

# Epigenetics of autism spectrum disorders

N. Carolyn Schanen<sup>1,2,3,\*</sup>

<sup>1</sup>Center for Pediatric Research, Nemours Biomedical Research, Wilmington, DE 19803, USA, <sup>2</sup>Biological Sciences, University of Delaware, Newark, DE 19716, USA and <sup>3</sup>Jefferson Medical College, Philadelphia, PA 19107, USA

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**The autism spectrum disorders (ASD) comprise a complex group of behaviorally related disorders that are primarily genetic in origin. Involvement of epigenetic regulatory mechanisms in the pathogenesis of ASD has been suggested by the occurrence of ASD in patients with disorders arising from epigenetic mutations (fragile X syndrome) or that involve key epigenetic regulatory factors (Rett syndrome). Moreover, the most common recurrent cytogenetic abnormalities in ASD involve maternally derived duplications of the imprinted domain on chromosome 15q11–13. Thus, parent of origin effects on sharing and linkage to imprinted regions on chromosomes 15q and 7q suggest that these regions warrant specific examination from an epigenetic perspective, particularly because epigenetic modifications do not change the primary genomic sequence, allowing risk epialleles to evade detection using standard screening strategies. This review examines the potential role of epigenetic factors in the etiology of ASD.**

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## INTRODUCTION

It is widely held that the autism spectrum disorders (ASD), including autism, Asperger disorder, childhood disintegrative disorder and pervasive developmental disorder-not otherwise specified (PDD-NOS) are largely genetic in origin, with a polygenic, epistatic model best fitting the family, twin and epidemiological data for non-syndromic forms. The number of interacting loci contributing to susceptibility has been estimated to range between two and 15 genes of varying effect (1,2). Despite considerable effort over the past decade, these underlying risk alleles have been remarkably elusive, with the exception of a handful of rare, large effect genes (3–5) and several single gene disorders associated with an increased risk for autism or ASD (6–11). Although this likely reflects the underlying genetic heterogeneity within and among the diagnostic categories in the ASD, the obstacles encountered in mapping the risk alleles have led a number of investigators to rethink the model of inheritance to include contributions of new mutations and/or epigenetic mechanisms such as genomic imprinting or epimutations in the underlying genetic susceptibility to ASD (12,13).

Epigenetic modifications including cytosine methylation and post-translational modification of histones provide a mechanism for modulation of gene expression that can be influenced by exposure to environmental factors and that may show parent of origin effects. Involvement of epigenetic

factors in ASD is demonstrated by the central role of epigenetic regulatory mechanisms in the pathogenesis of Rett syndrome and fragile X syndrome (FXS), single gene disorders commonly associated with ASD (12,14,15). Rett syndrome, included among the ASD in the Diagnostic and Statistical Manual, IV, is a complex neurological disorder that arises from mutation in the gene that encodes the methyl-CpG-binding protein 2 (MeCP2), one of the key mediators of epigenetic regulation of gene expression (9). As its name implies, MeCP2 binds methylated cytosine residues and interacts with chromatin remodeling complexes to generate repressive chromatin structures of the surrounding DNA (16–18). In contrast, FXS arises through a combination of genetic and epigenetic mutation, wherein expansion of a CGG repeat in the 5'-untranslated region of the *FMR1* gene, renders the region susceptible to epigenetic silencing, resulting in loss of expression of the gene (15).

Genomic imprinting is the classic example of regulation of gene expression via epigenetic modifications that leads to parent of origin-specific gene expression. In addition, a growing number of genes that are not imprinted are regulated by DNA methylation, including Reelin (*RELN*) (19–23), which has been considered as a candidate for autism (24–28). Because DNA methylation can be modified by mutation (10), maternal exposures (29–31) and postnatal experiences (32–34), it provides a tangible link between gene and environment. For example, in the viable yellow agouti mouse strain

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\*To whom correspondence should be addressed at: Nemours Biomedical Research, 1600 Rockland Road, Wilmington, DE 19803, USA. Tel: +1 3026516702; Fax: +1 3026516767; Email: schanen@medsci.udel.edu

**Table 1.** Summary of genome-wide linkage screens in ASD and relationship with known imprinted domains (135)

Cytogenetic location	Linked marker(s)	Position (cM)	Imprinted domain (cM)	References
1p13.1–21.1	D1S1631–D1S1675	136.88–149.2	NR	(2,39)
1p12–25	D1S1677	175.62	NR	(36)
1q21–22	D1S1653	149.2–164.09	NR	(39)
1q23.1–23.2	D1S2624–D1S2771	162.57–168.52	NR	(39)
2p12–13.1	D2S1351	103.04	NR	(43)
2q24.3–31.3	D2S2330–D2S364	169.41–186.21	NR	(46)
2q31–31.3	D2S2314–D2S2310	182.24–185.13	NR	(35)
2q33.1	D2S116–D2S309	198.65	NR	(45)
3p25.3	D3S3691	29.19	NR	(47)
3p25.3	D3S3594–D3S3589	32.36	NR	(136)
3p24–26	D3S3691	29.19	NR	(36)
3q13.12–26.1 <sup>word</sup>	D3S3045–D3S1763	124.16–176.54	NR	(114)
3q25–27	D3S3715–D3S3037	178.9–190.43	NR	(39)
4q21–31	D4S1591	106.89	Yes (~98)	(36,135)
5p13.1	D5S2494	58.91	NR	(37,38)
6q14–21	D6S1021	112.20	NR	(36)
6q16.3	D6S283	109.19	NR	(40)
7q21.3	D7S1813	103.63	Yes (~104–110)	(135,137)
7q21.2–q31.31	D7S2409–D7S480	110.57–125.95	Yes (~104–110)	(41,135)
7q22.3–q31.1	D7S496–D7S2418	119.81–122.48	Yes (~104–110)	(135,138)
7q22.1–22.2 (P > M)	D7S477–D7S2453	111.79–115.96	Yes (~104–110)	(35,135)
7q32.2	D7S530–D7S684	134.55–147.22	Yes (134.5–138)	(43,135)
7q22–q32	N/A	N/A	Yes (134.5–138)	(135,139)
7q32.2–32.3 (M > P)	D7S530–D7S640	134.55–137.83	Yes (134.5–138)	(35,135)
7q32–qter	N/A	N/A	Yes (134.5–138)	(135,139)
7q32.2–32.3 (P > M)	D7S2527–D7S495	128.99–144.72	Yes (134.5–138)	(115,135)
7q33–36.1	D7S483	165.18	Yes (134.5–138)	(36,37,135)
7q34–36.2	D7S1824–D7S3058	149.9–173.71	Yes (134.5–138)	(114,116,135)
7q36.1–36.3	D7S1805–D7S550	161.21–178.41	Yes (134.5–138)	(39,135)
8q22–24	D8S1832	132.49	Yes <sup>a</sup>	(36,135)
9p21.3–22.2 (M > P)	D9S157–D9S171	32.24–42.73	NR	(35)
9p21.3–21.2 (P > M)	D9S171–D9S161	42.73–51.81	NR	(35)
10p12–q11.1	N/A	N/A	NR	(139)
11p11.2–13	D11S1392–D11S1993	43.16–54.09	Yes (2–5 & ~38)	(38,135)
13q12.3	D13S217–D13S1229	17.21–21.51	Yes (~45)	(135,137)
13q21.33	D13S800	55.31	Yes (~45)	(135,137,140)
14q12	D14S80	26.59	NR	(43)
15q11.2–12	<i>GABRB3</i>	9.85	Yes (~6–18)	(74,135)
17p13.3–q21.1 <sup>phrase</sup>	D17S1298–D17S1299	10.72–62.01	NR	(114)
17p12–q21	D17S1294	50.74	NR	(36)
17p11.2–q11.1	N/A	N/A	NR	(139)
17q11.2	D17S1800	51.63	NR	(38)
17q11.2	D17S1294	50.74	NR	(47)
17q11–21 <sup>male only</sup>	D17S1880–D17S2180	53.41–66.85	NR	(141)
17q21.2	D17S1299	62.01	NR	(47)
17q23.1–25.2 <sup>word</sup>	D17S1290–D17S1301	82.0–100.02	NR	(114)
19p13.11–q13.1	D19S930–D19S113	44.41–56.69	Yes (~100)	(47,135)
19q12 <sup>b</sup>	D19S433	51.88	Yes (~100)	(37,135)
Xp11.3–q21.33	DXS6810–DXS6789	42.75–62.52	NR	(39)
Xq25	DXS1047	82.07	NR	(37)

Loci with lod scores over 2.0 are shown and parent of origin effects on sharing are noted as are endophenotypes included in the analyses. These include items from the Autism Diagnostic Interview—Revised: age at first word (word), age at first phrase (phrase) and gender of the affected sib-pair (male only). Markers at or bounding the linkage peak are noted and genetic positions in cM are relative to the Marshfield map (Center for Medical Genetics, Marshfield Medical Research Foundation). NR, none recorded; NA, not applicable. Reference is a meta-analysis of linkage data.

<sup>a</sup>Region of conserved synteny on mouse chromosome 15 contains imprinted *Peg13* gene but no human ortholog is known (142).

<sup>b</sup>Reference notes position at 19p but the marker mapped to 19q on current genome build. Imprinted loci are positioned relative to the nearest Marshfield markers and noted if they are <50 cM from the area of the linkage peak.

(*A<sup>vy</sup>*), coat color ranges from yellow to agouti based on the degree of methylation of an IAP element that lies in the promoter of the *Agouti* locus. The coat color trait is incompletely heritable and influenced by the mother's and not by the father's epigenotype (31). Moreover, modification of the maternal diet during gestation affects methylation of

the locus, reflected in variations in coat color in genetically identical mice (29,31).

In autism, the primary strategies for identifying susceptibility alleles have revolved around genome-wide and targeted analyses of allele sharing in sib-pairs, using association studies and transmission disequilibrium testing (TDT) of functional

and/or positional candidate genes and close scrutiny of novel and recurrent cytogenetic aberrations. A convergence of data from these studies implicate several genomic regions known to be subject to imprinting and the observation of parent of origin effects on allele sharing for markers or candidate genes as well as the potential for gene–environment interactions that could explain some of the enigmatic features of autism genetics make this notion particularly attractive. Moreover, because disruption of gene expression via epigenetic mechanisms is not reflected in the primary nucleotide sequence, epialleles may evade detection by standard mapping strategies. In this review, recent advances in the genetics of ASD are discussed with a focus on the emerging evidence for a role of epigenetic factors in susceptibility to autism and ASD.

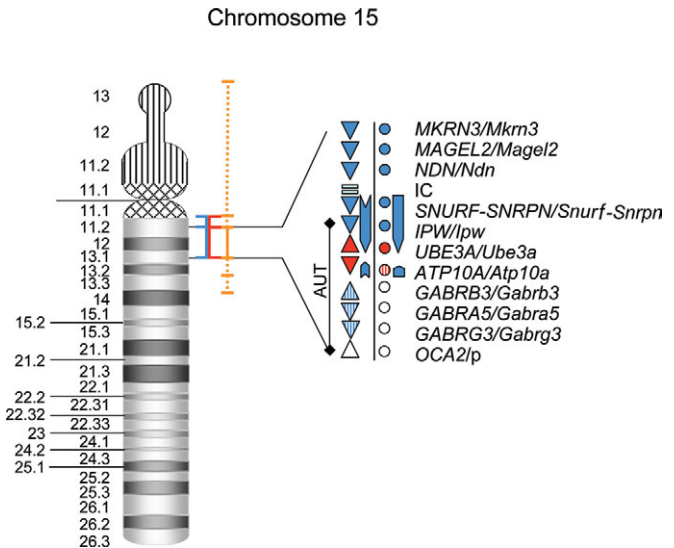
### EPIGENETIC HOTSPOTS IN AUTISM: CHROMOSOMES 15Q AND 7Q

Genome-wide scans using affected sib-pairs with autism or ASD reveal suggestive or possible linkage to numerous chromosomes, with loci on 1p, 2q, 3p, 7q, 15q and 17q showing the strongest evidence based on replication in independent patient cohorts (2,35–47) (Table 1). Curiously, several of the linkage peaks overlap or are in close proximity to regions that are subject to genomic imprinting on chromosomes 15q11–13, 7q21–31.31, 7q32.3–36.3 and possibly 4q21–31, 11p11.2–13 and 13q12.3, with the loci on chromosomes 15q and 7q demonstrating the most compelling evidence for a combination of genetic and epigenetic factors that confer risk for ASD.

### GROWING EVIDENCE FOR A ROLE FOR CHROMOSOME 15Q IN ASD: THE DUPLICATION CHROMOSOME 15 SYNDROME

Duplications of chromosome 15q11–13 are the only commonly recurrent cytogenetic aberration associated with ASD and occur in up to 5% of patients with ASD (48–50). These rearrangements primarily take two forms: an interstitial duplication [int dup(15)] or a supernumerary pseudodicentric chromosome 15 [idic(15)] and involve the imprinted genomic region that is deleted in Prader Willi syndrome (PWS) and Angelman syndrome (AS). In addition to ASD, the phenotype of the dup(15) syndrome includes variable degrees of cognitive impairment, motor delays, seizures and dysmorphic facial features, which may be subtle (48,50–53). Like PWS and AS, parent of origin effects on phenotypic expression are evident (48), and it is the maternally derived duplications that confer a high risk of ASD (>85%). In contrast, rare reports of paternally derived duplications suggest that they lead to mild developmental and cognitive deficits (48,52,54,55), with only one case reported who had PDD-NOS in conjunction with borderline mental retardation, which did not co-segregate with the interstitial duplication in the family (56).

The maternal origin of the duplicated chromosome in autistic patients with dup(15) syndrome focuses attention to the maternally expressed genes, although it is likely that misexpression of



**Figure 1.** Ideogram of chromosome 15 showing the imprinted domain. The direction of transcription is designated by the orientation of the triangle for the human genes. Maternal expression is indicated by red fill and paternal expression by blue fill. Those with conflicting or preliminary data are shown as striped. White fill indicates genes that are not imprinted and gray indicates that the imprinting status has not been determined. The complex *SNURF-SNRPN* *UBE3A* antisense transcript and *ATP10A* antisense transcripts are designated by the broad arrows to the right of the gene symbols. Circles represent the murine orthologs and their imprinting status is indicated by color. The vertical bar to the right of the ideogram represents the region deleted in AS (red) and PWS (blue) deletions. The orange bar represents the region involved in the duplications, with the solid line indicating the common region that predisposes to ASD in both interstitial duplications and idic(15) chromosomes. The dashed line represents regions that may be included in the duplications. The autism region is indicated next to the gene abbreviations. IC, imprinting center.

both imprinted and biallelically expressed genes contribute (57) (Fig. 1 and Table 2). Regulation of gene expression for this segment of the genome is particularly complex and involves differential methylation and expression of antisense and non-coding RNAs (58). A number of genes are subject to tissue and/or developmentally regulated monoallelic expression including at least two genes that are expressed preferentially from the maternal chromosome in brain: *UBE3A*, the gene that causes AS, and *ATP10A*, which lies 200 kb telomeric to *UBE3A* and encodes a putative human aminophospholipid-transporting ATPase (59). Within the duplicated segment in both idic(15) and int dup(15), imprinting is maintained and transcription of the additional maternal copies of *UBE3A* (60) translates into increased levels of the gene product, the E6-AP ubiquitin protein ligase, with a commensurate disturbance of ubiquitin-mediated protein turnover (57).

Notably, the commonly duplicated segment also encompasses several paternally expressed genes that might be considered functional candidates for ASD. These include two genes that are expressed in brain and encode proteins important for neuronal development, *NECDIN* (*NDN*) (61–63) and *MAGE*-like 2 (*MAGEL2*) (64–67). *NDN* is expressed in post-mitotic neurons and plays a critical role in the specification of inhibitory neurons in the brain via interaction with distal-less 5 (*DLX5*), the product of a maternally expressed gene that lies

**Table 2.** Genes in the imprinted cluster on chromosome 15q11–13

Gene	Imprinting status: human/mouse	Expressed allele: human/mouse	Functional relevance to autism or ASD	References
<i>MKRN3</i>	I/I	Pat/pat	Ubiquitously expressed, single exon gene encoding a RING Zinc finger protein. It is contained within the intron–exon of a paternally expressed gene ( <i>ZNF127AS</i> ) that is transcribed from the antisense strand	(143,144)
<i>ZNF127AS</i>	I/I	Pat/pat	Antisense transcript for <i>MKRN3</i>	(143,144)
<i>MAGEL2</i>	I/I	Pat/pat	Single exon gene expressed in brain, particularly strong in the hypothalamus. Has homology to necdin but differentially binds necdin interacting proteins at centrosomal regions. Important in PWS	(64,65,145,146)
<i>NDN</i>	I/I	Pat/pat	Encodes a nuclear protein expressed in postmitotic neurons that acts as a growth suppressor and promotes neurite outgrowth. Interacts with other proteins in centrosomal regions. Promotes GABAergic neuronal differentiation. Important in neurological dysfunction in PWS	(61–63,68,145,146)
<i>SNRPN-SNURF</i>	I/I	Pat/pat	This long, complex transcript encodes the small nucleolar RNA-binding protein N as well as a group of small nucleolar RNAs	(66,70,147)
<i>UBE3A-AS</i>	I/I	Pat/pat	<i>UBE3A</i> antisense transcript	(70)
<i>UBE3A</i>	I/I	Mat/mat	Encodes the E6-AP ubiquitin protein ligase. Loss of function leads to AS. Abnormal expression seen in brain in ASD and in lymphoblasts from dup(15) patients. Linkage to this gene has been detected in ASD but no mutations identified in a small group of subjects	(12,13,57,60,75,76)
<i>ATP10A</i>	I/I	Mat/mat	Encodes an aminophospholipid translocase, which actively transports phosphatidylserine and phosphatidylethanolamine across the cell membrane. Expressed in hippocampus and olfactory bulb. Linkage to this gene has been detected in ASD but no mutations identified in a small cohort of subjects	(59,76,77,148)
<i>GABRA5</i>	CD/NI	Pat/ni	Encodes the alpha 5 subunit of the GABA <sub>A</sub> receptor. <i>GABRA5</i> containing receptors mediate tonic inhibition in hippocampal neurons. Knockout of this subunit in mice leads to enhanced learning and memory	(78,91,149,150)
<i>GABRB3</i>	CD/NI	Pat/ni	Encodes the beta 3 subunit of the GABA <sub>A</sub> receptor. Linkage and association studies of this gene in ASD have given mixed results. This gene was mis-expressed in brain in ASD and Rett syndrome. Knockout is associated with cleft palate and neurological abnormalities and frequent early lethality	(12,78–81,91,92,151–153)
<i>GABRG3</i>	CD/NI	Pat/ni	Encodes the gamma 3 subunit of the GABA <sub>A</sub> receptor. Linkage and association of this gene in ASD have had mixed results, largely negative. Knockout of this gene does not have an overt phenotype	(78,91,93,152,154,155)

I, imprinted; NI, not imprinted; CD, conflicting data.

on chromosome 7q that has also been implicated in ASD (discussed subsequently) (68). *MAGEL2* is an intronless gene located in close proximity to the *NDN* locus. It is transcribed from the paternal allele and expressed predominantly in hypothalamus, making it a strong candidate for the eating disorder in PWS (64–66). Although these genes are strong candidates in PWS and potential functional candidates in ASD, it is difficult to rectify the apparent conflict between the consequences of additional copies of paternally expressed genes and the maternal-origin of the autism phenotype in dup(15) syndrome. However, given the complexities of regulation of gene expression in this region, it is possible that duplications on the maternal chromosome indirectly lead to misexpression of paternal genes by interfering with proper pairing (69) or by imbalances between sense and antisense transcripts (70).

Recent studies of autistic behaviors among patients with PWS arising from maternal uniparental disomy (UPD) compared with those with paternal deletions support the contribution of imprinted and non-imprinted genes on chromosome 15q in the development of ASD. Although patients with maternal UPD have significantly higher scores on measures of autistic behaviors, the frequency of ASD among these patients does not approach that seen in dup(15) syndrome (71–73). Because maternal UPD results in

expression of both copies of the maternally expressed genes but does not interfere with expression of non-imprinted genes in the region, this argues that, indeed, the maternally expressed transcripts contribute to the ASD susceptibility in dup(15) syndrome but are not, in and of themselves, sufficient to cause ASD. Consistent with this idea, genome-wide expression profiling using lymphoblasts from dup(15) patients identified over 100 consistently dysregulated transcripts, most of which arose from genes outside the duplication (57). Thus, even in dup(15) syndrome, it appears that the autism phenotype is an incompletely penetrant, multigenic trait.

### CHROMOSOME 15Q: EVIDENCE FOR A ROLE IN AUTISM IN CYTOGENETICALLY NORMAL PATIENTS

Perhaps surprisingly, genome-wide screens have not consistently identified evidence for linkage to chromosome 15 in ASD cohorts, although application of targeted high-density screening strategies or phenotypic subgroupings has defined a region of interest that extends from the PWS/AS critical region through the cluster of genes encoding  $\gamma$ -aminobutyric acid (GABA) receptor subunits (49,51,74–84). The numerous imprinted and biallelically expressed genes that lie in this

region are thus positional candidates, with the genes encoding GABA receptor subunits (*GABRB3*, *GABRA5*, *GABRG3*) of particular interest in ASD because of their function in the nervous system.

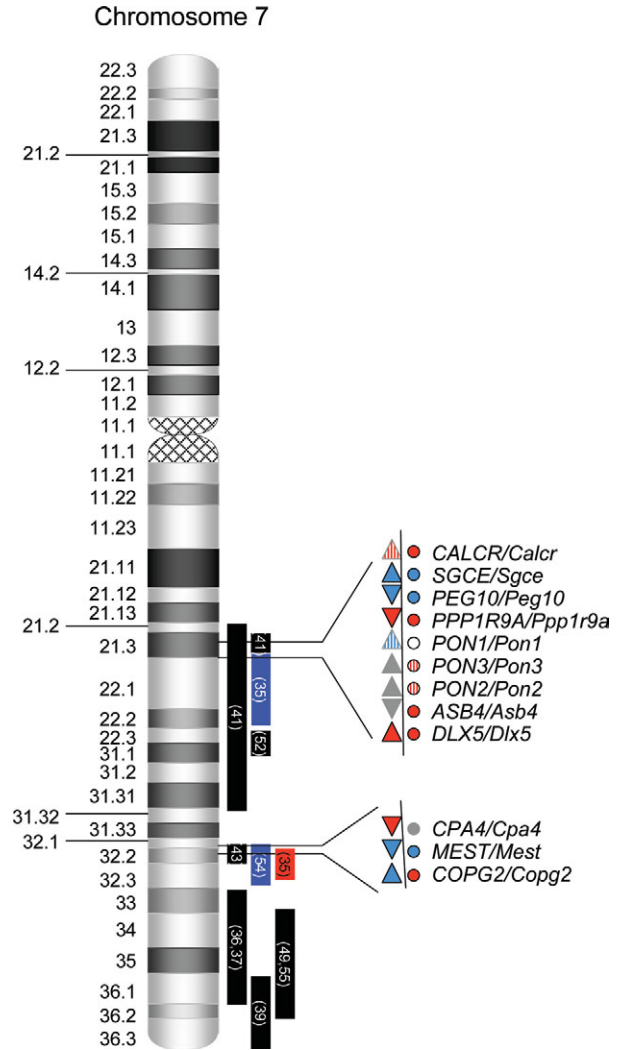
Both of the maternally expressed genes that lie toward the centromeric end of the candidate region have been specifically investigated in ASD. Using high-density, targeted screening strategies, linkage disequilibrium was detected at both the *UBE3A* and *ATP10A* loci in families with autism (75,76); however, mutation screening in two small cohorts of autistic patients did not identify clearly pathological mutations in these genes (77,85). Subsequent studies in postmortem brain samples from patients with autism revealed abnormalities in methylation of the *UBE3A* CpG island (13) as well as decreased *UBE3A*/E6-AP expression in autism, AS and Rett samples (12,13), suggesting that *UBE3A* misexpression may be a common mechanism for these phenotypically related disorders.

Similarly, the GABA receptor gene cluster (*GABRB3*, *GABRA5*, *GABRG3*), which lies toward the telomeric end of the ASD candidate region, has also received considerable attention. As the principal inhibitory neurotransmitter, GABA is a key regulator of excitability in the mammalian central nervous system. GABA receptor expression is regulated both regionally and developmentally and abnormalities in receptor expression have been detected in autistic brains (12,86–88). Disruption of development of GABAergic interneurons in mice leads to complex neurodevelopmental effects with similarity to ASD including deficits in socialization, seizures and anxiety (89). Further, autism and ASD are common features in succinic acid dehydrogenase deficiency, a rare autosomal recessive disorder that arises from loss of the enzyme that metabolizes GABA to succinic acid (90). Together, these data suggest that imbalances in GABAergic function may be a central deficit in the neurobiology of autism and ASD.

From the genetic standpoint, several studies identified evidence for linkage or association with the GABA receptor genes on chromosome 15q in autism samples, particularly *GABRB3* (49,78,79,83,91–93); although as commonly occurs in this field, not all groups replicated the finding (81,94). Recent studies of this group of genes in ASD focused on identification of potential interactions among alleles in different GABA receptor genes using a complex modeling system designed to reveal epistatic relationships. Although there was no strong evidence for interaction among the three genes on chromosome 15q12(99), extension of these analyses to GABA receptor genes on other chromosomes yielded significant results with positive associations between alleles for *GABRA4* and *GABRB1* on chromosome 4p and autism (93). This region has been previously implicated by linkage (43) and by the identification of chromosome rearrangements involving 4p12 in patients with autism (95,96).

**Imprinting and epigenetics of chromosome 7q in ASD**

One of the first regions identified in linkage studies in ASD encompassed most of the long arm of chromosome 7, and subsequent analyses indicated that this likely reflects linkage two



**Figure 2.** Ideogram of chromosome 7 showing the positions of the linkage peaks and imprinted domains. The direction of transcription is designated by the orientation of the triangle for the human genes. Maternal expression is indicated by red fill and paternal expression by blue fill. Those with conflicting or preliminary data are shown as striped. White fill indicates genes that are not imprinted and gray indicates that imprinting status is not known. Circles represent the murine orthologs and their imprinting status is indicated by color. The vertical bars represent the linkage peaks in genome-wide screens in ASD, with red and blue fill corresponding to increased maternal (red) or paternal (blue) sharing for the linkage peak.

(or more) susceptibility loci on chromosome 7q (Fig. 2). The more centromeric linkage peak(s) lie at 103.63–125.95 cM on 7q21–31.31. Using parent of origin linkage modeling, Lamb *et al.* identified a region of excess paternal sharing with a maximum lod score of 1.46 for identity by descent for paternal alleles at ~112 cM (35), near an imprinted gene cluster (~105–110 cM) in a region of conserved synteny with mouse chromosome 6 (97) (Table 3). In humans, the cluster includes two paternally expressed genes, *SGCE* (encoding sarcoglycan epsilon) and the retro-transposon derived paternally expressed gene 10 (*PEG10*), both of which were found to be binding targets for Mecp2 in mouse (98). Additionally, the paraoxonase 1 gene (*PON1*), which has

**Table 3.** Genes in the imprinted cluster on chromosome 7q21.3

Gene	Imprinting status: human/mouse	Expressed allele: human/mouse	Relevance to autism or ASD	References
<i>SGCE</i>	I/I	Pat/pat	Paternally derived mutations in <i>SGCE</i> lead to myoclonus-dystonia syndrome (DYT11, OMIM: 159900), which can be associated with obsessive-compulsive disorder and panic attacks	(156,157)
<i>PEG10</i>	I/I	Pat/pat	From overlapping reading frames, two proteins are generated that inhibit signaling from the transforming growth factor- $\beta$ (TGF- $\beta$ ) Type 1 receptor, activin receptor like kinase 1 (Alk1). The transcript is abundant in brain and overexpression of <i>PEG10</i> and Alk1 causes fibroblasts to acquire a neuronal-like morphology	(158,159)
<i>PPP1R9A</i>	I/I	Mat/mat	The protein phosphatase complex 1 collaborates with neurabin in dendritic development and maturation, and disruption of this system alters surface expression of glutamate receptors in hippocampal neurons	(160,161)
<i>DLX5</i>	I/I	Mat/mat	<i>DLX5</i> encodes a transcription factor, Dlx5, which is developmentally expressed and acts as critical mediator for the differentiation of GABAergic neurons in the forebrain. It shows complex regulation in concert with the adjacent <i>DLX6</i> gene that is regulated in part via MeCP2, the protein that is deficient in Rett syndrome	(68,98, 162–164)
<i>CALCR</i>	PD/I	Mat/mat	G-protein coupled receptor for calcitonin, involved in calcium metabolism	(102)
<i>ASB4</i>	NR/I	Un/mat	Expressed by the pro-opiomelanocortin and neuropeptide Y expressing neurons in the hypothalamus. Hypothesized to be a component of the central energy homeostatic circuit	(165)
<i>PON1</i>	PD/NI	Pat/ni	Developmentally regulated HDL-associated serum enzyme that hydrolyzes organophosphate pesticides and protects HDL from oxidation. Low levels of serum activity are associated with atherosclerosis. Investigation of SNPs in this gene in ASD have yielded mixed results. In a small cohort of subjects, serum activity was low in ASD	(100–103, 166–169)
<i>PON2</i>	NR/PD	Un/mat	Similar in function to <i>Pon1</i> but not associated with HDL and has cellular antioxidant activity. Potentially imprinted expression in mouse placenta	(107,167,168)
<i>PON3</i>	NR/PD	Un/mat	HDL associated, similar in function to <i>Pon1</i> . Potentially imprinted expression in mouse placenta	(107,167,168)

I, imprinted; NI, not imprinted; CD, conflicting data; NO, no ortholog; PD, preliminary data.

been examined as a candidate gene in ASD (99–101), was expressed preferentially from the paternal chromosome in human:mouse somatic cell hybrids (102). This gene has been extensively evaluated in cardiovascular disease and no evidence for parent of origin effects on allele transmission has been detected in humans or mice (103,104), arguing against imprinting *in vivo*.

Two genes are maternally expressed, *PPP1R9A* (protein phosphatase 1, regulatory subunit 9A) and *DLX5* (distal-less homeobox transcription factor 5), and there is evidence for maternal expression of the calcitonin receptor (*CALCR*) gene in both humans (102) and mice (105). Additionally, three other genes have some evidence for tissue-specific maternal expression in mice, but their imprinting status is not known in humans. These include the *Asb4* gene (Ankyrin repeat and SOCS box containing 4) (106) and two other paraoxonase paralogs, *Pon2* and *Pon3*, which were shown to be maternally expressed only in murine extraembryonic tissues (107).

*DLX5* has been examined as a candidate gene for ASD. *DLX5* is a particularly attractive candidate that is a member of a gene family that encodes a group of homeobox transcription factors that play critical roles in development of the nervous system. *DLX5* and *DLX2* directly regulate expression of glutamic acid decarboxylase, the enzyme that produces the neurotransmitter GABA (108). As such, these proteins are central to the specification and function of inhibitory GABAergic neurons in the mammalian forebrain, a population of

neurons that have been implicated in the pathogenesis of autism (109,110). The *DLX5* gene lies in a tail-to-tail configuration with another family member, *DLX6*, and on chromosome 2q, the *DLX1* and *DLX2* genes are similarly oriented and also in proximity to a region of interest in autism. An ultraconserved intergenic enhancer region that lies at the 3' end of the associated genes coordinates tissue specific and developmental expression of the gene pairs (111), and *Mecp2*, the protein that is deficient in the ASD, Rett syndrome, participates in the process (98). Because of the central role of the *DLX* proteins in GABAergic systems and the location of the gene pairs under ASD linkage peaks, Hamilton *et al.* examined the *DLX1/2* and *DLX5/6* pairs in a cohort of 161 patients with autism and 378 controls using a direct sequencing approach. A number of novel sequence variants were identified in both groups; however, none was *de novo* and examination of the segregation of the variations within and between families did not provide conclusive evidence supporting the genes as susceptibility alleles (112). Similarly, a recent study of the ultraconserved regions on chromosome 7q, including those surrounding *DLX5*, did not identify sequence variations that could be clearly associated with ASD (113). In both studies, the authors cautiously concluded that these loci could not be definitively associated with autism, although they each identified sequence variants that potentially affected expression or function of these genes. With this in mind and given the role for *MeCP2* and chromatin-based regulation for the *DLX5/DLX6*

**Table 4.** Genes in the imprinted cluster on chromosome 7q32.2

Gene	Imprinting status: human/mouse	Expressed allele: human/mouse	Functional relevance to autism or ASD	References
<i>CPA4</i>	I/NR	Mat/un	Metalloproteinase initially identified in prostate cancer cell line. Transcript is upregulated by histone deacetylase inhibitors	(117,170–173)
<i>MEST</i>	I/I	Pat/pat	Alternatively spliced transcript shows isoform-specific imprinting. Dysregulation of <i>MEST</i> expression alters cell growth and female <i>Mest</i> $-/-$ mice have impaired maternal behaviors	(117,170,174–178)
<i>MESTIT1</i>	I/NO	Pat/no	<i>MEST</i> antisense intronic transcript expressed in testis	(174,178,179)
<i>COPG2</i>	I/I	Pat/mat	The $\gamma 2$ subunit of the coatamer protein complex is involved in trafficking of proteins between the endoplasmic reticulum and Golgi. This subunit has been shown to directly associate with dopamine receptors	(170,171,178,180,181)
<i>COPG2IT1</i>	I/I	Pat/pat	<i>COPG2</i> intronic transcript	(180,181)

I, imprinted; NI, not imprinted; CD, conflicting data; NO, no ortholog; PD, preliminary data.

pair (98), however, it would be of interest to re-evaluate these genes from an epigenetic perspective.

On the distal long arm of chromosome 7 lies another region that has been linked to autism by several groups (35–37,39,43,114–116). Overlap of the linkage peaks suggests the possibility of two loci, one centered on 7q32.2 and the other on 7q35–36.2. Of note, two studies have detected parent of origin effects on sharing of alleles at chromosome 7q32.2, which encompasses another imprinted domain that includes one maternally expressed and four paternally expressed genes (Table 4). *CPA4*, which encodes a secreted metalloproteinase, is the only maternally expressed gene that has been identified in this region and is not an obvious functional candidate for autism. The four paternally expressed transcripts include (i) mesodermally expressed transcript (*MEST*); (ii) *MESTIT1*, which generates an antisense transcript from common promoter; (iii) the  $\gamma 2$  subunit of the coatamer protein complex (*COPG2*) and (iv) an intronic, antisense transcript from this gene (*COPG2IT1*). In a study that was the first of its kind, this gene cluster was examined from an epigenetic perspective in a subset of 46 autism families selected from the International Molecular Genetics Study of Autism Collaboration (IMGSAC) sample based on allele sharing for markers within the 7q32 region. Using sequence and TDT analyses, they did not detect evidence for pathological mutations nor did they detect differences in allele transmissions for *CPA1*, *CPA5*, *MEST* and *COPG2*. They extended the studies to examine potential epigenetic mutations using a Southern blot based strategy to examine the differentially methylated regions in *MEST* and *COPG2* and also examined replication timing across the region. Again, no abnormalities were detected in the autism group (117). Similar to the investigation of the *DLX* genes, the authors concluded that these data indicate that these genes are unlikely contributors to the etiology of autism. Nonetheless, it remains possible that epimutations are occurring in a tissue-specific manner or that important methylation sites were not detected using the Southern approach. Given the recurrence of linkage to this region and the parent of origin effect on sharing, further study of these genes is warranted including expression analysis and a thorough examination of methylation.

### Imprinting and the X chromosome

One of the truly enigmatic aspects of autism genetics has been rectifying the basis of the gender bias of these disorders, which show a roughly 4-fold excess of affected males across the ASD population. This gender bias is maintained even with exclusion of patients with mutations in known X-linked genes that can cause ASD (*FMRI* and *MECP2*), although it becomes less apparent in cohorts with more severe cognitive impairment. Although the most obvious explanation is involvement of an X-linked gene of major effect, the relative rarity of clearly X-linked pedigrees and lack of consistent linkage to the X chromosome suggest that the mechanism is more complex. Like ASD, many complex traits show gender differences in frequency, implying a difference in susceptibility that may be genetic and/or endocrine in origin. In a classic multigenic epistatic model for transmission, one would predict a higher rate of recurrence among families of probands of the less affected gender who presumably carry an increased number of susceptibility alleles. Family-based studies in ASD have consistently failed to support this prediction, however, with similar recurrence risks and an excess of affected males among relatives of male and female probands.

In 1997, Skuse *et al.* (118,119) proposed an intriguing hypothesis involving epigenetic mechanisms to explain the gender bias in ASD based on his work on social cognition and executive function in females with Turner syndrome (45,X). In a study of 80 females with monosomy X, the parent of origin of the X-chromosome impacted performance on measures of social cognition with better performance by females with paternally derived X chromosomes (118). The investigators proposed the existence of an imprinted locus, which they tentatively mapped to Xq or proximal Xp, that increased social behaviors in females and hence, conferred protection to females against ASD (and other disorders affecting social and language skills). In this model, the locus is silenced on the maternally derived X chromosome and thus not expressed in males, rendering them more vulnerable to impairments in social and communication skills (120–123). Consistent with this model, karyotypically normal females performed better on these measures than normal males and autism and/or ASD have been reported

in females with 45,X<sup>mat</sup> Turner syndrome (123) and Xp deletions (124).

This model has been somewhat controversial, in part, because the parent of origin effect on behavior has not been observed by other investigators (125), but also because of difficulties in mapping the locus. Thus, the recent discovery of a cluster of imprinted genes on the mouse X chromosome has generated considerable interest (126,127). These genes, *XI3b*, *XI4b* and *XI4c* are maternally expressed, escape X inactivation and lie in a region of conserved synteny with human chromosome Xq28, although human orthologs have not been identified. Notably, the model had predicted a gene that was expressed from the paternal X chromosome; however, it remains to be seen whether these genes directly contribute to behavior in mice and whether there are human counterparts that are similarly imprinted. Nonetheless, this discovery opens the door toward understanding whether there is indeed a role for one or more imprinted X-linked genes underlying the gender bias in ASD.

## CONCLUSION

The past few years have seen advances in our understanding of the genetic basis of autism and ASD, with the discovery of mutations in genes of major effect including *NLGN3* and *NLGN4* (5) and *CACNA1C*, which encodes the alpha-1c subunit of the type I voltage-dependent calcium channel (7). Although mutations in these genes may not be common among the autistic population (4,8,128–131), they shed light on pathways that are important in the ASD. Moreover, a growing body of data implicate a central role for GABAergic systems in these disorders (12,80,87–89,92,93,96,132–134). Additionally the role of epigenetic factors in the basic etiology of FXS and Rett syndrome, two single gene disorders associated with autism and ASD, clearly indicates that proper regulation of gene expression via epigenetic mechanisms is critical for development of the neural circuits involved in social behaviors, language and cognition in humans; the parent of origin effect on chromosome 15q duplications indicates that one (or more) of the imprinted genes in the region participate in the susceptibility to ASD associated with this disorder.

Methylation of DNA not only serves to mediate repression of gene expression in imprinted domains, but also provides a mechanism through which environmental factors can have long-lasting effects on the genome. How imprinting or methylation-based regulation of gene expression contribute to the loci that confer autism susceptibility remains to be seen. Because the standard approaches used in genome mapping do not assay DNA methylation, a risk conferred by variation of an epiallele would not be detected by sequence-based strategies. Thus, as the field continues to progress, with refinement of the areas of recurrent linkage by increasing sample sizes and the application of high resolution SNP typing and HapMap strategies in study of the ASD, it will be prudent to keep in mind the importance of epigenetic modifications in the regions of interest and to develop high throughput approaches to screen samples of adequate size to definitively determine their role in ASD.

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