple rearrangements of

mitochondrial DNA are often present, including duplications.

Mitochondria with deleted forms of DNA occur along with mitochondria with normal length DNA (wild type) and the degree of heteroplasmy varies in different cells and in different tissues in any one individual. Furthermore, deleted mitochondrial forms and wild type mitochondria may be passed on in different proportions to the offspring of a woman whose germline cells are heteroplasmic for different mitochondrial DNA forms.⁶⁴

Mitochondrial Maintenance and Depletion

Mitochondrial depletion is sometimes observed in autism; it may result from defects in mitochondrial maintenance. Shadel⁶⁵ reviewed expression and maintenances of mitochondrial DNA. He noted that expression, replication, and maintenance of mitochondrial DNA require factors encoded by nuclear genes. Nuclear-encoded signaling pathway genes play a role in mediating adaptive function of mitochondria under changing conditions. The target of Rapamycin (TOR) signaling pathway and the ataxia mutated ATM pathway play a role in regulation of mitochondrial gene expression. There is evidence that TOR signaling pathways impact mitochondrial respiratory function and ROS production. Shadel emphasized that approximately 1500 nuclear genes encode factors required by mitochondria for DNA transcription, RNA processing, translation, and for DNA synthesis and DNA repair. These factors are imported into mitochondria from the cytoplasm.

Enzymes in mitochondria are involved in the tricarboxylic acid cycle, in the catabolism and biosynthesis of fatty acids, heme and steroids. A key function of mitochondria is ATP production through action of the oxidative phosphorylation (OXPHOS) systems in the inner mitochondrial membrane. Of the 80 proteins in the OXPHOS complex, 13 are encoded by mitochondrial DNA. Impaired mitochon-

drial function may lead to enhanced production of reactive oxygen species and impaired ion homeostasis.

Shadel⁶⁵ noted that mechanisms of ribosome binding to mitochondrial RNA and mechanisms of translation initiation remain obscure.

Within each cell there are thousands of mitochondrial DNA strands, and tissue-specific factors regulate their number. Mitochondrial DNA synthesis requires a constant supply of deoxynucleoside triphosphates (dNTPs). Deoxynucleotide salvage pathways are therefore important in mitochondria. A number of mitochondrial DNA depletion syndromes are due to mutation in genes that encode cytoplasmic or mitochondrial deoxynucleotide salvage enzymes. Nucleotide synthesis in mitochondria likely occurs through the action of ribonucleotide reductase.

Mitochondrial depletion and impaired mitochondrial maintenance may be due to impaired mitochondrial DNA strand synthesis. This may occur as a consequence of mutations in specific polymerases or helicases (POLG1, POLG2 TWINKLE, and ANT).

Shadel⁶⁵ reported that null mutation in the ATM gene lead to mtDNA depletion and to disruption of mtDNA metabolism. The ATM gene encodes a serine threonine protein kinase. It plays a role in regulating ribonucleotide reductase. Mitochondrial depletion was reported in an autism patient by Pons and colleagues.⁵⁹

Nuclear Mitochondrial Interactions

Given that there are nuclear mitochondrial interactions, it is possible that mothers with a specific mitochondrial mutation or structural change may not show phenotypic variation; however, problems may result in the offspring where the nuclear gene or genes that interact with the mitochondria are altered. First, the aberrant nuclear gene in the offspring may originate from the X chromosome that was predominantly inactivated in the mother. Second, the father may donate a version of the nuclear gene that interacts aberrantly with the maternally derived mitochondrial gene.

Another consequence of intergenomic cross-talk may be that gene dosage changes in the nuclear genome that involve nuclear-encoded mitochondrial genes may not necessarily lead to frank metabolic disorders but may impair mitochondrial function and have consequences for tissues requiring high energy, such as brain tissue. Dosage changes in nuclear-encoded mitochondrial genes may not have deleterious consequences in the general population, but they may have effects in cases where a specific percentage of mitochondria are structurally abnormal or where mutations within mitochondrial DNA are present.

There is evidence that mitochondria play an important role in regulating developmental processes, including neurite outgrowth, axonal polarity, and synaptic plasticity.⁶⁶ It is therefore important to consider the role of mitochondrial genes in autism pathogenesis.

Hemizygoty for Nuclear-Encoded Mitochondrial Genes

We previously published molecular genetic and chromosome studies on autism patients; we delineated duplication regions in four patients and deletion regions in three patients. We initially used FISH analysis of chromosomes and analysis of polymorphic microsatellite repeat markers to search for unexpected differences between parents and autism-affected children to search for genomic imbalance in autism. Results of these studies led to identification in autistic subjects of 15q11–q13 duplications,⁵⁸ 15q22–q23 deletion,⁶⁷ 13q12–q13 deletion,⁶⁸ 4q34 deletion,⁶⁹ and 2q37.3 deletion.⁷⁰ We recently re-examined the genomic changes in these patients along with updated gene maps. In each of the cases described above the dosage change encompassed more than 20 genes.

In 2008 we carried out analysis of DNA from lymphoblastoid cell lines from a subset of autistic subjects and their parents using the Affymetrix 6.0 chip to refine estimations of the size and breakpoints of structural alterations.

In two of the subjects with 15q11–q13 duplications there was evidence of mitochondrial dysfunction based on clinical chemistry.⁵⁸ However, no specific gene has been identified in the duplication region that contributes to mitochondrial pathology.

In our 15q deletion patient, using the 2008 gene map and SNP microarray analysis we determined that the deletion extends from 70,750 Kb to 73,867 Kb, and the current gene map places this deletion within 15q24. We will discuss this patient again below in the context of other patients with 15q24 deletion. At this juncture we wish to point out, relevant to nuclear mitochondrial interactions, that the patient is hemizygous for COX5A. COX5A encodes a subunit of the multisubunit cytochrome c oxidase complex that couples the transfer of electrons from cytochrome c to molecular oxygen and contributes to a proton electrochemical gradient across the inner mitochondrial membrane. The cytochrome c oxidase complex consists of 13 mitochondrial- and nuclear-encoded subunits. Cox5A is expressed at higher levels in human brain than in chimpanzee and gorilla. It is particularly abundant in mitochondria of large size projection neurons.⁷¹

Updated analysis of the deletion breakpoint in the patient we described with autism and a chromosome 13q13 deletion, taking into account previous FISH data and microsatellite polymorphism analysis and the updated gene map, indicates that the deletion extends from 34,415 Kb to 43,5415 Kb on chromosome 13q13–q14.1. This deletion results in hemizygoty for Neurobeachin. This gene acts as a second messenger and Castermans and colleagues noted it to be disrupted by a translocation in an autism subject.⁷² Nuclear-encoded mitochondrial genes that are hemizygous in this patient by consequence of the deletion include MRPS31, mitochondrial ribosomal protein 31, and MTRF1 mitochondrial translation release protein.

The ribosomes present in mammalian mitochondria are 55–60 S particles and are composed of small (28 S) and large (39 S) subunits.

Compared with other organisms mammalian ribosomes contain a relatively low percentage of rRNA and there is a compensating increase in the number of ribosomal proteins. The small ribosome subunit is composed of 12 S rRNA and about 30 proteins. The large subunit contains 16 S rRNA and about 50 proteins.⁷³ There is evidence that the mitochondrial ribosomal proteins may also play a role in coordinating mitochondrial gene expression with the metabolic needs of the cell.

Termination of protein synthesis requires the action of several release factors. Zhang and Spremulli⁷⁴ identified the human mitochondrial translation release protein MTRF1 on the basis of its sequence and transcript homology to factors in lower organisms.

We described a patient with autism and a 19-Kb interstitial deletion of chromosome 4q32–q34.68.⁶⁹ The deletion led to hemizyosity for a number of genes, including neurotransmitter receptor genes and the nuclear-encoded mitochondrial gene known as electron-transferring-flavoprotein dehydrogenase (ETFHDH). This enzyme is located in the inner mitochondrial membrane. It accepts electrons from electron-transfer flavoprotein, located in the mitochondrial matrix, and reduces ubiquinone in the mitochondrial membrane. Homozygous deletions or mutations in this gene lead to secondary co-enzyme Q deficiency and to some forms of riboflavin responsive multiple CoA dehydrogenase deficiency.⁷⁵

There are a number of reports of deletions of chromosome 2q37.3 in autism. We previously published information on patients with this disorder.^{70,76} It is of interest to note that deletions in all three of these patients described in the paper by Wassink and colleagues⁷⁶ would result in hemizyosity for the gene NDUF10 that encodes NADH dehydrogenase ubiquinone 1 alpha subcomplex 10. The protein encoded by this gene constitutes a subunit of complex I, the first enzyme complex in the electron transport chain of mitochondria. This protein has NADH dehydrogenase activity and oxidoreductase activity. It transfers electrons from NADH to the respiratory chain.

De Novo Unique Sequence Nonpolymorphic Copy Number Changes in Nuclear and Mitochondrial Genomes

We analyzed lymphoblastoid DNA from eight triads composed of parents and a single autism affected child; we also analyzed two sets of autistic monozygotic twins and their parents. An initial goal of the studies was to search for de novo copy number changes (deletions or duplications) within unique sequence DNA. In one of the families we carried out studies to determine whether we could more precisely define the extent of the chromosome 15q24 deletion; in another family we sought to more precisely define the extent of a 15q11–q13 duplication. Another goal of the study was to determine whether or not differences existed in the genomic changes present in two members of a twin pair.

In addition to analysis of intensity of hybridization of sample to nuclear genomic DNA we examined signal intensity with mitochondrial DNA SNPs on the Affymetrix chip using the Partek GS analysis program (Fig. 1).

Results of our analyses are presented in Table 1 and in Figs. 1–6.

Table 1 contains a list of de novo copy number changes in probands; this list comprises changes that involve unique sequence DNA segments in which genes are located. Within the chromosomal region impacted as a result of large structural genomic changes that occurred in subject AU28-202 (15q24 deletion), there is a region rich in variable sequence elements, including GOLGIN and HERC2 elements. These are not listed in Table 1.

Chromosome 15q24 Deletion

Analysis of DNA using the Affymetrix 6.0 SNP chip enabled us to more precisely define the breakpoints in subject AU28-202, who was previously reported,⁵⁸ and to compare the extent of deletion with published data on other patients with chromosome 15q24 deletions analyzed using microarray analysis.^{77,78} In subject AU28-202 the deletion extended

Figure 1. Results in family AU28, visualized in the Partek GS software. The bottom half of the panel shows the heat maps for the father AU28-101, mother AU28-102, and autistic child AU28-202 (outlined in bold). The upper half shows the estimated copy number along the chromosome. The deletion (copy number of 1) in 15q24 can be clearly seen.

from 70,750,326 to 73,860,506 bp. This deletion is illustrated in Figure 1.

The centromeric deletion breakpoint in this subject most closely matches that of patient IMR371 reported by Sharp and colleagues,⁷⁷ where the deletion extends from 70.75–74.21 Mb. In the three other 15q24 deletion patients reported by these investigators the centromeric deletion breakpoint occurred at 72.15 Mb; in the patient reported by Klopocki and colleagues⁷⁸ it was at 72.2 Mb. In two of the patients reported by Sharp and colleagues the distal deletion breakpoint was at 76 MB; in the patient reported by Klopocki and colleagues it occurred at 75.9 Mb. The phenotype in our patient is apparently much more severe than phenotypes described in the reports by Sharp and colleagues and Klopocki and colleagues. Our patient has no speech, and her IQ at 6 years of age was reported as 14. It is interesting to note that in the three patients described by Sharp and colleagues where parental origin of the deletion was defined, that deletion was found to be maternal in origin. The parental origin of the 15q24 deletion in the Klopocki and colleagues case was not reported. In our

(female) patient the deletion was paternal in origin. Developmental delay and/or retardation are described in the patients described by Sharp and colleagues and Klopocki and colleagues; there is however, no mention of autism manifestations.

The severity of the phenotype in our patient may be due to the parent of origin of the deletion, implying that imprinting may occur in the 15q24 region. It is also important to note that dosage changes are demonstrable in other chromosome regions in our patient. She has de novo unique sequence copy number changes within genes in 22 other locations in the genome (see Tables 1 and 2). It is interesting to consider the possibility of genomic instability in this subject. In this context we note that as a consequence of the 15q24 deletion she is hemizygous for the NEIL1 that maps from 73,426,463 to 73,434,641 base pairs. NEIL1 is a recently characterized DNA glycosylase that plays a role in DNA repair and specifically in repair of damaged DNA in single strands or bubble forks that arise during replication or transcription. Maiti and colleagues⁷⁹ noted that downregulation of NEIL1

Figure 2. Results in family AU210, visualized in the Partek GS software. The bottom half of the panel shows the heat maps for the father AU210-101, mother AU210-102 and autistic child AU210-201 (outlined in bold). The upper half shows the estimated copy number along the chromosome. The duplication (copy number of 3) in 15q11–13 can be clearly seen.

enhanced mutations in cell lines, particularly when cells were grown under condition of oxidative stress. Hazra and colleagues⁸⁰ noted that given the preference of the two glycosylases NEIL1 and NEIL2 for repair of base lesions that arise during DNA replication or transcription these enzymes play a major role in maintaining structural integrity of the mammalian genome.

Relatively few genes that map within the 15q24 region that is deleted in our patient AU28-202 and in the patients described by Sharp and colleagues⁷⁷ and Klopocki and colleagues⁷⁸ and have location and function primarily in the central nervous system. There are three genes that meet these criteria: Neuroplastin (NPTN) located at 71.712 Mb to 71.716 Mb, Complexin 3 (CPX3) at 72.906–72.911 Mb, and Sorting Nexin 33 (SNX33) at 73.728–73.736 Mb.

The NPTN (SDFR1 gene) encodes neuroplastin, a glycoprotein abundant in the brain and enriched in synaptic membranes. It plays a role in synaptic plasticity. Contacts between pre- and postsynaptic neurons are mediated by synaptic adhesion proteins, such as

Figure 3. Genes GLTSCR1, EHD2, GLTSCR2, HBII-115, and SEPW map to chromosome 19q13.3 between 52874031 and 52989971 base pairs. This 115,940 base pair region underwent de novo duplication in autism subject AU44-201. A de novo duplication occurred in subject AU28-202 that encompassed the region that includes genes EHD2, GLTSCR2, HBII-115, and SEPW. This region was also duplicated in subject AU80-201 and in his unaffected father, AU80-101.

Figure 4. (A) Scatter plot of IQ versus the number of de novo CN changes that have no overlap with reported CNV polymorphisms, for the 12 autistic subjects. (B) Scatter plot of IQ versus the number of genes within de novo CN change regions that have no overlap with reported CNV polymorphisms, for the 12 autistic subjects.

neuroplastin. Bernstein and colleagues⁸¹ reported that neuroplastin is present in many neuronal cell types in forebrain and cerebellum. Two different isoforms are encoded by this gene, np65 (neuroplastin65) and np55. The np65 isoform plays an important role in long-term potentiation in the hippocampus. Empson and colleagues⁸² reported that neuroplastin65 modifies synaptic strength by altering p38 map kinase activity, and this leads to reduction in surface glutamate receptor expression.

Complexin 3 was identified by Reim and colleagues⁸³ on the basis of its homology to other complexins. It is expressed in the brain and in the eye. In the brain it is present in pyramidal cells of the hippocampus, in granule cells of the dentate gyrus, and in Purkinje

cells of the cerebellum. They determined that complexin 3 protein binds to proteins in synaptosomes, including syntaxin (STX1A), synaptosome-associated protein SNAP25, and synaptobrevin.

Sorting nexins play a role in endosomal trafficking and in function of the retromer, a structure that functions in the retromer and in retrograde transport of molecules within the endosomal system. SNX33 is present in the brain and plays a role in trafficking of protein complexes.⁸⁴

Copy number changes that occurred in subject AU28-202 included deletion in intron 1 of the CNTNAP2 gene, associated with autism through studies of Alarcon and colleagues⁴²; and Arking and colleagues.⁴³ As previously noted, in Figure 2 in the paper by Alarcon and colleagues⁴² the authors illustrate deletion within intron1 of CTNAP2 in an autism patient. Subject AU28-202 has a duplication of a 50.8-Kb region on 19p13.32 that contains 4 genes GLTSCR2, EHD2, HBIII 115, and SEPW1. Duplications in this region were present in two other subjects with autism in our study and the function of the brain expressed GLTSCR1 and GLTSCR 2 genes are described below.

Chromosome 15 q11–q13 Duplication

In subject AU210-201 we confirmed the presence of a duplication that spanned 4.07 megabases in 15q11–q13. This duplication is illustrated in Figure 2. Analysis of SNP alleles established that the duplication was paternal in origin. This subject met criteria for Asperger syndrome; she had impairment of social interactions and communication and IQ in the normal range. Our analyses did not reveal evidence of structural genomic changes on other chromosomes. There was also no evidence of mitochondrial deletions in this subject.

It is important to note that the duplication is paternally derived. Data presented in the literature indicate that it is only the maternally derived 15q11–q13 duplications that lead to autism spectrum disorders.³⁹ Subject

Figure 5. Image intensity for probes along the mitochondrial genome (from base pair 400 to 16,059) in family AU80 (from top to bottom: father AU80-101, mother AU80-102, and the autistic MZ twins AU80-202 and AU80-203). Decreased signal intensity is observed in the 9.7-Kb to 13.7-Kb region in both AU80-102 and AU80-202.

Figure 6. Image intensity for probes along the mitochondrial genome (from base pair 400 to 16,059) in the 10 autism families, plus 90 control Caucasian individuals from the HapMap CEU dataset (below the black line). Note the decreased signal intensity within a region between 9.7Kb and 13.7 Kb in many of the autism families, but few of the control samples.

AU210-201 therefore represents an exception to this general rule.

Chromosome 19q13.32 Duplication

Affymetrix SNP 6.0 analysis revealed that de novo duplication of a segment on chromosome 19q13.32 that encompassed four genes—GLTSCR2, EHD2, HBII-115, and SEPW1—was present in three patients in our study, AU28-202, AU80-202, and

AU44-201. This duplication was not detected in 90 HAPMAP samples we analyzed. The duplicated region in our subjects is illustrated in Figure 3. In AU44-201 the GLTSCR1 gene was also duplicated. HBII-115 is a small nucleolar RNA; SEPW1 is a selenoprotein and is a target of methylmercury in neuronal cells.⁸⁵ EHD2 is an EH domain protein with a nucleotide binding consensus site; it interacts with actin (Entrez Gene <http://www.ncbi.nlm.nih.gov>).

TABLE 2. Genes in the *de Novo* Deletion at 15q23–q24 in AU28-0202

7,0750,326–72,114,195 bp	72,114,195–72,285,241 bp
NEO1	repeated loci
HCN4	LOXL1
LOC283677	PML
NPTN	GOLGA6
CD276	
LOC388135	
72,285,241–73,103,000 bp	73,281,300–73,860,506 bp
CYP1A2	C15ORF39
CSK	COMMD4
LMAN1L	NEIL1
CPLX3	MAN2C1
ULK3	SIN3A
SCAMP2	PTPN9
MPI	SNUPN
C15ORF17	IMP3
COX5A	SH3PX3
RPP25	CSPG4
SCAMP5	ODF3L1
PPCDC	

GLTSCR2-(glioma tumor suppressor candidate region gene 2) encoded protein binds to the protein encoded by PTEN (phosphatase and tensin homolog) and stabilizes it. Loss of expression of GLTSCR2 occurs in neuroblastoma and leads to reduced PTEN expression. Okahara and colleagues⁸⁶ published evidence that the product of the GLTSCR2 gene plays a role in the phosphoinositol pathway PIP3, through stabilization of PTEN. PTEN was proposed as an autism candidate gene in several studies. Butler and colleagues⁸⁷ published results of studies on 18 autistic subjects with marked microcephaly; they identified PTEN mutations in 3 of these subjects.

It is interesting to note that the changes in 19q13.32 in our autism subjects are duplications. These may lead to increased expression of GLTSCR2. Yim and colleagues⁸⁸ reported that over expression of GLTSCR2 sensitizes cells to hypoxic injury and promotes apoptosis. They noted that cell death associated with induction of GLTSCR2 may involve PTEN but does not likely involve PTEN phosphorylation.

Protocadherin Variants

Protocadherins are sometimes referred to as *nonclassic cadherins*. Cadherins are structural proteins located in synapses. Neural development is characterized by synapse formation and by elaboration of dendritic arborization. Takeichi⁸⁹ reviewed the role of the cadherin superfamily of cell surface receptors in regulating neuronal recognition and connectivity. Cadherins are transmembrane proteins with repetitive subdomains, referred to as cadherin repeats that are present in the extracellular portions of the molecules. These extracellular domains bind calcium. The intracellular domains of cadherins exhibit greater variability. Many of the Protocadherin (PCDH) gene transcripts undergo extensive alternate splicing. Takeichi⁸⁹ reported that most of the protocadherins are expressed in neurons and some are localized at synapses. It is therefore possible that structural changes in protocadherins play a role in autism.

Two subjects in our study microarray analyses revealed deletions of unique sequence DNA adjacent to the 5′ end of the PCDH10 gene on chromosome 4q28.3. In AU44-201 we found an approximately 13-Kb deletion within 288 Kb of the 5′ end of PCDH10. In AU24-201 we found a 7-Kb deletion within 246 Kb of the 5′ end of PCDH10. Deletions or duplications of DNA segments adjacent to genes may lead to abnormal gene expression because they disrupt regulatory elements.

Variants in PCDH11X (apparent duplications) occurred in males in four families in our microarray study. No similar variants were detected in our analysis of 90 HapMap samples. It is, however, possible that the PCDH11X variants we detected in our autism probands may in fact represent polymorphisms or copy number changes in PCDH11Y. This is discussed in greater detail below. Genes on the X and Y chromosomes require careful consideration, given the male to female gender ratio of 4:1 among individuals with autisms.

Protocadherin H11 X and Y

Protocadherin H11X/Y, PCDH11X, and PCDH11Y are genes located on Xq21.3 and Yp11.3. This homology arose as a result of translocation of a region of Xq21.3 to Yp11.3. Analysis of genomic structure revealed that this translocation was followed by inversion of the translocated segment on Y and loss of part of the segment during inversion.⁹⁰

Through studies in primates this translocation event was determined to have taken place in the hominid lineage following divergence of chimpanzee and hominids. The X and Y protocadherin H pair are present only in hominids. PCDH11X is expressed only in brain. PCDH11Y is expressed primarily in brain. Some transcripts occur in testis. These genes are considered to be important candidates for brain asymmetry in the region of the planum temporale, emergence of handedness, and language-specific development in the human lineage and are therefore of significant interest in studies of autism. There is also evidence for accelerated positive selection on PCDH11X/Y.

Detailed analysis of genomic DNA and transcripts of PCDH11X and PCDH11Y established that PCDH11X has 17 exons and PCDH11Y has 15 exons, and is missing exons 7 and 8 and a large intron that lies between these exons. There is however evidence that transcripts of these genes undergo extensive alternate splicing.⁹¹ Protocadherin H11 is expressed from both the X and the Y chromosome and it escapes X inactivation in females. PCDH11X expression in females is higher than in males. XY pairing plays an important role in protection of these genes from inactivation.

Durand and colleagues⁹² noted that genes involved in synaptogenesis include protocadherins and neuroligins. They analyzed expression and genetic variability of PCDH11 activity. They demonstrated that following RT-PCR protocadherin X transcripts can be distinguished from protocadherin Y transcripts, since a site of cleavage for the restriction en-

donuclease Bst11 is present in the X transcript and absent from the Y transcript. On the basis of these studies the authors determined that the PCDH11Y gene is more abundantly expressed than the PCDH11X gene in males. Their studies demonstrated that PCDH11X and PCDH11Y transcripts are mainly present in the brain cortex. They identified two amino acid variations in the PCDH11Y products. They examined these allelic variants in controls and in subjects with autism or schizophrenia and found no differences between the different groups.

PCDH11X and PCDH11Y SNP

We determined that a number of the SNP probes used on the Affymetrix 6.0 chip that detected apparent duplications within PCDH11 on the X chromosome, when checked in Blast sequence analysis (BLASTN), each detect a single homologous sequence on the X chromosome and two different homologous but not identical sequences on the Y chromosome. The different Y sequences in the database that correspond to the SNP sequences may represent sequences derived from different individuals. Possible interpretations of our data are that in the microarray analysis of specific samples from male individuals typed as having PCDH11X segment duplication, there is a PCDH11Y polymorphism or copy number change.

Jamain and colleagues⁹³ used a set of Y polymorphisms to type control samples and samples from autistic families from France and Sweden. The polymorphisms typed included eight microsatellite polymorphisms and seven single nucleotide polymorphisms. They noted no differences in the frequency of specific haplotypes from patients and controls. They concluded, however, that a direct role of the Y chromosome in autism could not be ruled out on the basis of their haplotype analyses, particularly given the relatively high frequency of de novo point mutations and deletions on the Y chromosome.

GRID1

In autism subject AU93-202, a 6-Kb deletion on chromosome 10q23.1 occurred in intron 1 of the gene that encodes ionotropic glutamate receptor GRID1. It is possible that intronic deletions may disrupt splicing and binding of regulatory elements.

The delta glutamate receptors are classified as ionic receptors on the basis of their amino acid similarity to other ionotropic receptors. However, in *in vitro* analyses they do not form ion channels.⁹⁴ There is a close spatial relationship between GRID1 and GRID2 receptors and the AMPA glutamate receptors GluR2 and GluR3, and evidence for direct interaction. Linkage of schizophrenia to markers within the GRID1 receptor was found in studies by Fallin and colleagues.⁹⁵ They published evidence for association of schizophrenia with a 4 SNP haplotype in GRID1 in an Ashkenazi Jewish population. Guo and colleagues⁹⁴ carried out association studies in the Han Chinese schizophrenic subjects and controls. They discovered specific haplotypes within GRID1 that were significantly more common in subjects than in controls.

There are no published cases of GRID2 abnormalities in autism. However the finding of a GRID1 deletion in our patient is of interest given evidence of abnormalities in GRID1-interacting glutamate receptors, i.e., AMPA glutamate receptors, in autism.^{50,69}

Gene Dosage Changes in AU44-201

The high number of de novo unique sequence variants, 59, in one proband (AU44-201) was particularly striking. The question arises as to whether genomic instability existed in this subject. He had deletions in two genes that may be of interest in this regard. These included a 559 Kb deletion on 18p11.2 that encompasses the SMCDH1 (structural maintenance of chromosomes 1), and an intra-genic duplication encompasses part of intron 1, and all remaining introns and exons of the PRPS1 gene (phosphoribosyl-pyrophosphate

synthetase) on Xq22.3. PRPS1 enzyme is essential for nucleotide salvage.

The SMCHD1 gene contains an N-terminal ATPase domain and a C-terminal of maintenance of chromosomes hinge domain. The latter domain occurs in SMC proteins that play a role in chromosome condensation, DNA recombination and repair. It is of interest to note that in our study a structural abnormality in this gene occurred in an individual with multiple regions of genomic deletion.

Blewitt and colleagues⁹⁶ demonstrated that homozygous mutation in the SMCHD1 gene in the mouse is associated with impaired maintenance of hypermethylation of the inactive X chromosome. There is additional evidence that SMC and cohesin associate with Dnmt3b an enzyme involved in maintenance methylation of DNA. There is further evidence from plant studies that a maintenance of chromosomes hinge domain protein is required for RNA directed DNA methylation.⁹⁷ Together these studies link proteins associated with chromosome mechanics to epigenetic silencing.

The male subject AU44-201 in our study, showed a deletion within the EFHC2 gene on Xp12.3. We discussed this gene above in the description of Turner syndrome and autism. Weiss and colleagues¹⁶ demonstrated that this gene was most strongly associated with abnormal facial emotion recognition in subjects with autism symptoms and partial X chromosome deletions.

This subject also showed a deletion on chromosome 6p that results in hemizygosity for the gene DCDC2 that is a homolog of Doublecortin. DCDC2 was linked to developmental dyslexia by Parrachini and colleagues⁹⁸ Knockdown of expression of DCDC2 in embryonic rats led to marked disruption of neocortical migration.⁹⁹

Subject AU44-201 was found to have duplication of a segment on chromosome 1p36.31 in which the ACOT7 gene is located. This gene is expressed particularly in brain. The gene product acyl COA thioesterase plays a role in the hydrolysis of Acyl-CoA to free fatty acids;

it is present in the cytosol and in mitochondria.¹⁰⁰

Autism in Monozygotic Twins

In our study we carried out Affymetrix SNP 6.0 genotyping on two sets of twins. In one set of twins, AU80-202 and AU80-203, analysis of SNP alleles revealed that the twins were identical at 99.8% of loci; their phenotype was analyzed in some detail. The individual twins differed in the degree of autism severity. Both twins met diagnostic criteria for autism on ADOS (autism diagnostic observation schedule, Lord 1989).¹⁰¹ On clinical observation it was apparent that social interactions and communication skills were particularly impaired in twin AU80-202. There was a history of delayed development for both. The IQ scores in the two differed considerably. In AU80-202 the IQ score was 57, in AU80-203 the score was 92. It is of interest to note that the number of *de novo* unique sequence genomic changes was greater in the more severely affected twin; 7 of these unique changes impacted coding genes. One change was a duplication on chromosome 19q11.32 that led to increased gene dosage of the *GLTSCR2*, *EHD2*, *HBII-115*, and *SEWI* gene. These genes were discussed above. Other genes impacted by structural changes in this subject included deletion of *CDC73*, (cell division cycle gene 73) on 1q31.2, duplication of genes encoding *SLC39A13* Zn transporter and *PSMC3* (proteasome C3) on chromosome 11q23 and duplication of a region containing the *SP6* transcription factor on chromosome 17q21.32. He also had a deletion within an intron of *PDGFA* (platelet-derived growth factor A) on 4q12. None of these changes were observed in AU80-203.

Twin AU80-203 exhibited a gain that apparently encompassed part of the *PCDH11X* gene.

Within these twins and in other cases in our study we noticed a negative correlation between the number of *de novo* unique sequence copy number changes and IQ. We also observed

negative correlation between IQ and the number of genes involved in copy number changes (Fig. 4A and 4B).

The high degree of co-occurrence of autism in twins is often used as evidence for the importance of genetic factors in determining the etiology of this disorder. In their study of autism in twins Bailey and colleagues¹⁰² reported that 92% of monozygotic twins were concordant for autism and specifically for the broader spectrum of social and cognitive abnormalities. In their study only 10% of dizygotic twins were concordant for autism. Casanova⁵ noted that affected identical twins exhibit differences in severity of autism symptoms and in the specific manifestations and he concluded that these observations suggest that prenatal and postnatal influences differ in the two members of twin pairs.

Bruder and colleagues¹⁰³ published results of SNP genotyping on monozygotic twins. Their study was done on older adults and included a control sample and adults with late onset neurodegenerative disease, Parkinson disease. They demonstrated genomic copy number differences in twins concordant for neurodegenerative disease and in twins discordant for disease. It is important to note that the average age of their patient sample was approximately 70 years. The average age of their control population was mid-40s. They emphasized the importance of exercising caution when assigning disease causality to copy number changes.

Hu and colleagues¹⁰⁴ used microarray analysis to examine gene expression in lymphoblastoid cell lines in samples from two sets of control monozygotic twins and five sets of monozygotic twins discordant for severity of autism. In their analysis a number of genes showed quantitative differences in expression in severely versus the mildly affected members of the twin pairs. The authors observed that most differentially expressed genes operated in pathways critical to nervous system function and development. They noted that differential gene expression could be in part due to epigenetic effects. Hu and colleagues¹⁰⁴ assayed serotonin activity in

blood. They determined that the levels in the members of the two control sets of twins were the same. Measurements in the two members of each autism twin pair differed and the difference corresponded to the level of autism severity.

Mitochondrial Genome Analysis

Use of the Affymetrix 6.0 SNP microarray enabled us to assess hybridization intensity for DNA probes located between 410 and 16,141 base pairs of the mitochondrial DNA sequence. In 8 of 12 autistic probands (aged between 4 and 28 years) hybridization intensity was reduced in the 9.7–13.7 Kb region relative to that in the remaining region of the mitochondrial genome, indicative of deletions of variable length. Figure 5 illustrates mitochondrial SNP profiles in family AU80. There is evidence of decreased intensity of signal in the 9-Kb to 13-Kb region in the mother of the twins, AU80-102 and particularly in twin AU80-202, the more severely autistic and more severely cognitively impaired twin. In 7 of 90 Hap map samples there was some evidence of decrease in hybridization intensity within the 9.7-Kb to 13.7-Kb mitochondrial DNA region; however there were differences in the distribution of signal intensity changes across the region between the autism and HapMap samples, as can be seen in Figure 6. Samples from autism families appear in the top segment of the heat map.

These findings suggestive of the presence of mitochondria from which segments of DNA are apparently deleted are of interest. A number of interpretations are possible including that the findings indicate changes within the autism samples that impact mitochondrial DNA replication. There is evidence that increased oxidative stress impairs this process.⁶⁵

Conclusions

There is increasing evidence that autism is oligogenic or multigenic in nature. Autism pathogenesis may be determined by defec-

tive action of specific genes or by the collective effects of aberrant interactions of nuclear gene products or of nuclear and mitochondrial gene products. There is evidence that many of the affected pathways impact synapse structure and/or synaptic function.

Genetic studies can potentially elucidate basic mechanisms that may be perturbed in disease. These studies may also shed light on genotype–phenotype relationships. However the relationship between genotype and phenotype is often not linear. Moore¹⁰⁵ drew attention to the important role of gene–gene interactions, i.e., epistasis, in complex diseases. Moore noted that two different definitions of epistasis were proposed almost 100 hundred years ago and that these definitions still influence approaches to analysis of complex diseases. William Bateson¹⁰⁶ offered a biological definition and defined epistasis as “distortion in Mendelian ratios of segregation of a specific phenotype because effects of one gene were masked by effects of another.” R.A. Fisher¹⁰⁷ defined epistasis as “the case of deviations from additivity in linear statistical analyses.”

This shift in thinking about the etiology of autisms will require much greater effort to identify subgroups (endophenotypes) that share errors in the same genes and/or pathways. Investigations at both the genetic and phenotypic levels will be needed to take into account epistasis, heterogeneity and the multigenic nature of these disorders.

Role of Genomic Changes in the Etiology of Autism

In considering genomic changes and their role in the etiology of autism, there are several possibilities. One possibility is that a rare genomic change in a specific individual may be the primary cause of the disorder. In different individuals rare autism causing genomic changes may arise on different chromosomes and in different genes; occasionally they may arise in the same region or gene. The penetrance of

these rare mutations may be high. A second possibility is that in any one individual subtle mutation that arises in a number of different genes may act in concert to cause the disorder. Mutations may be base changes or structural genomic changes that impair function.

Morrow and colleagues¹⁰⁸ reported that dosage changes due to deletions or duplications of unique sequence DNA adjacent to genes might disrupt regulatory sequence elements and thereby impact gene expression and lead to autism. They identified, in consanguineous pedigrees, individuals with autism who were homozygous for deletions of noncoding unique sequence DNA segments adjacent to genes. Of particular interest in their study were large homozygous deletions on chromosome 3q24 adjacent to the NHE9 (SLC9A9) sodium channel gene and large homozygous deletions on chromosome 4q28.3 adjacent to the PCDH10 gene.

The key questions are whether or not a specific mutation or structural genomic dosage change has a functional effect and whether the altered function is causal in the disease.

Altered function may be implied based on the nature of the change or it may be possible to directly measure change. Structural genomic changes are considered candidate genes for a disorder if they occur with higher frequency in individuals with the disorder than in the control population. Furthermore, variants are considered as candidate disease genes if they arise in a gene that plays a role in the function of the key organ involved in the disease, or if they impact function of molecules involved in a physiological pathway known to be altered in the disease. Hence, we have devoted most of this review to genes in pathways that are related to the development, maintenance, and function of synapses, viewed as the most likely site of pathology in autism spectrum disorders.

The role of the X and Y chromosomes in autisms should be more actively considered. PCDH11X and PCDHY remain of interest in this regard. Precise analyses of Y-located genes are often difficult because of the high degree of

homology of X- and Y-related genes. However, efforts to explain consistent gender difference observed in autisms may be very fruitful for increasing our understanding of this complex set of disorders.

It is important to consider whether environmental factors play a role in generating the nuclear and mitochondrial genomic instability we have observed. Such factors may exert their effect preconceptually, prenatally, or during infancy. Others and we have found evidence for such instability in a peripheral tissue, lymphocytes, of subjects with autism. It will be important to examine other tissues.

The changes we observed in mitochondrial DNA may be due to increased concentrations of reactive oxygen species due to metabolic factors or environmental factors. Genetic factors are known to play a role in determining increased propensity to produce reactive oxygen species or to decreased ability to metabolize them. Nuclear and mitochondrial genomic replications are influenced by alterations in the nucleotide pool. The nucleotide pool is impacted by dietary factors and by genetic variation in nucleotide scavenging pathways. Thus, all of these may contribute to the developments of autism spectrum disorders in different individuals, making our search for the etiological factors more difficult.

Understanding of the etiology and pathophysiology of the autisms and autism spectrum disorders will not only lead directly to better treatment of and intervention with these impaired children, but it will also contribute greatly to our basic understanding of the way the brain influences behavior. However, success in the study of these complex disorders best referred to as autisms, rather than autism, will require that we abandon our dependence on limited set diagnostic criteria and turn to systematic studies of the wide range of phenotypic presentations to identify subtypes (endophenotypes). Simultaneously, a move to the study of pathways and multigene clusters for the ultimate identification of subtypes that share a specific genetic etiology will be advantageous.

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Conflicts of Interest

The authors declare no conflicts of interest.

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