Autism risk factors: genes, environment, and gene-environment interactions

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The aim of this review is to summarize the key findings from genetic and epidemiological research, which show that autism is a complex disorder resulting from the combination of genetic and environmental factors. Remarkable advances in the knowledge of genetic causes of autism have resulted from the great efforts made in the field of genetics. The identification of specific alleles contributing to the autism spectrum has supplied important pieces for the autism puzzle. However, many questions remain unanswered, and new questions are raised by recent results. Moreover, given the amount of evidence supporting a significant contribution of environmental factors to autism risk, it is now clear that the search for environmental factors should be reinforced. One aspect of this search that has been neglected so far is the study of interactions between genes and environmental factors.

Dialogues Clin Neurosci. 2012;14:281-292.

Keywords: *autism; risk; genetic; environment; interaction*

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Introduction

Autism was originally defined by Leo Kanner in 1943 as an innate inability to create normal, biologically determined, emotional contact with others. The primacy of the social deficit is widely recognized, and lack of social reciprocity is a central part of the diagnosis. Beyond that, there have been great changes in the past decade in the conceptualization of autism and related disorders, eventually reflected in the draft of the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-5*, www.dsm5.org). Indeed, proposed revisions of the precedent edition of the manual (*DSM IV-TR*)[1] include the combination of specific *DSM-IV-TR* diagnoses into a single broad autism spectrum disorder (ASD), and the identification of two domains of impairment (social communication and interaction, and restricted repetitive behavior) instead of three (social interaction, communication, and restricted repetitive and stereotyped patterns of behavior, interests and activities). These issues are discussed in detail by Volkmar et al in this issue of the journal.

Because of the high heritability estimates in autism, a major focus of research in autism has been on finding the underlying genetic causes, with less emphasis on potential environmental triggers or causes. Although remark-

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able advances in our knowledge of genetic causes have resulted from these great efforts made in the field of genetics, recent debates about increasing prevalence[2] or heritability[3] have highlighted the necessity to expand the research on environmental factors. In this review, we summarize the findings regarding genetic, epigenetic, and environmental risk factors identified in autism, and discuss the issue of gene × environment interactions (G×E).

Genetic risk factors

Genetic epidemiology

Heritability

The recurrence risk of pervasive developmental disorder in siblings of children with autism is 2% to 8%,[4] and it rises to 12% to 20% if one takes into account the siblings showing impairment in one or two of the three domains impaired in autism respectively.[5] Moreover, several twin studies have suggested that this aggregation within families is best explained by shared genes as opposed to shared environment.[6-8] Interestingly, the variation of autistic traits in the general population has been shown to be highly heritable, at a similar level of genetic influence to autism itself, even though the results are heterogeneous (heritability 40% to 80%).[9,10] These results have led to a huge effort in research to try to unravel the genetic factors underlying the disorder. However, two recent twin studies have provided intriguing results. One study showed that monozygotic twins had higher concordance rates than dizygotic twins for ASDs, attention deficit hyperactivity disorder (ADHD), developmental coordination disorder, and tic disorder with differences in cross-disorder effects between monozygotic and dizygotic twins, raising the question of the specificity of the underlying genetic factors.[8] Another study recently challenged the high heritability model of autism, estimating the heritability of autism to be 55%.[3] This study generated considerable discussion, the main criticisms concerning the very large confidence interval of the odds ratio (9% to 81%) and the low participation rate. However, this study is the largest population-based twin study of autism that used contemporary standards for the diagnosis of autism.

The independent heritability of each of the domains of autistic symptomatology is still a matter of debate. While some argue that different autistic symptoms, to a considerable extent, have separate genetic influences,[11,12] others argue that there is strong evidence in favor of the hypothesis that symptom domains represent correlated behavioral manifestations of a single underlying quantitative neurodevelopmental impairment.[13]

Transmission in simplex and multiplex families

According to two studies, the prevalence of de novo chromosomal rearrangements is higher in subjects from simplex families (one affected individual) compared with subjects from multiplex families,[14,15] which is consistent with the high rate of notable de novo mutations identified in probands from simplex families.[16] This is also consistent with the results of studies which have shown that familial aggregation of subclinical autistic traits may occur only in multiplex families, suggesting differential mechanisms of genetic transmission of autism in the population.[17,18]

Several biological pathways identified

Individuals with ASD vary in language ability, ranging from absent speech to fluent language, and in cognitive development, ranging from profound intellectual disability to above-average intellectual functioning. Individuals may also show associated medical comorbidities including epilepsy and minor physical anomalies, as well as psychiatric comorbidities, thus showing a wide clinical heterogeneity. The clinical heterogeneity of autism has long been a hindrance to understanding the pathophysiological mechanisms involved. However, although many questions remain and new questions are being raised, the last several years of investigation have brought important pieces to the autism puzzle. Indeed, the identification of specific alleles contributing to ASD has shed light on pathogenic mechanisms.

The only consensus regarding the mode of inheritance of autism is that it is not Mendelian, at least in a vast majority of cases. Several studies were initially in favor of a polygenic model.[19-21] Therefore, the initial strategy to unravel genetic factors increasing autism risk was to build large cohorts for linkage and association studies. Given the lack of replication of the results, consortia gathering several cohorts were created to increase the power of the studies, but without clear results. With regard to nonparametric linkage, the largest study to date included 1181 multiplex families[22] and did not iden-

tify highly significant evidence for linkage. Moreover, the three large studies using genome-wide association that have been published thus far each highlight a single, non-overlapping risk locus.[23-25] These findings led some authors to predict that few, if any, common variants have a substantial impact on risk (odds ratio >1.2), but many common variants could have a more modest impact.[26]

Going back to an individual approach, already used in mental retardation, the search for rare mutations or chromosomal rearrangements was then used, allowing new hypotheses about the mechanisms involved in autism. While the existence of many genetic syndromes associated with autism first led to considering the existence of genetic heterogeneity mirroring the clinical variability, genetic studies in idiopathic autism confirmed the existence of different defects in common pathways. The results suggest that autism may be caused by a multitude of genetic alterations that ultimately affect only limited biological pathways of brain development and plasticity.

First, specific genetic syndromes like Rett syndrome or Fragile-X syndrome, or cytogenetic abnormalities, the most common being the 15q11–q13 duplication of the maternal allele, associated with ASD affect synaptic plasticity. Moreover, the first mutations identified in idiopathic autism involve synaptic genes like *NLGN3* and *NLGN4X*,[27,28] or *SHANK3*.[29-31] Results were enriched by the development of whole-genome screening methodologies which have shown that genetic structural variation contributes significantly to autism. The detection of copy number variations (CNVs), with constantly increasing resolution, consistently confirmed the importance of the synaptic function in autism.[22] Several subsequent studies showed CNV in the NLGN-NRXN-SHANK pathway, and other synaptic genes such as *SynGAP* and *DLGAP2*[15,32,33] *(Table I)*.

The analysis of genes affected by rare CNVs has confirmed the crucial role of abnormalities in synapse formation and maintenance, but also identified other affected pathways, including cellular proliferation and motility, GTPase/Ras signaling, and neurogenesis.[33-35] It is interesting to note that some de novo or inherited CNVs associated with ASD, which recur at the same locus among unrelated individuals, have so far resisted identification of specific ASD genes. One of the most frequent of these involves the 16p11 region. Moreover, as techniques are improving very fast, the first results of large-scale studies using whole-exome sequencing, ie, the mapping of every base of DNA across the exome, were recently released.[36-38] These three studies report de novo mutations with a twofold to fourfold increase in de novo nonsense variants among affected subjects over that expected by chance. Interestingly, two of these studies report that spontaneous changes are correlated with paternal age.[36-38] One of these studies strongly suggests the involvement of brain signaling as a new biological pathway.[37]

It is now clear that there is a huge genetic heterogeneity in ASD, involving both a locus heterogeneity and an allelic heterogeneity. The exome sequencing studies suggest that the recent results predicting up to 234 loci contributing to ASD risk[39] are probably even an underestimation.[37,38] Some important Web resources cataloguing genetic contributors in ASD include the SFARI Gene database (https://gene.sfari.org/autdb/), the AutDB database (http://www.mindspec.org/autdb.html), and the Autism Chromosome Rearrangement Database (http://projects.tcag.ca/autism/).

Remaining questions

Genotype/phenotype correlations

One of the most important remaining unsolved issues is the understanding of the relationships between genetic variation and phenotype, given the recent observations that identical mutations may be associated with highly divergent phenotype. Indeed, identical CNVs have been associated with autism and schizophrenia, notably 16p11 rearrangements.[15,40-43] *SHANK3* and *NRXN1* genes were also suggested to be involved in schizophrenia,[44-46] and genes implicated in autism and/or schizophrenia were significantly enriched in ADHD CNV genes in one study.[47] A first hypothesis to explain this phenotypic heterogeneity is that a secondary insult is necessary during development to result in the phenotype, as in the "two-hit model" proposed in developmental delay.[48] Several studies recently supported the existence of such a combination of rare variants in some cases.[39,49,50] Another hypothesis is the contribution of both rare and frequent variants. This would be consistent with the observations of broader subthreshold traits in siblings.[51] Although, as we have already mentioned, association studies have not provided clear evidence of the contribution of common variants in autism, a recent analysis of genetic variations associated with ASD suggests that common and rare

Study ref	Patients	Controls	Array	Candidate region/ gene identified	Genetic result region/ gene	Other results
112	350 cases (SSC)	337 NIMH controls	CGH array	*FOXP1* *DPP6, SCN4A, WNT3* and *WNT9B*	De novo deletion	Patients with autism without mental retardation show only modest increases in large CNV burden compared with controls
39	852 quartets and 252 trios (SSC)	852 unaffected siblings	Illumina 1M	7q11.23 (Williams Beuren region), 1q21.1, 16p13.2, *CDH13*	Recurrent de novo deletions/duplications	Only modest correlation with IQ Rare inherited CNVs equally represented in patients and unaffected siblings Estimation of >234 distinct genomic regions contributing to large ASD-related de novo structural variations
113	510 quartets and 277 trios		NimbleGen HD2 2.1 million probe microarray	7q11.23 (Williams Beuren region), 16p13.2, *COMMD1*, *CACNA2D4*	Recurrent de novo deletions/duplications Rare homozygous deletions	
33	996 cases (AGP)	1287	Illumina 1M	*SHANK2, SynGAP*, *DLGAP2, PTCHD1*	De novo deletion Maternal transmission (X chromosome)	N de novo CNV multiplex = simplex Common pathways/ intellectual disability
114	859 cases (ACC), 1336 cases (AGRE)	1409	Ilumina 550K	*PARK2, UBE3A*, *RFWD2*, and *FBXO40 (ubiquitination), NLGN1 CNTN4, 15q11, 22q11, NRXN1*	Statistically significant association Inherited del/dup absent in controls	
115	859 cases (ACC), 912 families (AGRE)	1448	Illumina 550K	*CNTNAP2, NRXN1, PCDH9, BZRAP1, MDGA2, RAI1, TSC2, NLGN1*	Inherited deletion absent in controls Statistically significant association	
116	104 including 88 consanguineous		Affymetrix 500K et CGH array	22q11 *PCDH10, CNTN3*	De novo deletion homozygous inherited deletion	Role of inherited CNVs
117	397 cases AGRE	372	CGH array	15q11, 22q11, 16p11	De novo deletion	
15	427 (Can.)	500	Affymetrix 500K	16p11 *NLGN4, DLGAP2, SHANK3*, 22q11,15q11-q13 16p11 *PTCHD1* *NRXN1*	Statistically significant Association De novo deletion De novo deletion Maternal transmission (X chromosome) Statistically significant Association	N de novo CNV simplex>multiplex
43	1441 AGRE	1420 parents, 2814 controls	Affymetrix 5.0/Affymetrix 500K	16p11	Statistically significant Association	

Table I. Main copy number variation (CNV) studies.

variants contribute to ASD by perturbation of common neuronal networks.[35] The last hypothesis, which is not mutually exclusive with other hypotheses, is the contribution of environmental factors which modify the phenotype.

Sex ratio

Autism affects males four times more than females,[52] and the cause for this difference is not well understood. Several theories have been proposed, among which the involvement of the sex chromosome in the etiology of ASD, and the role of hormonal influences in utero (for review see ref 53). However, none of these theories has been confirmed yet.

Intellectual disability

Intellectual disability (ID) is present in 65% to 75% of individuals with a strict diagnosis of autistic disorder, and in 30% to 55% if all ASDs are considered.[54,55] Two different models are proposed to explain this overlap. The first model proposes that intellectual disability and ASD share common genetic bases, common genes causing a continuum of developmental disorders that manifest in different ways depending on other genetic or environmental factors. This model is supported by the observation that all recurrent genetic defects reported in autism, including autism without mental retardation, have been causally implicated in intellectual disability,[56] and that analysis of the genes affected by rare CNVs reveal that they are strongly functionally related to genes previously implicated in intellectual disability.[33,34] The second model assumes that in patients with intellectual disability, the general cognitive disability unmasks the limitations in the individual's capacity for social reciprocity.[57] This model is supported by evidence that indicates a continuous distribution of autistic traits in normal population[9] and etiological similarity across ASD and autistic traits in the general population.[58] However it is tempered by the results of a recent large-scale CNV study showing a strong effect of large rare genic de novo CNVs on the presence or absence of an ASD diagnosis, but did not support IQ as a useful predictor for probands carrying these risk variants.[39]

Environmental risk factors

Indirect evidence suggesting a contribution of environmental factors

Prevalence

Prevalence studies of autism spectrum disorders conducted in recent years have been the source of an important debate because of a steady and highly significant increase of estimates of the total prevalence of pervasive developmental disorders. Indeed, while the prevalence was estimated at 6 per 1000 in a population of school children in 2005,[52] recent studies have gone so far as to estimate the prevalence to be one child in 38.[59] The last prevalence estimates in the United States, released by the Centers for Disease Control recently,[60] reached 1 in 88 child in 2008, while their previous estimate was one in 110 in 2006. However, most of the studies are not comparable in method or in the populations studied. One hypothesis is that this increase is the result of enlargement of diagnostic criteria, and the growing importance of screening for ASDs. The results of an epidemiological study from England, based on a national

40	712 AGRE + NIMH	837	CGH array	16p11	Statistically significant Association	
14	264 families AGRE	99 families	Agilent 85K	*FLJ16237, SLC4A10, A2BP1, FHIT* 15q11-q13,22q13.33, 16p11.2	De novo deletion De novo deletion/ duplication	N CNV de novo greater in probands from simplex families
22	1496 families AGP	Unaffected relatives	Affymetrix 10K	*NRXN1*	De novo deletion in two affected sisters	
118	29 (Fr)	-	CGH array	*GRIA3* (Xq25), 15q11-q13	Maternal transmission (X chromosome) De novo duplication	

Table I. Continued.

sample from 2007, support this hypothesis. Indeed the authors found a rate of about 1% in adults across the entire age range, without a significant reduction in the older part of the sample, as one would expect if the prevalence had increased in recent years.[61] However, another study suggested that diagnostic substitution, especially for the most severe cases, and better ascertainment, especially for children at the less severe end of the spectrum, explain only a part of the linear increase observed in the California registry.[62]
While the hypothesis of an increased incidence in relation to environmental factors could not be confirmed nor excluded definitely, studies using the same protocol several years apart are required.[63] Nevertheless, it seems reasonable to think that there may be both a real increase in the number of cases and an increase in the detection of affected children, and one should not wait for the results of these studies to search for environmental factors increasing risk for autism.

Immune dysfunction

Several lines of evidence support the hypothesis of immune changes in autism. First, several studies have shown abnormalities in the peripheral immune system such as T-cell dysfunction, autoantibody production, increase in the number of activated B cells and NK cells, and increase in proinflammatory cytokines.[64-66] Moreover, a landmark study provided evidence for microglial and astroglial activation in brain of patients with ASD.[67] The most prominent microglial reaction was observed in the cerebellum and cerebral white matter. The authors also found, in the cerebrospinal fluid of other patients, an increase of proinflammatory and modulatory cytokines. Another study consistently reported microglial activation in the dorsolateral prefrontal cortex in brains of patients with ASD.[68] This neuroglial response may result from either a primary disturbance of neuroglial function or unknown factors that disturb prenatal or postnatal CNS development.

Transcriptome

The first comprehensive gene-expression analysis of brains of patients with ASD recently reported differences in transcriptome organization between autistic and normal brain.[69] The measure of messenger RNA levels, using Illumina microarrays, in three regions of postmortem brains from patients with autism and controls, showed 444 genes differentially expressed between the cerebral cortices of the autistic and control brains. Moreover, the authors identified two discrete modules of coexpressed genes associated with autism. While the first module, which is related to synaptic function and neuronal projection, was underexpressed in autism cases, the second module, which was enriched for immune genes and glial markers, was overexpressed. These results are consistent with the findings mentioned above, implicating synaptic dysfunction as well as immune dysregulation in autism. Interestingly, the first module shows a highly significant enrichment for variants genetically associated with autism, further supporting the genetic basis of synaptic dysfunction in ASD. On the contrary, the authors did not find any evidence for a genetic etiology for the upregulation of the genes of the second module, suggesting that it is probably a nongenetic, adaptive, or environmental process.

Epigenetic dysregulation in autism

Epigenetic marks define chromatin state and regulate the expression of many genes without affecting primary DNA sequence. These include DNA methylation, and histone methylation and acetylation, and can be modified in response to either genetic mutations or environmental exposure. Several elements indicate the existence of epigenetic dysregulation in autism. First, several syndromes associated with autism are caused by mutations in genes involved in epigenetic regulation. For example, there are abnormalities of transcriptional regulation in Rett syndrome, caused by a mutation of methyl-CpG-binding protein 2 (MeCP2). Indeed, MeCP2 binds to methylated DNA and represses the transcription of target genes.[70] Second, several chromosomal regions subject to parental imprinting (transcriptional regulation of either the maternal allele or the paternal allele inducing monoallelic expression) were associated with autism. Notably, microduplications or microdeletions of the region 15q11q13, which is subject to parental imprinting, have been repeatedly reported in subjects with autism.[71,72] Moreover, in Turner's syndrome, women who have monosomy of the X chromosome (X0), often have autistic traits which are correlated with the parental origin of the X chromosome they received.[73] Regarding common variants, several studies have reported an association of ASD with single-nucleotide polymorphisms in

a gene which is directly involved in methylation.[74,75] Last, direct changes in DNA methylation profile in lymphoblastoid cells of autistic patients were reported recently[76] showing a decreased expression of retinoic acid-related orphan receptor alpha gene (*RORA*) and B-cell lymphoma 2 (BCL-2).

Although most of the epigenetic modifications described above are underpinned by genetic mechanisms, the evidence of the contribution of epigenetic dysregulation in autism raises the issue of the role of epigenetic modifications by environmental factors. An example is assisted conception. Indeed, while it was shown that in vitro fertilization and ovulation induction can result in abnormal methylation and dysregulation of imprinted genes,[77] epidemiologic studies on the use of assisted reproductive technology and the risk of autism found conflicting results.[78]

Direct evidence for the contribution of environmental factors

There has been much discussion about the initial suggestion that MMR (measles mumps, rubella) vaccine.[79] However there is now a scientific consensus that the evidence favors rejection of a causal relationship between thimerosal-containing vaccines and autism, based on multiple epidemiologic studies which did not support a link between thimerosal-containing vaccines and ASD (see the review by Parker in ref 80). However, other environmental factors are likely to contribute to a significant proportion of ASD risk.

Prenatal and perinatal factors

A recent meta-analysis of prenatal factors, limited to pregnancy-related factors, identified few significant risk factors.[81] The main factors are maternal gestational diabetes, maternal bleeding during pregnancy, and maternal medication. The latter issue will be further discussed later. Moreover, increased risk was also found in this meta-analysis for first-born children compared with children born third or later, and, in Nordic countries, for offspring of mothers born abroad. Exposure to intrauterine infections was associated with a significant increase in risk for autism in the analysis limited to the four studies that controlled for multiple covariates or used sibling controls. The association between maternal infection and autism risk is further supported by the results with rodent models of the maternal infection. In these animal models, gestational viral infection is mimicked by systemic administration of Poly I:C, a synthetic double-stranded RNA, which elicits an innate immune response. It seems that gestational viral infections trigger a maternal immune response, which can perturb fetal brain development, at least in part through interleukin-6.[82]

In another meta-analysis focusing on the perinatal and neonatal period,[83] the same authors identified several potential risk factors, the main being fetal presentation, umbilical-cord complications, fetal distress, birth injury or trauma, multiple birth, maternal hemorrhage, summer birth, low birth weight, small for gestational age, low 5-minute Apgar score, meconium aspiration, neonatal anemia, ABO or Rh incompatibility, and hyperbilirubinemia. Feeding difficulties and congenital malformation that are also mentioned should rather be considered as symptoms of an underlying cause of autism. The identification of summer birth as a risk factor is consistent with the results of a recent study showing that maternal infection in the first trimester increases autism risk.[84]

Overall, preterm birth was not associated with the risk of autism. However, a recent study based on rigorous diagnostic assessment using validated instruments suggested an association between preterm birth and risk for ASD consistent with the results of most previous prospective studies.[85] This study estimated the prevalence to be 5% in adolescents who had a birth weight <2000 g in the US, which is significantly greater than the last national prevalence estimates. Most of these subjects were born preterm (96.7%); however, 32.3% were small for gestational age and the authors did not use multivariate analyses to simultaneously control for birth weight and gestational age. This methodological issue concerns most of the studies on perinatal and neonatal risk factors, which makes it difficult to interpret the results of these studies, since many of the events studied are likely to occur at the same time. Another limitation to the interpretation of the results is that some studies have suggested that increased rates of birth and pregnancy complications are likely secondary to familial factors associated with autism.[86]

Socioeconomic status

Although one study did not find any association between risk of autism and socioeconomic status[87] including maternal education level, the latter may sig-

nificantly influence the age of first single words.[88] Moreover, as already mentioned, autism risk was found to be significantly increased for the offspring of mothers born abroad in a meta-analysis.[81] This risk was further specified in a very recent study[89] showing that children of migrant parents are at an increased risk of autism with intellectual disability, especially when parents migrated to Sweden from regions with a low human development index, and a decreased risk of high-functioning autism. The risk for low-functioning autism peaked when migration occurred around the time of pregnancy. Different mechanisms can be proposed to explain these results, such as the high level of maternal stress or low immunity regarding common infections.

Drugs and toxic exposure

As previously mentioned, exposure to medication during pregnancy was found to increase autism risk in the most recent meta-analyses.[81] Prenatal exposure to valproate is a recognized risk factor for ASD, especially in the first trimester of pregnancy. Children exposed in utero to valproate have 8–fold increased risk to have ASD.[90] Interestingly, a downregulation of NLGN3 was observed in hippocampal subregions and the somatosensory cortex of mice prenatally exposed to valproate.[91] Moreover, one of the major concerns regarding medication exposure during pregnancy concerns the use of antidepressants, since selective serotonin reuptake inhibitor medication during pregnancy increased from 1.5% in 1996 to 6.4% in 2004 and 6.2% in 2005.[92] It was suggested that antidepressant exposure during pregnancy modestly increases the risk of ASD, especially in the first semester.[93] Lastly, exposure in utero to an organophosphate insecticide, chlorpyrifos, was found to increase ASD risk and it was suggested that synthetic chemicals should be far more explored.[94]

Gene-environment interaction

Genetic heterogeneity can be one explanation for the absence of replication of association studies in autism. However, these results could also be interpreted within the framework of a G×E interaction model.[95] If, for example, an association has been found in a sample with subjects frequently exposed to a particular environmental risk but not in those infrequently exposed, and exposure was not ascertained, the source of nonreplication will remain elusive.

The existence of interactions between genetic background and environmental factors in autism was first suggested for perinatal complications. Indeed, in an epidemiological study on autism that included a comparison group of siblings,[96] unaffected siblings had fewer prenatal and perinatal complications than their affected siblings, but more than control subjects. This suggested that individuals with autism may react differently to the same environmental stimuli and may have less tolerance to the prenatal experience compared with their siblings. Moreover, studies of animal models have suggested that genetic defects in synaptic function may alter sensitivity to the environment. Indeed a study has shown that neuroligin-deficient mutants of *C. elegans* nematodes are hypersensitive to oxidative stress.[97] Another study reported that the hippocampal slices from MecP2- deficient mice are more susceptible to hypoxia.[98] Conversely, it was shown in an animal models that the most significant pathology of the extremely premature brain is the disruption of synaptic development.[99] It was thus hypothesized that synaptic gene defects could interact with environmental factor to increase autism risk. Another hypothesis is the interaction between genetic variations melatonin pathway genes and oxidative stress. Indeed, low plasma melatonin concentration is a frequent trait in ASD patients,[100,101]caused by a primary deficit in acetylserotonin-methyl-transferase (ASMT) activity. It was suggested that genetic variations contribute to the enzymatic deficit.[100] Several studies have suggested an antioxidant effect of melatonin in vitro,[102,103] and it was shown that the administration of melatonin reduces oxidative stress in newborn infants exposed to infection or fetal distress,[104] and promotes oligodendroglial maturation in the newborn rat with abnormal white matter related to fetal hypoxia.[105] Thus it could have a neuroprotective effect in the newborn exposed to fetal distress. Interestingly Gardener et al[81] noted that several of the perinatal and neonatal risk factors they identified may be associated with an increased risk of hypoxia. We can thus hypothesize that a deficit of melatonin could be taken into account in the consequences of perinatal distress.

Beyond these observations, available evidence for the contribution of G×E to autism risk comes from animal models. In a first study,[106] mice haploinsufficient for the TSC2 gene demonstrated a lack of normal social approach behavior only when exposed to maternal immune activation. The authors propose that disinhibited

TSC/mTOR signaling downstream of mediators of gestational immune activation effects amplifies their impact on the mutant mice fetal brain; or that the immune activation may be more pronounced in mutants because of the role of TSC/mTOR signaling in the regulation of the adaptive immune response. Moreover, exploring further the possible interaction between tuberous sclerosis and maternal immune activation in a cohort of individuals with tuberous sclerosis, the authors found an association of late gestation with peak seasonal flu activity specifically in individuals affected by ASD. These results suggest that late gestation is the main period of vulnerability of neurodevelopment to flu infection, which is in contradiction with results, discussed earlier, suggesting that summer birth and maternal infection during the first trimester are risk factors for ASD. However, we can reasonably hypothesize that the period of main vulnerability to infection during gestation may vary according to genetic factors, and that there is a specific period of vulnerability of neurodevelopment during late gestation in tuberous sclerosis. In another animal model,[107] prenatal maternal immune activation and expression of a mutant DISC1 protein interacted to produce an altered pattern of sociability. This neurobehavioral profile was absent in untreated mice expressing the mutant.

Although these results are very encouraging, family and population-based association studies in autism have not been extended for G×E interaction yet. One of the main problems with this kind of study is that power to detect G×E interactions is even lower than power to detect genetic or environmental main effects, and the enthusiasm for G×E research in other psychiatric disorders has recently been tempered by the absence of replication of many positive results.[108] Nevertheless, these studies are needed since they might help us to understand the inconsistency in results found in classical association studies and provide useful hints with regard to prevention. Two large-scale prospective epidemiological studies aiming at exploring environmental factors and G×E interaction were recently launched. The National Children's study will follow 100 000 children in the US from conception to age 21.[109] Biological samples are collected from each mother and child. The Autism Birth Cohort will follow 100 000 children from conception to age 7.[110] Biological samples are collected from children and their parents. Interestingly, an encouraging result came from an association study in attention deficit with hyperactivity disorder (ADHD), which found G×E effects on ASD symptoms in children with ADHD. Multiple regression analyses for GxE effects showed that 5-HTTLPR S/S genotype interacted with maternal smoking during pregnancy, increasing problems in social interaction, and also interacted with low birth weight, increasing rigid behavior.[111] Last, given the new understanding of the genetic architecture of autism, further study of the interaction of rare variants associated with ASD and environmental factors in populations carrying identical mutations would be useful but are difficult to perform due to the small number of carriers.

Conclusion

Contrary to the frequent assertion that we know only little of the risk of autism, major advances have been made in the past decade in this domain. In particular, recent advances in genetics have allowed a new conceptualization of molecular and cellular mechanisms of the pathology. At the same time new questions are raised, including the role of common variants and the relationship between genotype and phenotype. The contribution of environmental factors through additive or multiplicative effect needs to be further explored. New funding will need to be dedicated to this domain of research, which has been sparsely funded until very recently. ❑

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Factores de riesgo del autismo: genes, ambiente e interacciones genes-ambiente

El objetivo de esta revisión es resumir los principales hallazgos de la investigación genética y epidemiológica, que muestran que el autismo es un trastorno complejo que resulta de la combinación de factores genéticos y ambientales. Gracias los grandes esfuerzos que se han realizado en el campo de la genética se cuenta con destacados avances en el conocimiento de este tipo de causas en el autismo. La identificación de alelos específicos que contribuyen al espectro autista ha aportado piezas importantes para el puzzle del autismo. Sin embargo, aun hay muchas dudas sin responder y han surgido nuevas preguntas a partir de resultados recientes. Además, considerando la cantidad de evidencia que sustenta una contribución significativa de los factores ambientales al riesgo de autismo, hoy es claro que se debe reforzar la investigación de los factores ambientales. Un aspecto de esta investigación que se ha descuidado hasta ahora es el estudio de las interacciones entre los genes y los factores ambientales.

Facteurs de risque d'autisme : gènes, environnement et interactions gène-environnement

Le but de cet article est de résumer les résultats importants de la recherche génétique et épidémiologique qui montrent que l'autisme est un trouble complexe issu de la combinaison de facteurs génétiques et environnementaux. Les grands efforts réalisés dans le domaine de la génétique ont permis des progrès remarquables dans la connaissance des causes génétiques de l'autisme. L'identification d'allèles spécifiques contribuant au développement des troubles du spectre autistique a fourni des pièces importantes pour le puzzle de l'autisme. Cependant, de nombreuses questions restent sans réponse et de nouvelles sont soulevées par des résultats récents. Par ailleurs, les résultats suggérant une participation significative des facteurs environnementaux au risque d'autisme, engagent à maintenant insister sur leur recherche. L'étude des interactions entre les gènes et les facteurs environnementaux est un aspect de la recherche qui a été négligé jusqu'à maintenant.

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