Cadherins in Autism Spectrum Disorders

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Abstract

Autism spectrum disorders (ASD) are developmental disorders with a multiple genetic background. Many genes have been shown to be associated with ASD, including genes coding for cell-adhesion molecules. These molecules are important in developmental processes, such as brain development. Mutations in these genes coding for cell-adhesion molecules can lead to altered brain development and altered synapse formation causing ASD. The underlying mechanisms are largely unknown, however knowledge on the functions of cell-adhesion molecules suggests their implications in the development of ASD.

A family of cell-adhesion molecules associated with ASD is the cadherin family. Cadherins are highly expressed in the central nervous system and involved in synapse formation. The cadherin family can be further divided in subfamilies based on their homology; classical cadherins (type I and type II), protocadherins (pcdh), seven-pass transmembrane cadherins and miscellaneous cadherins. The extracellular domain of cadherin contains a cadherin repeat sequence able to form dimers with other cadherin molecules across the synapse. Subtypes of the cadherin family have different intracellular domains, resulting in differences in signal transduction. Classical cadherins bind β-catenin and p120-catenin and interact with the actin cytoskeleton via α-catenin. Furthermore, they are cleaved by ADAM10, presenilin 1 and MT5-MMP, creating an intracellular domain that translocates to the nucleus and regulates gene transcription. Classical cadherins also interact with other receptors with common signalling molecules. Eight cadherin subtypes have been shown to be associated with ASD: cadherin 8, -9, -10, -15, and -18, and protocadherin 9, -10 and -19. Unfortunately, their roles in brain development and the development of ASD are largely unknown, but some functions are suggested in this thesis.

Contents

1. Introduction

Autism spectrum disorders (ASD) are developmental diseases, characterised by difficulties in social interaction and by communication disabilities. Furthermore, autistic patients show restricted, repetitive and stereotyped patterns of behaviour.[1] The autism spectrum disorders can be distinguished in five disorders according to the DSM IV; Autistic Disorder, PDD-NOS (Pervasive Developmental Disorder, Not Otherwise Specified), Asperger's Disorder, Rett's Disorder, Childhood Disintegrative Disorder.[1]

> **Autism**
> Pronunciation: /ˈɔːtɪz(ə)m/
> *noun*
> A mental condition, present from early childhood, characterized by great difficulty in communicating and forming relationships with other people and in using language and abstract concepts.
>
> *From: Oxford dictionairies*

In the USA about 1 in 88 (1,14%) children aged eight years are diagnosed with an autism spectrum disorder. ASD is about five times more prevalent in boys than in girls (one in 54 compared to one in 252). The onset occurs in the first three years of life.[2] Family studies show that autism spectrum disorders have a high heritability. Concordance-rates in monozygotic twins have been reported to be as high as 82-92% [3, 4] and 1-10% in dizygotic twins.[3] Moreover, copy-number variation studies show that many different genes may be affected in autism. Approximately 5-15% of the ASD patients have a known chromosomal rearrangement or single gene disorder and 5-10% of the idiopathic ASD patients have rare de novo or inherited copy number variations (CNVs).[5] This highly variable genetic background and diversity of symptoms makes ASD a complex disorder. The pathogenesis (especially the molecular mechanisms) is poorly understood, and it is thought to be a multi-domain disorder, rather than a single anomaly.[3] Several neurobiological theories exist which all include altered neurodevelopment as the underlying cause of ASD, the four most interesting are explained below.

First of all there is the theory of neural connectivity; both increased and decreased neural connectivity has been shown to be associated with autism and may underlie the deficits in communicational and emotional function observed in autism.[3] Next is the theory of neural migration; defects in neural migration may lead to cortical malformations causing the symptoms of ASD. Next, there is the hypothesis that an unbalance between excitatory and inhibitory neural activity is involved in the development of autism. GABA and glutamate receptors would cause this effect, however, the exact mechanism is unknown. Calcium signalling might be responsible for the imbalance between excitatory and inhibitory networks. In addition, altered calcium signalling can also lead to dysfunctional synaptogenesis resulting in an altered neural circuitry in the brain. Furthermore, abnormal dendritic spine morphology due to the lack of a scaffolding protein has been shown in autistic patients.[3] Last, there is the theory that improper synapse formation - due to affected cell-adhesion molecules - might lead to neurodevelopmental disorders, such as autism.[6] The existence of several different theories indicates that there is no direct pathway pointing towards a simple pathogenesis also several theories be combined to explain the development of ASD.[3] The genetic background of the theories is investigated and it has been shown that all of these theories are supported by rare genetic variants in a variety of genes, for example genes coding for cell-adhesion molecules.[7] These molecules will be explained in more detail in this thesis. I will focus on cadherins since this group of cell-adhesion molecules is shown to be involved in ASD, but their mechanisms are largely unknown.

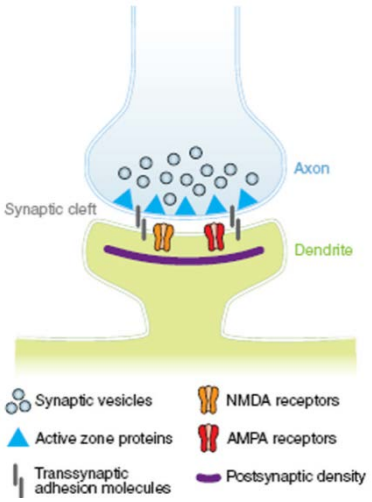

Figure 1 Synapse
The presynaptic site (axon terminal) contains vesicles filled with neurotransmitters and many different proteins, the active zone proteins. The axon terminal is separated from the dendrite by the synaptic cleft. Cell-adhesion molecules span this cleft and connect the axon with the dendrite. At the postsynaptic site (or dendrite) neurotransmitter receptors are found in the membrane and receive the chemical signal of the neurotransmitters. The receptors are connected to signalling molecules and scaffolding proteins, which together form the postsynaptic density.[6]

1.1. Synapse

A synapse in the central nervous system is an asymmetrical specialised junction between two nerve cells for unidirectional transmission of information by a neurotransmitter, a chemical transmitter agent.[8] (Figure 1[6]) The axon terminal of one neuron forms the presynaptic side, containing vesicles with neurotransmitters. These neurotransmitters can be released in the synaptic cleft, and bind to receptors at the dendrite i.e. postsynaptic membrane of the other neuron. Receptors will interact with cell-signalling molecules, leading to an appropriate response.[9] Formation of these synapses is a complex process; the human brain contains 100 billion of neurons, which can each potentially form thousands of connections with other neurons. The brain is estimated to have 10^{15} specific interconnections between neurons. The formation of this precise complex network requires controlled spatial and temporal expression of specific adhesion molecules in the neurons.[10] Besides these synaptic connections between neurons, other neural adhesive contacts such as glial cells, axon fasciculation and connections with cells outside the nervous system, e.g. muscles, are also necessary for a functional nervous system.[10] Furthermore, there are many types of synapses in the brain depending on the neurotransmitter they release. Although most research is done on glutamatergic synapses and it is presumed that mechanisms of synaptogenesis (the development of a synapse) of glutamatergic synapses are similar for other types of synapses.[9] More research is needed to understand these complex processes.

1.2. Synaptogenesis

Synaptogenesis is the process of formation of a synapse, when an axon communicates with the dendrite of a postsynaptic neuron to create a stable synapse.[11] Synaptogenesis includes adhesion between pre- and postsynaptic neurons, binding to the extracellular matrix and interaction with neighbouring glial cells. The molecular cues involved are now beginning to be discovered and include scaffolding proteins, neurotransmitter receptors and adhesion molecules. However, the exact molecular mechanism has yet to be determined.[11]

Synapse formation occurs in several steps (Figure 2)[12]; initiation, induction, differentiation, maturation and maintenance.[9, 12, 13] The initial contact is the first physical contact between proteins, such as cell-adhesion molecules, on two opposing neurons. This initial contact is mediated by spatiotemporal signals that guide axons towards their postsynaptic targets. Temporal signals can be intrinsic and genetically encoded, for example an intracellular clock; guiding neuronal growth and differentiation.[14] After the initial contact, the formation of the synapse is induced by pre- and postsynaptic components such as signalling molecules and cell-adhesion molecules. Next, synapse differentiation occurs by recruitment of neurotransmitter receptors, neurotransmitter release machinery and other proteins to form anatomical and functional different synapses.[15] In order to form a mature functional synapse cytoskeletal arrangements and stabilisation of the synapse are required. The last step is the maintenance of the functional synapse by exchange of proteins between different synapses.[13] The maintenance of synapses also depends on synaptic activity, both during development and in the adult brain.[14] Synapse formation is an important process during foetal development and in early postnatal life. However, it also occurs in adults where it is implicated in learning and memory.[14]

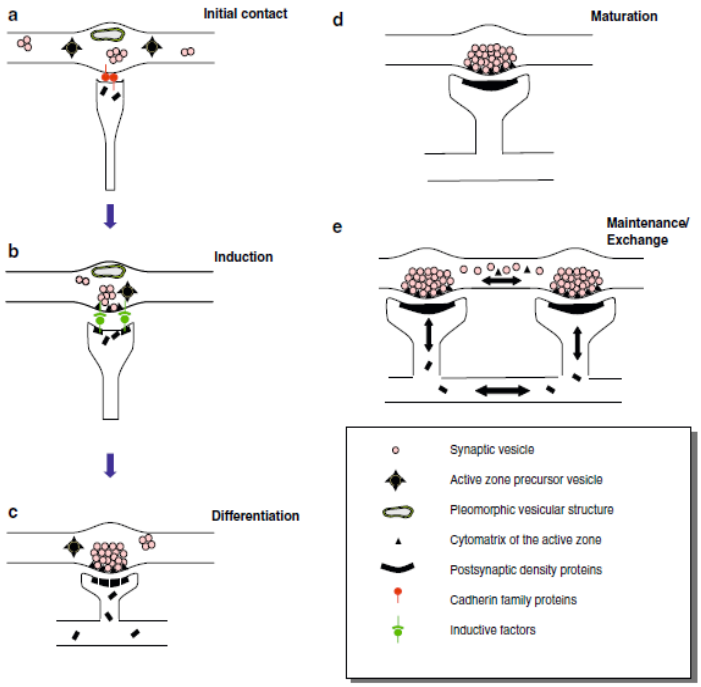

Figure 2 Synaptogenesis
Formation of synapses occur in several steps. A) The initial contact between two cells is mediated by cell-adhesion molecules, such as cadherins. B) Proteins are recruited to the presynaptic and postsynaptic zones through inductive factors, this leads to the formation presynaptic active zone and the postsynaptic density. C) The next step is differentiation of the synapse by guiding neurotransmitter receptors to the postsynaptic membrane. D) The final protein composition, synapse morphology and synapse stability is formed during the process of maturation. E) Maintenance of synapses over a long period of time can be enabled by the exchange of pre- and postsynaptic proteins between synapses. [12]

Cell-adhesion molecules (CAMs) are necessary for several steps in synaptogenesis (Figure 3).[16] Precise spatial and temporal expression of CAMs is required for the initial contact between pre- and postsynaptic cells and thus for the first step in synaptogenesis.[7, 12] When target cells are found, the CAMs are important to stabilise the contact between axons and dendrites[16] and induce the formation of intracellular signalling in the pre- and postsynaptic terminals by recruiting synaptic proteins.[6, 16] Maturation can be induced by activation of intracellular signalling, including the anchoring of scaffolding molecules to CAMs, which in turn interact with the actin cytoskeleton and lead to changes in synapse morphology.[7, 16] In the mature synapse, cell-adhesion molecules are implicated in the maintenance of the synapse and they can modulate synaptic functioning and plasticity.[13, 16] Several studies show that disruption of the adhesive properties of cadherins in immature synapses reduce their stability and in mature synapses they affect their function.[12] CAMs have been shown to be important in the formation of the complex network of the nervous system.[10] Disruption of the adhesion between neurons can lead to structural and functional differences in the brain implicated in neural diseases, such as autism and neurodegeneration. Therefore, studying the implication of cell-adhesion molecules in the development of the nervous system is important for the understanding of the pathogenesis of several brain disorders.[13]

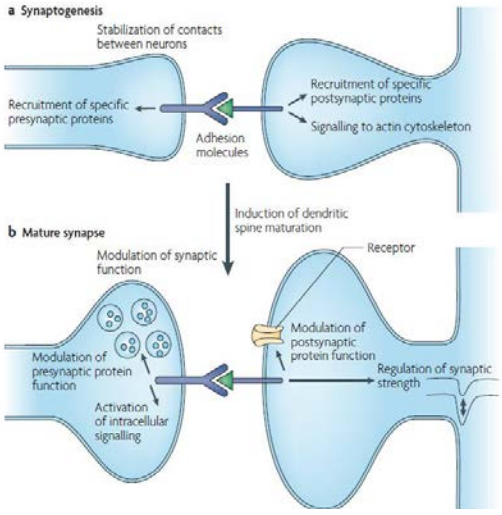

Figure 3 CAM function in synapse
Cell-adhesion molecules (CAMs) function throughout the life of a synapse. They are important during synaptogenesis, but also in the mature synapse. Different functions of cell-adhesion molecules are shown in Figure a and b. [16]

Cell-adhesion molecules can be divided into several families based on their structure, function and localisation. (Figure 4[7]) In this thesis I will focus on the cadherin family, since this subtype is implicated in some causes of ASD and incompletely understood.

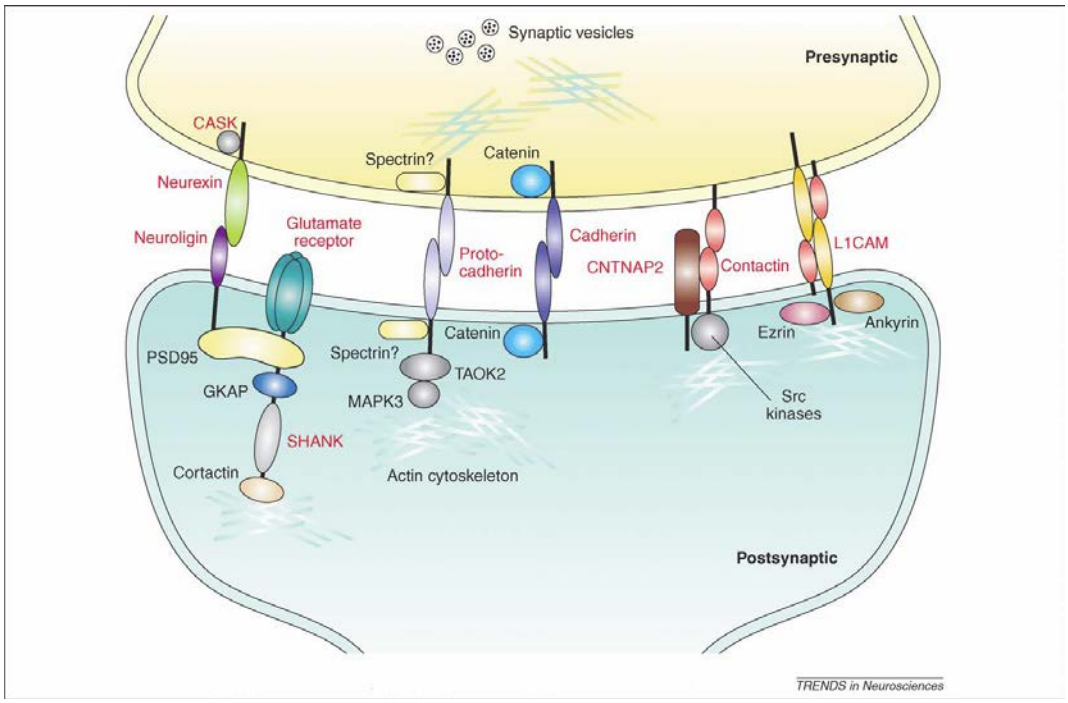

Figure 4 Synaptic cell-adhesion molecules

Different families of cell-adhesion molecules (CAMs) in the synapse. CAMs are divided in groups based on their structure, function and localization. [7]

2. Cadherin family

Cell-adhesion molecules (CAMs) mediate cell-cell interactions throughout the body. In this thesis I will only address CAMs in the nervous system focussing on the cadherin family. The cadherin family contains more than 100 different proteins, divided into several subfamilies, based on their morphology (Figure 5 [10]); the classical cadherins (type I and type II), protocadherins (pcdh), seven-pass transmembrane cadherins and miscellaneous cadherins.[17, 18] Most research is done on classical cadherins and by lack of counterarguments it is assumed that knowledge obtained for classical cadherins also applies to other cadherins. Therefore, in this thesis I will speak of classical cadherins as cadherins in general. Besides the classification in subfamilies, cadherins are also named to the location where they are expressed; epithelial (E-) cadherin (*CDH 1*), neural (N-) cadherin (*CDH 2*), placental (P-) cadherin (*CDH 3*), retinal (R-) cadherin (*CDH 4*), vascular endothelial (VE-) cadherin (*CDH 5*), kidney (K-) cadherin (*CDH 6*).[19]

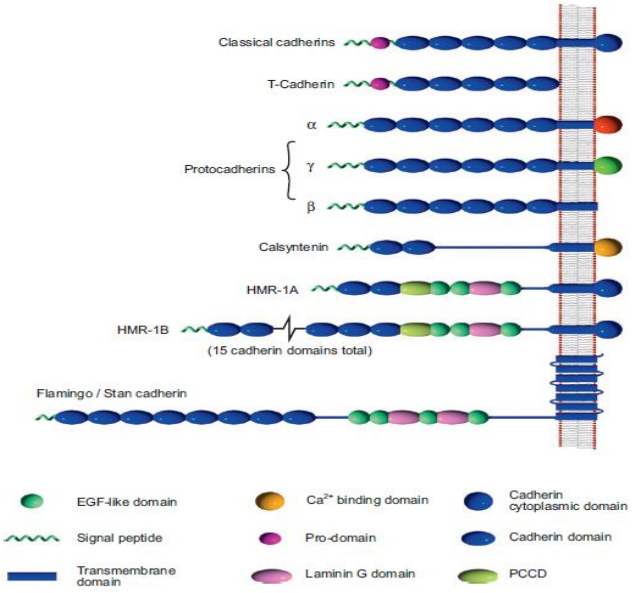

Figure 5 Cadherin family proteins. Schematic overview of the structure of cadherin proteins. Classical cadherins contain a prodomain, five extracellular cadherin (EC) domains, a transmembrane domain and a cytoplasmic domain that is able to bind catenins. Protocadherins have six or seven EC domains and a different intracellular domain, protocadherins lack the prodomain. Other cadherin subtypes have a different number of EC domains, a similar transmembrane domain and a different intracellular domain. The Flamingo subtype has a complex extracellular domain and a seven-pass transmembrane part.[10]

Cadherin proteins are characterised by a cadherin repeat sequence in the extracellular domain.[10] The classical cadherins contain five extracellular repeat domains (EC1-EC5), a single transmembrane domain and an intracellular domain (Figure 6[20]). [20, 21] Binding of three calcium ions between each repeat domain pair stiffens the molecule and is necessary for the adhesive function.[10] The calcium-binding site between EC1 and EC2 has a very high specificity for Ca^{2+}; interactions only occur at high Ca^{2+} concentrations.[22] Furthermore, binding of calcium prevents them for proteolytic degradation by several proteolytic enzymes, which is important in the signal transduction mechanism.[13, 23] The proteolytic cleavage will be explained in section 2.1. The EC1 domain is able to insert its tryptophan 2 (Trp2) side chain into the hydrophobic core of another cadherin protein, preferably of the same subtype, thereby forming dimers (Figure 6[20]).[20, 24] It has been thought that these proteins form *cis* dimers; dimerisation between cadherins on the same cell.[21] However, Zhang *et al.* showed that cadherins do not form *cis* dimers, but only form *trans* dimers (interaction with a protein on a neighbouring cell) through their EC1 domains.[25] Though this is the only study indicating that *cis* dimers do not form, additional evidence is still lacking. Clustering of cadherin molecules on a cell membrane increases the chance to form *trans* dimers and eventually increases the adhesion strength.[25] Mutations in the Trp2 side disrupt cadherin adhesion properties.[26] The number of cadherin proteins at the presynaptic membrane is similar to the amount on the postsynaptic membrane, supporting the idea of homophilic dimerisation of cadherin proteins on opposing cells[27], leading to cell-adhesion.[10] However, the cadherin-cadherin dimerisation interaction is weak and lasts only for approximately two seconds. Thus, to obtain a stable cell-cell interaction many cadherin dimers are necessary.[10]

Classical cadherins can be divided in type I and type II cadherins based on the difference in an extracellular amino acid sequence in the EC1-domain. Type I classical cadherins contain an amino acid sequence within the EC1-domain; HAV (His-Ala-Val).[28, 29] Experiments blocking this sequence by an exogenous HAV sequence reduce axon growth of these neurons.[29] In contrast, type II cadherins contain glutamine instead of histadine in this sequence, making it a QAV sequence.[29] Blocking of cadherin 8, a type II cadherin, by an endogenous QAV sequence increases axon growth.[29] Type I cadherin is shown to mainly form homophilic interactions, whereas type II cadherin interactions are more complicated and also heterophilic.[20] In addition, type II cadherins are different from type I in that they contain two tryptophan domains involved in dimerisation and that adhesion between type II cadherins is seven-times weaker than to type I cadherins.[28]

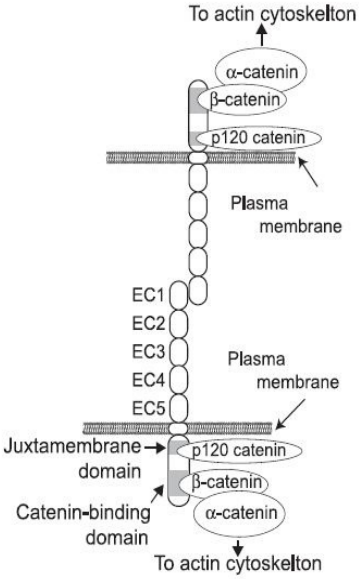

Figure 6 Cadherin dimer
Cadherins interact with their EC1 domain to form *trans* dimers with opposing cells. The intracellular domain binds p120-catenin and β-catenin. The latter interacts with α-catenin to the actin cytoskeleton. [20]

The intracellular domain of cadherins is not conserved among the subfamilies.[30] However, the general intracellular signal transduction mechanism is that they bind to p120-catenin and β-catenin (Figure 6[20]). [20, 21] These molecules interact with α-actenin which links to the actin cytoskeleton.[21] Through interaction with the actin cytoskeleton via catenin proteins, classical cadherins play a role in regulating neural structure.[10] The different intracellular domains of the cadherin types lead to diverse functions of cadherins in cell-cell adhesion and signalling.[30] The signal transduction mechanism of cadherins will be explained in section 2.1 in more detail.

T-cadherin (truncated cadherin), also known as H-cadherin (Hearth) or cadherin 13[31], is another type of cadherin belonging to the miscellaneous family of cadherins. T-cadherin has a completely different structure; it lacks the transmembrane and the intracellular domains, explaining the name; truncated-cadherin.[19, 32] T-cadherin is attached to the cell membrane via a glycosylphosphatidylinositol (GPI)-anchor.[19, 32] Despite the lack of transmembrane and intracellular domains, T-cadherin is involved in calcium-dependent cell-adhesion. This was demonstrated by treatment of cells with GPI-specific phospholipase X which removes the GPI anchor and results in reduced cell-adhesion.[19] In addition, T-cadherin inhibits axon outgrowth in cultured neurons.[31] Besides, it has been shown that loss of T-cadherin correlates with the development of cancer, and administering of T-cadherin cDNA in tumor cells decreases proliferation *in vivo* and *in vitro*.[31] However, the exact mechanism and the role in the developing brain remains to be unravelled.

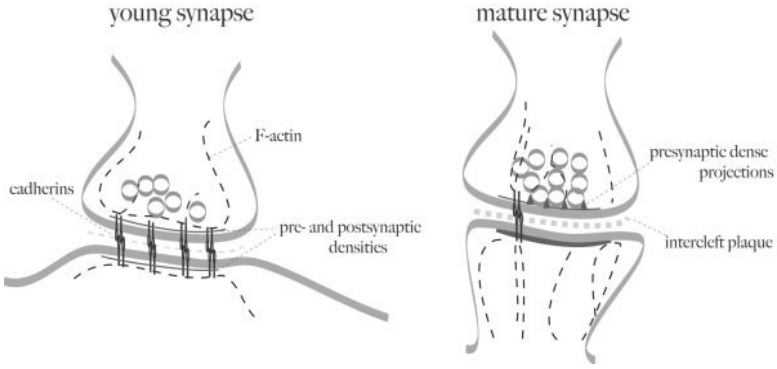

Figure 7 Distribution of cadherin in young and mature synapses
Cadherins are evenly distributed along the entire length of the young synapse. In contrast, cadherins are clustered at the mature synapse. [27]

Cadherins are more important in the target recognition and axon-target adhesion leading to the formation of a synapse, than for the stabilisation and maturation.[9, 33] Disruption of classical cadherin adhesion has an effect on immature synapses; they require cadherin adhesion to maintain the initial synaptic contact. However, disruption of cadherin adhesion in mature synapses has smaller effects. Thus, mature synapses do not require cadherin adhesion to maintain stability.[12] In the mature synapses, cadherin molecules are involved in the maintenance of dendritic spine shapes, in modulation of presynaptic vesicle releases and in synaptic plasticities.[27] Distribution of cadherins along the synapse depends on the stage of maturation; young neurons express cadherins along the entire surface of the synapse, whereas mature synapses have cadherin clusters in, and at the side of, the active zone. (Figure 7)[27] During maturation, cadherin complexes migrate to lateral sites of the active zone of the synapse. [13] This region is called the puncta adherentia and is a closely opposed contact site of the synapse for mechanical adhesion.[34] (Figure 8)[35]

Synaptic activity influences cell-adhesion by stabilising dimers of cadherin at the synapse, thus modulating synaptic strength.[10, 13, 36] In the mature brain, cadherin is preserved at excitatory synapses and lost from inhibitory synapses.[13, 17] Furthermore, NMDA receptor activation induces the dimerisation of N-cadherin. Synaptic activity regulates distribution of N-cadherin, αN-catenin and β-catenin at the synapse.[17] This is shown by blocking of neural activity by administration of TTX (tetrodotoxin), a toxic that binds to voltage-gated sodium channels in nerve cell membranes and thereby blocks action potentials. Blocking of the neural activity results in a decrease of αN-catenin in the synapse.[37] In contrast, neural activity is promoted by treatment with bicuculline, a GABA-antagonist. The inhibitory signal of GABA was inhibited, leading to an increase in neural activity. Treatment with bicuculline increases the amount of N-cadherin, αN-catenin and β-catenin at the synapse.[37] Association of β-catenin with cadherin is also induced by depolarisation of the postsynaptic membrane.[17] Thus, synaptic activity enhances synaptic strength.

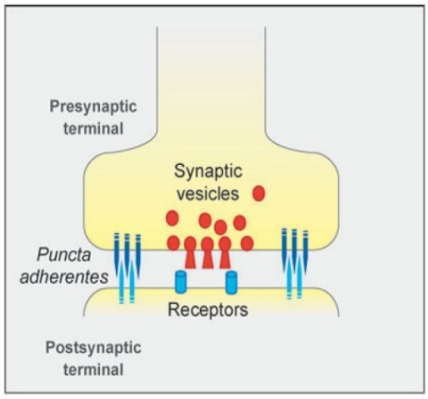

Figure 8 Puncta adherentia
Cell-adhesion molecules cluster at sites lateral to the active zone; the puncta adherentia. [35]

Cadherin expression is regulated at different levels in the cell. First of all there are the general mechanisms of regulation; gene transcription, RNA translation and protein synthesis. Besides, cadherins expression is also regulated by methylation[36], endocytosis[22] and proteolytic cleavage[22] of the protein. Addition of a methylgroup to cytosine residues suppresses promoter activity and thereby inhibits transcription of cadherin.[36] Cadherin molecules at the membrane can be temporally internalised by activity-regulated endocytosis, thereby decreasing cell-adhesion strength. Furthermore, activity and expression of cadherin molecules at the cell-surface is regulated by proteolytic cleavage and is important in the signal transduction mechanism. This proteolytic cleavage is explained in section 2.1. In addition, classical cadherins contain an extracellular prodomain when synthesised in the endoplasmatic reticulum to prevent them from early adhesion to other cadherin proteins.[28] This prodomain is cleaved by furin-like proteases in the Golgi-network in order to activate their adhesion capacity.[24, 28]

2.1. Signal transduction mechanism

The signal transduction mechanism of cadherins is complex and not completely understood. However, an overview of existing knowledge is provided in Figure 9. Cadherins bind intracellularly at their C-terminal domain (CBP) to β-catenin via its armadillorepeat and at their juxtamembrane domain (JMD) to p120-catenin and δ-catenin.[6, 38] B-catenin contains a PDZ domain that interacts with IQGAP or α-catenin.[38] Interaction of β-catenin with IQGAP prevents binding of α-catenin[38], which will

bind to and actively regulate the structure of the actin cytoskeleton.[6, 13, 24] This interaction with the actin cytoskeleton is required for cell-adhesion. In addition, in the presynaptic neuron β-catenin interacts with a scaffold complex containing different proteins; Lin-7, CASK and Mint1.[17] This interaction localises synaptic vesicles to future synaptic sites.[17] Tyrosine phosphorylation of β-catenin by protein tyrosine kinases alters the binding affinity for α-catenin and cadherin.[36] Depending on the site of phosphorylation, binding affinity will be enhanced or reduced.[28] For example, phosphorylation of β-catenin at S29, T102 or T112 by CKII (Casein Kinase II) enhances binding affinity for α-catenin, whereas phosphorylation of Y142 results in dissociation of α-catenin.[28] Moreover, serine phosphorylation of the cadherin intracellular domain increases the binding affinity for the β-catenin.[36] Phosphorylation of cadherin at S690 by CKI or at Y755 by Src enhances endocytosis of cadherin and thereby reduces adhesion.[28] In contrast, NMDA receptor activation decreases the tyrosine phosphorylation of β-catenin at Y654 (located in the armadillo repeat sequence required for binding to cadherins), thereby increasing the binding of N-cadherin and β-catenin and thus stabilising N-cadherin at the cell-surface. [28, 36]

Besides the interaction with the actin cytoskeleton, α-catenin also interacts with Afadin, another actin-binding protein.[39] Afadin binds another protein, Kalirin-7, via its PDZ domain leading to activation of P21-activated kinase (PAK), resulting in spine growth and increased AMPA receptor content.[39] Afadin is also able to bind directly to cadherin through a PDZ domain.[39] In addition, Afadin binds nectins, another type of cell-adhesion molecules.[35] In conclusion, Afadin is involved in the interaction of cadherin with other type of cell-adhesion molecules.

P120-catenin is an Armadillo repeat domain protein that binds to a GGGEED sequence in the juxtamembrane domain of the cadherin molecule. It is able to enhance cadherin stability and regulates cytoskeletal changes by Rho-family of GTPase signalling.[6] Cadherins with defects in this juxtamembrane domain are unable to bind p120-catenin and have weak adhesion properties.[38] P120-catenin is present in the cell bound to cadherin and unbound in the cytoplasm. Cytoplasmic p120-catenin inhibits RhoA and activates Rac1 and Cdc42. Activation of Rac1 and Cdc42 dissociates IQGAP from β-catenin, making it possible for α-catenin to bind to β-catenin, resulting in actin polymerisation. These actions result in increased motility and migration of the cell.[38] Overexpression of p120-catenin gives rise to morphological changes, which is independent of cadherin, since cadherin-deficient cells show the same effect.[38] Thus, attachment of p120-catenin to cadherins at the cell-cell contact, buffers cytoplasmic p120-catenin, thereby decreasing cell motility and increasing cell-cell adhesion.[38]

Cadherins also interact with AMPA receptors via δ-catenin (also known as NPRAP; neural plakophilin-related arm protein).[39] This molecule interacts with ABP (AMPA-receptor binding protein) and GRIP which in turn bind to the GluR2 and GluR3 subunit of the AMPA receptor respectively.[39] Furthermore, this δ-catenin binds PSD-95, interacting with NMDA receptor and neuroligin.[40] Via this mechanism, different cell-adhesion molecules are coupled to common cytoplasmic proteins and may thereby trigger recruitment, stabilisation or adhesion of the other.[41]

Besides, cadherin function is regulated by proteolytic cleavage.[42] Two membrane-bound matrix metalloproteinases (MMPs), ADAM10 and MT5-MMP, are able to cleave the extracellular domain of cadherins. If that occurs cell-cell adhesion will decrease. MT5-MMP associates with ABP/GRIP proteins at the cytoplasmic domain.[42] Intracellularly, cadherins are cleaved by Presenilin-1 (PS-1) upon NMDA receptor activation. The released cytoplasmic tail can promote degradation of CBP (CREB-binding protein), a protein required for the transcription of stress-response genes in order to cope with environmental challenges.[22, 36]

To conclude, cadherins bind to several scaffolding proteins in the nerve terminal, including β-catenin, p120-catenin and δ-catenin. These proteins are involved in many other cell-signalling processes and

interact with many other proteins. Cadherin is stabilised at the membrane by binding of p120-catenin intracellularly and Ca^{2+} extracellularly. Cadherins interact with other receptors, such as AMPA and NMDA-receptors, through their signalling molecules.[42] Interaction of β-catenin with α-catenin regulates neural structure by bridging cadherins to the actin cytoskeleton. In the presynaptic terminal the cadherin-catenin complex tethers the neurotransmitter release machinery, whereas in the postsynaptic terminal it anchors glutamate- and other receptors and interacts with the actin cytoskeleton.[40]

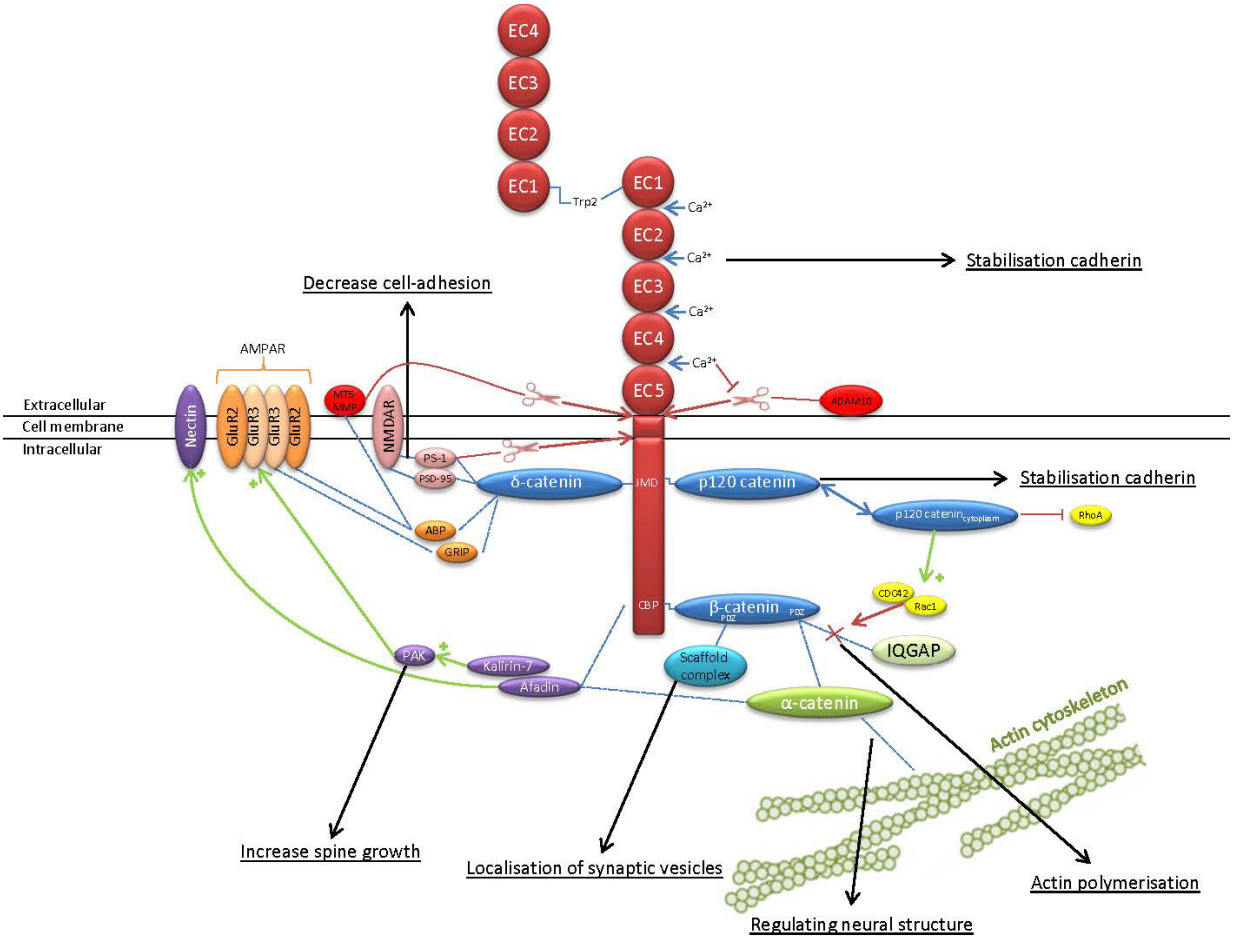

Figure 9 Cadherin signal transduction mechanism overview
Abbreviations: ABP = AMPA-receptor binding protein, CBP = catenin-binding domain, GRIP = glutamate receptor interacting protein, JMD = Juxtamembrane domain, PAK = p21-activated kinase, PS1 = presenilin 1, PSD-95 = scaffolding protein postsynaptic density 95.

2.2. Protocadherins

The protocadherin (PCDH) subfamily is the largest subgroup of the cadherin family, consisting of more than 70 members.[9, 43] Protocadherins can be divided into clustered and non-clustered protocadherins (Figure 10[43]). Clustered protocadherins have a specific genomic organisation in a small genome locus and they can be further divided into α-, β- and γ-protocadherins.[43] The non-clustered protocadherins do not have a specific genome locus and can be divided into δ-PCDH and other protocadherins.[43] The large gene family of protocadherins undergo alternative splicing in order to make different protocadherin molecules.[14]

Structurally, protocadherins are different from classical cadherins in that they have a variable number of cadherin repeats[13] and lack the hydrophobic tryptophan pocket in the extracellular cadherin 1 domain, which is required for adhesion in classical cadherins.[43] Nevertheless, they do contain a hydrophobic region, although smaller compared to classical cadherins.[43] It is suggested that the homophilic adhesion mechanism is different in protocadherins compared to classical cadherins.[43] Protocadherins are also able to form heterophilic interactions with other receptors on the cell membrane.[44] For example, *PCDH α4* is able to interact with β1-integrin and *PCDH 8* and *PCDH 15* are able to interact with classical cadherins.[45] Furthermore, the strength of adhesion is weaker in protocadherin interactions compared to adhesion of classical cadherins.[18] Indeed, replacing the cytoplasmic tail of protocadherins with the cytoplasmic tail of E-cadherin strengthened the adhesion. This suggests that the weak adhesion is due to the inability of the cytoplasmic tail to stabilise the molecule, and not caused by the properties of the extracellular domain.[36] The cytoplasmic domains of protocadherins differ from each other and protocadherins lack the β-catenin binding domain.[45] Since protocadherins have weaker adhesion properties and they are able to form heterophilic interactions with other receptors, it is suggested that protocadherins are involved in cell-recognition and -signalling and in the modulation of cell-adhesion, rather than maintaining the physical interaction between cells.[36] Furthermore, protocadherins are suggested to act as regulators for other receptors.[45] For example, the cytoplasmic domain of *PCDH 8* interacts with TAO2β (thousand and one amino acid kinase 2 β) which activates the p38 MAPK pathway, enhancing endocytosis of N-cadherin.[45] Besides, protocadherins contain a Cys-X_5-Cys sequence that is implicated in protein-protein interactions in glycoproteins, this region could function as a novel adhesion site for protocadherins.[43] Another structural difference between classical cadherins and protocadherins is that protocadherins lack the prodomain[46], that is important for cell-adhesion in classical cadherins.[10]

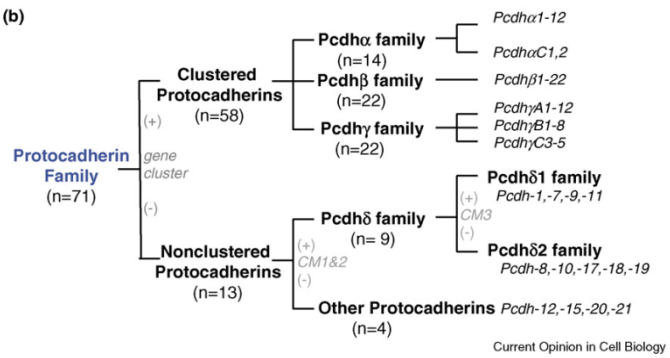

Figure 10 Protocadherin family
The protocadherin family can be divided in the clustered and non-clustered protocadherins. The well-known clustered protocadherins can be further subdivided into the α-, β-, and γ-*PCDHs*. The non-clustered protocadherins are δ-*PCDHs* and others. [43]

PCDH-δ proteins are a subfamily of the protocadherins. They contain two highly conserved cytoplasmic domains (CM1 and CM2).[43] On basis of homology the *PCDH-δ* family can be further divided in *PCDH-δ1* and *PCDH-δ2*.[43] The first has seven extracellular cadherin repeats, whereas group 2 has six extracellular cadherin repeats.[43] Another difference between the *PCDH-δ1* and *PCDH-δ2* family is the absence of a CM3 domain, required for the binding to PP1α (protein phosphatise-1α), in *PCDH-δ2*.[46]

Members of the protocadherin family are expressed in specific brain regions[13, 14], for example *PCDH 10* is highly expressed in the olfactory and limbic system.[46] Protocadherins are expressed in neurons, localised in synaptic junctions[13] and are involved in brain development.[43] The expression decreases after maturation and myelinisation of neurons.[43]

2.3. Cadherins in development

Cadherins are involved in many developmental processes in the body (Figure 11).[36] First of all cadherins are required for cell-cell adhesion in different tissues, including the nervous system. (Figure 11A)[36] The cell-cell adhesion is performed by homophilic and heterophilic interactions between cadherin molecules and opposing cells. Homophilic interactions of cadherin proteins are

also required for cell-sorting; overexpression of a specific cadherin in Purkinje cell progenitors results in redistribution of the cells to regions of the cerebellum that expresses that specific cadherin. (Figure 11B[36]) In addition, cadherins are required for cell polarity in epithelial tissues mainly. (Figure 11E[36]) In early embryogenesis, during the formation of the neural plate, switching the expression of cadherin subtypes (N-cadherin for E-cadherin)[47] enables forming of the neural tube by losing the cell-cell adhesion properties with the mesoderm. (Figure 11C[36]) N-cadherin knockout mice die around embryonic day 10.[28] However, mice with conditional knockout of N-cadherin in specific neural regions survive despite of severe disorganisation of that region.[47] In the developing nervous system, specific cadherins localise to different regions, leading to correct cell-sorting in the developing brain.[36] Moreover, N-cadherin is important in neural crest cell formation. In the neural tube, N-cadherin is cleaved by ADAM10 (a matrix metalloprotease) in the extracellular domain, thereby down-regulating the adhesive function of N-cadherin and generating a cytoplasmic fragment that is processed by presenilin 1 (PS1).[28] This cytoplasmic fragment in turn translocates to the nucleus and stimulates transcription mediated by β-catenin.[47] Figure 11F shows a mature neuronal synapse in which cadherins form puncta adherents, surrounding the active zone.[36] Thus cadherins have developmental roles throughout the body and are also implicated in correct structural and functional brain development.[36]

Specific cadherin subtypes can mediate specific functions during development. For example, N-cadherin and R-cadherin activate FGFR and thereby stimulate neurite outgrowth[36] and cadherin-11 stimulates axon elongation[35] , whereas cadherin-13 acts as a repellant cue for growth cones.[35, 36]

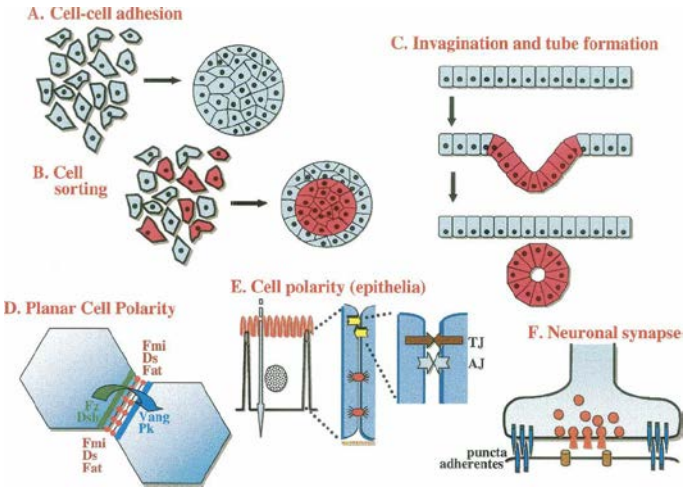

Figure 11 Developmental roles of cadherins
Cadherins are involved in many developmental processes, such as cell-cell adhesion, cell-sorting, formation of polarised epithelia, formation of the neural tube and formation of neural synapses. [36]

2.4. Loss-of-function studies

Studies using transgenic mice are useful for investigating the function of different proteins. Several cadherin mutant mice are studied and the outcome will be explained below.

Dominant-negative N-cadherin mutant constructs are DNA constructs of N-cadherin lacking the extracellular domain. They are used in cultured neurons and expressed in mutant mice. These mice show abnormal morphology of dendritic spines, increased spine length and reduced presynaptic densities.[18] The effects of the N-cadherin mutant constructs is bigger in younger cells, suggesting a more active role of N-cadherin in young neurons.[18] Blocking of the extracellular domain of N-cadherin using blocking-antibodies, resulted in increased distance between axonal and dendritic membranes and a decrease in synaptic function.[35] A point mutation in the extracellular domain, especially in the first extracellular cadherin domain, had small effects on the adhesive properties, however, depolarising and repolarising responses of the synapse were altered.[35]

Several studies show that deletion of the *PCDH-γ* cluster results in loss of spinal interneurons due to apoptosis.[35, 43] The role of *PCDH-γ* genes in synaptogenesis cannot be investigated using these knockout mice since the interneurons undergo apoptotic degeneration leading to neonatal death.[13, 41] Minimising cell death by removing BAX, a pro-apoptotic gene, ensures the survival of the neurons.[43] Thus, *PCDH-γ* genes are involved in the survival of interneurons. These mutant mice show reduced density of the puncta adherentia and decreased synaptic activity.[13, 41, 43] Additionally, these mutated neurons made significantly fewer synapses than wild-type neurons[9], indicating a role of *PCDH-γ* in synaptogenesis and activity and a requirement for neuronal survival.[41] General effects on axonal growth, adhesion and migration are not observed and might be caused by compensation by *PCDH-α* and –β.[13, 41, 43]

3. Cadherins implicated in ASD

Genome-wide association studies have identified several mutations in cadherin genes implicated in ASD. These include classical cadherins; cadherin 8 (*CDH 8*), *CDH 9*, *CDH 10*, *CDH 15*, *CDH 18*, and δ-protocadherins; protocadherin 9 (*PCDH 9*), *PCDH 10* and *PCDH 19*. Functions of these genes are largely unknown, available knowledge is explained below and summarised in Table 1.

3.1. Classical cadherins involved in ASD

The classical cadherins correlated with autism spectrum disorders are all type I or type II cadherins. No defects are found in other subtypes of the cadherin family except protocadherins, which will be explained later.

3.1.1. Cadherin 8

Cadherin 8 (*CDH 8*) is a classical cadherin type II, widely expressed in the central nervous system, especially in the striatum, cortex and hippocampus.[48] Mutations in this gene increase the susceptibility for ASD and learning disabilities.[49] Cadherin 8 is different from other classical cadherin in that it does not associate very well with alpha-catenin and gamma-catenin.[32] Furthermore, cadherin degradation is prevented by Ca^{2+}. However, presence of Ca^{2+} does not protect *CDH 8* to be digested by trypsin.[32] The exact function of *CDH 8* is unknown, although some roles are suggested below. Blocking of *CDH 8* with an exogenous QAV sequence (binding to the first extracellular cadherin domain of *CDH 8*) enhances axon growth.[29] Thus *CDH 8* might be involved in the regulation of axon growth. It has been shown that a few splice variants of *CDH 8* are expressed in the rat and chicken brain.[32, 50] The longest isoform has the structure of classical cadherins with five EC domains, a transmembrane domain and a cytoplasmic tail[32], and is highly similar to the human cadherin 8 protein.[50] On the other hand, the splice variants have a truncated structure and lack the transmembrane domain and the cytoplasmic tail.[32, 50] The truncated form appears to be soluble, and therefore cannot serve as a cell adhesion protein.[32] Expression of these isoforms is regulated temporally and regionally in the brain[50] and it is suggested that these *CDH 8* isoforms play a role in the modulation of cell adhesion or neurite outgrowth.[32] These results show that cadherin 8 is somehow involved in brain development, however the mechanism in which cadherin 8 increases the susceptibility for ASD and learning deficits is not yet understood.

3.1.2. Cadherin 9

Cadherin 9 is a classical cadherin type II, selectively expressed in the hippocampus in dentate gyrus (DG) and CA3 neurons and also expressed in the striatum and the cortex (Figure 12).[48] The hippocampus is part of the limbic system and involved in memory processes.[51] Knockdown of *CDH 9* in CA3 or DG neurons results in a decrease in the number of synapses between DG and CA3 neurons.[52] Thus presynaptic and postsynaptic expression of *CDH 9* is required for correct synapse formation, suggesting homophilic binding interactions of *CDH 9* in the synapse.[52] However, knockdown of *CDH 9* in DG neurons resulted in no observed defects in the number of DG synapses onto CA1 neurons in vitro. In addition, knockdown of *CDH 9* in CA3 neurons did not affect the amount of CA-CA synapses.[52] This indicates that *CDH 9* is involved in a specific class of synapses and that *CDH 9* knockdown does not cause general synaptic defects.[52] In order to investigate whether *CDH 9* can induce synapse formation, effects of overexpression of *CDH 9* are studied. Overexpression of *CDH 9* in cultured hippocampal neurons had no effects on synapse formation. Thus, *CDH 9* is required for synapse formation between DG and CA3 neurons but is not able to induce this formation.[52] Memory deficits are shown in patients with ASD[51] and thus, defects in cadherin 9 in patients with ASD might be the underlying cause of these learning deficits seen in these ASD patients.

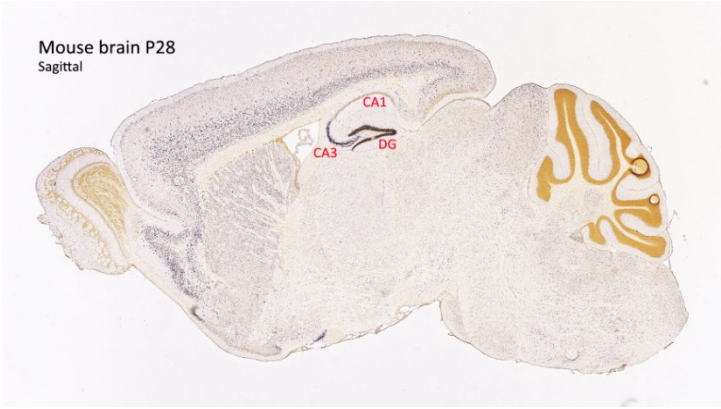

Figure 12 Expression profile *CDH9*
In situ hybridisation for *Cadherin 9* in a mouse brain of postnatal day 28 shows that *CDH 9* is specifically expressed in the CA3 and DG neurons in the hippocampus. Furthermore it is expressed in the cortex and in the striatum. [48]

3.1.3. Cadherin 10

Cadherin 10 is a type II cadherin.[4] It is expressed in the CA1 and CA3 region of the hippocampus.[29, 53] *In situ* hybridisation data of the Allen Brain Atlas also shows that *CDH 10* is expressed in the olfactory areas.[48] Furthermore, high expression is seen in the frontal cortex, a region known to be important in ASD.[4] However, little is known about this cadherin type, and thus the implications for ASD are unknown.

3.1.4. Cadherin 15

Cadherin 15 is a type I classical cadherin, also known as M-cadherin (myotubule) and *CDH 14*. It is specifically expressed in the cerebellum[48] and in skeletal muscle and shown to be involved in patients with ASD and intellectual disability (ID). None of these ASD or ID patients with mutations in *CDH 15* have a skeletal muscle disorder.[54] Mutations found in the *CDH 15* gene are all functionally critical regions; one mutation is located in the signal peptide region and three mutations are in the extracellular cadherin domain 1, crucial for cell-adhesion.[54] Cell lines containing these mutations are studied; they show normal amounts of cadherins at the cell surface, however the cell-cell adhesion properties was decreased by 80%.[54] This decrease in cell-adhesion might be the underlying mechanism of ASD and ID. Patients with mutations in *CDH 15* also suffer from facial dysmorphisms and structural abnormalities of the brain and seizures.[55]

3.1.5. Cadherin 18

Cadherin 18 is a type II cadherin, also known as *CDH 14* or *CDH 24*. The gene *CDH 14* is only used as a synonym for both *CDH 15* and *CDH 18*, however these names do refer to different genes. A *CDH 14* gene is found in rat but not in human. Using this name as a synonym for *CDH 15* or *CDH 18* can be confusing, since *CDH 15* and *CDH 18* are different genes, expressed on different chromosomes and coding for other cadherin proteins. *CDH 18* is expressed specifically in the central nervous system in the cortex and the cerebellar cortex.[48] It is shown to be associated with schizophrenia and autism spectrum disorders.[56] However, nothing is known about the specific molecular mechanisms of this type of cadherins.

3.2. Protocadherins involved in ASD

Dysfunctioning of non-clustered-protocadherins is associated with cognitive dysfunctioning.[45] The protocadherins involved in ASD are *PCDH 9*, *PCDH 10* and *PCDH 19*. These are all members of the δ-protocadherin family.[43]

3.2.1. Protocadherin 9

Protocadherin 9 is a δ1-protocadherin weakly expressed in the central nervous system.[48] Expression is found in the cranial ganglia, especially during development.[46] Experiments with protocadherin 9

knockout mice give insight in the possible roles of *PCDH 9* in autism spectrum disorders. *PCDH 9*$^{-/-}$ mice show reduction in cortical thickness. This anatomical abnormality is also observed in post-mortem studies of psychiatric disorders, including ASD.[57] This reduction in cortical thickness is due to increased cell density and reduces the processing of sensory information relevant for social adaptation.[57] Behavioural testing of the *PCDH 9*$^{-/-}$ mice showed that social long-term memory is affected but non-social long-term memory is not affected.[57] It is clear that *PCDH 9* is involved in clinical features of ASD, probably due to reduced cortical thickness, Although the underlying molecular mechanism is poorly understood.

3.2.2. Protocadherin 10

Protocadherin 10 (also known as OL-protocadherin) is a δ2-protocadherin[43] widely expressed in the central nervous system[58], it is specifically expressed in the olfactory bulb, most parts of the limbic system and the cerebellum.[44, 46] *PCDH 10* is associated with ASD.[7] *PCDH 10* act as a cell-adhesion molecule, although much weaker than classical cadherins.[59] *PCDH 10* interacts with Nap1 (Nck-associated protein 1) and a protein called Wave.[45] This Nap1/Wave complex interacts with actin and regulates cell migration.[45] Knockout studies of *PCDH 10* have shown the importance of *PCDH 10* in axon guidance through the ventral telencephalon. Mice lacking *PCDH 10* show misguiding of axons in the ventral telencephalon.[60] Furthermore, *PCDH 10* is required for cerebellar development and synapse formation of cerebellar Purkinje cells.[44] *PCDH 10* is required for axon guidance in the developing brain, defects in this mechanism might cause symptoms of ASD.

3.2.3. Protocadherin 19

Protocadherin 19 (also known as KIAA 1313) is located on the X-chromosome and codes for a δ2-protocadherin. It has been shown to be associated with epilepsy and mental retardation limited to females (EFMR).[26] This disorder is X-linked dominant, in which females are affected. Males are not affected, but are able to transmit the mutation.[61] This is thought to be due to X inactivation of the *PCDH 19* gene; males have a homogeneous population of *PCDH 19*-negative cells while affected females have *PCDH 19*-negative and –positive cells. *PCDH 19* cell-adhesion is suggested to be homophilic, thus females with the two cell types will have more severe disrupted cell-adhesion than a simple loss of *PCDH 19.*[7] *PCDH 19* is a δ-protocadherin highly expressed in the hippocampus (CA1 and DG neurons) and the cortex of the mouse brain.[48] It is able to form a cis-complex with N-cadherin.[26] Two mutations are found, one leading to a premature termination codon of the gene and another missense mutation disrupting the adhesion properties of *PCDH 19.*[7] Mutations in *PCDH 19* results in impaired calcium-dependent adhesion of the cis-complex, indicating that *PCDH 19* has a major role in mediating cell-adhesion within the *PCDH 19* - N-cadherin complex.[26] Since *PCDH 9* and *PCDH 10* are implicated in ASD, and *PCDH 19* is involved in cell-adhesion it has been suggested that *PCDH 19* might also be involved in the pathogenesis of ASD. However, Hynes et al showed no correlation between *PCDH 19* mutations and ASD.[62]

Gene	Subtype	Expression	Mutation	-/- phenotype	ASD patients
CDH 8	Classical type II	Striatum, Cx, hipp.	Deletion > 1.6 Mb	↑ axon growth	Low IQ / learning disabilities
CDH 9	Classical type II	Hipp. DG+CA3, Striatum, Cortex	Common SNPs	↓ number synapse DG-CA3	unknown
CDH 10	Classical type II	Hip. CA1+CA3, olf, frontal cx.	Common SNPs	unknown	unknown
CDH 15	Classical type I	Cerebellum	Chromosomal abnormalities, rare mutations	↓ cell-cell adhesion	ID, facial dysmorphisms, structural brain abnormalities, seizures
CDH 18	Classical type II	Cortex, cerebellar cortex	Chromosomal abnormalities	unknown	Schizophrenia
PCDH 9	δ1-PCDH	Weak expression, overall during development	CNVs	↓ cortical thickness ↑ cell density Social long-term memory affected	unknown
PCDH 10	δ2-PCDH	Widely expressed, specifically olf, limbic system, cerebellum	Homozygous deletion	Misguided axon guidance in ventral telencephalon	unknown
PCDH 19	δ2-PCDH	Hippocampus: CA1+DG, cortex	Rare mutations	Disrupted adhesion	EFMR

Table 1 Genes associated with ASD
Characteristics of cadherin genes associated with ASD. Abbreviations; Cx = cortex, hipp = hippocampus, DG = dentate gyrus, olf = olfactory areas, SNPs = single nucleotide polymorphisms, CNVs = copy-number variations, ID = intellectual disabilities, EFMR = epilepsy and mental retardation limited to females.

4. Discussion and conclusion

Autism spectrum disorders (ASD) are common disorders with a high heritability.[3] Using genome-wide association studies, many genes are discovered to be associated with the development of ASD.[5] A family of genes implicated are the genes coding for synaptic cell-adhesion molecules.[7] Synaptically localised cell adhesion molecules are able to control synapse formation, regulate dendritic spine morphology, modify synaptic receptor function and modulate synaptic plasticity.[16] It is expected that mutations in these genes can lead to defects in these processes, and eventually lead to the development of ASD.[7] Unfortunately, little is known about the specific molecular and cellular functions of these genes, and how they contribute to the development of ASD.

Cadherins are cell-adhesion molecules that are expressed in several tissues such as the central nervous system. The cadherin proteins are characterised by extracellular cadherin repeat domains.[10] Ca^{2+} is required for interaction with other cadherin proteins at a neighbouring cell, thereby mediating cell-cell adhesion.[10] Cadherins interact with the actin cytoskeleton via β- and α-catenin in order to modulate neuronal structure.[10] Modulation of neuronal structure is important for the formation and maintenance of synapses in the brain. Cadherins are important in target recognition, synaptogenesis and stabilisation of synapses in the brain.[9] Most knowledge about cadherins is gained by research of classical cadherins. It is assumed that the characteristics of classical cadherins apply to all the cadherin subtypes since counterarguments are lacking. Furthermore, most research is performed on the E- (endothelial) cadherin subtype, since endothelial cells are most widely available.

Protocadherins are the largest subfamily of cadherins and are able to form homophilic and heterophilic interactions with other receptors. They have weak binding properties and are thought to be involved in the modulation of synaptic adhesion rather than implicated in the physical interaction itself.[45]

Eight subtypes of cadherins are shown to be correlated with ASD and are explained further. However, more affected genes are discovered rapidly by recent genetic studies. Thus, these eight cadherin subtypes associated with ASD, might not be all cadherin genes affected in ASD. *CDH 8, CDH 9, CDH 10, CDH 15 and CDH 18* are all classical cadherins and the protocadherins associated with ASD are all δ-protocadherins. This suggest that the molecular mechanisms of these subtypes are important for the development of ASD. The common feature of the classical cadherins is the extracellular domain. However, when these domains are causative for ASD, other classical cadherins would also be associated. The δ-protocadherins on the other hand share common cytoplasmic domains; CM1 and CM2. These domains might be important in the association with ASD. However, other δ-protocadherins containing these domains are not associated with ASD.

Cadherin 8 increases the susceptibility for ASD and specifically learning disabilities.[49] *CDH 8* is shown to be involved in regulation of axon growth, since blocking of *CDH 8* increases axon growth.[29] This indicates that *CDH 8* is involved in brain development and it could be possible that its function in axon growth is required for proper brain development. Although, the underlying mechanism still has to be determined. SNPs (single nucleotide polymorphisms) are found between *CDH 9* and *CDH 10* on chromosome location 5p14.1 which affects both genes.[7] CDH *9* is another cadherin subtype which is selectively expressed in the hippocampus in the dentate gyrus and CA3 neurons.[48] Knockout studies for *CDH 9* are performed and show a decrease in the number of synapses between the DG and CA3 when *CDH 9* is absent.[15] Thus, *CDH 9* is required for synapse formation in the hippocampus. The hippocampus is involved in memory and thus *CDH 9* deficiency might be the underlying cause of learning deficits in some ASD patients. Another cadherin subtype associated with ASD is *CDH 10*. The function and molecular mechanism of this cadherin subtype is unknown. However, it is known to be highly expressed in the frontal cortex, a region known to be important for ASD development.[4] Next,

mutations in the cadherin 15 gene is correlated with ASD.[7] Studies using cell culture with these mutations show a decrease of 80% in cell-cell adhesion properties, although the amounts of cadherin 8 at the cell surface was unaffected.[54] Patients with mutations in this gene suffer from facial dysmorphisms and structural abnormalities of the brain.[55] This may be caused by the decrease in cell-adhesion and might be the origin of ASD in these patients. Cadherin 18 is the last classical cadherin associated with ASD. *CDH 18* is also known to be correlated with schizophrenia.[56] These disorders might share a common molecular pathway for their development. However, nothing is known about the molecular mechanism or function of *CDH 18*.

In addition to these classical cadherins, another subfamily of cadherins is associated with the development of ASD; the protocadherins (PCDH). Three protocadherins are affected; *PCDH 9, PCDH 10* and *PCDH 19.* These are all members of the non-clustered, δ-protocadherin family.[43] Dysfunction of non-clustered protocadherins was previously shown to be associated with cognitive dysfunctions including autism.[45] Deletion of *PCDH 9* in mice results in increased cortical cell density leading to a diminished cortical thickness, an anatomical abnormality that is also observed in post-mortem studies on psychatric patients.[57] These knockout mice show deficits in social long-term memory which might be caused by a reduction in the processing of social sensory information.[57] It is obvious that *PCDH 9* is involved in ASD. Although, the mechanism through which it contributes to abnormal brain development is still unknown. Protocadherin 10 is largely studied for its function in axon guidance.[60] Axons of the ventral telencephalon are misguided in the absence of *PCDH 10*, thus defects in the gene coding for this protein cause improper formation of circuitry in the brain.[60] Deficits in these pathways can cause cognitive dysfunctions such as autism. Protocadherin 19 is the last gene discussed. Mutations in this gene are found in families with EFMR (epilepsy, female-restricted with mental retardation).[7] *PCDH 19* mutations causes disrupted homophilic cell-adhesion properties in affected females. However, although these patients have mental retardation, they do not significantly show symptoms of ASD.[62]

To conclude, classical cadherins and δ-protocadherins are implicated in some cases of ASD. They might share a common mechanism in affecting brain development in ASD. This mechanism is unknown, but might take place in the shared extracellular domain of the classical cadherins and the shared intracellular domain of the δ-protocadherins. More research has to be done to investigate the molecular mechanisms. Identifying these mechanisms, could help discovering new therapeutics in the field of personalised medicine. Patients suffering from ASD with mutations in these genes could be treated with novel medicines targeting the affected molecular mechanisms of cadherins.

References

1. Diagnostic and statistical manual of mental disorders. Fourth Edition, Text Revision ed. Washington, DC: American Psychiatric Association; 2000. .

2. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators. Prevalence of autism spectrum disorders - autism and developmental disabilities monitoring network, 14 sites, united states, 2008. MMWR Surveill Summ 2012 Mar 30;61(3):1-19.

3. Watts TJ. The pathogenesis of autism. Clin Med Pathol 2008;1:99-103.

4. Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS, Salyakina D, Imielinski M, Bradfield JP, Sleiman PMA, et al. Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature 2009 05/28;459(7246):528-33.

5. Devlin B, Scherer SW. Genetic architecture in autism spectrum disorder. Curr Opin Genet Dev 2012 Mar 29.

6. McAllister AK. Dynamic aspects of CNS synapse formation. Annu Rev Neurosci 2007;30:425-50.

7. Betancur C, Sakurai T, Buxbaum JD. The emerging role of synaptic cell-adhesion pathways in the pathogenesis of autism spectrum disorders. Trends Neurosci 2009 Jul;32(7):402-12.

8. Purves D, Augustine GJ, Fitzpatrick D, Hall WC, LaMantia A, White LE. Neuroscience. Fifth Edition ed. Sinauer; 2012. .

9. Gerrow K, El-Husseini A. Cell adhesion molecules at the synapse. Front Biosci 2006 Sep 1;11:2400-19.

10. Shapiro L, Love J, Colman DR. Adhesion molecules in the nervous system: Structural insights into function and diversity. Annu Rev Neurosci 2007;30:451-74.

11. Akins MR, Biederer T. Cell-cell interactions in synaptogenesis. Curr Opin Neurobiol 2006 Feb;16(1):83-9.

12. Garner CC, Waites CL, Ziv NE. Synapse development: Still looking for the forest, still lost in the trees. Cell Tissue Res 2006 Nov;326(2):249-62.

13. Giagtzoglou N, Ly CV, Bellen HJ. Cell adhesion, the backbone of the synapse: "vertebrate" and "invertebrate" perspectives. Cold Spring Harb Perspect Biol 2009 Oct;1(4):a003079.

14. Waites CL, Craig AM, Garner CC. Mechanisms of vertebrate synaptogenesis. Annu Rev Neurosci 2005;28:251-74.

15. Williams ME, de Wit J, Ghosh A. Molecular mechanisms of synaptic specificity in developing neural circuits. Neuron 2010 Oct 6;68(1):9-18.

16. Dalva MB, McClelland AC, Kayser MS. Cell adhesion molecules: Signalling functions at the synapse. Nat Rev Neurosci 2007 Mar;8(3):206-20.

17. Salinas PC, Price SR. Cadherins and catenins in synapse development. Curr Opin Neurobiol 2005 Feb;15(1):73-80.

18. Piechotta K, Dudanova I, Missler M. The resilient synapse: Insights from genetic interference of synaptic cell adhesion molecules. Cell Tissue Res 2006 Nov;326(2):617-42.

19. Angst BD, Marcozzi C, Magee AI. The cadherin superfamily: Diversity in form and function. J Cell Sci 2001 Feb;114(Pt 4):629-41.

20. Suzuki SC, Takeichi M. Cadherins in neuronal morphogenesis and function. Dev Growth Differ 2008 Jun;50 Suppl 1:S119-30.

21. Arikkath J, Reichardt LF. Cadherins and catenins at synapses: Roles in synaptogenesis and synaptic plasticity. Trends Neurosci 2008 Sep;31(9):487-94.

22. Tai CY, Kim SA, Schuman EM. Cadherins and synaptic plasticity. Curr Opin Cell Biol 2008 Oct;20(5):567-75.

23. Shimoyama Y, Tsujimoto G, Kitajima M, Natori M. Identification of three human type-II classic cadherins and frequent heterophilic interactions between different subclasses of type-II classic cadherins. Biochem J 2000 Jul 1;349(Pt 1):159-67.

24. Shapiro L, Weis WI. Structure and biochemistry of cadherins and catenins. Cold Spring Harb Perspect Biol 2009 Sep;1(3):a003053.

25. Zhang Y, Sivasankar S, Nelson WJ, Chu S. Resolving cadherin interactions and binding cooperativity at the single-molecule level. Proc Natl Acad Sci U S A 2009 Jan 6;106(1):109-14.

26. Emond MR, Biswas S, Blevins CJ, Jontes JD. A complex of protocadherin-19 and N-cadherin mediates a novel mechanism of cell adhesion. J Cell Biol 2011 Dec 26;195(7):1115-21.

27. Elste AM, Benson DL. Structural basis for developmentally regulated changes in cadherin function at synapses. J Comp Neurol 2006 Mar 20;495(3):324-35.

28. Stemmler MP. Cadherins in development and cancer. Mol Biosyst 2008 Aug;4(8):835-50.

29. Bekirov IH, Nagy V, Svoronos A, Huntley GW, Benson DL. Cadherin-8 and N-cadherin differentially regulate pre- and postsynaptic development of the hippocampal mossy fiber pathway. Hippocampus 2008;18(4):349-63.

30. Takeichi M. The cadherin superfamily in neuronal connections and interactions. Nat Rev Neurosci 2007 Jan;8(1):11-20.

31. Ivanov DB, Philippova MP, Tkachuk VA. Structure and functions of classical cadherins. Biochemistry (Mosc) 2001 Oct;66(10):1174-86.

32. Kido M, Obata S, Tanihara H, Rochelle JM, Seldin MF, Taketani S, Suzuki ST. Molecular properties and chromosomal location of cadherin-8. Genomics 1998 Mar 1;48(2):186-94.

33. Craig AM, Graf ER, Linhoff MW. How to build a central synapse: Clues from cell culture. Trends Neurosci 2006 Jan;29(1):8-20.

34. Sanes DH, Reh TA, Harris WH. Synapse formation and function. In: Development of the nervous system. third edition ed. Academic Press; 2011. .

35. Yamada S, Nelson WJ. Synapses: Sites of cell recognition, adhesion, and functional specification. Annu Rev Biochem 2007;76:267-94.

36. Halbleib JM, Nelson WJ. Cadherins in development: Cell adhesion, sorting, and tissue morphogenesis. Genes Dev 2006 Dec 1;20(23):3199-214.

37. Abe K, Chisaka O, Van Roy F, Takeichi M. Stability of dendritic spines and synaptic contacts is controlled by alpha N-catenin. Nat Neurosci 2004 Apr;7(4):357-63.

38. Anastasiadis PZ, Reynolds AB. Regulation of rho GTPases by p120-catenin. Curr Opin Cell Biol 2001 Oct;13(5):604-10.

39. Tallafuss A, Constable JR, Washbourne P. Organization of central synapses by adhesion molecules. Eur J Neurosci 2010 Jul;32(2):198-206.

40. Silverman JB, Restituito S, Lu W, Lee-Edwards L, Khatri L, Ziff EB. Synaptic anchorage of AMPA receptors by cadherins through neural plakophilin-related arm protein AMPA receptor-binding protein complexes. J Neurosci 2007 Aug 8;27(32):8505-16.

41. Washbourne P, Dityatev A, Scheiffele P, Biederer T, Weiner JA, Christopherson KS, El-Husseini A. Cell adhesion molecules in synapse formation. J Neurosci 2004 Oct 20;24(42):9244-9.

42. Restituito S, Khatri L, Ninan I, Mathews PM, Liu X, Weinberg RJ, Ziff EB. Synaptic autoregulation by metalloproteases and gamma-secretase. J Neurosci 2011 Aug 24;31(34):12083-93.

43. Morishita H, Yagi T. Protocadherin family: Diversity, structure, and function. Curr Opin Cell Biol 2007 Oct;19(5):584-92.

44. Frank M, Kemler R. Protocadherins. Curr Opin Cell Biol 2002 Oct;14(5):557-62.

45. Kim SY, Yasuda S, Tanaka H, Yamagata K, Kim H. Non-clustered protocadherin. Cell Adh Migr 2011 Mar-Apr;5(2):97-105.

46. Redies C, Vanhalst K, Roy F. Delta-protocadherins: Unique structures and functions. Cell Mol Life Sci 2005 Dec;62(23):2840-52.

47. Stepniak E, Radice GL, Vasioukhin V. Adhesive and signaling functions of cadherins and catenins in vertebrate development. Cold Spring Harb Perspect Biol 2009 Nov;1(5):a002949.

48. Allen Brain Atlas Resources [Internet]Seattle (WA): Allen Institute for Brain Science; c2009. Available from: http://www.brain-map.org.

49. Pagnamenta AT, Khan H, Walker S, Gerrelli D, Wing K, Bonaglia MC, Giorda R, Berney T, Mani E, Molteni M, et al. Rare familial 16q21 microdeletions under a linkage peak implicate cadherin 8 (CDH8) in susceptibility to autism and learning disability. J Med Genet 2011 Jan;48(1):48-54.

50. Lin J, Luo J, Redies C. Molecular cloning and expression analysis of three cadherin-8 isoforms in the embryonic chicken brain. Brain Res 2008 Mar 27;1201:1-14.

51. Ben Shalom D. Memory in autism: Review and synthesis. Cortex 2003 Sep-Dec;39(4-5):1129-38.

52. Williams ME, Wilke SA, Daggett A, Davis E, Otto S, Ravi D, Ripley B, Bushong EA, Ellisman MH, Klein G, et al. Cadherin-9 regulates synapse-specific differentiation in the developing hippocampus. Neuron 2011 Aug 25;71(4):640-55.

53. Bekirov IH, Needleman LA, Zhang W, Benson DL. Identification and localization of multiple classic cadherins in developing rat limbic system. Neuroscience 2002;115(1):213-27.

54. Bhalla K, Luo Y, Buchan T, Beachem MA, Guzauskas GF, Ladd S, Bratcher SJ, Schroer RJ, Balsamo J, DuPont BR, et al. Alterations in CDH15 and KIRREL3 in patients with mild to severe intellectual disability. Am J Hum Genet 2008 Dec;83(6):703-13.

55. Willemsen MH, Fernandez BA, Bacino CA, Gerkes E, de Brouwer AP, Pfundt R, Sikkema-Raddatz B, Scherer SW, Marshall CR, Potocki L, et al. Identification of ANKRD11 and ZNF778 as candidate genes for autism and variable cognitive impairment in the novel 16q24.3 microdeletion syndrome. Eur J Hum Genet 2010 Apr;18(4):429-35.

56. Singh SM, Castellani C, O'Reilly R. Autism meets schizophrenia via cadherin pathway. Schizophr Res 2010 Feb;116(2-3):293-4.

57. Bruining H, Matsui A, Stiedl O, van Engeland H, Kahn RS, Laarakker MC, Ramakers GMJ, Fernandes C, van Lith HA, Nonkens L, et al. Genetic mapping of social memory components in mice specifies the role of PCDH9 variants in human psychopathology. 2012.

58. Hirano S, Yan Q, Suzuki ST. Expression of a novel protocadherin, OL-protocadherin, in a subset of functional systems of the developing mouse brain. J Neurosci 1999 Feb 1;19(3):995-1005.

59. Nakao S, Platek A, Hirano S, Takeichi M. Contact-dependent promotion of cell migration by the OL-protocadherin-Nap1 interaction. J Cell Biol 2008 Jul 28;182(2):395-410.

60. Uemura M, Nakao S, Suzuki ST, Takeichi M, Hirano S. OL-protocadherin is essential for growth of striatal axons and thalamocortical projections. Nat Neurosci 2007 Sep;10(9):1151-9.

61. Scheffer IE, Turner SJ, Dibbens LM, Bayly MA, Friend K, Hodgson B, Burrows L, Shaw M, Wei C, Ullmann R, et al. Epilepsy and mental retardation limited to females: An under-recognized disorder. Brain 2008 April 01;131(4):918-27.

62. Hynes K, Tarpey P, Dibbens LM, Bayly MA, Berkovic SF, Smith R, Raisi ZA, Turner SJ, Brown NJ, Desai TD, et al. Epilepsy and mental retardation limited to females with PCDH19 mutations can present de novo or in single generation families. J Med Genet 2010 Mar;47(3):211-6.