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Epigenetics of autism spectrum disorders

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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.

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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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^{word}	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[a]	(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	*GABRB3*	9.85	Yes (∼6–18)	(74,135)
17p13.3–q21.1^{phrase}	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$^{male\ only}$	D17S1880–D17S2180	53.41–66.85	NR	(141)
17q21.2	D17S1299	62.01	NR	(47)
17q23.1–25.2^{word}	D17S1290–D17S1301	82.0–100.02	NR	(114)
19p13.11–q13.1	D19S930–D19S113	44.41–56.69	Yes (∼100)	(47,135)
19q12[b]	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.
[a]Region of conserved synteny on mouse chromosome 15 contains imprinted *Peg13* gene but no human ortholog is known (142).
[b]Reference notes position at 19p but the marker mapped to 19q on current genome build. Imprinted loci are positioned relative to the nearest Marshfiield markers and noted if they are <50 cM from the area of the linkage peak.

(A^{vy}), 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

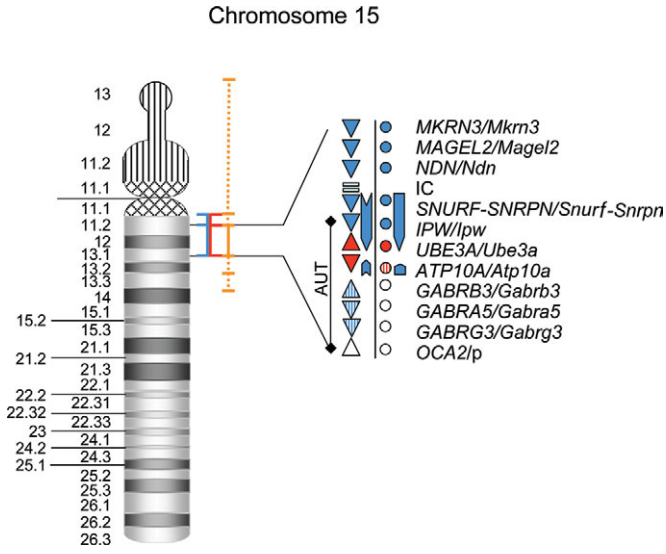

Figure 1. Ideogram of chromosome 15 showing the imprinted domain. The direction of transcription is designated by the orientation of the triangle for the human genes. Maternal expression is indicated by red fill and paternal expression by blue fill. Those with conflicting or preliminary data are shown 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 noncoding 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 postmitotic 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
MKRN3	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 (*ZNF127AS*) that is transcribed from the antisense strand	(143,144)
ZNF127AS	I/I	Pat/pat	Antisense transcript for *MKRN3*	(143,144)
MAGEL2	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)
NDN	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)
SNRPN-SNURF	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)
UBE3A-AS	I/I	Pat/pat	*UBE3A* antisense transcript	(70)
UBE3A	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)
ATP10A	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)
GABRA5	CD/NI	Pat/ni	Encodes the alpha 5 subunit of the $GABA_A$ receptor. GABRA5 containing receptors mediate tonic inhibition in hippocampal neurons. Knockout of this subunit in mice leads to enhanced learning and memory	(78,91,149,150)
GABRB3	CD/NI	Pat/ni	Encodes the beta 3 subunit of the $GABA_A$ receptor. Linkage and association studies of this gene in ASD have given mixed results. This gene was misexpressed 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)
GABRG3	CD/NI	Pat/ni	Encodes the gamma 3 subunit of the $GABA_A$ 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 γ-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

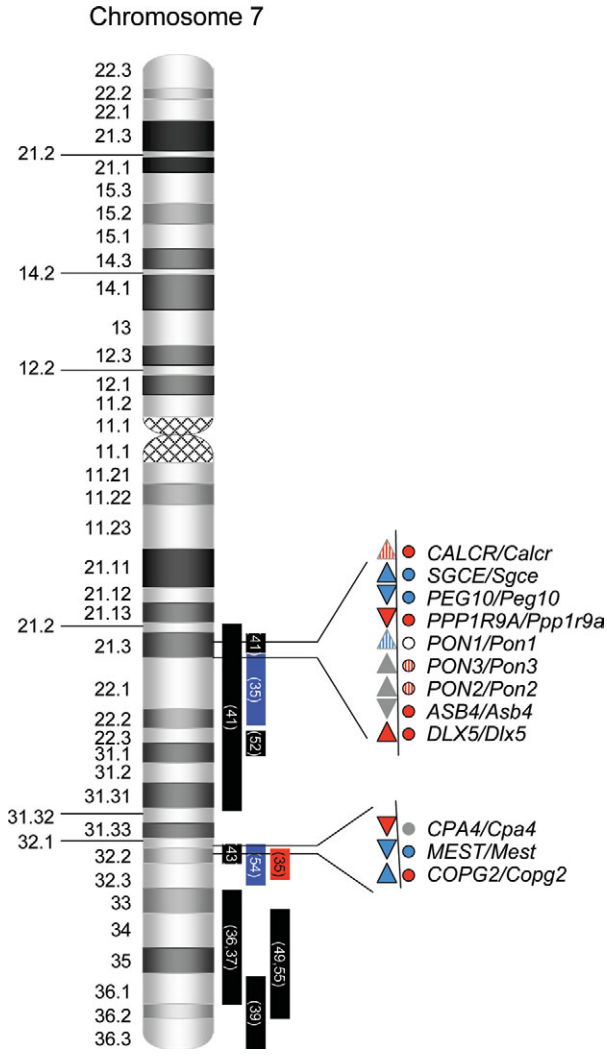

Figure 2. Ideogram of chromosome 7 showing the positions of the linkage peaks and imprinted domains. The direction of transcription is designated by the orientation of the triangle for the human genes. Maternal expression is indicated by red fill and paternal expression by blue fill. Those with conflicting 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
SGCE	I/I	Pat/pat	Paternally derived mutations in SGCE lead to myoclonus-dystonia syndrome (DYT11, OMIM: 159900), which can be associated with obsessive–compulsive disorder and panic attacks	(156,157)
PEG10	I/I	Pat/pat	From overlapping reading frames, two proteins are generated that inhibit signaling from the transforming growth factor-β (TGF-β) Type 1 receptor, activin receptor like kinase 1 (Alk1). The transcript is abundant in brain and overexpression of PEG10 and Alk1 causes fibroblasts to acquire a neuronal-like morphology	(158,159)
PPP1R9A	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)
DLX5	I/I	Mat/mat	*DLX5* encodes a transcription factor, Dlx5, which is developmentally expressed and acts as critical mediator for the differentiation of GABergic neurons in the forebrain. It shows complex regulation in concert with the adjacent *DLX6* gene that is regulated in part via MeCP2, the protein that is deficient in Rett syndrome	(68,98, 162–164)
CALCR	PD/I	Mat/mat	G-protein coupled receptor for calcitonin, involved in calcium metabolism	(102)
ASB4	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)
PON1	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)
PON2	NR/PD	Un/mat	Similar in function to Pon1 but not associated with HDL and has cellular antioxidant activity. Potentially imprinted expression in mouse placenta	(107,167,168)
PON3	NR/PD	Un/mat	HDL associated, similar in function to Pon1. 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
CPA4	I/NR	Mat/un	Metallocarboxypeptidase initially identified in prostate cancer cell line. Transcript is upregulated by histone deacetylase inhibitors	(117,170–173)
MEST	I/I	Pat/pat	Alternatively spliced transcript shows isoform-specific imprinting. Dysregulation of MEST expression alters cell growth and female *Mest* −/− mice have impaired maternal behaviors	(117,170,174–178)
MESTIT1	I/NO	Pat/no	*MEST* antisense intronic transcript expressed in testis	(174,178,179)
COPG2	I/I	Pat/mat	The γ2 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)
COPG2IT1	I/I	Pat/pat	*COPG2* 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 metallocarboxypeptidase, 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 γ2 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 (*FMR1* 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^{mat} 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, *Xl3b*, *Xl4b* and *Xl4c* 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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REFERENCES

1. Pickles, A., Bolton, P., Macdonald, H., Bailey, A., Le Couteur, A., Sim, C.H. and Rutter, M. (1995) Latent-class analysis of recurrence risks for complex phenotypes with selection and measurement error: a twin and family history study of autism. *Am. J. Hum. Genet.*, **57**, 717–726.
2. Risch, N., Spiker, D., Lotspeich, L., Nouri, N., Hinds, D., Hallmayer, J., Kalaydjieva, L., McCague, P., Dimiceli, S., Pitts, T. *et al*. (1999) A genomic screen of autism: evidence for a multilocus etiology. *Am. J. Hum. Genet.*, **65**, 493–507.
3. Jamain, S., Quach, H., Betancur, C., Rastam, M., Colineaux, C., Gillberg, I.C., Soderstrom, H., Giros, B., Leboyer, M., Gillberg, C. *et al*. (2003) Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nat. Genet.*, **34**, 27–29.
4. Vincent, J.B., Kolozsvari, D., Roberts, W.S., Bolton, P.F., Gurling, H.M. and Scherer, S.W. (2004) Mutation screening of X-chromosomal neuroligin genes: no mutations in 196 autism probands. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, **129**, 82–84.
5. Laumonnier, F., Bonnet-Brilhault, F., Gomot, M., Blanc, R., David, A., Moizard, M.P., Raynaud, M., Ronce, N., Lemonnier, E., Calvas, P. *et al*. (2004) X-linked mental retardation and autism are associated with a mutation in the *NLGN4* gene, a member of the neuroligin family. *Am. J. Hum. Genet.*, **74**, 552–557.
6. Butler, M.G., Dasouki, M.J., Zhou, X.P., Talebizadeh, Z., Brown, M., Takahashi, T.N., Miles, J.H., Wang, C.H., Stratton, R., Pilarski, R. *et al*. (2005) Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. *J. Med. Genet.*, **42**, 318–321.
7. Splawski, I., Timothy, K.W., Sharpe, L.M., Decher, N., Kumar, P., Bloise, R., Napolitano, C., Schwartz, P.J., Joseph, R.M., Condouris, K. *et al*. (2004) Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell*, **119**, 19–31.
8. Splawski, I., Yoo, D.S., Stotz, S.C., Cherry, A., Clapham, D.E. and Keating, M.T. (2006) CACNA1H mutations in autism spectrum disorders. *J. Biol. Chem.*, **281**, 22085–22091.
9. Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U. and Zoghbi, H.Y. (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.*, **23**, 185–188.
10. Pieretti, M., Zhang, F.P., Fu, Y.H., Warren, S.T., Oostra, B.A., Caskey, C.T. and Nelson, D.L. (1991) Absence of expression of the *FMR-1* gene in fragile X syndrome. *Cell*, **66**, 817–822.
11. Verkerk, A.J., Pieretti, M., Sutcliffe, J.S., Fu, Y.H., Kuhl, D.P., Pizzuti, A., Reiner, O., Richards, S., Victoria, M.F., Zhang, F.P. *et al*. (1991) Identification of a gene (*FMR-1*) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*, **65**, 905–914.
12. Samaco, R.C., Hogart, A. and LaSalle, J.M. (2005) Epigenetic overlap in autism-spectrum neurodevelopmental disorders: MECP2 deficiency causes reduced expression of UBE3A and GABRB3. *Hum. Mol. Genet.*, **14**, 483–492.
13. Jiang, Y.H., Sahoo, T., Michaelis, R.C., Bercovich, D., Bressler, J., Kashork, C.D., Liu, Q., Shaffer, L.G., Schroer, R.J., Stockton, D.W. *et al*. (2004) A mixed epigenetic/genetic model for oligogenic inheritance of autism with a limited role for UBE3A. *Am. J. Med. Genet. A*, **131**, 1–10.
14. Lopez-Rangel, E. and Lewis, M.E. (2006) Further evidence for epigenetic influence of MECP2 in Rett, autism and Angelman's syndromes. *Clin. Genet.*, **69**, 23–25.

15. Hagerman, R.J., Ono, M.Y. and Hagerman, P.J. (2005) Recent advances in fragile X: a model for autism and neurodegeneration. *Curr. Opin. Psychiatry*, **18**, 490–496.
16. Klose, R.J. and Bird, A.P. (2006) Genomic DNA methylation: the mark and its mediators. *Trends Biochem. Sci.*, **31**, 89–97.
17. Kriaucionis, S. and Bird, A. (2003) DNA methylation and Rett syndrome. *Hum. Mol. Genet.*, **12** (Spec no. 2), R221–R227.
18. LaSalle, J.M., Hogart, A. and Thatcher, K.N. (2005) Rett syndrome: a Rosetta stone for understanding the molecular pathogenesis of autism. *Int. Rev. Neurobiol.*, **71**, 131–165.
19. Chen, Y., Sharma, R.P., Costa, R.H., Costa, E. and Grayson, D.R. (2002) On the epigenetic regulation of the human reelin promoter. *Nucleic Acids Res.*, **30**, 2930–2939.
20. Noh, J.S., Sharma, R.P., Veldic, M., Salvacion, A.A., Jia, X., Chen, Y., Costa, E., Guidotti, A. and Grayson, D.R. (2005) DNA methyltransferase 1 regulates reelin mRNA expression in mouse primary cortical cultures. *Proc. Natl Acad. Sci. USA*, **102**, 1749–1754.
21. Grayson, D.R., Chen, Y., Costa, E., Dong, E., Guidotti, A., Kundakovic, M. and Sharma, R.P. (2006) The human reelin gene: Transcription factors (+), repressors (−) and the methylation switch (+/−) in schizophrenia. *Pharmacol. Ther.*, **111**, 272–286.
22. Sato, N., Fukushima, N., Chang, R., Matsubayashi, H. and Goggins, M. (2006) Differential and epigenetic gene expression profiling identifies frequent disruption of the RELN pathway in pancreatic cancers. *Gastroenterology*, **130**, 548–565.
23. Numachi, Y., Yoshida, S., Yamashita, M., Fujiyama, K., Naka, M., Matsuoka, H., Sato, M. and Sora, I. (2004) Psychostimulant alters expression of DNA methyltransferase mRNA in the rat brain. *Ann. NY Acad. Sci.*, **1025**, 102–109.
24. Serajee, F.J., Zhong, H. and Mahbubul Huq, A.H. (2006) Association of Reelin gene polymorphisms with autism. *Genomics*, **87**, 75–83.
25. Huang, C.H. and Chen, C.H. (2006) Absence of association of a polymorphic GGC repeat at the 5′ untranslated region of the reelin gene with schizophrenia. *Psychiatry Res.*, **142**, 89–92.
26. Skaar, D.A., Shao, Y., Haines, J.L., Stenger, J.E., Jaworski, J., Martin, E.R., DeLong, G.R., Moore, J.H., McCauley, J.L., Sutcliffe, J.S. *et al*. (2005) Analysis of the RELN gene as a genetic risk factor for autism. *Mol. Psychiatry*, **10**, 563–571.
27. Li, J., Nguyen, L., Gleason, C., Lotspeich, L., Spiker, D., Risch, N. and Myers, R.M. (2004) Lack of evidence for an association between WNT2 and RELN polymorphisms and autism. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, **126**, 51–57.
28. Bonora, E., Beyer, K.S., Lamb, J.A., Parr, J.R., Klauck, S.M., Benner, A., Paolucci, M., Abbott, A., Ragoussis, I., Poustka, A. *et al*. (2003) Analysis of reelin as a candidate gene for autism. *Mol. Psychiatry*, **8**, 885–892.
29. Dolinoy, D.C., Weidman, J.R., Waterland, R.A. and Jirtle, R.L. (2006) Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environ. Health Perspect.*, **114**, 567–572.
30. Waterland, R.A. (2006) Assessing the effects of high methionine intake on DNA methylation. *J. Nutr.*, **136**, 1706S–1710S.
31. Wolff, G.L., Kodell, R.L., Moore, S.R. and Cooney, C.A. (1998) Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J.*, **12**, 949–957.
32. Weaver, I.C., Champagne, F.A., Brown, S.E., Dymov, S., Sharma, S., Meaney, M.J. and Szyf, M. (2005) Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. *J. Neurosci.*, **25**, 11045–11054.
33. Weaver, I.C., Meaney, M.J. and Szyf, M. (2006) Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc. Natl Acad. Sci. USA*, **103**, 3480–3485.
34. Weaver, I.C., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M. and Meaney, M.J. (2004) Epigenetic programming by maternal behavior. *Nat. Neurosci.*, **7**, 847–854.
35. Lamb, J.A., Barnby, G., Bonora, E., Sykes, N., Bacchelli, E., Blasi, F., Maestrini, E., Broxholme, J., Tzenova, J., Weeks, D. *et al*. (2005) Analysis of IMGSAC autism susceptibility loci: evidence for sex limited and parent of origin specific effects. *J. Med. Genet.*, **42**, 132–137.
36. Ylisaukko-oja, T., Alarcon, M., Cantor, R.M., Auranen, M., Vanhala, R., Kempas, E., von Wendt, L., Jarvela, I., Geschwind, D.H. and Peltonen, L. (2006) Search for autism loci by combined analysis of Autism Genetic Resource Exchange and Finnish families. *Ann. Neurol.*, **59**, 145–155.
37. Liu, J., Nyholt, D.R., Magnussen, P., Parano, E., Pavone, P., Geschwind, D., Lord, C., Iversen, P., Hoh, J., Ott, J. *et al*. (2001) A genomewide screen for autism susceptibility loci. *Am. J. Hum. Genet.*, **69**, 327–340.
38. Yonan, A.L., Alarcon, M., Cheng, R., Magnusson, P.K., Spence, S.J., Palmer, A.A., Grunn, A., Juo, S.H., Terwilliger, J.D., Liu, J. *et al*. (2003) A genomewide screen of 345 families for autism-susceptibility loci. *Am. J. Hum. Genet.*, **73**, 886–897.
39. Auranen, M., Vanhala, R., Varilo, T., Ayers, K., Kempas, E., Ylisaukko-Oja, T., Sinsheimer, J.S., Peltonen, L. and Jarvela, I. (2002) A genomewide screen for autism-spectrum disorders: evidence for a major susceptibility locus on chromosome 3q25–27. *Am. J. Hum. Genet.*, **71**, 777–790.
40. Philippe, A., Martinez, M., Guilloud-Bataille, M., Gillberg, C., Rastam, M., Sponheim, E., Coleman, M., Zappella, M., Aschauer, H., Van Maldergem, L. *et al*. (1999) Genome-wide scan for autism susceptibility genes. Paris Autism Research International Sibpair Study. *Hum. Mol. Genet.*, **8**, 805–812.
41. International Molecular Genetic Study of Autism Consortium (IMGSAC). (2001) Further characterization of the autism susceptibility locus AUTS1 on chromosome 7q. *Hum. Mol. Genet.*, **10**, 973–982.
42. Shao, Y., Wolpert, C.M., Raiford, K.L., Menold, M.M., Donnelly, S.L., Ravan, S.A., Bass, M.P., McClain, C., von Wendt, L., Vance, J.M. *et al*. (2002) Genomic screen and follow-up analysis for autistic disorder. *Am. J. Med. Genet.*, **114**, 99–105.
43. International Molecular Genetic Study of Autism Consortium (IMGSAC). (1998) A full genome screen for autism with evidence for linkage to a region on chromosome 7q. *Hum. Mol. Genet.*, **7**, 571–578.
44. Buxbaum, J.D., Silverman, J., Keddache, M., Smith, C.J., Hollander, E., Ramoz, N. and Reichert, J.G. (2004) Linkage analysis for autism in a subset families with obsessive-compulsive behaviors: evidence for an autism susceptibility gene on chromosome 1 and further support for susceptibility genes on chromosome 6 and 19. *Mol. Psychiatry*, **9**, 144–150.
45. Shao, Y., Raiford, K.L., Wolpert, C.M., Cope, H.A., Ravan, S.A., Ashley-Koch, A.A., Abramson, R.K., Wright, H.H., DeLong, R.G., Gilbert, J.R. *et al*. (2002) Phenotypic homogeneity provides increased support for linkage on chromosome 2 in autistic disorder. *Am. J. Hum. Genet.*, **70**, 1058–1061.
46. Buxbaum, J.D., Silverman, J.M., Smith, C.J., Kilifarski, M., Reichert, J., Hollander, E., Lawlor, B.A., Fitzgerald, M., Greenberg, D.A. and Davis, K.L. (2001) Evidence for a susceptibility gene for autism on chromosome 2 and for genetic heterogeneity. *Am. J. Hum. Genet.*, **68**, 1514–1520.
47. McCauley, J.L., Li, C., Jiang, L., Olson, L.M., Crockett, G., Gainer, K., Folstein, S.E., Haines, J.L. and Sutcliffe, J.S. (2005) Genome-wide and ordered-subset linkage analyses provide support for autism loci on 17q and 19p with evidence of phenotypic and interlocus genetic correlates. *BMC Med. Genet.*, **6**, 1.
48. Cook, E.H., Jr, Lindgren, V., Leventhal, B.L., Courchesne, R., Lincoln, A., Shulman, C., Lord, C. and Courchesne, E. (1997) Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. *Am. J. Hum. Genet.*, **60**, 928–934.
49. Cook, E.H., Jr, Courchesne, R.Y., Cox, N.J., Lord, C., Gonen, D., Guter, S.J., Lincoln, A., Nix, K., Haas, R., Leventhal, B.L. *et al*. (1998) Linkage-disequilibrium mapping of autistic disorder, with 15q11–13 markers. *Am. J. Hum. Genet.*, **62**, 1077–1083.
50. Schroer, R.J., Phelan, M.C., Michaelis, R.C., Crawford, E.C., Skinner, S.A., Cuccaro, M., Simensen, R.J., Bishop, J., Skinner, C., Fender, D. *et al*. (1998) Autism and maternally derived aberrations of chromosome 15q. *Am. J. Med. Genet.*, **76**, 327–336.
51. Bass, M.P., Menold, M.M., Wolpert, C.M., Donnelly, S.L., Ravan, S.A., Hauser, E.R., Maddox, L.O., Vance, J.M., Abramson, R.K., Wright, H.H. *et al*. (2000) Genetic studies in autistic disorder and chromosome 15. *Neurogenetics*, **2**, 219–226.
52. Bolton, P.F., Dennis, N.R., Browne, C.E., Thomas, N.S., Veltman, M.W., Thompson, R.J. and Jacobs, P. (2001) The phenotypic manifestations of interstitial duplications of proximal 15q with special reference to the autistic spectrum disorders. *Am. J. Med. Genet.*, **105**, 675–685.

53. Bolton, P.F., Veltman, M.W., Weisblatt, E., Holmes, J.R., Thomas, N.S., Youings, S.A., Thompson, R.J., Roberts, S.E., Dennis, N.R., Browne, C.E. *et al*. (2004) Chromosome 15q11–13 abnormalities and other medical conditions in individuals with autism spectrum disorders. *Psychiatr. Genet.*, **14**, 131–137.
54. Mohandas, T.K., Park, J.P., Spellman, R.A., Filiano, J.J., Mamourian, A.C., Hawk, A.B., Belloni, D.R., Noll, W.W. and Moeschler, J.B. (1999) Paternally derived *de novo* interstitial duplication of proximal 15q in a patient with developmental delay. *Am. J. Med. Genet.*, **82**, 294–300.
55. Browne, C.E., Dennis, N.R., Maher, E., Long, F.L., Nicholson, J.C., Sillibourne, J. and Barber, J.C. (1997) Inherited interstitial duplications of proximal 15q: genotype–phenotype correlations. *Am. J. Hum. Genet.*, **61**, 1342–1352.
56. Veltman, M.W., Thompson, R.J., Craig, E.E., Dennis, N.R., Roberts, S.E., Moore, V., Brown, J.A. and Bolton, P.F. (2005) A paternally inherited duplication in the Prader-Willi/Angelman syndrome critical region: a case and family study. *J. Autism. Dev. Disord.*, **35**, 117–127.
57. Baron, C.A., Tepper, C.G., Liu, S.Y., Davis, R.R., Wang, N.J., Schanen, N.C. and Gregg, J.P. (2006) Genomic and functional profiling of duplicated chromosome 15 cell lines reveal regulatory alterations in UBE3A-associated ubiquitin–proteasome pathway processes. *Hum. Mol. Genet.*, **15**, 853–869.
58. Nicholls, R.D. and Knepper, J.L. (2001) Genome organization, function, and imprinting in Prader-Willi and Angelman syndromes. *Annu. Rev. Genomics Hum. Genet.*, **2**, 153–175.
59. Herzing, L.B., Kim, S.J., Cook, E.H., Jr. and Ledbetter, D.H. (2001) The human aminophospholipid-transporting ATPase gene *ATP10C* maps adjacent to UBE3A and exhibits similar imprinted expression. *Am. J. Hum. Genet.*, **68**, 1501–1505.
60. Herzing, L.B., Cook, E.H., Jr. and Ledbetter, D.H. (2002) Allele-specific expression analysis by RNA-FISH demonstrates preferential maternal expression of UBE3A and imprint maintenance within 15q11–q13 duplications. *Hum. Mol. Genet.*, **11**, 1707–1718.
61. Jay, P., Rougeulle, C., Massacrier, A., Moncla, A., Mattei, M.G., Malzac, P., Roeckel, N., Taviaux, S., Lefranc, J.L., Cau, P. *et al*. (1997) The human necdin gene, *NDN*, is maternally imprinted and located in the Prader-Willi syndrome chromosomal region. *Nat. Genet.*, **17**, 357–361.
62. Nakada, Y., Taniura, H., Uetsuki, T., Inazawa, J. and Yoshikawa, K. (1998) The human chromosomal gene for necdin, a neuronal growth suppressor, in the Prader-Willi syndrome deletion region. *Gene*, **213**, 65–72.
63. MacDonald, H.R. and Wevrick, R. (1997) The necdin gene is deleted in Prader-Willi syndrome and is imprinted in human and mouse. *Hum. Mol. Genet.*, **6**, 1873–1878.
64. Boccaccio, I., Glatt-Deeley, H., Watrin, F., Roeckel, N., Lalande, M. and Muscatelli, F. (1999) The human *MAGEL2* gene and its mouse homologue are paternally expressed and mapped to the Prader-Willi region. *Hum. Mol. Genet.*, **8**, 2497–2505.
65. Lee, S., Kozlov, S., Hernandez, L., Chamberlain, S.J., Brannan, C.I., Stewart, C.L. and Wevrick, R. (2000) Expression and imprinting of MAGEL2 suggest a role in Prader-Willi syndrome and the homologous murine imprinting phenotype. *Hum. Mol. Genet.*, **9**, 1813–1819.
66. Lee, S., Walker, C.L. and Wevrick, R. (2003) Prader-Willi syndrome transcripts are expressed in phenotypically significant regions of the developing mouse brain. *Gene Expr. Patterns*, **3**, 599–609.
67. Chibuk, T.K., Bischof, J.M. and Wevrick, R. (2001) A necdin/MAGE-like gene in the chromosome 15 autism susceptibility region: expression, imprinting, and mapping of the human and mouse orthologues. *BMC Genet.*, **2**, 22.
68. Kuwajima, T., Nishimura, I. and Yoshikawa, K. (2006) Necdin promotes GABAergic neuron differentiation in cooperation with Dlx homeodomain proteins. *J. Neurosci.*, **26**, 5383–5392.
69. LaSalle, J.M. and Lalande, M. (1995) Domain organization of allele-specific replication within the *GABRB3* gene cluster requires a biparental 15q11–13 contribution. *Nat. Genet.*, **9**, 386–394.
70. Runte, M., Huttenhofer, A., Gross, S., Kiefmann, M., Horsthemke, B. and Buiting, K. (2001) The IC-SNURF-SNRPN transcript serves as a host for multiple small nucleolar RNA species and as an antisense RNA for UBE3A. *Hum. Mol. Genet.*, **10**, 2687–2700.
71. Veltman, M.W., Thompson, R.J., Roberts, S.E., Thomas, N.S., Whittington, J. and Bolton, P.F. (2004) Prader-Willi syndrome–a study comparing deletion and uniparental disomy cases with reference to autism spectrum disorders. *Eur. Child Adolesc. Psychiatry*, **13**, 42–50.
72. Dykens, E.M., Sutcliffe, J.S. and Levitt, P. (2004) Autism and 15q11–q13 disorders: behavioral, genetic, and pathophysiological issues. *Ment. Retard. Dev. Disabil. Res. Rev.*, **10**, 284–291.
73. Descheemaeker, M.J., Govers, V., Vermeulen, P. and Fryns, J.P. (2006) Pervasive developmental disorders in Prader-Willi syndrome: The Leuven experience in 59 subjects and controls. *Am. J. Med. Genet. A.*, **140**, 1136–1142.
74. Shao, Y., Cuccaro, M.L., Hauser, E.R., Raiford, K.L., Menold, M.M., Wolpert, C.M., Ravan, S.A., Elston, L., Decena, K., Donnelly, S.L. *et al*. (2003) Fine mapping of autistic disorder to chromosome 15q11–q13 by use of phenotypic subtypes. *Am. J. Hum. Genet.*, **72**, 539–548.
75. Nurmi, E.L., Bradford, Y., Chen, Y., Hall, J., Arnone, B., Gardiner, M.B., Hutcheson, H.B., Gilbert, J.R., Pericak-Vance, M.A., Copeland-Yates, S.A. *et al*. (2001) Linkage disequilibrium at the Angelman syndrome gene *UBE3A* in autism families. *Genomics*, **77**, 105–113.
76. Nurmi, E.L., Amin, T., Olson, L.M., Jacobs, M.M., McCauley, J.L., Lam, A.Y., Organ, E.L., Folstein, S.E., Haines, J.L. and Sutcliffe, J.S. (2003) Dense linkage disequilibrium mapping in the 15q11–q13 maternal expression domain yields evidence for association in autism. *Mol. Psychiatry*, **8**, 624–634, 570.
77. Kim, S.J., Herzing, L.B., Veenstra-VanderWeele, J., Lord, C., Courchesne, R., Leventhal, B.L., Ledbetter, D.H., Courchesne, E. and Cook, E.H., Jr. (2002) Mutation screening and transmission disequilibrium study of ATP10C in autism. *Am. J. Med. Genet.*, **114**, 137–143.
78. Menold, M.M., Shao, Y., Wolpert, C.M., Donnelly, S.L., Raiford, K.L., Martin, E.R., Ravan, S.A., Abramson, R.K., Wright, H.H., Delong, G.R. *et al*. (2001) Association analysis of chromosome 15 gabaa receptor subunit genes in autistic disorder. *J. Neurogenet.*, **15**, 245–259.
79. Martin, E.R., Menold, M.M., Wolpert, C.M., Bass, M.P., Donnelly, S.L., Ravan, S.A., Zimmerman, A., Gilbert, J.R., Vance, J.M., Maddox, L.O. *et al*. (2000) Analysis of linkage disequilibrium in gamma-aminobutyric acid receptor subunit genes in autistic disorder. *Am. J. Med. Genet.*, **96**, 43–48.
80. Nurmi, E.L., Dowd, M., Tadevosyan-Leyfer, O., Haines, J.L., Folstein, S.E. and Sutcliffe, J.S. (2003) Exploratory subsetting of autism families based on savant skills improves evidence of genetic linkage to 15q11–q13. *J. Am. Acad. Child Adolesc. Psychiatry*, **42**, 856–863.
81. Salmon, B., Hallmayer, J., Rogers, T., Kalaydjieva, L., Petersen, P.B., Nicholas, P., Pingree, C., McMahon, W., Spiker, D., Lotspeich, L. *et al*. (1999) Absence of linkage and linkage disequilibrium to chromosome 15q11–q13 markers in 139 multiplex families with autism. *Am. J. Med. Genet.*, **88**, 551–556.
82. Chagnon, Y.C. (2006) Shared susceptibility region on chromosome 15 between autism and catatonia. *Int. Rev. Neurobiol.*, **72**, 165–178.
83. Curran, S., Roberts, S., Thomas, S., Veltman, M., Browne, J., Medda, E., Pickles, A., Sham, P. and Bolton, P.F. (2005) An association analysis of microsatellite markers across the Prader-Willi/Angelman critical region on chromosome 15 (q11–13) and autism spectrum disorder. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, **137**, 25–28.
84. Maddox, L.O., Menold, M.M., Bass, M.P., Rogala, A.R., Pericak-Vance, M.A., Vance, J.M. and Gilbert, J.R. (1999) Autistic disorder and chromosome 15q11–q13: construction and analysis of a BAC/PAC contig. *Genomics*, **62**, 325–331.
85. Veenstra-VanderWeele, J., Gonen, D., Leventhal, B.L. and Cook, E.H., Jr. (1999) Mutation screening of the UBE3A/E6-AP gene in autistic disorder. *Mol. Psychiatry*, **4**, 64–67.
86. Sinkkonen, S.T., Homanics, G.E. and Korpi, E.R. (2003) Mouse models of Angelman syndrome, a neurodevelopmental disorder, display different brain regional GABA(A) receptor alterations. *Neurosci. Lett.*, **340**, 205–208.
87. Blatt, G.J. (2005) GABAergic cerebellar system in autism: a neuropathological and developmental perspective. *Int. Rev. Neurobiol.*, **71**, 167–178.
88. Schmitz, C., van Kooten, I.A., Hof, P.R., van Engeland, H., Patterson, P.H. and Steinbusch, H.W. (2005) Autism: neuropathology, alterations of the GABAergic system, and animal models. *Int. Rev. Neurobiol.*, **71**, 1–26.
89. Levitt, P. (2005) Disruption of interneuron development. *Epilepsia*, **46** (Suppl. 7), 22–28.

90. Pearl, P.L., Gibson, K.M., Acosta, M.T., Vezina, L.G., Theodore, W.H., Rogawski, M.A., Novotny, E.J., Gropman, A., Conry, J.A., Berry, G.T. *et al*. (2003) Clinical spectrum of succinic semialdehyde dehydrogenase deficiency. *Neurology*, **60**, 1413–1417.
91. McCauley, J.L., Olson, L.M., Delahanty, R., Amin, T., Nurmi, E.L., Organ, E.L., Jacobs, M.M., Folstein, S.E., Haines, J.L. and Sutcliffe, J.S. (2004) A linkage disequilibrium map of the 1-Mb 15q12 GABA(A) receptor subunit cluster and association to autism. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, **131**, 51–59.
92. Buxbaum, J.D., Silverman, J.M., Smith, C.J., Greenberg, D.A., Kilifarski, M., Reichert, J., Cook, E.H., Jr, Fang, Y., Song, C.Y. and Vitale, R. (2002) Association between a GABRB3 polymorphism and autism. *Mol. Psychiatry*, **7**, 311–316.
93. Ma, D.Q., Whitehead, P.L., Menold, M.M., Martin, E.R., Ashley-Koch, A.E., Mei, H., Ritchie, M.D., Delong, G.R., Abramson, R.K., Wright, H.H. *et al*. (2005) Identification of significant association and gene–gene interaction of GABA receptor subunit genes in autism. *Am. J. Hum. Genet.*, **77**, 377–388.
94. Maestrini, E., Lai, C., Marlow, A., Matthews, N., Wallace, S., Bailey, A., Cook, E.H., Weeks, D.E. and Monaco, A.P. (1999) Serotonin transporter (5-HTT) and gamma-aminobutyric acid receptor subunit beta3 (*GABRB3*) gene polymorphisms are not associated with autism in the IMGSA families. The International Molecular Genetic Study of Autism Consortium. *Am. J. Med. Genet.*, **88**, 492–496.
95. Sabaratnam, M., Turk, J. and Vroegop, P. (2000) Case report: autistic disorder and chromosomal abnormality 46, XX duplication (4) p12–p13. *Eur. Child Adolesc. Psychiatry*, **9**, 307–311.
96. Vincent, J.B., Horike, S.I., Choufani, S., Paterson, A.D., Roberts, W., Szatmari, P., Weksberg, R., Fernandez, B. and Scherer, S.W. (2006) An inversion inv(4)(p12–p15.3) in autistic siblings implicates the 4p GABA receptor gene cluster. *J. Med. Genet.*, **43**, 429–434.
97. Piras, G., El Kharroubi, A., Kozlov, S., Escalante-Alcalde, D., Hernandez, L., Copeland, N.G., Gilbert, D.J., Jenkins, N.A. and Stewart, C.L. (2000) Zac1 (Lot1), a potential tumor suppressor gene, and the gene for epsilon-sarcoglycan are maternally imprinted genes: identification by a subtractive screen of novel uniparental fibroblast lines. *Mol. Cell. Biol.*, **20**, 3308–3315.
98. Horike, S., Cai, S., Miyano, M., Cheng, J.F. and Kohwi-Shigematsu, T. (2005) Loss of silent-chromatin looping and impaired imprinting of DLX5 in Rett syndrome. *Nat. Genet.*, **37**, 31–40.
99. D'Amelio, M., Ricci, I., Sacco, R., Liu, X., D'Agruma, L., Muscarella, L.A., Guarnieri, V., Militerni, R., Bravaccio, C., Elia, M. *et al*. (2005) Paraoxonase gene variants are associated with autism in North America, but not in Italy: possible regional specificity in gene–environment interactions. *Mol. Psychiatry*, **10**, 1006–1016.
100. Pasca, S.P., Nemes, B., Vlase, L., Gagyi, C.E., Dronca, E., Miu, A.C. and Dronca, M. (2006) High levels of homocysteine and low serum paraoxonase 1 arylesterase activity in children with autism. *Life Sci.*, **78**, 2244–2248.
101. Serajee, F.J., Nabi, R., Zhong, H. and Huq, M. (2004) Polymorphisms in xenobiotic metabolism genes and autism. *J. Child Neurol.*, **19**, 413–417.
102. Okita, C., Meguro, M., Hoshiya, H., Haruta, M., Sakamoto, Y.K. and Oshimura, M. (2003) A new imprinted cluster on the human chromosome 7q21-q31, identified by human–mouse monochromosomal hybrids. *Genomics*, **81**, 556–559.
103. Costa, L.G., Cole, T.B., Jarvik, G.P. and Furlong, C.E. (2003) Functional genomic of the paraoxonase (PON1) polymorphisms: effects on pesticide sensitivity, cardiovascular disease, and drug metabolism. *Annu. Rev. Med.*, **54**, 371–392.
104. Shih, D.M., Gu, L., Xia, Y.R., Navab, M., Li, W.F., Hama, S., Castellani, L.W., Furlong, C.E., Costa, L.G., Fogelman, A.M. *et al*. (1998) Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature*, **394**, 284–287.
105. Hoshiya, H., Meguro, M., Kashiwagi, A., Okita, C. and Oshimura, M. (2003) Calcr, a brain-specific imprinted mouse calcitonin receptor gene in the imprinted cluster of the proximal region of chromosome 6. *J. Hum. Genet.*, **48**, 208–211.
106. Mizuno, Y., Sotomaru, Y., Katsuzawa, Y., Kono, T., Meguro, M., Oshimura, M., Kawai, J., Tomaru, Y., Kiyosawa, H., Nikaido, I. *et al*. (2002) Asb4, Ata3, and Dcn are novel imprinted genes identified by high-throughput screening using RIKEN cDNA microarray. *Biochem. Biophys. Res. Commun.*, **290**, 1499–1505.
107. Ono, R., Shiura, H., Aburatani, H., Kohda, T., Kaneko-Ishino, T. and Ishino, F. (2003) Identification of a large novel imprinted gene cluster on mouse proximal chromosome 6. *Genome Res.*, **13**, 1696–1705.
108. Stuhmer, T., Anderson, S.A., Ekker, M. and Rubenstein, J.L. (2002) Ectopic expression of the Dlx genes induces glutamic acid decarboxylase and Dlx expression. *Development*, **129**, 245–252.
109. Blatt, G.J., Fitzgerald, C.M., Guptill, J.T., Booker, A.B., Kemper, T.L. and Bauman, M.L. (2001) Density and distribution of hippocampal neurotransmitter receptors in autism: an autoradiographic study. *J. Autism. Dev. Disord.*, **31**, 537–543.
110. Casanova, M.F., Buxhoeveden, D. and Gomez, J. (2003) Disruption in the inhibitory architecture of the cell minicolumn: implications for autisim. *Neuroscientist*, **9**, 496–507.
111. Zerucha, T., Stuhmer, T., Hatch, G., Park, B.K., Long, Q., Yu, G., Gambarotta, A., Schultz, J.R., Rubenstein, J.L. and Ekker, M. (2000) A highly conserved enhancer in the Dlx5/Dlx6 intergenic region is the site of cross-regulatory interactions between Dlx genes in the embryonic forebrain. *J. Neurosci.*, **20**, 709–721.
112. Hamilton, S.P., Woo, J.M., Carlson, E.J., Ghanem, N., Ekker, M. and Rubenstein, J.L. (2005) Analysis of four DLX homeobox genes in autistic probands. *BMC Genet.*, **6**, 52.
113. Richler, E., Reichert, J.G., Buxbaum, J.D. and McInnes, L.A. (2006) Autism and ultraconserved non-coding sequence on chromosome 7q. *Psychiatr. Genet.*, **16**, 19–23.
114. Alarcon, M., Yonan, A.L., Gilliam, T.C., Cantor, R.M. and Geschwind, D.H. (2005) Quantitative genome scan and ordered-subsets analysis of autism endophenotypes support language QTLs. *Mol. Psychiatry*, **10**, 747–757.
115. Ashley-Koch, A., Wolpert, C.M., Menold, M.M., Zaeem, L., Basu, S., Donnelly, S.L., Ravan, S.A., Powell, C.M., Qumsiyeh, M.B., Aylsworth, A.S. *et al*. (1999) Genetic studies of autistic disorder and chromosome 7. *Genomics*, **61**, 227–236.
116. Alarcon, M., Cantor, R.M., Liu, J., Gilliam, T.C. and Geschwind, D.H. (2002) Evidence for a language quantitative trait locus on chromosome 7q in multiplex autism families. *Am. J. Hum. Genet.*, **70**, 60–71.
117. Bonora, E., Bacchelli, E., Levy, E.R., Blasi, F., Marlow, A., Monaco, A.P. and Maestrini, E. (2002) Mutation screening and imprinting analysis of four candidate genes for autism in the 7q32 region. *Mol. Psychiatry*, **7**, 289–301.
118. Skuse, D.H., James, R.S., Bishop, D.V., Coppin, B., Dalton, P., Aamodt-Leeper, G., Bacarese-Hamilton, M., Creswell, C., McGurk, R. and Jacobs, P.A. (1997) Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function. *Nature*, **387**, 705–708.
119. Scourfield, J., McGuffin, P. and Thapar, A. (1997) Genes and social skills. *Bioessays*, **19**, 1125–1127.
120. Skuse, D.H. (2000) Imprinting, the X-chromosome, and the male brain: explaining sex differences in the liability to autism. *Pediatr. Res.*, **47**, 9–16.
121. El Abd, S., Patton, M.A., Turk, J., Hoey, H. and Howlin, P. (1999) Social, communicational, and behavioral deficits associated with ring X turner syndrome. *Am. J. Med. Genet*, **88**, 510–516.
122. Telvi, L., Lebbar, A., Del Pino, O., Barbet, J.P. and Chaussain, J.L. (1999) 45,X/46,XY mosaicism: report of 27 cases. *Pediatrics*, **104**, 304–308.
123. Donnelly, S.L., Wolpert, C.M., Menold, M.M., Bass, M.P., Gilbert, J.R., Cuccaro, M.L., Delong, G.R. and Pericak-Vance, M.A. (2000) Female with autistic disorder and monosomy X (Turner syndrome): parent-of-origin effect of the X chromosome. *Am. J. Med. Genet.*, **96**, 312–316.
124. Thomas, N.S., Sharp, A.J., Browne, C.E., Skuse, D., Hardie, C. and Dennis, N.R. (1999) Xp deletions associated with autism in three females. *Hum. Genet.*, **104**, 43–48.
125. Ross, J., Zinn, A. and McCauley, E. (2000) Neurodevelopmental and psychosocial aspects of Turner syndrome. *Ment. Retard. Dev. Disabil. Res. Rev.*, **6**, 135–141.
126. Davies, W., Isles, A., Smith, R., Karunadasa, D., Burrmann, D., Humby, T., Ojarikre, O., Biggin, C., Skuse, D., Burgoyne, P. *et al*. (2005) Xlr3b is a new imprinted candidate for X-linked parent-of-origin effects on cognitive function in mice. *Nat. Genet.*, **37**, 625–629.
127. Raefski, A.S. and O'Neill, M.J. (2005) Identification of a cluster of X-linked imprinted genes in mice. *Nat. Genet.*, **37**, 620–624.

128. Blasi, F., Bacchelli, E., Pesaresi, G., Carone, S., Bailey, A.J. and Maestrini, E. (2006) Absence of coding mutations in the X-linked genes neuroligin 3 and neuroligin 4 in individuals with autism from the IMGSAC collection. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, **141**, 220–221.
129. Gauthier, J., Bonnel, A., St-Onge, J., Karemera, L., Laurent, S., Mottron, L., Fombonne, E., Joober, R. and Rouleau, G.A. (2005) *NLGN3*/*NLGN4* gene mutations are not responsible for autism in the Quebec population. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, **132**, 74–75.
130. Yan, J., Oliveira, G., Coutinho, A., Yang, C., Feng, J., Katz, C., Sram, J., Bockholt, A., Jones, I.R., Craddock, N. *et al*. (2005) Analysis of the neuroligin 3 and 4 genes in autism and other neuropsychiatric patients. *Mol. Psychiatry*, **10**, 329–332.
131. Ylisaukko-oja, T., Rehnstrom, K., Auranen, M., Vanhala, R., Alen, R., Kempas, E., Ellonen, P., Turunen, J.A., Makkonen, I., Riikonen, R. *et al*. (2005) Analysis of four neuroligin genes as candidates for autism. *Eur. J. Hum. Genet.*, **13**, 1285–1292.
132. Collins, A.L., Ma, D., Whitehead, P.L., Martin, E.R., Wright, H.H., Abramson, R.K., Hussman, J.P., Haines, J.L., Cuccaro, M.L., Gilbert, J.R. *et al*. (2006) Investigation of autism and GABA receptor subunit genes in multiple ethnic groups. *Neurogenetics*, **7**, 167–174.
133. Hussman, J.P. (2001) Suppressed GABAergic inhibition as a common factor in suspected etiologies of autism. *J. Autism Dev. Disord.*, **31**, 247–248.
134. Dhossche, D., Applegate, H., Abraham, A., Maertens, P., Bland, L., Bencsath, A. and Martinez, J. (2002) Elevated plasma gamma-aminobutyric acid (GABA) levels in autistic youngsters: stimulus for a GABA hypothesis of autism. *Med. Sci. Monit.*, **8**, PR1–6.
135. Morison, I.M., Ramsay, J.P. and Spencer, H.G. (2005) A census of mammalian imprinting. *Trends Genet.*, **21**, 457–465.
136. Lauritsen, M.B., Als, T.D., Dahl, H.A., Flint, T.J., Wang, A.G., Vang, M., Kruse, T.A., Ewald, H. and Mors, O. (2006) A genome-wide search for alleles and haplotypes associated with autism and related pervasive developmental disorders on the Faroe Islands. *Mol. Psychiatry*, **11**, 37–46.
137. Barrett, S., Beck, J.C., Bernier, R., Bisson, E., Braun, T.A., Casavant, T.L., Childress, D., Folstein, S.E., Garcia, M., Gardiner, M.B. *et al*. (1999) An autosomal genomic screen for autism. Collaborative linkage study of autism. *Am. J. Med. Genet.*, **88**, 609–615.
138. Hutcheson, H.B., Bradford, Y., Folstein, S.E., Gardiner, M.B., Santangelo, S.L., Sutcliffe, J.S. and Haines, J.L. (2003) Defining the autism minimum candidate gene region on chromosome 7. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, **117**, 90–96.
139. Trikalinos, T.A., Karvouni, A., Zintzaras, E., Ylisaukko-oja, T., Peltonen, L., Jarvela, I. and Ioannidis, J.P. (2006) A heterogeneity-based genome search meta-analysis for autism-spectrum disorders. *Mol. Psychiatry*, **11**, 29–36.
140. Veenstra-VanderWeele, J., Kim, S.J., Lord, C., Courchesne, R., Akshoomoff, N., Leventhal, B.L., Courchesne, E. and Cook, E.H., Jr. (2002) Transmission disequilibrium studies of the serotonin 5-HT2A receptor gene (*HTR2A*) in autism. *Am. J. Med. Genet.*, **114**, 277–283.
141. Cantor, R.M., Kono, N., Duvall, J.A., Alvarez-Retuerto, A., Stone, J.L., Alarcon, M., Nelson, S.F. and Geschwind, D.H. (2005) Replication of autism linkage: fine-mapping peak at 17q21. *Am. J. Hum. Genet.*, **76**, 1050–1056.
142. Smith, R.J., Dean, W., Konfortova, G. and Kelsey, G. (2003) Identification of novel imprinted genes in a genome-wide screen for maternal methylation. *Genome Res.*, **13**, 558–569.
143. Jong, M.T., Gray, T.A., Ji, Y., Glenn, C.C., Saitoh, S., Driscoll, D.J. and Nicholls, R.D. (1999) A novel imprinted gene, encoding a RING zinc-finger protein, and overlapping antisense transcript in the Prader-Willi syndrome critical region. *Hum. Mol. Genet.*, **8**, 783–793.
144. Jong, M.T., Carey, A.H., Caldwell, K.A., Lau, M.H., Handel, M.A., Driscoll, D.J., Stewart, C.L., Rinchik, E.M. and Nicholls, R.D. (1999) Imprinting of a RING zinc-finger encoding gene in the mouse chromosome region homologous to the Prader-Willi syndrome genetic region. *Hum. Mol. Genet.*, **8**, 795–803.
145. Kuwako, K., Taniura, H. and Yoshikawa, K. (2004) Necdin-related MAGE proteins differentially interact with the E2F1 transcription factor and the p75 neurotrophin receptor. *J. Biol. Chem.*, **279**, 1703–1712.
146. Lee, S., Walker, C.L., Karten, B., Kuny, S.L., Tennese, A.A., O'Neill, M.A. and Wevrick, R. (2005) Essential role for the Prader-Willi syndrome protein necdin in axonal outgrowth. *Hum. Mol. Genet.*, **14**, 627–637.
147. Runte, M., Varon, R., Horn, D., Horsthemke, B. and Buiting, K. (2005) Exclusion of the C/D box snoRNA gene cluster HBII-52 from a major role in Prader-Willi syndrome. *Hum. Genet.*, **116**, 228–230.
148. Kashiwagi, A., Meguro, M., Hoshiya, H., Haruta, M., Ishino, F., Shibahara, T. and Oshimura, M. (2003) Predominant maternal expression of the mouse Atp10c in hippocampus and olfactory bulb. *J. Hum. Genet.*, **48**, 194–198.
149. Collinson, N., Kuenzi, F.M., Jarolimek, W., Maubach, K.A., Cothliff, R., Sur, C., Smith, A., Otu, F.M., Howell, O., Atack, J.R. *et al*. (2002) Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABAA receptor. *J. Neurosci.*, **22**, 5572–5580.
150. Caraiscos, V.B., Elliott, E.M., You-Ten, K.E., Cheng, V.Y., Belelli, D., Newell, J.G., Jackson, M.F., Lambert, J.J., Rosahl, T.W., Wafford, K.A. *et al*. (2004) Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. *Proc. Natl Acad. Sci. USA*, **101**, 3662–3667.
151. Borgatti, R., Piccinelli, P., Passoni, D., Romeo, A., Viri, M., Musumeci, S.A., Elia, M., Cogliati, T., Valseriati, D., Grasso, R. *et al*. (2003) Peripheral markers of the gamma-aminobutyric acid (GABA)ergic system in Angelman's syndrome. *J. Child Neurol.*, **18**, 21–25.
152. Culiat, C.T., Stubbs, L.J., Montgomery, C.S., Russell, L.B. and Rinchik, E.M. (1994) Phenotypic consequences of deletion of the gamma 3, alpha 5, or beta 3 subunit of the type A gamma-aminobutyric acid receptor in mice. *Proc. Natl Acad. Sci. USA*, **91**, 2815–2818.
153. Culiat, C.T., Stubbs, L.J., Woychik, R.P., Russell, L.B., Johnson, D.K. and Rinchik, E.M. (1995) Deficiency of the beta 3 subunit of the type A gamma-aminobutyric acid receptor causes cleft palate in mice. *Nat. Genet.*, **11**, 344–346.
154. Ashley-Koch, A.E., Mei, H., Jaworski, J., Ma, D.Q., Ritchie, M.D., Menold, M.M., Delong, G.R., Abramson, R.K., Wright, H.H., Hussman, J.P. *et al*. (2006) An analysis paradigm for investigating multi-locus effects in complex disease: examination of three GABA receptor subunit genes on 15q11–q13 as risk factors for autistic disorder. *Ann. Hum. Genet.*, **70**, 281–292.
155. Meguro, M., Mitsuya, K., Sui, H., Shigenami, K., Kugoh, H., Nakao, M. and Oshimura, M. (1997) Evidence for uniparental, paternal expression of the human GABAA receptor subunit genes, using microcell-mediated chromosome transfer. *Hum. Mol. Genet.*, **6**, 2127–2133.
156. Asmus, F., Zimprich, A., Tezenas Du Montcel, S., Kabus, C., Deuschl, G., Kupsch, A., Ziemann, U., Castro, M., Kuhn, A.A., Strom, T.M. *et al*. (2002) Myoclonus-dystonia syndrome: epsilon-sarcoglycan mutations and phenotype. *Ann. Neurol.*, **52**, 489–492.
157. Zimprich, A., Grabowski, M., Asmus, F., Naumann, M., Berg, D., Bertram, M., Scheidtmann, K., Kern, P., Winkelmann, J., Muller-Myhsok, B. *et al*. (2001) Mutations in the gene encoding epsilon-sarcoglycan cause myoclonus-dystonia syndrome. *Nat. Genet.*, **29**, 66–69.
158. Ono, R., Kobayashi, S., Wagatsuma, H., Aisaka, K., Kohda, T., Kaneko-Ishino, T. and Ishino, F. (2001) A retrotransposon-derived gene, *PEG10*, is a novel imprinted gene located on human chromosome 7q21. *Genomics*, **73**, 232–237.
159. Lux, A., Beil, C., Majety, M., Barron, S., Gallione, C.J., Kuhn, H.M., Berg, J.N., Kioschis, P., Marchuk, D.A. and Hafner, M. (2005) Human retroviral gag- and gag-pol-like proteins interact with the transforming growth factor-beta receptor activin receptor-like kinase 1. *J. Biol. Chem.*, **280**, 8482–8493.
160. Carmody, L.C., Bauman, P.A., Bass, M.A., Mavila, N., DePaoli-Roach, A.A. and Colbran, R.J. (2004) A protein phosphatase-1gamma1 isoform selectivity determinant in dendritic spine-associated neurabin. *J. Biol. Chem.*, **279**, 21714–21723.
161. Terry-Lorenzo, R.T., Roadcap, D.W., Otsuka, T., Blanpied, T.A., Zamorano, P.L., Garner, C.C., Shenolikar, S. and Ehlers, M.D. (2005) Neurabin/protein phosphatase-1 complex regulates dendritic spine morphogenesis and maturation. *Mol. Biol. Cell*, **16**, 2349–2362.
162. Eisenstat, D.D., Liu, J.K., Mione, M., Zhong, W., Yu, G., Anderson, S.A., Ghattas, I., Puelles, L. and Rubenstein, J.L. (1999) DLX-1, DLX-2 and DLX-5 expression define distinct stages of basal forebrain differentiation. *J. Comp. Neurol.*, **414**, 217–237.

163. Chen, X., Li, X., Wang, W. and Lufkin, T. (1996) Dlx5 and Dlx6: an evolutionary conserved pair of murine homeobox genes expressed in the embryonic skeleton. *Ann. NY Acad. Sci.*, **785**, 38–47.
164. McLarren, K.W., Litsiou, A. and Streit, A. (2003) DLX5 positions the neural crest and preplacode region at the border of the neural plate. *Dev. Biol.*, **259**, 34–47.
165. Li, J.Y., Kuick, R., Thompson, R.C., Misek, D.E., Lai, Y.M., Liu, Y.Q., Chai, B.X., Hanash, S.M. and Gantz, I. (2005) Arcuate nucleus transcriptome profiling identifies ankyrin repeat and suppressor of cytokine signalling box-containing protein 4 as a gene regulated by fasting in central nervous system feeding circuits. *J. Neuroendocrinol.*, **17**, 394–404.
166. Cole, T.B., Jampsa, R.L., Walter, B.J., Arndt, T.L., Richter, R.J., Shih, D.M., Tward, A., Lusis, A.J., Jack, R.M., Costa, L.G. *et al*. (2003) Expression of human paraoxonase (PON1) during development. *Pharmacogenetics*, **13**, 357–364.
167. Draganov, D.I., Teiber, J.F., Speelman, A., Osawa, Y., Sunahara, R. and La Du, B.N. (2005) Human paraoxonases (PON1, PON2 and PON3) are lactonases with overlapping and distinct substrate specificities. *J. Lipid Res.*, **46**, 1239–1247.
168. Leviev, I. and James, R.W. (2000) Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. *Arterioscler. Thromb. Vasc. Biol.*, **20**, 516–521.
169. Richter, R.J. and Furlong, C.E. (1999) Determination of paraoxonase (PON1) status requires more than genotyping. *Pharmacogenetics*, **9**, 745–753.
170. Yamada, T., Mitsuya, K., Kayashima, T., Yamasaki, K., Ohta, T., Yoshiura, K., Matsumoto, N., Yamada, H., Minakami, H., Oshimura, M. *et al*. (2004) Imprinting analysis of 10 genes and/or transcripts in a 1.5-Mb MEST-flanking region at human chromosome 7q32. *Genomics*, **83**, 402–412.
171. Bentley, L., Nakabayashi, K., Monk, D., Beechey, C., Peters, J., Birjandi, Z., Khayat, F.E., Patel, M., Preece, M.A., Stanier, P. *et al*. (2003) The imprinted region on human chromosome 7q32 extends to the carboxypeptidase A gene cluster: an imprinted candidate for Silver–Russell syndrome. *J. Med. Genet.*, **40**, 249–256.
172. Kayashima, T., Yamasaki, K., Yamada, T., Sakai, H., Miwa, N., Ohta, T., Yoshiura, K., Matsumoto, N., Nakane, Y., Kanetake, H. *et al*. (2003) The novel imprinted carboxypeptidase A4 gene (*CPA4*) in the 7q32 imprinting domain. *Hum. Genet.*, **112**, 220–226.
173. Wei, S., Segura, S., Vendrell, J., Aviles, F.X., Lanoue, E., Day, R., Feng, Y. and Fricker, L.D. (2002) Identification and characterization of three members of the human metallocarboxypeptidase gene family. *J. Biol. Chem.*, **277**, 14954–14964.
174. Hayashida, S., Yamasaki, K., Asada, Y., Soeda, E., Niikawa, N. and Kishino, T. (2000) Construction of a physical and transcript map flanking the imprinted MEST/PEG1 region at 7q32. *Genomics*, **66**, 221–225.
175. El Kharroubi, A., Piras, G. and Stewart, C.L. (2001) DNA demethylation reactivates a subset of imprinted genes in uniparental mouse embryonic fibroblasts. *J. Biol. Chem.*, **276**, 8674–8680.
176. Kobayashi, S., Kohda, T., Miyoshi, N., Kuroiwa, Y., Aisaka, K., Tsutsumi, O., Kaneko-Ishino, T. and Ishino, F. (1997) Human PEG1/MEST, an imprinted gene on chromosome 7. *Hum. Mol. Genet.*, **6**, 781–786.
177. Lefebvre, L., Viville, S., Barton, S.C., Ishino, F., Keverne, E.B. and Surani, M.A. (1998) Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene Mest. *Nat. Genet.*, **20**, 163–169.
178. Nakabayashi, K., Bentley, L., Hitchins, M.P., Mitsuya, K., Meguro, M., Minagawa, S., Bamforth, J.S., Stanier, P., Preece, M., Weksberg, R. *et al*. (2002) Identification and characterization of an imprinted antisense RNA (MESTIT1) in the human MEST locus on chromosome 7q32. *Hum. Mol. Genet.*, **11**, 1743–1756.
179. Reule, M., Krause, R., Hemberger, M. and Fundele, R. (1998) Analysis of Peg1/Mest imprinting in the mouse. *Dev. Genes Evol.*, **208**, 161–163.
180. Lee, Y.J., Park, C.W., Hahn, Y., Park, J., Lee, J., Yun, J.H., Hyun, B. and Chung, J.H. (2000) Mit1/Lb9 and Copg2, new members of mouse imprinted genes closely linked to Peg1/Mest(1). *FEBS Lett.*, **472**, 230–234.
181. Yamasaki, K., Hayashida, S., Miura, K., Masuzaki, H., Ishimaru, T., Niikawa, N. and Kishino, T. (2000) The novel gene, gamma2-COP (COPG2), in the 7q32 imprinted domain escapes genomic imprinting. *Genomics*, **68**, 330–335.