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REVIEW

Triggers for Autism: Genetic and Environmental Factors

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Abstract: This report reviews the research on the factors that cause autism. In several studies, these factors have been verified by reproducing them in autistic animal models. Clinical research has demonstrated that genetic and environmental factors play a major role in the development of autism. However, most cases are idiopathic, and no single factor can explain the trends in the pathology and prevalence of autism. At the time of this writing, autism is viewed more as a multi-factorial disorder. However, the existence of an unknown factor that may be common in all autistic cases cannot be ruled out. It is hoped that future biological studies of autism will help construct a new theory that can interpret the pathology of autism in a coherent manner. To achieve this, large-scale epidemiological research is essential.

Keywords: autism, prevalence, susceptibility genes, environmental factors, animal models

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Introduction

Autism is a developmental disorder that is clinically characterized by deficits in social reciprocity and communication, and by unusual restricted, repetitive behaviors, hyperesthesia, and hyperactivity.[1,2] Autism has been the focus of debate in recent years, largely as a result of multinational reports of increasing prevalence.[3] However, the precise mechanism underlying the pathophysiology of this disorder remains to be determined.[1,2]

In this review, we focus on the possible etiological factors that cause autism: genetic and environmental factors. Most candidates have been verified by reproducing them in animal models. We also discuss the challenges of creating autistic animal models based on several topical factors.

Study of Genetic Factors in Autism

Autism has a strong genetic basis. Several lines of evidence support genetic factors as a predominant cause of the autistic spectrum disorders. Subsequent twin studies have provided additional support for a complex genetic etiology. In a combined sample, 60% of monozygotic (MZ) pairs were concordant for autism versus no dizygotic (DZ) pairs, and 92% of MZ pairs were concordant for a broader spectrum of related cognitive or social abnormalities versus 10% of DZ pairs.[4] A twin study in a northern European community has shown that the concordance for autism by pairs was 91% in MZ and 0% in DZ pairs.[5] These findings indicate that autism is under a high degree of genetic control and suggest the involvement of multiple genetic loci.[4,5] It is a unique characteristic of autism among other psychiatric diseases.[6–8]

The search methods of disease susceptibility genes are roughly divided into a candidate gene approach and genome wide approach. The former identifies the gene by examining families with genetic diseases that have a high rate of certain complications or a particular chromosomal abnormality. The latter uses linkage analysis and large-scale genome screening to investigate chromosomal regions. Currently, in the study of susceptibility genes in genetic diseases, the most commonly used method is positional cloning, in which linkage analysis of samples taken from affected families is followed by mutational analysis of candidate genes. These technical advances made it possible to perform the first candidate gene association studies and re-sequencing efforts in the late 1990s. Whole-genome linkage studies were used to identify additional loci of potential interest. Although the application of genome-wide techniques to assess copy number variation (CNV) has only just begun, these studies have already identified a large number of potentially important novel candidate loci.[6] The following chromosomal regions and candidate genes are considered important (See Table 1).

Chromosome 15

Findings from various genetic studies of autism have strongly suggested that the chromosome 15q11-q13 is a candidate region for autism.[9] Maternal duplication anomalies of this region have been observed in many cases, and these findings are either insertional duplication or excessive pseudoduplication of the 15q11-q13 region. The 15q proximal side is the causative gene locus of Angelman syndrome (caused by a maternal defect) and Prader-Willi syndrome (caused by a paternal defect).[10] This region includes ATPase type 10C (ATP10C), a GABA-A receptor subunit β3 (GABRB3), small nuclear ribonucleoprotein polypeptide N (SNRPN), and ubiquitin protein ligase E3A (UBE3A).

Linkage analysis has suggested a link between autism and the GABRB3 gene.[11] A correlation with the GABRB3 gene was later clarified by the detailed mapping analysis of gene susceptibility focusing on the insistence on sameness observed in the autistic subjects.[12] A strong correlation between autism and UBE3A, the responsible gene for the Angelman syndrome, has been demonstrated in a recent large-scale search of CNV in the genome.[13] On the other hand, a correlation between autism and the ATP10C gene, involved in Angelman syndrome, still remains controversial. Similarly, there are no data that actively support the correlation between autism and the SNRPN gene, which has been suggested to be involved in Prader-Willi syndrome.

Takumi et al used genetic engineering technology to produce a mouse model with a duplicated 15q11-q13 region and they investigated its phenotype.[14] Their results revealed social disorders, stereotyped movement, perseverative tendency, retarded development of ultrasonic vocalization, increased anxiety, as well as abnormal functioning of serotonin neurons in a mouse model with paternal duplication.[14] On the other hand, they also showed that a mouse model with maternal

Table 1. Susceptibility genes of autistic spectrum disorder in this review.

Gene name	Symbol	Chromosomal location	Study type	Reference
Gamma-aminobutyric acid (GABA) A receptor, beta 3	GABRB3	15q11	Linkage analysis	11,12
Ubiquitin protein ligase E3A	UBE3A	15q11	Large-scale search of CNV	13
ATPase, class V, type 10C	ATP10C	15q11	**In controversy**	
Small nuclear ribonucleoprotein polypeptide N	SNRPN	15q11	**In controversy**	
Distal-less homeobox 5	DLX5	7q22	Allele specific expression analysis	17
Reelin	RELN	7q22	Family-based association analyses	18,19
Ca++-dependent secretion activator 2	CADPS2	7q31	RT-PCR analysis	20
Forkhead box P2	FOXP2	7q31	Bioinformatic analyses, RT-PCR analysis, expression analyses, translocation mapping	21,22
IMP2 inner mitochondrial membrane peptidase-like	IMMP2L	7q31	High-density association analysis	24
Wingless-type MMTV integration site family member 2	WNT2	7q31	Genome-wide linkage screen	26
Homeobox A1	HOXA1	7p15	SNP-based linkage analysis	28
EF-hand domain (C-terminal) containing 2	EFHC2	Xp11	Dense mapping, quantitative trait analysis, long-range haplotype analysis	30
Fragile X mental retardation 1	FMR1	Xq27	**In controversy**	
Methyl CpG binding protein 2	MECP2	Xq28	**In controversy**	
Neuroligin	NLGN	Xp22	RT-PCR analysis	34
Neurexin 1	NRXN1	2p16	Array comparative genomic hybridization	36
Contactin associated protein-like 2	CNTNAP2	7q35	Linkage, association, and gene-expression analyses	37,38
SH3 and multiple ankyrin repeat domains 3	SHANK3	22q13	Genome-wide association analysis	39
SH3 and multiple ankyrin repeat domains 2	SHANK2	11q13	Genome-wide association analysis, dense genotyping array	42,43
Neuropilin 2	NRP2	2q33	PCR-RFLP analysis	45
Synaptic Ras GTPase activating protein 1	SYNGAP1	6p21	RT-PCR analysis	46
Glutamate receptor, ionotropic, kainate 2	GLUR6/GRIK2	6q21	Affected sib-pair method, transmission disequilibrium test	47
Serotonin transporter; solute carrier family 6 member 4	SLC6A4	17q11	Transmission disequilibrium test	50,51

duplication anomaly was not significantly different from the wild-type.[14]

Chromosome 7

The chromosome 7q has emerged as a candidate region as a result of multiple genome wide screening reports that suggest its involvement. Genetic studies indicate that chromosome 7q is likely to contain an autism susceptibility locus (AUTS1).[15] It has the strongest statistical support for involvement in the etiology of autism and it has been identified by each of the three large-scale screening studies conducted by

the International Molecular Genetic Study of Autism Consortium (IMGSAC).[15,16] This region includes Ca2+-dependent activator protein for secretion 2 (CADPS2), distal-less homeobox 5 (DLX5), DLX6, forkhead box P2 (FOXP2), IMP2 inner mitochondrial membrane peptidase-like (IMMP2L), suppression of tumorigenicity 7 (RAY1/ST7), reelin (RELN), as well as wingless-type MMTV integration site family member 2 (WNT2).

DLX5 and DLX6 located in the 7q22 region are the homolog of the homeobox gene DLX1, and its correlation with autism was recently reported.[17] RELN in the same region regulates neuronal migration in cortical lamina formation, and several gene analysis reports have suggested the possibility that the RELN gene causes autism.[18,19] CADPS2 in the 7q31 region is necessary in the exocytosis of brain-derived growth factor (BDNF) secretion granules, and a mouse model lacking this gene displays a lack of social skills, hyperactive tendencies and reduced ability to raise offspring. A splicing anomaly in this gene can be found in some autistic patients.[20] FOXP2 in the same region is a gene related to language disorders and used to be called speech and language disorder 1 (SPCH1). A point mutation of FOXP2 was discovered in families with severe speech and language disability.[21] However, a later report suggested that FOXP2 is not a significant susceptibility gene for autism.[22] IMMP2L in the 7q31 translocation breakpoint was discovered as a gene related to Gilles de la Tourette syndrome (GTS), and this suggests a link with autism, but no coding mutations have been found in either GTS or autistic patients.[23] Later, association and copy number variant analysis highlighted several genes that warrant further investigation, including IMMP2L on chromosome 7.[24] Similarly, RAY1/ST7 in the 7q31 translocation breakpoint was discovered as a tumor suppressor gene.[25] WNT2 is adjacent to RAY1 and is related to the development of the nervous system. Mutation in WNT2 was observed in symptomatic brothers in a study of autistic families.[26] WNT signal transmission depends on dishevelled (DVL), and a mouse lacking this gene presents with a lowered social cross-reaction.[27] Additionally, HOXA1 in the 7p15 region (not in 7q) is noteworthy of mention. This gene is essential in the development of the hindbrain, and mutation in the coding region, which is peculiar to autism, has been observed.[28]

X chromosome

The fact that autism occurs more in males than in females suggests several genetic mechanisms of autism based on the research of sex chromosome genetic disorders. Skuse et al identified EFHC2 in Xp11 as a new QTL in a study on Turner syndrome, which showed that impaired social interaction is more frequent where the X chromosome was inherited from the mother.[29,30] They therefore assumed that the gene locus related to cognitive behavior is located in the X chromosome. Among the genetic disorders that cause mental retardation and resemble autism is fragile X syndrome. This disorder occurs more in girls, and manifests itself with mental retardation, facial characteristics, attention deficit, hyperactivity, hyperesthesia, as well as emotional instability. The gene responsible for fragile X syndrome, fragile X mental retardation 1 (FMR1), exists in Xq27 and codes RNA binding protein FMRP.[31] This abnormal gene sequence induces the methylation of DNA, which inhibits the expression of FMRP, thus causing fragile X syndrome.[31] Another congenital developmental disorder is Rett syndrome. The responsible gene for this syndrome is transcriptional repressor methyl CpG binding protein 2 (MECP2) in Xq28, which codes protein that specifically binds itself to methylated DNA.[32] However, previous studies offered only modest support for a susceptibility locus for autism within the Xq27-q28 region. Further genetic investigations of these region are warranted.

Last, there is the X-linked gene neuroligin (NLGN), which has received attention in recent years. Neuroligins and neurexins are synaptic cell-adhesion molecules that connect pre- and postsynaptic neurons at synapses, mediate trans-synaptic signaling, and shape neural network properties by specifying synaptic functions.[33] NLGN gene mutation was found in two autistic pairs of Swedish brothers and caused a sensation.[34] Südhof et al who discovered the gene reproduced the mutation in a mouse model, which confirmed weakened social skills, abnormal learned behavior, and an augmentation of inhibitory synapses.[35] The NRXN1 gene (neurexin: 2p16.3) and its superfamily member contactin-associated protein-like 2 (CNTNAP2) in 7q35 have also been identified as a susceptibility gene to autism.[36–38] In addition, a characteristic deletion of synaptic scaffolding protein SHANK3 in 22q13 was observed in

autistic patients.[39] This discovery, together with the study report of NLGN, has become the basis of the hypothesis that the cause of autism lies in abnormalities of synapses.[40,41] Recent genome-wide association studies identified de novo copy number variations in the SHANK2 synaptic scaffolding gene in 11q13 as a susceptibility gene region to autism.[42,43]

Other chromosomal regions

The chromosomes 2, 6, 11, 17 and 22 have also been demonstrated in multiple genome screening reports. A study from Holland pointed out the importance of 2q and 6p in autism.[44] Neuropilin (NRP2) exists in 2q33 with a specific SNP for autism.[45] Synaptic Ras GTPase activating protein 1 (SYNGAP1) has been found in 6p21, a gene mutation that has recently been discovered to be a genetic cause of autosomal non-syndromic mental retardation.[46] A significant link to autism has been demonstrated in the gene polymorphism of glutamate receptor 6 (GLUR6/GRIK2) in 6q21.[47] The Autism Genome Project Consortium discovered chromosome 11 in the largest linkage analysis ever carried out with more than 1,000 families as the subjects. The results revealed that 11p12-13 is linked to the development of autism.[48] Chromosomes 17 and 22 have drawn the attention of researchers in recent years as a result of a Finnish report.[49] SLC6A4, the gene coding serotonin transporter, located in 17q11, possesses a repeat sequence within its promoter (HTTLPR) sequence. A possible association between the polymorphism of the promoter region and autism was first reported by Cook et al[50] After this time, an excess of the long/long 5-HTTLPR genotype was observed in 35 autistic families.[51]

Study of Environmental Factors in Autism

Various countries have reported an increase in the prevalence of autism. While its cause is uncertain, many researchers of psychiatry are examining environmental factors as the reason for the increase. Since there has never been a study report with a 100% agreement rate for monozygotic twins to date, the possibility of environmental factors contributing to the prevalence of autism cannot be ruled out. However, some researchers consider that the major reason for the increase of the morbidity rate is due to relaxing diagnostic criteria and applying it to lower levels of intelligence, other medical conditions, and chromosomal abnormalities.[52] When considering environmental factors in autism, the key issue is at what point do they start to affect the central nervous system, causing the onset of abnormal changes in development? Since it was discovered that autism manifests itself before the age of 3 years, environmental risk factors from conception to immediately after birth have been investigated. The following examples are some of the known risk factors.

Thalidomide and valproic acid

The critical period for exposure to teratogens shown to increase the risk of autism is early in the first trimester of pregnancy. Thalidomide (THAL) and valproic acid (VPA) have been verified as teratogenic drugs related to the risk of autism in epidemiological studies. The probability of an autistic disorder significantly rises when the mother takes either of these drugs during pregnancy.

Disabilities in children born to pregnant women who took THAL received a lot of attention in the 1960s. Strömland et al reported that 5% of the THAL victims also developed autism.[53] A well-known disability caused by this drug is malformed neonates characterized by brachymelia. However, children with associated autism only have deformed ears and no brachymelia, and this led to the assumption that the abnormal changes causing autism occur in the first trimester of pregnancy when the brainstem is formed.[53]

A disability in children born to pregnant women who took VPA is known as fetal VPA syndrome and it is associated with a characteristic facial and head appearance. These characteristic appearances include ocular hypertelorism, brachygnathia, protrusion of the forehead, a low and flattened nose, as well as malformation of the auricle. The probability of developing autism is also high in individuals with this syndrome.[54] A review of the postmortem brain of an autistic patient by Rodier et al revealed a decrease in nerve cells in the facial motor nucleus and the nucleus olivaris, leading to the assumption that the onset time of the disease is immediately after the neural tube is closed (the first pregnancy trimester).[55] They further conducted an experiment to cause a pathological change in the cranial nerve nucleus of a fetus by administering VPA to a pregnant rat. In their experiment, early exposure to the drug caused pathological changes

in the trigeminal nucleus and hypoglossal nerve nucleus, while in later exposure, changes were seen in the nucleus of the oculomotor nerve and abducens nucleus. Although they failed to reproduce the same pathological changes found in the postmortem brain of an autistic patient,[56] this animal model reproduced a decrease of Purkinje cells in the cerebellar vermis, which paralleled the previous report for human cases of autism.[56,57] Furthermore, VPA-exposed animal models have shown autistic behavior such as hyperesthesia, hyperactivity, learning difficulty, impaired social interaction, and non-exploratory activities.[58,59] Therefore, this model has been used most frequently as the autistic model of environmental factors in recent years. Other findings that focus on the intracerebral synaptic transmission of this model include the overexpression of NMDA receptor subunits and accompanying long-term potentiation enhancement,[60] and decreased expression of NLGN3, among others.[61]

Vaccines

Vaccinations for measles, mumps, rubella (MMR), diphtheria, pertussis, and tetanus (DPT) have long been claimed as evidence of the recent increase in morbidity rate of autism. Of these environmental factors, MMR vaccine has drawn particular attention since the study conducted by Wakefield et al. They postulated that the MMR vaccine may be a causative factor in the development of autism spectrum disorder.[62] Since this initial publication, immunization remains controversial for some parents and the uptake of the MMR vaccine has fallen in some countries, despite much discussion regarding the safety of MMR, a lack of evidence for an association between MMR and autism, and the risks of insufficient protection against wild measles virus infection.[63] However, studies in autism and MMR immunization in California have demonstrated no correlation between increased prevalence rates of autism (373%) and increased rates of immunization (14%) for MMR.[64] Another recent, large-scale study showed no increased risk for autism for children who had been vaccinated with a thimerosal-containing pertussis vaccine compared with children who had been vaccinated with the same pertussis vaccine formulated without thimerosal.[64] The Lancet has fully retracted the paper by Wakefield and his colleagues because it is now clear that several elements are incorrect, contrary to the findings of an earlier investigation.[65]

Viral infection

There have been numerous studies indicating a correlation between autism and immune system disorders such as autoimmune disorders and cerebral inflammation.[66] Since the 1970s, congenital viral infection has been a topic of various pathological studies of autism. Rubella virus and cytomegalovirus both cause social dysfunction in children born to mothers who contracted them during pregnancy.[67,68] Influenza and Borna viruses are now being investigated for their correlation with autism since infected animal models display the same characteristics.[69,70] However, there have been no clinical reports supporting this to date.

Thyroid hormones

The fact that thyroid hormone during fetal life is essential in the development of the central nervous system has led to the view that decreased thyroid function in either the mother or fetus might be related to autism. Gillberg et al have suggested that hypothyroidism in the mother and congenital hypothyroidism are related to autism.[71] Later reports on intelligence tests conducted among 62 children born to mothers with an elevated thyrotropin level[72] and those in 83 cases with congenital hypothyroidism[73] have demonstrated a clear correlation between low thyroid function during fetal life and impaired central nervous system development. In the recent Collaborative Programs of Excellence in Autism Study (CPEA Study), the only specific autoimmune disorder found to be associated with regression was autoimmune thyroid disease.[74]

Oxytocin

Oxytocin has been regarded as a peptide hormone peculiar to female sexual functions. However, recent reports have revealed its role in forming human bonds and enhancing trust, and have drawn the attention of autism researchers as an important factor in social development.[75] Several animal studies have suggested a relationship between oxytocin and social behavior. Comparative studies of monogamous and nonmonogamous voles demonstrated species differences in the regional expression of oxytocin receptors in the brain, which revealed that cerebral oxytocin receptors play a major role in forming a pair.[76] Disruption of CD38 produces impairment of maternal behavior and male social recognition, and a reduction in oxytocin secretion in mice.[77] Genome-wide screening

has provided data supporting a correlation between the oxytocin receptor gene and autism,[78,79] followed by various reports on SNP in this gene region (3p25) that show a strong correlation with autism.[80–83] Some study groups have claimed that the low level of serum oxytocin in autistic patients[84] suggests that oxytocin could be successfully administered in the treatment of autistic patients.[85–87] An experimental economics study of oxytocin in a trust game exercise conducted among a healthy population reported an interesting effect of oxytocin in enhancing trust.[88]

Conclusion

Previous studies have demonstrated that genetic and environmental factors play a major role in the development of autism. However, no single neurobiological factor currently dominates the mechanism, pathology and prevalence of autism.[2] This suggests that interactions between multiple genes cause "idiopathic" autism but that epigenetic factors and exposure to environmental modifiers may contribute to variable expression of autism-related traits. Although these factors independently account for few cases, environmental factors may interact with genetic susceptibility to increase the likelihood of autism. For example, some data implicate a possible role of immune factors, including an increased family history of autoimmune diseases and presence of autoantibodies to neural antigens.[89,90] Epidemiological studies have linked prenatal stress to increases in the incidence of neurodevelopmental disorders, including autism spectrum disorders, and these associations are often sex dependent.[91,92] Autism often displays sex differences in prevalence, presentation, or therapeutic outcomes.[93] The contribution of epigenetic modifications on the pathophysiology of autism has also been championed. Therefore, many studies have focused on genome imprinting as the research strategy. However, the manner and extent of their involvement remains to be defined. As additional genetic risk factors of autism are identified, the way by which these molecules interact with the environment can finally be addressed.

It is hoped that future studies of the molecular biology of autism will help construct a new theory that can interpret the pathology of autism in a coherent manner. To investigate the genetic and environmental causative factors of autism, large-scale epidemiological research is essential. A recent example of such epidemiological research is the Childhood Autism Risk of Gene and Environment (CHARGE) project, funded by the NIH in the United States and it was launched in 2002. This is an autism-specific research of causative factors covering a wide range of factors such as genetics, infections, food, and products, as well as air. In this clinical study, autistic children between the age of 2 to 5 years are compared with a control group of the same ages.[94] The CHARGE project aims to collect the data of 1000 to 2000 children, and it has been reported that there is already sufficient data indicating the involvement of immunological abnormalities. Further progress in the biological research of autism is awaited.

Author Contributions

Wrote the first draft of the manuscript: HM. Contributed to the writing of the manuscript: HM, KI. Made critical revisions and approved final version: HM, TM. All authors reviewed and approved of the final manuscript.

Disclosures

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