Neuroendocrine pathways altered in autism. Special role of reelin

Silvia Kelemenova, Daniela Ostatnikova
Comenius University, Faculty of Medicine, Institute of Physiology, Bratislava, Slovakia;

Correspondence to: Silvia Kelemenova, MSc.
Institute of Physiology, Faculty of Medicine,
Comenius University, Sasinkova 2, 81372 Bratislava, Slovakia
PHONE: +421-2-59357520; E-MAIL: skelemenova@gmail.com

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Abstract

Autism is the most genetically influenced neuropsychiatric disorder with heritability of approximately 90%. Since genetic factors seem to play a crucial role in autism etiology, enormous attention is focused on genetic analyses of the disorder. Reelin, one of the autism candidates, is necessary in regulation of neuronal migration during brain development and also in maintaining synaptic plasticity during postnatal life period. Reduced reelin levels were observed in sera and brain cortices of autistic patients. In this review, abnormalities in reelin signaling and the relationship between reelin deficiency and principal neuroendocrine pathways are discussed.

REVIEW

Introduction

Autism is a neuropsychiatric disorder with very high heritability and an unclear etiology. e The incidence of autism spectrum is 0.1%. The most pronounced symptoms include social deficits, impaired speech and communication, obsessive and repetitive behaviors. A number of research teams attempted to determine the etiology and pathogenesis of the disorder. Autistic disorder can be syndromic, that means associated with a known monogenic disorder such as tuberous sclerosis or fragile X syndrome (Folstein & Rosen-Sheidley, 2001), or nonsyndromic, idiopathic autism. Autism spectrum disorders belong to one of the most genetically influenced neuropsychiatric diseases with a heritability of approximately 90% (Folstein & Rosen-Sheidley, 2001; Veenstra-Vander Weele & Cook, 2004). Relative risk of first relatives is about 100-fold higher than the risk in the normal population and concordance in monozygotic twins is about 82–92%. Heritability of autism spectrum disorders approaches but does not reach 100%. This suggests the involvement of environmental factors in autism etiology. The interaction between genetic and environmental factors classifies autism as a complex disease. Important environmental components include vaccinations with live virus and thimerosal (toxic mercury component) and prenatal toxic teratogenic exposures (Hviid *et al.* 2003; Geier & Geier, 2006). Another environmental circumstance very strongly related to autism is prenatal exposure to ethanol, thalidomide, valproic acid, misoprostol and maternal rubella infection (Arndt *et al.* 2005). In addition, food containing casein and gluten is discussed as an environmental factor in autism since casein and gluten intolerance is often associated with severity of autistic traits (Millward *et al.* 2004).

Since a genetic component is cardinal in autism etiology, strong attention is devoted to genetic studies of autism. Recent genetic studies using whole genome analyses identified several candidate genes for autism. Most of them are related to synaptic events, namely genes involved in cell adhesion,

Abbreviations :

APOE-R2 - gene encoding apolipoprotein E receptor 2
FMRP - fragile X mental retardation protein
GABA - gamma-aminobutyric acid
GAD1 - gene encoding glutamate decarboxylase 1
$(GGC)_n$ - repeat of triplet of GGC nucleotides
HTR1A - gene encoding 5-hydroxytryptamine (serotonin) receptor 1A
HTR2A - gene encoding 5-hydroxytryptamine (serotonin) receptor 2A
HTR7 - gene encoding 5-hydroxytryptamine (serotonin) receptor 7
HVC - high vocal center
LTD - long term depression
LTP - long term potentiation
MeCP2 - gene encoding methyl CpG binding protein
RELN - gene encoding reelin
SERT - gene encoding serotonin transporter
UBE3A - gene encoding ubiquitin protein ligase E3A
VLDLR - gene encoding very low density lipoprotein receptor
5HT - 5-hydroxytryptamine (serotonin)
5MT - 5-metoxytryptamine
5'UTR - 5' untranslated region
5,7diHT - 5,7-dihydroxytryptamine

migration, neurotransmission, apoptosis, cellular signaling, cytoskeleton dynamics, chromatin remodeling and regulation of gene expression (Persico & Bourgeron, 2006). The products of these genes regulate processes at the synapse, such as synapse formation, synapse maintenance, synapse elimination as well as functions provided by synaptic receptors, integrins, cell adhesive molecules, proteins secreted by neurons, cell signaling molecules that affect axonal outgrowth. Dysfunction of these genes was described to contribute to the development of an autistic phenotype.

Reelin pathway and synaptic events

RELN (chromosomal location 7q22) is one of the candidate genes involved in synaptic processes. A number of studies analyzed the association between reelin, a product of the RELN gene and autism. Reelin mRNA and protein levels are decreased in serum and frontal and cerebellar cortices of autistic patients (Fatemi *et al.* 2002; Fatemi *et al.* 2005). These findings might have a genetic association. Long variants of RELN $(GGC)_n$ repeats in 5'UTR were associated with decreased RELN expression (Lugli *et al.* 2003; Persico *et al.* 2006). A higher frequency of these long variants was observed in autistic populations. (Persico *et al.* 2001).

Reelin is produced by Cajal-Retzius cells in the hippocampal cortex during neurodevelopment and secreted into the circulation. It has a major role in neuronal migration and in prenatal development of neuronal connections (D'Arcangelo *et al.* 1995). Neuronal migration during development could be performed by radial or tangential motions. Pyramidal or glutamatergic cortical neurons migrate radially from the ventricular zone towards the pial surface. Radial migration requires radial glia as supportive guides for migrating neurons. While migrating through the marginal zone, where the Cajal-Retzius neurons express reelin, it binds to their receptors and initiates the biochemical cascade resulting in proper laminar positioning. Thus, neurons born at the same time become positioned at the same cortical layer (in radial dimension). For instance, GABAergic interneurons use tangential migration that does not require specific interactions with radial glial cells. It has been shown that also tangentially migrating neurons require reelin for their proper laminar determination (Yabut *et al.* 2007). In addition, cortical interneurons can dynamically switch between tangential and radial modes of migration. Reeler mice lacking the RELN gene, show altered neuronal migration and altered cytoarchitectonics (D'Arcangelo *et al.* 1995). Migrating neurons of the developing reeler brains traveling through the marginal zone cannot acquire the signal for proper layer positioning normally acquired by reelin.

Reelin exerts its function via two pathways: proteolysis and receptor-dependent signaling. During neurodevelopment, the proteolysis of extracellular matrix proteins by reelin as a serin protease is crucial for migrating neurons. Postnatally, the proteolytic activity of reelin contributes to the development of the neuromuscular junction, motor end-plate maturation and proper nerve-muscle connectivity (Quattrocchi *et al.* 2002). Reelin, however, also binds to its receptors on migrating neurons activating intracellular signaling cascades. The reelin pathway involves reelin receptors: VLDLR (very low density lipoprotein receptor), APOE-R2 (apolipoprotein E receptor 2), a3b1 integrins (Hiesberger *et al.* 1999). For a detailed description of the reelin signaling pathway see ref. (Kelemenova & Ostatnikova, 2008).

The reeler phenotype includes impaired neuronal migration, disrupted organization of cerebellar and frontal cerebral cortices and also impaired brain lamination. In addition, reeler mice and reelin receptor-knock outs showed hyperphosphorylation of Tau protein resulting in impaired axonal growth and synaptic plasticity. It is remarkable that impairments in the reelin pathway at the level of receptors and terminal molecules of reelin signalling (e.g. Tau) result in a similar phenotype observed in reeler mice indicating the importance of the reelin pathway in control of neurodevelopment.

Although reelin is considered to act mainly during neurodevelopment, it has been shown that it participates in the regulation of synaptic plasticity and memory processes in the adult brain (Alcantra *et al.* 1998; Weeber *et al.* 2002; Panteri *et al.* 2006; Zhao *et al.* 2007). The reelin producing Cajal-Retzius neurons degenerate within three weeks after birth (Weeber *et al.* 2002). Reelin expression in the adult brain is restricted to GABAergic neurons in the cerebral cortex and hippocampus and to glutamatergic neurons (granule cells) (Pesold *et al.* 1998) and GABAergic neurons in the

cerebellum. Cortical GABAergic interneurons expressing reelin form a heterogenous cell population with a number of inhibitory synaptic contacts. Disruption of these inhibitory pathways together with other synaptic impairments might cause the overreactivity to external stimuli, one of the features of autism.

Reelin and animal models of autism

Several animal models have been created in attempt to clarify the etiology of autism. The most common approaches involved the knock-out of candidate genes or lesions of candidate brain regions (Andres, 2002). Behavioral phenotyping is required in completion of the phenotype profiling. The reeler mouse is an example of an autism animal model. Reeler behavioral pattern includes motor impairments, action tremors, dystonia, and ataxic gait. Loss of reelin also caused increased anxiety and deficits in memory and learning (Marrone *et al.* 2006), features similar to those seen in autism. In addition to reeler similarities with autism, maternal separation was followed by decreased ultrasonic vocalization in male reeler pups, the phenomenon that is a common expression of social deficits after maternal separation (Ognibene *et al.* 2007). However, lack of reelin in humans causes lissencephaly with severe mental retardation resembling neither reeler nor autism (Persico & Bourgeron, 2006).

Other candidate gene knock-out models of autism involves Fmr1 –/–, MeCP2 –/– and UBE3A –/– mice (reviewed by Moy & Nadler, 2008). These three candidate genes are necessary in regulation of synaptic plasticity, the process impaired in autism. Synaptic plasticity involves the regulation of long term depression (LTD) and long term potentiation (LTP). LTP is the long lasting enhancement in communication between two neurons considered as the major cellular mechanism of learning and memory processes. Dysregulation of synaptic protein synthesis and degradation causes impaired synaptic transmittion and thus synaptic plasticity impairments. Fmr1 gene deficiency is associated with fragile X syndrome, the disease with frequent co-occurrence with autism (Belmonte & Bourgeron, 2006). FMRP (fragile X mental retardation protein) loss causes enhanced LTD in the hippocampus and decreased LTP in cortical regions (Hou *et al.* 2006; Li *et al.* 2002). MeCP2 protein regulates the transcription of the synaptic genes (Monteggia & Kavalali, 2009). UBE3A encodes ubiquitin ligase, an enzyme responsible for synaptic protein degradation (Zoghbi, 2003). Loss of MeCP2 or UBE3A is followed by deficits in LTP, the phenomenon observed in autism. Additionally, one of the aformentioned functions of reelin is the regulation of synaptic plasticity in the adult brain. Reelin via binding to its receptors (ApoER2 and VLDLR) enhances LTP and thus regulates long lasting forms of synaptic plasticity involved in processes of learning and memory retention (Weeber *et al.* 2002). In addition, reeler mice showed aberrant striatal LTP (Marrone *et al.* 2006).

Serotonin and reelin

Another approach in animal modeling of autism is to intervene in the suspected pathway. An example of a highly suspected pathway that is abnormal in autism is the serotonergic system. Many authors have reported an increase in blood serotonin levels in autistic patients (Schain & Freedman, 1961; Anderson *et al.* 1987; Anderson *et al.* 1990; Cook *et al.* 1993, Herault *et al.* 1996). According to the autism hyperserotonemia phenomenon, another animal model of autism was created based on the manipulation of serotonin (5-hydroxytryptamine, 5HT). Newborn rats whose mothers were treated with serotonin receptor agonist (5metoxytryptamine; 5MT) during pregnancy showed brain metabolic and behavioral patterns that mimicked the autistic phenotype in humans (Kahne *et al.* 2002). These pups were found to be over reactive to auditory stimuli, displayed motor dysfunctions and decreased ultrasonic vocalization induced by maternal separation, patterns resembling autistic-like behavior (Kahne *et al.* 2002). Furthermore, Janusonis with colleagues (2004) demonstrated that administration of 5MT resulted in decreased RELN expression in blood and brain lysates detected by western blotting. This effect was enabled via synaptic contacts among 5HT neurons and Cajal-Retzius cells expressing reelin (Janusonis *et al.* 2004). Postnatally, reelin is expressed mainly by GABAergic neurons which create synaptic contacts with 5HT neurons (Freund *et al.* 1990; DeFelipe *et al.* 1991; Van Bockstaele *et al.* 1996; Varga *et al.* 2001). GABAergic and serotonergic connections were reported to be abnormal in mutant reeler mice lacking the RELN gene. (Gilerovich & Grigor'ev, 2005), suggesting the existence of a functional linkage among GABA, serotonergic systems and the reelin pathway, which are all abnormal in autism. In addition, daily injections of 5MT caused a marked decrease of reelin expression in the adult female rat cerebellum and prefrontal cortex, the effect supposedly mediated by the physical connections between GABAergic and serotonergic neuronal cells (Kelemenova *et al.*, unpublished data). In addition to the importance of GABAergic pathway in autism, autistic patients have decreased expression of glutamate decarboxylase (GAD1), an enzyme converting glutamate to GABA (Yip *et al.* 2007). Studies on post mortem autistic brains showed impairment of the cerebellar structure with marked reduction in Purkinje cell number and the GAD1 expression. A decreased number of Purkinje cells with decreased GABA synthesis might cause impaired signalling from the cerebellar nuclei towards higher association areas in the cerebral cortex resulting in cognitive and/or motor abnormalities (Yip *et al.* 2007).

Another animal model with impaired an serotonergic pathway used the elimination of serotonergic fibers by specific neurotoxin 5,7diHT at birth followed by numerous lesions in the hippocampus and cortex together with deficits in social learning and increased repetitive behavior (Boylan *et al.* 2007). In addition, the number of knock-out mice lacking serotonergic receptors (HTR2A, HTR7) or serotonin transporter (SERT) has been created possessing the behavioral patterns of depression, anxiety, aggression and also hypolocomotion, reduced social interaction, altered spatial learning and memory deficits (Moy & Nadler, 2008). SERT–/– mice have increased 5HT in extracellular space leading to altered cortical layer thickness and neuronal cell density, since serotonin plays a morphogenetic role during neurodevelopment (Altamura *et al.* 2007). Serotonin receptors (HTR1A and HTR2A) were found to be differentially expressed in brains of male and female rats and also showed region-specific expression patterns (Zhang *et al.* 1999). These authors provided evidence of the modulatory role of testosterone in serotonin receptor expression. Perinatal testosterone intake results in masculinization of the serotonin nerve fiber distribution (Simerly *et al.* 1985). Gonadectomy together with serotonin depletion reduced disinhibitory behavior such as aggression (Svensson *et al.* 2000). As mentioned above, testosterone modulates the expression of serotonin receptors and transporters (Fink *et al.* 1999; Zhang *et al.* 1999; Keleta *et al.* 2007) and it modulates serotonergic activity (Briger *et al.* 2003; Dominguez *et al.* 2003). These facts underlie the renowned importance of testosterone in regulation of neurodevelopment. Furthermore, testosterone has been implicated in autism etiology, since the incidence of this disorder is fourfold higher in males than females.

Testosterone and reelin

Testosterone plays an important role in regulation of brain development. It affects special brain regions and provides sexual differentiation also at the level of the central nervous system. Testosterone binding to androgen receptors results in receptor complex formation that serves in the nucleus as a transcription factor (Moilanen *et al.* 1998). It controls the expression of target genes involved in processes such as neurotransmitter production and release, synapse conformation changes, controlling of the neuronal apoptosis, and altering neurochemical profiles (for review see ref. Alonso-Solis *et al.* 1996). Androgen receptors are present in the brain regions crucial for memory and learning such as the hippocampus, amygdala and prefrontal cortex, but are not present in other cortical regions of the brain (Finley & Kritzer, 1999; Beyenburg *et al.* 2000). The evidence that testosterone modulates spatial cognition is given by the fact that these functions are improved after supplementation therapy in hypogonadal older men (Cherrier *et al.* 2001). The spatial cognitive effects of testosterone were shown to be mediated also by its conversion to estradiol by the enzyme aromatase (Cherrier *et al.* 2005). In addition, salivary testosterone levels were positively correlated with spatial abilities in women and negative correlation was found in men, suggesting the existence of optimal free testosterone levels contributing to optimal spatial performance (Ostatnikova *et al.* 2002). Spatial performance is one of the psychological attributes of masculine behavior. One of the theories attempting to explain the causes of autism development is the pronounced extreme male brain – testosterone theory. It suggests that autism represents an exaggeration of the male pattern. Exposure to high levels of prenatal testosterone results in masculinized social behavior in the areas of spatial performance and cognitive ability (Knickmeyer *et al.* 2005). The aforementioned study showed a negative correlation between fetal testosterone levels and social relationships that are impaired in autism. Furthermore, children with high fetal testosterone measured in amniotic fluid exhibit strong autistic traits, strong systemizing, and deficits in empathy (Baron-Cohen & Belmonte, 2005; Chapman *et al.* 2006; Knickmeyer *et al.* 2006; Auyeung *et al.* 2009). Other evidence of this theory is that autistic traits are increased in congenital adrenal hyperplasia patients (Knickmeyer *et al.* 2006).

In songbirds, the special role of testosterone was found in facilitating the quality and the frequency of birdsongs (Ball *et al.* 2003). Testosterone controls the song behavior directly via androgen receptors or by its metabolite estradiol via estrogen receptors that are expressed in many specialized forebrain song control nuclei. Furthermore, testosterone was found to influence the expression of reelin in the brain of male European starlings (Absil *et al.* 2003). Levels of testosterone change seasonally in the lifecycle of these birds. Reelin contributes to the incorporation of new neurons to the song control nucleus (HVC, high vocal center) of the songbird brain. Reelin expression in the songbird brain varies seasonally and could thus mediate seasonal incorporation of new neurons into the HVC. In addition, testosterone administration sharply decreased reelin expression in these birds (Absil *et al.* 2003). These facts hint, that testosterone and reelin pathways may be connected in humans. Autistic patients have altered levels of testosterone and reelin (Fatemi *et al.* 2002; Fatemi *et al.* 2005; Knickmeyer & Baron-Cohen, 2006). We speculate that the possible relationship between elevated testosterone and decreased reelin levels might be an important factor in the pathogenesis of autism.

Oxytocin and reelin

Testosterone has a special role in regulation of reproduction-related and behavioral functions mediated by oxytocin. The mechanism includes the modulation of the oxytocin binding sites by testosterone expressed in the forebrain and spinal cord in rats (Uhl-Bronner *et al.* 2008).

Oxytocin and vasopressin are neuropeptides with sexually dimorphic effects on brain and behavior especially during in utero development (Carter, 2007). Oxytocin receptors are localized especially in the brainstem regions crucial for regulation of social and reproductive behavior. Single nucleotide polymorphisms of gene encoding oxytocin receptor are associated with autism spectrum disorders (Wu *et al.* 2005; Jacob *et al.* 2007; Lerer *et al.* 2008). Autistic patients have low blood levels of oxytocin (Carter, 2007). Oxytocin plays an important role in the neurodevelopment and modulation of synaptic plasticity. Oxytocin producing neurons are capable of changing their shape and thus forming new synaptic connections especially in early life (Theodosis *et al.* 2006).

Reelin protein is also involved in the oxytocin pathway. In reeler mice oxytocin receptor expression is decreased in brain cortical regions such as the piriform cortex, neocortex, retrosplenial cortex and certain regions of the hippocampus (Liu *et al.* 2005). These mice show behavioral deficiencies similar to oxytocin-deficient mice (Ferguson *et al.* 2002). Down regulation of reelin expression together with decreased oxytocin receptor expression could be proposed as a possible contribution to the development of autistic disorders. In addition, there are hypotheses about reciprocal regulation of reelin expression by neuropeptides such as oxytocin (Carter, 2007). Reelin expression is regulated by epigenetic methylation or histone deacetylation of the RELN gene promoter (Chen *et al.* 2002). Events, such as the exposure to neuropeptides during neurodevelopment and differential level of maternal care might influence reelin expression via hypermethylation or gene silencing (Carter, 2007). Low levels of maternal care induced a decrease and high levels of maternal care induced an increase in reelin expression. These events were reversed in adulthood by histone deacetylase inhibitor and L-methionin treatment respectively, substances epigenetically regulating RELN gene promoter (Weaver *et al.* 2005). In summary, it is hypothesized that neuropeptides such as oxytocin might be involved in epigenetic regulation of reelin expression. High levels of maternal care up regulate the expression of reelin. Furthermore, oxytocin positively modulates maternal care behavior itself. Down regulation of oxytocin and/or reelin pathways might contribute to autism development.

Conclusions

Impairment of several neuroendocrine pathways has been reported in autism. In this review we have focused on testosterone, serotonin and oxytocin systems (Figure 1). As suggested by the extreme male brain theory of autism (Baron-Cohen & Belmonte, 2005), elevated testosterone levels in autistic patients (prenatally and also postnatally) lead to masculine types of social and cognitive behaviors (Baron-Cohen & Belmonte, 2005; Knickmeyer *et al.* 2005; Capman *et al.* 2006; Knickmeyer *et al.* 2006; Knickmeyer & Baron-Cohen, 2006; Geier & Geier, 2007; Auyeung *et al.* 2009). Plasma and platelets serotonin levels are also elevated in autistic subjects (Schain & Freedman, 1961; Anderson *et al.* 1987; Anderson *et al.* 1990; Cook *et al.* 1993, Herault *et al.* 1996). Neuropeptide oxytocin has been described as a protective against autism, since it reduces repetitive behavior, one of the main features of autism. Autistic patients have decreased oxytocin levels (Carter, 2007). The importance of these abnormalities in the pathogenesis of autism is given by the fact that all these systems are connected in many ways. Testosterone masculinizes serotonin nerve fibers distribution

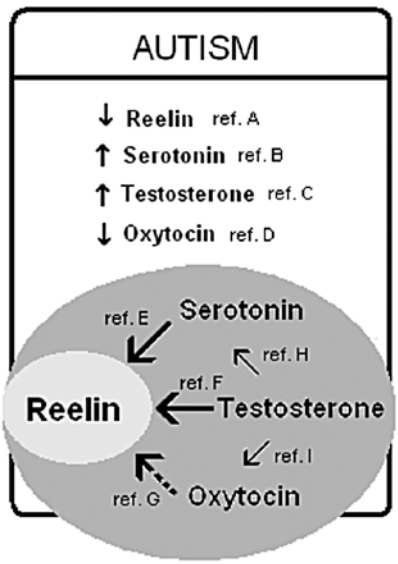

Figure 1. Neuroendocrine pathways impaired in autism. Reduced reelin levels measeured in autistic sera and brain cortices might be the result of deregulated neuroendocrine pathways in which serotonin, testosterone and oxytocin are involved. See the text and references for details. Ref. A: Persico *et al.* 2001; Fatemi *et al.* 2002; Weeber *et al.* 2002; Lugli *et al.* 2003; Fatemi *et al.* 2005; Marrone *et al.* 2006; Persico *et al.* 2006. Ref. B: Schain & Freedman, 1961; Anderson *et al.* 1987; Anderson *et al.* 1990; Cook *et al.* 1993, Herault *et al.* 1996. Ref. C: Baron-Cohen & Belmonte, 2005; Knickmeyer *et al.* 2005; Capman *et al.* 2006; Knickmeyer *et al.* 2006; Knickmeyer & Baron-Cohen, 2006; Geier & Geier, 2007; Auyeung *et al.* 2009. Ref. D: Carter, 2007. Ref. E: Janusonis *et al.* 2004. Ref. F: Absil *et al.* 2003. Ref. G: Chen *et al.* 2002; Weaver *et al.* 2005; Carter, 2007. Ref. H: Fink *et al.* 1999; Zhang *et al.* 1999; Svensson *et al.* 2000; Birger *et al.* 2003; Dominguez *et al.* 2003; Keleta *et al.* 2007. Ref. I: Uhl-Bronner *et al.* 2008.

and also affects the expression of serotonin receptors and transporters (Simerly *et al.* 1985; Fink *et al.* 1999; Zhang *et al.* 1999; Keleta *et al.* 2007). The testosterone – serotonin link is strengthened by the fact that gonadectomy together with decreased serotonin stimulation reduced aggressive behaviors (Svensson *et al.* 2000). Testosterone also affects the expression of oxytocin receptors and thus masculinizes oxytocin binding sites in the central nervous system (Uhl-Bronner *et al.* 2008). In autistic subjects, decreased levels of reelin have been reported in sera and in the frontal and cerebellar cortices (Fatemi *et al.* 2002; Fatemi *et al.* 2005). Genetic studies confirmed the RELN gene as a candidate for autism. Reelin regulates neuronal migration during brain development prenatally and synaptic plasticity postnatally. It seems that serotonin, testosterone and oxytocin pathways are connected with reelin (Figure 1). Prenatally, serotonin down regulates reelin expression in the rat brain (Janusonis *et al.* 2004). This effect is mediated also in adulthood (unpublished data). Postnatally, testosterone sharply decreases reelin expression in the songbird brain (Absil *et al.* 2003). Prenatal testosterone influence on reelin expression seems to be present also in mammals (unpublished data). Oxytocin is supposed to epigenetically regulate the expression of reelin (Weaver *et al.* 2005; Carter, 2007). In addition, reelin expression is influenced by different levels of maternal care, a phenomenon that may involve oxytocin. It is obvious that reelin plays an important role in autism pathogenesis. Much attention is dedicated to reelin function in specific aspects, such as exploration of neurological or behavioral patterns followed by reelin deficiency. However, further research employing complex approaches is needed to uncover the complicated pathogenesis of autism. Research should also be conducted considering the relationship between deeper neuroendocrine and neuropsychological processes related to autism.

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