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A question of balance: a proposal for new mouse models of autism

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Abstract

Autism spectrum disorder (ASD) represents a major mental health problem with estimates of prevalence ranging from 1/500 to 1/2000. While generally recognized as developmental in origin, little to nothing is certain about its etiology. Currently, diagnosis is made on the basis of a variety of early developmental delays and/or regressions in behavior. There are no universally agreed upon changes in brain structure or cell composition. No biomarkers of any type are available to aid or confirm the clinical diagnosis. In addition, while estimates of the heritability of the condition range from 60 to 90%, as of this writing no disease gene has been unequivocally identified. The prevalence of autism is three- to four-fold higher in males than in females, but the reason for this sexual dimorphism is unknown. In light of all of these ambiguities, a proposal to discuss potential animal models may seem the heart of madness. However, parsing autism into its individual genetic, behavioral, and neurobiological components has already facilitated a 'conversation' between the human disease and the neuropathology and biochemistry underlying the disorder. Building on these results, it should be possible to not just replicate one aspect of autism but to connect the developmental abnormalities underlying the ultimate behavioral phenotype. A reciprocal conversation such as this, wherein the human disease informs on how to make a better animal model and the animal model teaches of the biology causal to autism, would be highly beneficial.

Keywords: Autism; PDDs; ASD

1. The human biology of autistic disorder

In humans, the range of pervasive developmental disorders (PDDs) covers a broad spectrum; from the restricted interests and repetitive behaviors of Asperger syndrome to the complete lack of social interaction found in patients with classical autism. This spectrum of severity, although classified as independent disorders, may in fact represent variation over a continuum rather than a series of diseases. While there are clinical distinctions among the PDDs, the accurate diagnosis of an individual child still represents a clinical challenge.

1.1. Neuropathology of autistic disorder

In many human neurological disorders, there are structural changes in the brain that are apparent on pathological exam. In some instances, such as Alzheimer's disease, information on the details of the neuropathology drives the final diagnosis and serves as the 'gold standard' by which behavioral and clinical interpretations are validated. The situation for autism is the exact opposite. The behavioral and neurological symptoms serve as the sole basis of the diagnosis as there are no disease-related structural defects for which a consensus exists. Having said this, however, pathological studies have revealed an association between autistic symptoms and the appearance of certain pathological changes in brain structure or cellularity in a number of different brain regions. The value of these findings is mitigated somewhat by the fact that in the entire pathological literature only a few dozen different

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Table 1
Neuropathological changes associated with autistic disorder

Measurement	Finding	References
Total brain weight	Increased	Bauman and Kemper (1985), Kemper and Bauman (1998), Bailey et al. (1998)
Cerebellum		
Purkinje cells	Decreased numbers	Ritvo et al. (1986), Kemper and Bauman (1993), Bailey et al. (1998)
Deep cerebellar nuclei	Cell size decrease	Kemper and Bauman (1993)
	No change	Bailey et al. (1998)
Inferior olive	Focal cell loss	Bauman and Kemper (1985)
Limbic system		
Amygdala (medial nuclei)	Cell size decrease	Kemper and Bauman (1993)
	No change	Bailey et al. (1998)
Hippocampal formation	Cell size decrease	Kemper and Bauman (1993)
	Reduced dendrites	
	Cell density increase	Bailey et al. (1998)
Mammillary nuclei	Cell size decrease	Kemper and Bauman (1993)
Brain stem		
Facial nucleus, superior olive	Cell loss	Rodier et al. (1996)
Inferior olive	Cell density increase	Kemper and Bauman (1993)
Cortex	Enlarged	Bailey et al. (1998)
Minicolumns	Smaller, more numerous	Casanova et al. (2002)

cases have come to autopsy. This is changing rapidly, but given the heterogeneity that is likely inherent in the disorder, larger studies are clearly needed. Of the studies to date, there have been consistent findings in the olivo-cerebellar system, the limbic system, the brainstem, and the cortex. These are summarized in Table 1.

In addition to histopathological changes, differences in brain volume (measured by modern methods of imaging) have also been noted. These anomalies are summarized in Table 2; each has been documented in one or more studies. As autism is likely to be a common outcome initiated by a number of different insults to different brain areas at different developmental times (genetic analysis suggests on the order of a dozen different genes could be involved), even those changes that are found in only a minority of the cases of autism might nonetheless hold one or more keys to the biology of the disorder. For an animal model of autism to be truly useful, however, it will be necessary for it to replicate at least a subset of the reported pathologies.

1.2. The genetics of autism

While the precise anatomical defects are yet unspecified, twin and family genetic studies have shown a robust genetic component for the disorder. The concordance rate for monozygotic twins varies from 60 to 95%, while that of dizygotic twins ranges from 0 to 24% (Ritvo et al., 1985; Steffenburg et al., 1989; Bailey et al., 1995). The prevalence rate of non-twin siblings of children with autism varies from 1 to 6% (Hallmayer et al., 2002). Differences in diagnostic criteria and inclusion of ''spectrum'' phenotypes lends to the variability in estimates

Table 2
Volumetric findings in autism

Measurement	Finding	References
Total brain volume	Increased	Piven et al. (1995), Davidovitch et al. (1996), Piven et al. (1996), Lainhart et al. (1997), Courchesne et al. (2001), Bailey et al. (1998), Aylward et al. (2002), Sparks et al. (2002), Herbert et al. (2003)
White matter	Increased	Courchesne et al. (2001), Herbert et al. (2003)
Limbic system volume	Decreased	Aylward et al. (1999), Herbert et al. (2003)
Amygdala	Decreased	Aylward et al. (1999), Herbert et al. (2003), Pierce et al. (2001)
	Increased	Howard et al. (2000)
	Variable	Abell et al. (1999), Sparks et al. (2002)
Hippocampus	Decreased	Aylward et al. (1999), Herbert et al. (2003), Saitoh et al. (2001)
	No change	Piven et al. (1998), Saitoh et al. (1995)
Other limbic cortex	Decreased	Abell et al. (1999)
Cerebellar cortex	Focal decrease	Courchesne et al. (1988)
(Lobules VI and VII)	Focal increase	Saitoh and Courchesne (1998)
	No decrease	Holttum et al. (1992)

of heritability, prevalence and concordance. In keeping with the concept of a prominent genetic component, one study found chromosomal abnormalities in 9% of individuals with autism who were cytogenetically analyzed (Wassink et al., 2001). Another study found an increase in fragile sites among autistic individuals and that these sites were not randomly distributed (Arrieta et al., 2002).

The hunt for specific genes involved in the origins of autism is still a work in progress. One would assume that given a heritability estimate of greater than 90%, finding causative mutations in major susceptibility genes would not be a difficult undertaking (Bailey et al., 1996). However, it has been estimated that from 2 to more than 15 genes can contribute to the etiology of autism (Pickles et al., 1995; Risch et al., 1999). Further complicating matters, the results of several genome-wide scans completed to date have each suggested the involvement of a set of overlapping but non-identical set of genetic loci (reviewed in Nicolson and Szatmari, 2003). Those most frequently reaching the threshold of at least suggestive linkage are chromosomes 7q, 2q and 16p (Nicolson and Szatmari, 2003). Even though the methods of detecting linkage and criteria of diagnosis differed among the studies, the most frequent candidate region, chromosome 7q, has been identified in a number of genome scans (IMGSAC, 1998; Barrett et al., 1999; Philippe et al., 1999; IMGSAC, 2001). Chromosomal rearrangements and translocations also support the genetic linkage found on chromosome 7q (Lopreiato and Wulfsberg, 1992; Vincent et al., 2000; Warburton et al., 2000; Tentler et al., 2001; Sultana et al., 2002). Evidence for the involvement of chromosome 2q comes from both mapping studies (Buxbaum et al., 2001; IMGSAC, 2001) and chromosomal deletions in some autistic individuals (Smith et al., 2001; Borg et al., 2002; Gallagher et al., 2003). A schematic of the ranges or peaks of suggestive linkage from six different mapping studies are shown in Fig. 1. While not a key player denoted in the genome scans, structural aberrations of chromosome 15 have been implicated in a number of cases of autism (Wassink et al., 2001).

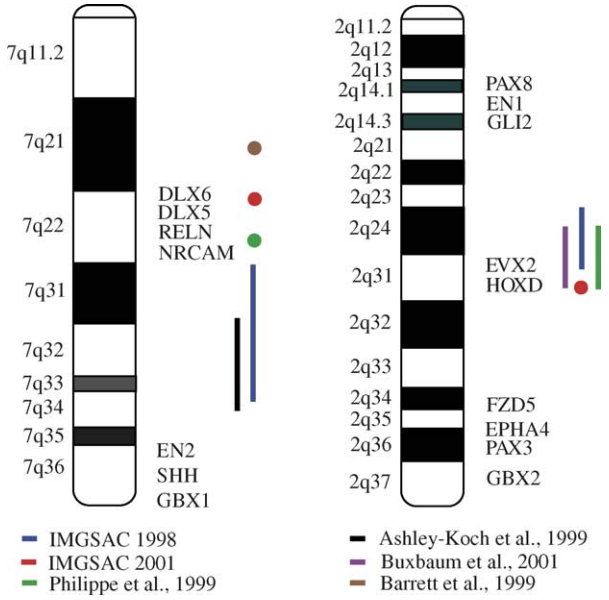

Fig. 1. Results of mapping studies for autism susceptibility genes. Ideograms of the q arms of chromosomes 7 and 2 are labeled with important developmental genes as well as the peak (circle) or range (line) of suggestive linkage from the corresponding mapping studies.

1.3. The 'chemistry' of autism

There is a small, but significant human literature that documents a correlation between exposure to teratogens early in development and the emergence of symptoms of autism after birth. Exposure to either thalidomide (Stromland et al., 1994) or valproate (Christianson et al., 1994; Williams and Hersh, 1997) during the first weeks of gestation is associated with autistic like symptoms in the child. These observations have led Rodier et al. (1996) and others to hypothesize that the problems in autism are not only developmental in origin but may arise in the very first stages of embryonic development.

Alterations in neuropeptides have also been reported in association with autistic symptoms. BDNF is a neurotrophic factor that is increasingly recognized as playing a neuromodulatory role in CNS function (Castren et al., 1993; Levine et al., 1995; Kang and Schuman, 1995) in addition to its role in the development and stabilization of certain classes of neurons during development. In a study of blood samples taken within a few days of birth as part of a routine program of neonatal screening, Nelson et al. (2001) discovered levels of BDNF as well as VIP, CGRP, and NT4/5 were significantly elevated in the children who later developed autism. The exact meaning of this finding is not clear. A comparable elevation was also observed in children who later developed mental retardation not associated with autism. While few new details have emerged since the original publication, the correlations nonetheless represent potentially important clues. Further human and animal studies will assist in developing this story.

Oxytocin has been implicated as well. Oxytocin and vasopressin are neurohypophyseal peptides that may contribute to the phenotypes associated with autism (Insel et al., 1999). Normally, the posterior pituitary releases these peptides into the bloodstream, where they function developmentally in social behavior and cognition through receptors throughout the forebrain and brainstem. Chromosomal abnormalities have been identified in the region of the *oxytocin* gene (20p 11.1–11.2) in autistic individuals (Cook et al., 1998). Additionally, abnormalities in oxytocin or vasopressin levels have been reported in several autistic individuals (Modahl et al., 1998; Green et al., 2001; Gale et al., 2003), and oxytocin infusion has been shown to reduce repetitive behaviors in humans with autism (Hollander et al., 2003).

2. An animal model of autism: theoretical considerations

2.1. Animal models and complex socio-behavioral disorders

Although several studies of autism have been carried out in primates and rodents, no generally accepted model system has yet been developed. Implicitly, the challenge lies in the fact that autism is a strictly socio-behavioral disorder, with clinical hallmarks difficult or impossible to replicate in a model organism, particularly a rodent. Discussion of the full range of social behavior in the mouse and its relevance to human behaviors is beyond the scope of this paper, but those behaviors that pertain to the clinical manifestations of autism are reviewed in Table 3. In each section, the human trait is listed as a main heading while the proposed rodent behaviors are listed underneath.

Based on Table 3, a mouse displaying autistic-like symptoms would display impairments in aspects of social interaction and communication as well as a pattern of repetitive behaviors. However, an animal model of autism based solely on behavioral assays would be incomplete. To be useful, any animal model of autism must accurately replicate a combination of the behavioral, neuropathological, biochemical and genetic basis for autistic disorder. The greater the number of features that are represented, the closer the model's approximation to autism spectrum disorder (ASD) will be.

3. Current animal models of autism

3.1. Lesion induced behavioral abnormalities

Lesion models suggest that brain damage in specific brain regions leads to the development of specific or general behavioral abnormalities that are quite similar to those seen in autism. These models are thus quite useful even if they do not reproduce the underlying genetic or developmental pathways of autistic spectrum disorders. Often surgical in nature, one of the first attempts purportedly to develop an autism model was from Bachevalier (1996), who lesioned the amygdala of rhesus monkeys and recorded the resultant behavioral abnormalities. Wolterink et al. (2001) repeated these experiments in rats, finding similar abnormalities.

Table 3
Behavioral measures of an autistic mouse

Social interaction
Huddle, groom, barber
Play behavior (chase, spar, wrestle, pin)
Social investigation (approach, investigate, nose groom)
Aggression (threat, attack, bite, chase, aggressive-groom, full aggressive)
Sexual activity (follow, sniff, attempted mount, mount, genital groom)
Communication
Pup distress call
Mating call
Submissive call
Restricted, repetitive and stereotyped patterns of behavior
Repeated locomotor activity (explore, circle, etc.)
Self-involvement (wash, self-groom, scratch, dig, shake, jump, eat, drink)
Self-injurious behavior (excessive self-grooming)

A more recent lesion study has particular relevance to the model system proposed below (Bobee et al., 2000). These authors created midline cerebellar lesions in 10-day-old rats. The attentional capabilities, spontaneous motor activities, and anxiety behavior of 15 of these animals was assessed and compared to 16 controls. The lesioned rats appeared less anxious as evidenced by behavioral response to an elevated maze, electrode stimulation, and in response to tonal stimulation. The spontaneous motor activity of the lesioned rats was increased even though they showed no changes in exploratory behavior. Several previous investigators (Leaton and Supple, 1991; Joyal et al., 1996) had lesioned the cerebellar midline in adult rats. As has been previously demonstrated (Auvray et al., 1989; Wolterink et al., 2001; Daenen et al., 2002), the age at which the lesion is made has a significant impact on the phenotypic outcome.

The results described above are typical of this category of model: experimentally induced abnormalities produce behavioral changes reminiscent of those associated with autism. However, as the injuries producing the abnormal behavior destroy entire regions (and all the constituent cells) they bear little relationship to the specific pathologies listed in Table 1. Consequently, no thorough investigation of genetic or developmental mechanism can occur in a lesion produced by a knife or an electrode. Nevertheless, such models are useful for demonstrating the ability of defects in a particular region to produce altered behaviors of a specific nature.

Other lesion-induced models use chemical or infectious agents to produce behavioral changes. Immunological abnormalities have long been suspected involved in autism (reviewed recently in Krause et al., 2002 and Torres, 2003), and studies of the offspring of female mice infected with *Trichinella spiralis* during gestation (Rau, 1983) revealed long ago a potential role for infection in altered CNS development. More recently, Pletnikov et al. (1999) and Hornig et al. (1999) independently published on behavioral abnormalities resulting from neonatal exposure to Borna virus (reviewed in Pletnikov et al., 2003). Several other groups have also published on behavioral abnormalities resulting from immunological abnormalities or challenges (Patterson, 2002; Shi et al., 2003; Vojdani et al., 2003). Similarly, Rodier et al. (1997a) and Ingram et al. (2000a) used chemical perturbations to produce autism-like phenotypes in animal models. In all of these cases, the environmental nature of the perturbations potentially reproduces the conditions experienced by developing humans. Additionally, the action of these chemical and infectious agents is usually global in nature, as opposed to specifically focused on one brain region. This combination

creates both a more complete model of autism and the need for a more complex interpretation of the results.

3.2. Genetically induced behavioral abnormalities

Another class of potential rodent models of autistic spectrum disorders attempts to use genetics to mimic certain biochemical abnormalities found in autistic individuals. In this group, a mutation in a gene connected to the biochemical abnormality is created and the behavior of the animal is assessed. In some cases, such as those with mutations in the serotonin and oxytocin–vasopressin systems, autistic-like behavioral abnormalities are found. The neuropathological abnormalities summarized in Table 1, however, are either not present in these animals or not sufficient to explain the behavior. One such example of behavioral abnormalities without known structural defects is the *Dishevelled-1* (*Dvl1*) null mouse. Deficits in social interaction as measured by whisker trimming, nest-building and huddling behaviors are found in mice homozygous for a targeted deletion of *Dvl1* (Lijam et al., 1997; Long et al., 2004). These mice show no motor, sensory, spatial learning or social memory deficits, nor are there overt abnormalities in the brain.

Brattleboro rats have a spontaneous mutation in the arginine vasopressin (*Avp*) gene, resulting in an inability to secrete this neuropeptide (Birkett and Pickering, 1988). Based on the abnormalities in arginine vasopressin seen in some human autisitic patients, these rats have been used as an animal model for autism. Supporting this model, Brattleboro rats show less social memory than controls (Englemann, 1994). Other animal studies reveal oxytocin knockout pups emit fewer separation distress calls (Young et al., 1997) and fail to develop social memory (Ferguson et al., 2000) as a result of an oxytocin deficit in the amygdala (Ferguson et al., 2001). Additionally, these animals exhibit decreased investigative behaviors and increased aggression (Insel et al., 1999). Similarly, rodents with serotonin-related deficits (discussed in Scott and Deneris, this issue) or increases (Kahne et al., 2002) also display behavioral abnormalities. However, this model system presents no inherent mechanism through which to generate the neuropathological abnormalities associated with autistic disorder.

Perhaps the most realistic autism model in this class is the guinea pigs of Caston et al. (1998). These animals had naturally occurring cerebellar defects first described at 3 weeks of age. At this time, the cerebella contained 20–25% fewer granule and Purkinje cells as compared to controls. The losses were not uniform, but instead appeared to favor a reduction in the sizes of lobules VI and VII (Lev-Ram et al., 1993). Neocortical abnormalities were also described, including decreases in dendritic arborization and a mild shrinkage of cell somas. These changes were apparent within the first 3 weeks of development. By one year of age, cerebellar lobule VIII was found to be missing while lobules VI and VII were compressed into one large folium. Due to the similarity of these neuropathological abnormalities to those described by Courchesne in autistic children (1988), this line was assayed for their behavior. Social interactions, response to familiar and unfamiliar sounds, spatial exploration tendencies, and their ability to perform a motor learning task were all abnormal compared to controls (Caston et al., 1998). Unfortunately, connecting these malformations to the specific genetic abnormalities thought to produce them is now impossible; this line has been reported to have died out (Andres, 2002).

3.3. Pathological models of autism in animals

In all of the examples of animal models cited thus far, the metric used to determine the fidelity of the model to the symptoms of autism is behavior. This focus on a behavioral model is understandable considering the poor consensus on the neuropathology of autism. Nonetheless, as indicated in Tables 1 and 2, there are some consistent changes in autistic individuals. The loss of cerebellar cells, Purkinje cells for certain, is nearly universal in the published studies of human autopsy material. There also seems to be some consensus on the involvement of limbic structures, particularly the amygdala. While researchers vary on the direction of the change, there is a reasonable concordance of both neuropathology and imaging to suggest a place to begin. Despite the opportunities presented by the areas of agreement on brain structure changes in autism, there is probably only one animal model published specifically linking neuropathology with autism. In 1997, Rodier et al. reported on a deceased autistic patient whose neuropathology included significant loss of brainstem structures such as, the facial nucleus and the superior olive. The authors observed that this phenotype is reminiscent of the deficits seen in the *Hoxa1* knockout mouse (Rodier et al., 1997b). Pursuing this lead, a subsequent study by the same group found evidence suggesting HOXA1 and HOXB1 (on chromosomes 7 and 17, respectively) were susceptibility loci for autism (Ingram et al., 2000b). Additional studies suggest that this correlation does not hold true for all populations as no linkage has been found in six more recent studies (Devlin et al., 2002; Li et al., 2002; Talebizadeh et al., 2002; Collins et al., 2003; Romano et al., 2003; Gallagher et al., 2004). Most recently, Conciatori et al. (2004) linked a specific Hoxa1 variant to cranial morphology in autistic individuals.

From this brief review of animal models of autism, it is apparent that there is a lack of animal models attempting to model the neuropathology of autism (with or without linkage to the various behavioral phenotypes). Given the variability of the neuropathology in human autistic patients this is hardly unexpected. However, without such models it will be difficult to validate the significance of any given treatment or genetic mutation. Models focusing on the neuropathology of autism will thus help us to bridge the gap between genetics and behavior even where no behavioral phenotype results in individual models.

4. Autistic disorder: a theory of balance

This is an exciting but frustrating time in autism research. There is rapid progress toward the identification of susceptibility genes, strong growth in the use of modern imaging techniques to define the structural correlates of autism in living individuals, and increasingly refined diagnostic criteria for identifying the various forms of the disorder. Yet thus far the emerging picture of autism is more like a fractured mosaic than a coherent image. The information presented in Table 2 is but one example: the same brain structure (e.g., the amygdala) viewed by different groups at different times appears larger, smaller or unchanged in individuals with autism. The genetic data are equally elusive. We have defined large regions of chromosomes as candidates; however, as we try to narrow in on specific genes (e.g., *Engrailed*) they appear as strong candidates in one study, but are excluded in others. It strikes us that in light of all of this seemingly conflicting data, autism is best seen not as a disease of a single CNS structure, but rather as a failure of the inter-relationships among CNS structures—a question of balance.

Viewed from many different perspectives, it seems apparent that the organization of the CNS places a high premium on a proper balance among its many components. Consider the precision with which neuron and target populations achieve numerical balance in the adult CNS. Through the process of target-related cell death a near linear relationship is achieved between a neuronal population and the size of its target (reviewed in Williams and Herrup, 1988). The relationship is maintained over a wide range of target sizes and functions to adjust neuronal populations both outside and inside the CNS—local circuit and projection neurons alike. A second type of balance was highlighted by Rubenstein and Merzenich (2003). These authors have presented an elegant case for tracing the origins of autism to a failure of balance between excitation and inhibition in the brain. They stress the problem of signal to noise and the propensity of 'noisy' circuits to malfunction in ways that seem compatible with the observed neurological features of autism. As only one example, an imbalance caused by a deficiency in the GABAergic system might be anticipated to reduce cortical inhibitory circuits and thus increase the noise. This particular scenario is especially compelling in light of the converging lines of evidence implicating a genetic locus containing a cluster of GABA receptors in the 15q11–13 region—a locus with a long association with familial autism (see above).

Herbert et al. (2003) highlight yet a third type of balance in their imaging study of the brains of autistic children. They propose that the large-scale balance of the various brain regions is perturbed in autism and lies at the root of the behavioral symptoms. We concur with this interpretation and have based much of our modeling on its implications. Consider the association of autism with very early insults to the CNS (e.g., thalidomide Stromland et al., 1994; Rodier, 2002) and the suggestive association of chromosome 7q, which contains pattern formation genes such as, *WNT2*, *GBX1*, *RELN*, and *EN2*. We agree with the insight of Herbert et al. (2003) that a failure to achieve a proper balance among the major brain vesicles can lead to subtle changes that might well lead to complex behavioral problems such as those found in autism. These changes would be difficult to detect within the bounds of a single cell population or even by a cursory examination of an entire brain. Even more challenging would be to detect a transient developmental imbalance that was corrected by adulthood, but which forever compromised the ability of the brain to wire correctly. The temporary overgrowth of the cerebral cortex reported in some autistic children might be a symptom of such a developmental problem.

One plausible place to look for defects in the specification of this regional balance is in the pattern formation genes that subdivide the early neuroepithelium into a series of regions, each fated to produce a different structure in the adult. In approaching the problem in this way, we have been drawn to the possible participation of the two *Engrailed* genes (*EN1* and *EN2*) in the generation of the symptoms of autism. The reproduction of the described neuropathology is particularly intriguing and we would propose that these mice deserve significant attention from the community of autism researchers as a welcome addition to the human/mouse 'conversation'.

5. The *Engrailed* genes as candidates for mouse models of autism

If the balance among brain regions is the culprit in autism, then the fulcrum for this balance is most likely the midbrain/hindbrain boundary of the early neural tube. The existence of cerebellar abnormalities in autistic individuals, as a number of groups have described, is a direct prediction of this model and the disruptions of genes that are responsible for patterning in this region become clear candidates for the sources of autism.

5.1. A brief history of the origins of the cerebellum

The cerebellum develops from the ordered sequence of gene expression at the mid/hindbrain junction established by the apposition of *Otx2* and *Gbx2* expression domains. From this isthmic organizer develops a domain of expression of the major players in early cerebellum development: *Wnt1*, *Fgf8*, *Pax2*, *Pax5* and the two *Engrailed* genes. By perturbing this sequence, either in space or in time, changes occur in the structure and organization of the cerebellum and the midbrain. Hypomorphic and conditional null alleles of *Fgf8* (Meyers et al., 1998; Chi et al., 2003), targeted deletions of *Wnt1* and *En1* (McMahon and Bradley, 1990; Thomas and Capecchi, 1990; Wurst et al., 1994) and double mutants of *En1/En2* or *Pax2/Pax5* (Joyner, 1996; Schwarz et

al., 1997) result in large deletions of the midbrain and cerebellum.

More subtle phenotypes are found in *Pax5* and *En2* single mutants and *En1* mutants on an inbred C57BL/6 background. *Pax5* homozygous null mice have changes in the foliation pattern of the anterior cerebellum and reduction of the inferior colliculus (Urbanek et al., 1994). The *En2* mutant cerebellum is smaller than normal and has consistent folial abnormalities of the posterior cerebellum (Joyner et al., 1991; Millen et al., 1994; Kuemerle et al., 1997; Bilovocky et al., 2003). Careful analysis has also shown that while the cells are properly positioned in these mutants, there is a 30–40% deficit in cells of the olivocerebellar circuit; including Purkinje, granule, inferior olive and deep nuclei. At the molecular level, region-specific banded expression of Zebrin II and Ppath is disrupted in *En2* mutants suggesting that the intricacies of fine patterning have not been preserved (Kuemerle et al., 1997). *En1* homozygous null mice on a C57BL/6 background have relatively normal cerebellar architecture with no cellular deficits. The only structural change in these mutants is a fusion of lobules IV and V in the anterior cerebellum (Bilovocky et al., 2003).

These focal cerebellar patterning abnormalities—the apparent hypoplasia of the posterior cerebellum of the *En2* mutant and the anterior cerebellum of the C57BL/6-*En1*—were the features that initially drew us to the possibility that the *Engrailed* mutations might serve as pathological mimics of the autistic brain. The cellular changes in the *En2* mutant further strengthened this view. The losses in the Purkinje, deep nuclear, and inferior olive populations seem very much in keeping with the findings from the human autistic brain (Table 1). The *Engrailed-1* mutant is a less perfect pathological model as there are no significant cell losses reported. Nonetheless, the strong background dependence of the phenotype (Bilovocky et al., 2003) seems to us to describe a developmental process where the expression of a pattern formation gene is modulated by the state of many other genes. In the end it is the combined effect of this entire network of genes that is needed for the full definition of the mutant phenotype. This situation mirrors that described for the genetic basis of autism: strong heritability, but no single, easily identifiable, disease locus.

Further evidence strengthening the candidacy of the mouse *Engrailed* genes as models of human autism comes from the human gene mapping studies. As mentioned previously, chromosomes 2 and 7 are among the top candidate regions for autism susceptibility genes. Mapping adjacent to the region of interest on chromosome 7 is *Engrailed-2* (*EN2*), located at 7q36 (Logan et al., 1989; Poole et al., 1989). Based on its location as well as role in CNS development, *EN*2 has been investigated for a link to autism. To date, studies have both supported and refuted the validity of *EN2* as a susceptibility gene for autism (Petit et al., 1995; Zhong et al., 2003). Most recently, Gharani et al. (2004) found an association between ASD and two polymorphisms in intronic regions of *EN2*. The human *EN1* gene is also located near a region of interest, mapping to chromosome 2q14 (Matsui et al., 1993). The potential of *Engrailed-1* (*EN1*) as a candidate gene has yet to be fully explored; this probably owes to the fact that *EN1* does not map precisely within the region on chromosome 2 with the strongest linkage from the mapping studies. Interestingly, as shown in Fig. 1, the region encompassing the evolutionary duplication of the *EN* homologues contains other important developmental genes maintained in proximity to the *EN* genes (Gibert, 2002).

With structural changes that show strong analogy to the human material and promising genetic evidence, the next test of an autism model is whether or not it captures any features of the behavioral abnormalities. Gerlai et al. (1996) assessed several aspects of the behavior of *En2* mutant mice. They found that while *En2* mutants performed normally in several tests of motor function, both hetero- and homozygotes performed below the level of wild type mice on the rotorod. Rotorod tests on *En1* heterozygotes also show changes in performance from that of wild type (CLM, unpublished data). Another recent study found that mice with *Otx2* replacing one copy of *En1* ($En1^{+/Otx2}$) are hyperactive in open field tests (Brodski et al., 2003). While these tests do not speak to the social interaction phenotype of autism, they do suggest that subtle changes in cerebellar architecture can have read outs in behavioral symptoms. It would certainly be worthwhile to test either of these mutants for autistic-like behaviors, either without manipulation or in conjunction with known environmental effectors such as valproate exposure.

6. Conclusions

The proposal to consider the mutations in the two *Engrailed* genes as models of human autism continues the ‘conversation’ between mouse and human begun with the chemical lesion studies of Rodier, the explorations of the oxytocin/vasopressin mutations of Englemann, Young, Ferguson, Insel, and the *Pet-1* mutants described by Deneris elsewhere in this volume. Each of these models brings a unique contribution to the discussion and each seems to capture at least one of the pieces of the autism puzzle. None of these will likely prove to be a perfect mouse model of the disorder. The next steps should be to make the conversation more interactive. Each of the rodent models should be explored more fully in light of both the human data as well as the other rodent findings. For example, do the *Pet-1* or *oxytocin* mutants have defects in any part of the limbic or olivocerebellar systems? Similarly, do the *Engrailed* mutants have social interaction deficits? From the standpoint of the human condition, does the expression of any of these genes change noticeably in any subset of the individuals with autism? The ‘conversation’ is just beginning, but a lively discussion seems easy to predict.

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References

Abell, F., Krams, M., Ashburner, J., Passingham, R., Friston, K., Frackowiak, R., Happe, F., Frith, C., Frith, U., 1999. The neuroanatomy of autism: a voxel-based whole brain analysis of structural scans. Neuroreport 10, 1647–1651.

Andres, C., 2002. Molecular genetics and animal models in autistic disorder. Brain Res. 57, 109–119.

Arrieta, I., Nunez, T., Martinez, B., Perez, A., Telez, M., Criado, B., Gainza, I., Lostao, C.M., 2002. Chromosomal fragility in a behavioral disorder. Behav. Genet. 32, 397–412.

Auvray, N., Caston, J., Reber, A., Stelz, T., 1989. Role of the cerebellum in the ontogenesis of the equilibrium behavior in the young rat: a behavioral study. Brain Res. 505, 291–301.

Aylward, E.H., Minshew, N.J., Goldstein, G., Honeycutt, N.A., Augustine, A.M., Yates, K.O., Barta, P.E., Pearlson, G.D., 1999. MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. Neurology 53, 2145–2150.

Aylward, E.H., Minshew, N.J., Field, K., Sparks, B.F., Singh, N., 2002. Effects of age on brain volume and head circumference in autism. Neurology 59, 175–183.

Bachevalier, J., 1996. Brief report: medial temporal lobe and autism: a putative animal model in primates. J. Autism Dev. Disord. 26, 217–220.

Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E., Rutter, M., 1995. Autism as a strongly genetic disorder: evidence from a British twin study. Psychol. Med. 25, 63–77.

Bailey, A., Phillips, W., Rutter, M., 1996. Autism: towards an integration of clinical, genetic, neuropsychological, and neurobiological perspectives. J. Child Psychol. Psychiatry 37, 89–126.

Bailey, A., Luthert, P., Dean, A., Harding, B., Janota, I., Montgomery, M., Rutter, M., Lantos, P., 1998. A clinicopathological study of autism. Brain 121 (Pt 5), 889–905.

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., Gilman, S., Haines, J.L., Hopkins, K., Landa, R., Meyer, N.H., Mullane, J.A., Nishimura, D.Y., Palmer, P., Piven, J., Purdy, J., Santangelo, S.L., Searby, C., Sheffield, V., Singleton, J., Slager, S., 1999. An autosomal genomic screen for autism: collaborative linkage study of autism. Am. J. Med. Genet. 88, 609–615.

Bauman, M., Kemper, T.L., 1985. Histoanatomic observations of the brain in early infantile autism. Neurology 35, 866–874.

Bilovocky, N.A., Romito-Digiacomo, R.R., Murcia, C.L., Maricich, S.M., Herrup, K., 2003. Factors in the genetic background suppress the engrailed-1 cerebellar phenotype. J. Neurosci. 23, 5105–5112.

Birkett, S.D., Pickering, B.T., 1988. The vasopressin precursor in the Brattleboro (di/di) rat. Int. J. Pept. Protein Res. 32, 565–572.

Bobee, S., Mariette, E., Tremblay-Leveau, H., Caston, J., 2000. Effects of early midline cerebellar lesion on cognitive and emotional functions in the rat. Behav. Brain Res. 112, 107–117.

Borg, I., Squire, M., Menzel, C., Stout, K., Morgan, D., Willatt, L., O'brien, P.C., Ferguson-Smith, M.A., Ropers, H.H., Tommerup, N., Kalscheuer, V.M., Sargan, D.R., 2002. A cryptic deletion of 2q35 including part of the *PAX3* gene detected by breakpoint mapping in a child with autism and a de novo 2;8 translocation. J. Med. Genet. 39, 391–399.

Brodski, C., Weisenhorn, D.M., Signore, M., Sillaber, I., Oesterheld, M., Broccoli, V., Acampora, D., Simeone, A., Wurst, W., 2003. Location and size of dopaminergic and serotonergic cell populations are controlled by the position of the midbrain-hindbrain organizer. J. Neurosci. 23, 4199–4207.

Buxbaum, J.D., Silverman, J.M., Smith, C.J., Kilifarski, M., Reichert, J., Hollander, E., Lawlor, B.A., Fitzgerald, M., Greenberg, D.A., 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, (erratum appears in Am. J. Hum. Genet. 2001 69 (2) 470).

Casanova, M.F., Buxhoeveden, D.P., Switala, A.E., Roy, E., 2002. Minicolumnar pathology in autism. Neurology 58, 428–432.

Caston, J., Yon, E., Mellier, D., Godfrey, H.P., Delhaye-Bouchaud, N., Mariani, J., 1998. An animal model of autism: behavioral studies in the GS guinea-pig. Eur. J. Neurosci. 10, 2677–2684.

Castren, E., Pitkanen, M., Sirvio, J., Parsadanian, A., Lindholm, D., Thoenen, H., Riekkinen, P.J., 1993. The induction of LTP increases BDNF and NGF mRNA but decreases NT-3 mRNA in the dentate gyrus. Neuroreport 4, 895–898.

Chi, C.L., Martinez, S., Wurst, W., Martin, G.R., 2003. The isthmic organizer signal FGF8 is required for cell survival in the prospective midbrain and cerebellum. Development 130, 2633–2644.

Christianson, A.L., Chesler, N., Kromberg, J.G., 1994. Fetal valproate syndrome: clinical and neuro-developmental features in two sibling pairs. Dev. Med. Child Neurol. 36, 361–369.

Collins, J.S., Schroer, R.J., Bird, J., Michaelis, R.C., 2003. The HOXA1 A218G polymorphism and autism: lack of association in white and black patients from the South Carolina Autism Project. J. Autism Dev. Disord. 33, 343–348.

Conciatori, M., Stodgell, C.J., Hyman, S.L., O'bara, M., Militerni, R., Bravaccio, C., Trillo, S., Montecchi, F., Schneider, C., Melmed, R., Elia, M., Crawford, L., Spence, S.J., Muscarella, L., Guarnieri, V., D'agruma, L., Quattrone, A., Zelante, L., Rabinowitz, D., Pascucci, T., Puglisi-Allegra, S., Reichelt, K.L., Rodier, P.M., Persico, A.M., 2004. Association between the HOXA1 A218G polymorphism and increased head circumference in patients with autism. Biol. Psychiatry 55, 413–419.

Cook Jr., E.H., Courchesne, R.Y., Cox, N.J., Lord, C., Gonen, D., Guter, S.J., Lincoln, A., Nix, K., Haas, R., Leventhal, B.L., Courchesne, E., 1998. Linkage-disequilibrium mapping of autistic disorder, with 15q11-13 markers. Am. J. Hum. Genet. 62, 1077–1083.

Courchesne, E., Yeung-Courchesne, R., Press, G.A., Hesselink, J.R., Jernigan, T.L., 1988. Hypoplasia of cerebellar vermal lobules VI and VII in autism. N. Engl. J. Med. 318, 1349–1354.

Courchesne, E., Karns, C.M., Davis, H.R., Ziccardi, R., Carper, R.A., Tigue, Z.D., Chisum, H.J., Moses, P., Pierce, K., Lord, C., Lincoln, A.J., Pizzo, S., Schreibman, L., Haas, R.H., Akshoomoff, N.A., Courchesne, R.Y., 2001. Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. Neurology 57, 245–254.

Daenen, E.W., Wolterink, G., Gerrits, M.A., Van Ree, J.M., 2002. Amygdala or ventral hippocampal lesions at two early stages of life differentially affect open field behavior later in life; an animal model of neurodevelopmental psychopathological disorders. Behav. Brain Res. 131, 67–78.

Davidovitch, M., Patterson, B., Gartside, P., 1996. Head circumference measurements in children with autism. J. Child Neurol. 11, 389–393.

Devlin, B., Bennett, P., Cook Jr., E.H., Dawson, G., Gonen, D., Grigorenko, E.L., Mcmahon, W., Pauls, D., Smith, M., Spence, M.A., Schellenberg, G.D., 2002. No evidence for linkage of liability to autism to HOXA1 in a sample from the CPEA network. Am. J. Med. Genet. 114, 667–672.

Englemann, G.L., 1994. Direct effects of the adrenergic system on cardiac hypertrophy: molecular analysis of the heterotopically transplanted heart. J. Lab. Clin. Med. 124, 744–745.

Ferguson, J.N., Young, L.J., Hearn, E.F., Matzuk, M.M., Insel, T.R., Winslow, J.T., 2000. Social amnesia in mice lacking the *oxytocin* gene. Nat. Genet. 25, 284–288.

Ferguson, J.N., Aldag, J.M., Insel, T.R., Young, L.J., 2001. Oxytocin in the medial amygdala is essential for social recognition in the mouse. J. Neurosci. 21, 8278–8285.

Gale, S., Ozonoff, S., Lainhart, J., 2003. Brief report: pitocin induction in autistic and nonautistic individuals. J. Autism. Dev. Disord. 33, 205–208.

Gallagher, L., Becker, K., Kearney, G., Dunlop, A., Stallings, R., Green, A., Fitzgerald, M., Gill, M., 2003. Brief report: a case of autism associated with del(2)(q32.1q32.2) or (q32.2q32.3). J. Autism. Dev. Disord. 33, 105–108.

Gallagher, L., Hawi, Z., Kearney, G., Fitzgerald, M., Gill, M., 2004. No association between allelic variants of HOXA1/HOXB1 and autism. Am. J. Med. Genet. 124B, 64–67.

Gerlai, R., Millen, K.J., Herrup, K., Fabien, K., Joyner, A.L., Roder, J., 1996. Impaired motor learning performance in cerebellar *En-2* mutant mice. Behav. Neurosci. 110, 126–133.

Gharani, N., Benayed, R., Mancuso, V., Brzustowicz, L.M., Millonig, J.H., 2004. Association of the homeobox transcription factor, ENGRAILED 2, with autism spectrum disorder. Mol. Psychiatry 9, 474–484.

Gibert, J.M., 2002. The evolution of *engrailed* genes after duplication and speciation events. Dev. Genes Evol. 212, 307–318.

Green, L., Fein, D., Modahl, C., Feinstein, C., Waterhouse, L., Morris, M., 2001. Oxytocin and autistic disorder: alterations in peptide forms. Biol. Psychiatry 50, 609–613.

Hallmayer, J., Glasson, E.J., Bower, C., Petterson, B., Croen, L., Grether, J., Risch, N., 2002. On the twin risk in autism. Am. J. Hum. Genet. 71, 941–946.

Herbert, M.R., Ziegler, D.A., Deutsch, C.K., O'brien, L.M., Lange, N., Bakardjiev, A., Hodgson, J., Adrien, K.T., Steele, S., Makris, N., Kennedy, D., Harris, G.J., Caviness Jr., V.S., 2003. Dissociations of cerebral cortex, subcortical and cerebral white matter volumes in autistic boys. Brain 126, 1182–1192.

Hollander, E., Novotny, S., Hanratty, M., Yaffe, R., Decaria, C.M., Aronowitz, B.R., Mosovich, S., 2003. Oxytocin infusion reduces repetitive behaviors in adults with autistic and Asperger's disorders. Neuropsychopharmacology 28, 193–198.

Holttum, J.R., Minshew, N.J., Sanders, R.S., Phillips, N.E., 1992. Magnetic resonance imaging of the posterior fossa in autism. Biol. Psychiatry 32, 1091–1101.

Hornig, M., Weissenbock, H., Horscroft, N., Lipkin, W.I., 1999. An infection-based model of neurodevelopmental damage. Proc. Natl. Acad. Sci. U.S.A. 96, 12102–12107.

Howard, M.A., Cowell, P.E., Boucher, J., Broks, P., Mayes, A., Farrant, A., Roberts, N., 2000. Convergent neuroanatomical and behavioral evidence of an amygdala hypothesis of autism. Neuroreport 11, 2931–2935.

IMGSAC, 1998. A full genome screen for autism with evidence for linkage to a region on chromosome 7q. Hum. Mol. Genet. 7, 571–578.

IMGSAC, 2001. A genomewide screen for autism: strong evidence for linkage to chromosomes 2q, 7q, and 16p. Am. J. Hum. Genet. 69, 570–581.

Ingram, J.L., Peckham, S.M., Tisdale, B., Rodier, P.M., 2000a. Prenatal exposure of rats to valproic acid reproduces the cerebellar anomalies associated with autism. Neurotoxicol. Teratol. 22, 319–324.

Ingram, J.L., Stodgell, C.J., Hyman, S.L., Figlewicz, D.A., Weitkamp, L.R., Rodier, P.M., 2000b. Discovery of allelic variants of HOXA1 and HOXB1: genetic susceptibility to autism spectrum disorders. Teratology 62, 393–405.

Insel, T.R., O'brien, D.J., Leckman, J.F., 1999. Oxytocin, vasopressin, and autism: is there a connection? Biol. Psychiatry 45, 145–157.

Joyal, C.C., Meyer, C., Jacquart, G., Mahler, P., Caston, J., Lalonde, R., 1996. Effects of midline and lateral cerebellar lesions on motor coordination and spatial orientation. Brain Res. 739, 1–11.

Joyner, A.L., Herrup, K., Auerbach, B.A., Davis, C.A., Rossant, J., 1991. Subtle cerebellar phenotype in mice homozygous for a targeted deletion of the En-2 homeobox. Science 251, 1239–1243.

Joyner, A.L., 1996. *Engrailed*, *Wnt* and *Pax* genes regulate midbrain–hindbrain development. Trends Genet. 12, 15–20.

Kahne, D., Tudorica, A., Borella, A., Shapiro, L., Johnstone, F., Huang, W., Whitaker-Azmitia, P.M., 2002. Behavioral and magnetic resonance spectroscopic studies in the rat hyperserotonemic model of autism. Physiol. Behav. 75, 403–410.

Kang, H., Schuman, E.M., 1995. Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science 267, 1658–1662.

Kemper, T.L., Bauman, M.L., 1993. The contribution of neuropathologic studies to the understanding of autism. Neurol. Clin. 11, 175–187.

Kemper, T.L., Bauman, M., 1998. Neuropathology of infantile autism. J. Neuropathol. Exp. Neurol. 57, 645–652.

Krause, I., He, X.S., Gershwin, M.E., Shoenfeld, Y., 2002. Brief report: immune factors in autism: a critical review. J. Autism. Dev. Disord. 32, 337–345.

Kuemerle, B., Zanjani, H., Joyner, A., Herrup, K., 1997. Pattern deformities and cell loss in *Engrailed-2* mutant mice suggest two separate patterning events during cerebellar development. J. Neurosci. 17, 7881–7889.

Lainhart, J.E., Piven, J., Wzorek, M., Landa, R., Santangelo, S.L., Coon, H., Folstein, S.E., 1997. Macrocephaly in children and adults with autism. J. Am. Acad. Child Adolesc. Psychiatry 36, 282–290.

Leaton, R.N., Supple Jr., W.F., 1991. Medial cerebellum and long-term habituation of acoustic startle in rats. Behav. Neurosci. 105, 804–816.

Levine, E.S., Dreyfus, C.F., Black, I.B., Plummer, M.R., 1995. Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. Proc. Natl. Acad. Sci. U.S.A. 92, 8074–8077.

Lev-Ram, V., Valsamis, M., Masliah, E., Levine, S., Godfrey, H.P., 1993. A novel non-ataxic guinea pig strain with cerebrocortical and cerebellar abnormalities. Brain Res. 606, 325–331.

Li, J., Tabor, H.K., Nguyen, L., Gleason, C., Lotspeich, L.J., Spiker, D., Risch, N., Myers, R.M., 2002. Lack of association between *HoxA1* and *HoxB1* gene variants and autism in 110 multiplex families. Am. J. Med. Genet. 114, 24–30.

Lijam, N., Paylor, R., Mcdonald, M.P., Crawley, J.N., Deng, C.X., Herrup, K., Stevens, K.E., Maccaferri, G., Mcbain, C.J., Sussman, D.J., Wynshaw-Boris, A., 1997. Social interaction and sensorimotor gating abnormalities in mice lacking Dvl1. Cell 90, 895–905.

Logan, C., Willard, H.F., Rommens, J.M., Joyner, A.L., 1989. Chromosomal localization of the human homeo box-containing genes, *EN1* and *EN2*. Genomics 4, 206–209.

Long, J.M., Laporte, P., Paylor, R., Wynshaw-Boris, A., 2004. Expanded characterization of the social interaction abnormalities in mice lacking *Dvl1*. Genes Brain Behav. 3, 51–62.

Lopreiato, J.O., Wulfsberg, E.A., 1992. A complex chromosome rearrangement in a boy with autism. J. Dev. Behav. Pediatr. 13, 281–283.

Matsui, T., Hirai, M., Hirano, M., Kurosawa, Y., 1993. The HOX complex neighbored by the *EVX* gene, as well as two other homeobox-containing genes, the GBX-class and the EN-class, are located on the same chromosomes 2 and 7 in humans. FEBS Lett. 336, 107–110.

McMahon, A.P., Bradley, A., 1990. The *Wnt-1* (*int-1*) proto-oncogene is required for development of a large region of the mouse brain. Cell 62, 1073–1085.

Meyers, E.N., Lewandoski, M., Martin, G.R., 1998. An *Fgf8* mutant allelic series generated by Cre- and Flp-mediated recombination. Nat. Genet. 18, 136–141.

Millen, K.J., Wurst, W., Herrup, K., Joyner, A.L., 1994. Abnormal embryonic cerebellar development and patterning of postnatal foliation in two mouse *Engrailed-2* mutants. Development 120, 695–706.

Modahl, C., Green, L., Fein, D., Morris, M., Waterhouse, L., Feinstein, C., Levin, H., 1998. Plasma oxytocin levels in autistic children. Biol. Psychiatry 43, 270–277.

Nelson, K.B., Grether, J.K., Croen, L.A., Dambrosia, J.M., Dickens, B.F., Jelliffe, L.L., Hansen, R.L., Phillips, T.M., 2001. Neuropeptides and neurotrophins in neonatal blood of children with autism or mental retardation. Ann. Neurol. 49, 597–606.

Nicolson, R., Szatmari, P., 2003. Genetic and neurodevelopmental influences in autistic disorder. Can. J. Psychiatry 48, 526–537.

Patterson, P.H., 2002. Maternal infection: window on neuroimmune interactions in fetal brain development and mental illness. Curr. Opin. Neurobiol. 12, 115–118.

Petit, E., Herault, J., Martineau, J., Perrot, A., Barthelemy, C., Hameury, L., Sauvage, D., Lelord, G., Muh, J.P., 1995. Association study with two markers of a human homeogene in infantile autism. J. Med. Genet. 32, 269–274.

Philippe, A., Martinez, M., Guilloud-Bataille, M., Gillberg, C., Rastam, M., Sponheim, E., Coleman, M., Zappella, M., Aschauer, H., Van Maldergem, L., Penet, C., Feingold, J., Brice, A., Leboyer, M., Van Malldergerme, L., 1999. Genome-wide scan for autism susceptibility genes. Hum. Mol. Genet. 8, 805–812.

Pickles, A., Bolton, P., Macdonald, H., Bailey, A., Le Couteur, A., Sim, C.H., 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.

Pierce, K., Muller, R.A., Ambrose, J., Allen, G., Courchesne, E., 2001. Face processing occurs outside the fusiform 'face area' in autism: evidence from functional MRI. Brain 124, 2059–2073.

Piven, J., Arndt, S., Bailey, J., Havercamp, S., Andreasen, N.C., Palmer, P., 1995. An MRI study of brain size in autism. Am. J. Psychiatry 152, 1145–1149.

Piven, J., Arndt, S., Bailey, J., Andreasen, N., 1996. Regional brain enlargement in autism: a magnetic resonance imaging study. J. Am. Acad. Child Adolesc. Psychiatry 35, 530–536.

Piven, J., Bailey, J., Ranson, B.J., Arndt, S., 1998. No difference in hippocampus volume detected on magnetic resonance imaging in autistic individuals. J. Autism Dev. Disord. 28, 105–110.

Pletnikov, M.V., Rubin, S.A., Schwartz, G.J., Moran, T.H., Sobotka, T.J., Carbone, K.M., 1999. Persistent neonatal Borna disease virus (BDV) infection of the brain causes chronic emotional abnormalities in adult rats. Physiol. Behav. 66, 823–831.

Pletnikov, M.V., Rubin, S.A., Moran, T.H., Carbone, K.M., 2003. Exploring the cerebellum with a new tool: neonatal Borna disease virus (BDV) infection of the rat's brain. Cerebellum 2, 62–70.

Poole, S.J., Law, M.L., Kao, F.T., Lau, Y.F., 1989. Isolation and chromosomal localization of the human *En-2* gene. Genomics 4, 225–231.

Rau, M.E., 1983. The open-field behavior of mice infected with *Trichinella spiralis*. Parasitology 86 (Pt 2), 311–318.

Risch, N., Spiker, D., Lotspeich, L., Nouri, N., Hinds, D., Hallmayer, J., Kalaydjieva, L., Mccague, P., Dimiceli, S., Pitts, T., Nguyen, L., Yang, J., Harper, C., Thorpe, D., Vermeer, S., Young, H., Hebert, J., Lin, A., Ferguson, J., Chiotti, C., Wiese-Slater, S., Rogers, T., Salmon, B., Nicholas, P., Myers, R.M., 1999. A genomic screen of autism: evidence for a multilocus etiology. Am. J. Hum. Genet. 65, 493–507.

Ritvo, E.R., Freeman, B.J., Mason-Brothers, A., Mo, A., Ritvo, A.M., 1985. Concordance for the syndrome of autism in 40 pairs of afflicted twins. Am. J. Psychiatry 142, 74–77.

Ritvo, E.R., Freeman, B.J., Scheibel, A.B., Duong, T., Robinson, H., Guthrie, D., Ritvo, A., 1986. Lower Purkinje cell counts in the cerebella of four autistic subjects: initial findings of the UCLA-NSAC Autopsy Research Report. Am. J. Psychiatry 143, 862–866.

Rodier, P.M., Ingram, J.L., Tisdale, B., Nelson, S., Romano, J., 1996. Embryological origin for autism: developmental anomalies of the cranial nerve motor nuclei. J. Comp. Neurol. 370, 247–261.

Rodier, P.M., Ingram, J.L., Tisdale, B., Croog, V.J., 1997a. Linking etiologies in humans and animal models: studies of autism. Reprod. Toxicol. 11, 417–422.

Rodier, P.M., Bryson, S.E., Welch, J.P., 1997b. Minor malformations and physical measurements in autism: data from Nova Scotia. Teratology 55, 319–325.

Rodier, P.M., 2002. Converging evidence for brain stem injury in autism. Dev. Psychopathol. 14, 537–557.

Romano, V., Cali, F., Mirisola, M., Gambino, G.R.D.A., Di Rosa, P., Seidita, G., Chiavetta, V., Aiello, F., Canziani, F., De Leo, G., Ayala, G.F., Elia, M., 2003. Lack of association of *HOXA1* and *HOXB1* mutations and autism in Sicilian (Italian) patients. Mol. Psychiatry 8, 716–717.

Rubenstein, J.L., Merzenich, M.M., 2003. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav. 2, 255–267.

Saitoh, O., Courchesne, E., Egaas, B., Lincoln, A.J., Schreibman, L., 1995. Cross-sectional area of the posterior hippocampus in autistic patients with cerebellar and corpus callosum abnormalities. Neurology 45, 317–324.

Saitoh, O., Courchesne, E., 1998. Magnetic resonance imaging study of the brain in autism. Psychiatry Clin Neurosci. 52 (Suppl.), S219–222.

Saitoh, O., Karns, C.M., Courchesne, E., 2001. Development of the hippocampal formation from 2 to 42 years: MRI evidence of smaller area dentata in autism. Brain 124, 1317–1324.

Schwarz, M., Alvarez-Bolado, G., Urbanek, P., Busslinger, M., Gruss, P., 1997. Conserved biological function between Pax-2 and Pax-5 in midbrain and cerebellum development: evidence from targeted mutations. Proc. Natl. Acad. Sci. U.S.A. 94, 14518–14523.

Shi, L., Fatemi, S.H., Sidwell, R.W., Patterson, P.H., 2003. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. J. Neurosci. 23, 297–302.

Smith, M., Escamilla, J.R., Filipek, P., Bocian, M.E., Modahl, C., Flodman, P., Spence, M.A., 2001. Molecular genetic delineation of 2q37.3 deletion in autism and osteodystrophy: report of a case and of new markers for deletion screening by PCR. Cytogenet. Cell Genet. 94, 15–22.

Sparks, B.F., Friedman, S.D., Shaw, D.W., Aylward, E.H., Echelard, D., Artru, A.A., Maravilla, K.R., Giedd, J.N., Munson, J., Dawson, G., Dager, S.R., 2002. Brain structural abnormalities in young children with autism spectrum disorder. Neurology 59, 184–192.

Steffenburg, S., Gillberg, C., Hellgren, L., Andersson, L., Gillberg, I.C., Jakobsson, G., Bohman, M., 1989. A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden.. J. Child. Psychol. Psychiatry 30, 405–416.

Stromland, K., Nordin, V., Miller, M., Akerstrom, B., Gillberg, C., 1994. Autism in thalidomide embryopathy: a population study. Dev. Med. Child Neurol. 36, 351–356.

Sultana, R., Yu, C.E., Yu, J., Munson, J., Chen, D., Hua, W., Estes, A., Cortes, F., De La Barra, F., Yu, D., Haider, S.T., Trask, B.J., Green, E.D., Raskind, W.H., Disteche, C.M., Wijsman, E., Dawson, G., Storm, D.R., Schellenberg, G.D., Villacres, E.C., 2002. Identification of a novel gene on chromosome 7q11.2 interrupted by a translocation breakpoint in a pair of autistic twins. Genomics 80, 129–134.

Talebizadeh, Z., Bittel, D.C., Miles, J.H., Takahashi, N., Wang, C.H., Kibiryeva, N., Butler, M.G., 2002. No association between *HOXA1* and *HOXB1* genes and autism spectrum disorders (ASD). J. Med. Genet. 39, e70.

Tentler, D., Brandberg, G., Betancur, C., Gillberg, C., Anneren, G., Orsmark, C., Green, E.D., Carlsson, B., Dahl, N., 2001. A balanced reciprocal translocation t(5;7)(q14;q32) associated with autistic disorder: molecular analysis of the chromosome 7 breakpoint. Am. J. Med. Genet. 105, 729–736.

Thomas, K.R., Capecchi, M.R., 1990. Targeted disruption of the murine *int-1* proto-oncogene resulting in severe abnormalities in midbrain and cerebellar development. Nature 346, 847–850.

Torres, A.R., 2003. Is fever suppression involved in the etiology of autism and neurodevelopmental disorders? BMC Pediatr. 3, 9.

Urbanek, P., Wang, Z.Q., Fetka, I., Wagner, E.F., Busslinger, M., 1994. Complete block of early B cell differentiation and altered patterning of the posterior midbrain in mice lacking Pax5/BSAP. Cell 79, 901–912.

Vincent, J.B., Herbrick, J.A., Gurling, H.M., Bolton, P.F., Roberts, W., Scherer, S.W., 2000. Identification of a novel gene on chromosome 7q31 that is interrupted by a translocation breakpoint in an autistic individual. Am. J. Hum. Genet. 67, 510–514.

Vojdani, A., Pangborn, J.B., Vojdani, E., Cooper, E.L., 2003. Infections, toxic chemicals and dietary peptides binding to lymphocyte receptors and tissue enzymes are major instigators of autoimmunity in autism. Int. J. Immunopathol. Pharmacol. 16, 189–199.

Warburton, P., Baird, G., Chen, W., Morris, K., Jacobs, B.W., Hodgson, S., Docherty, Z., 2000. Support for linkage of autism and specific language

impairment to 7q3 from two chromosome rearrangements involving band 7q31. Am. J. Med. Genet. 96, 228–234.
Wassink, T.H., Piven, J., Patil, S.R., 2001. Chromosomal abnormalities in a clinic sample of individuals with autistic disorder. Psychiatr. Genet. 11, 57–63.
Williams, P.G., Hersh, J.H., 1997. A male with fetal valproate syndrome and autism. Dev. Med. Child Neurol. 39, 632–634.
Williams, R.W., Herrup, K., 1988. The control of neuron number. Annu. Rev. Neurosci. 11, 423–453.
Wolterink, G., Daenen, L.E., Dubbeldam, S., Gerrits, M.A., Van Rijn, R., Kruse, C.G., Van Der Heijden, J.A., Van Ree, J.M., 2001. Early amygdala damage in the rat as a model for neurodevelopmental psychopathological disorders. Eur. Neuropsychopharmacol. 11, 51–59.
Wurst, W., Auerbach, A.B., Joyner, A.L., 1994. Multiple developmental defects in *Engrailed-1* mutant mice: an early mid-hindbrain deletion and patterning defects in forelimbs and sternum. Development 120, 2065–2075.
Young, L.J., Winslow, J.T., Wang, Z., Gingrich, B., Guo, Q., Matzuk, M.M., Insel, T.R., 1997. Gene targeting approaches to neuroendocrinology: oxytocin, maternal behavior, and affiliation. Horm Behav. 31, 221–231.
Zhong, H., Serajee, F.J., Nabi, R., Huq, A.H., 2003. No association between the *EN2* gene and autistic disorder. J. Med. Genet. 40, e4.