Chapter 24

The Role of Cadherins in Ca^{2+}-Mediated Cell Adhesion and Inherited Photoreceptor Degeneration

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Cadherins are Ca^{2+}-binding, transmembrane proteins involved in cell adhesion. Recently, three cadherin molecules, cadherin-23, protocadherin-15, and cadherin-3 were found to be defective in various human diseases, many of them with photoreceptor degeneration and/or sensorineural hearing loss as major featuresUsher syndrome type 1D (USH1D), USH1F, and hypotrichosis with juvenile macular dystrophy, respectively. The process, by which mutations lead to photoreceptor degeneration is still not fully understood. Data from the inner ear phenotype of USH1 mouse models suggest that loss of cell adhesion is a crucial event.

Cadherins: Features and Functions

Cadherins are Ca^{2+}-binding, transmembrane proteins involved in cell adhesion. The cadherin superfamily consists presently of more than 300 proteins (in vertebrates alone) and represents, with the immunoglobulin-type molecules, a major group of cell adhesion molecules both in vertebrates and invertebrates. In human, more than 80 different cadherins have been identified to date. Some members of the cadherin protein family gained special attention due to their involvement in different forms of cancer.[1] The identification of mutations in three cadherin genes in patients with various sensory disorders, most of them with photoreceptor degeneration as a major feature, revealed the importance of cadherins for retinal integrity. This Chapter will focus on the cadherin molecules implicated in human retinal disease.

Common to all cadherins are the multiple (5 to 34) cadherin domains (extracellular domains, EC), tandemly repeated, ~100-amino-acid stretches connected by ~10-amino-acid linker regions. ECs contain the evolutionarily highly conserved, negatively charged DXD, DRE, and DXNDN motifs that mediate Ca^{2+}-dependent homophilic binding between cadherin molecules (Fig. 1). Cadherins can be grouped based on the number and sequence of their ECs (as to the latter by comparing the first N-terminal EC, called EC1) and other domains, e.g., the cytoplasmic domain which may provide information on interacting partners of members of a given subfamily (for overview of phylogenetic classification, see Ref. 2). Presently, members of the cadherin superfamily fall into six groups according to size, number and feature of domains, function, and binding partners. We distinguish classical cadherins, desmosomal cadherins, protocadherins, cadherins with tyrosine-kinase domains (pointing towards a role of cadherins in signal transduction pathways), Fat-like cadherins (large cadherins with similarity to Fat, a cadherin with 34 ECs), and seven-pass transmembrane cadherins (Flamingo)[3,4] (Fig. 2).

The so-called classical cadherins possess five ECs (Fig. 1). In addition, classical cadherins share a similar intracellular peptide sequence that, in turn, gives clue to interacting partners.

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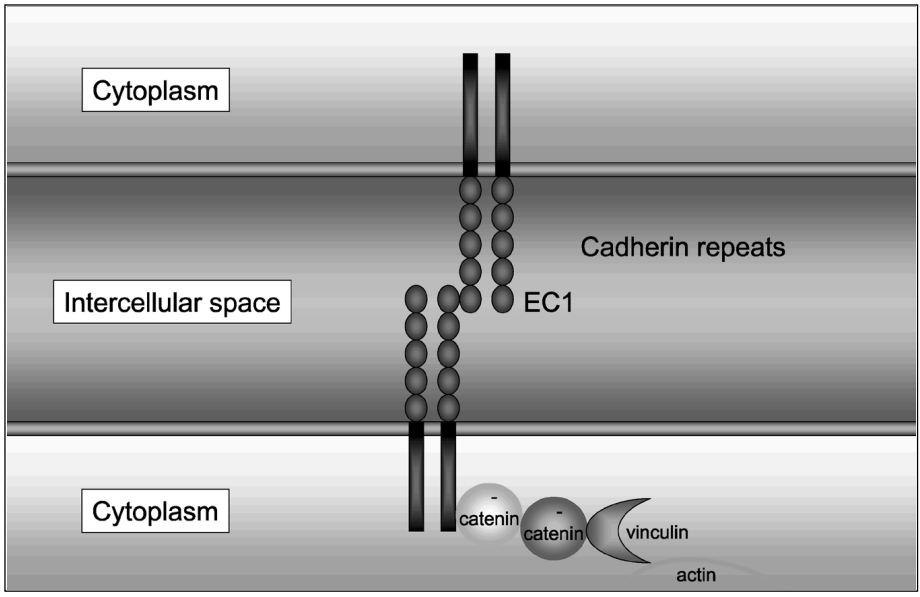

Figure 1. Cartoon of classical cadherins. Two opposite cells are shown. Five extracellular cadherin domains (interspersed linker regions are not shown) of each cadherin molecule protrude from the cells, form dimers with the horizontally neighbouring molecule and bridge the intercellular space by interacting laterally with dimers of the other cell (see text for details). By the cytoplasmic tail, classical cadherins are linked to the actin cytoskeleton via α- and β-catenins.

Indeed, all classical cadherins interact with the actin cytoskeleton via catenins. The intracellular domain is thought to play a regulatory role for the adhesive state of the extracellular domains.[5] The Ca^{2+}-binding pocket is formed by residues of EC1 and EC2 and those of the linker region (Fig. 3a). Each cadherin dimer associates with six Ca^{2+} ions primarily via the residues of the linker region between EC1 and EC2, whereas the interaction with amino acids from the ECs is also required (Fig. 3b). Ca^{2+} -binding seems to provide the molecule with a rigid and proteolysis-resistant arrangement of the Ecs.[6]

The structural basis of cell adhesion and molecular interaction has been studied so far mainly for classical cadherins. The cadherin fold consists of a seven-strand β-sheet. It seems that cadherins of the same cell, by lateral association, form parallel *cis*-dimers which are today considered "building blocks" for lateral clustering and thereby form the basis for stable cell adhesion.[7] *Trans*-dimerization results from contacts between the N-terminal ECs of cadherin molecules from both opposite cells involved.[8] More recent studies suggested the possibility of a variable degree of anti-parallel overlap, to an extent where all five ECs may overlap.[9] Remarkably, the predicted distance (20-25 nm) at maximal overlap between two classical cadherin molecules of adjacent membranes corresponds well to the cell-cell distance at adherens junctions, at which those cadherins play an important role, supporting the assumption of a possible overlap to a greater extent than just the N-terminal ECs.

Cadherins mediate two types of adhesive contacts: Firstly, oligomers of *trans*-dimers form diffuse adhesive contacts between neighboring cells. Secondly, a greater density of adhesive contacts is reached in clusters of *trans*-dimers that are found in specialized adherens junctions such as the zonula adherens. Although homophilic *cis*-binding seems typical, heteromeric binding has also been observed.[10]

Cadherins are also implicated in determining cell polarity, that is particularly important in highly organized tissue structures such as epithelial layers.[11] Initial cell-cell contacts are stabilized

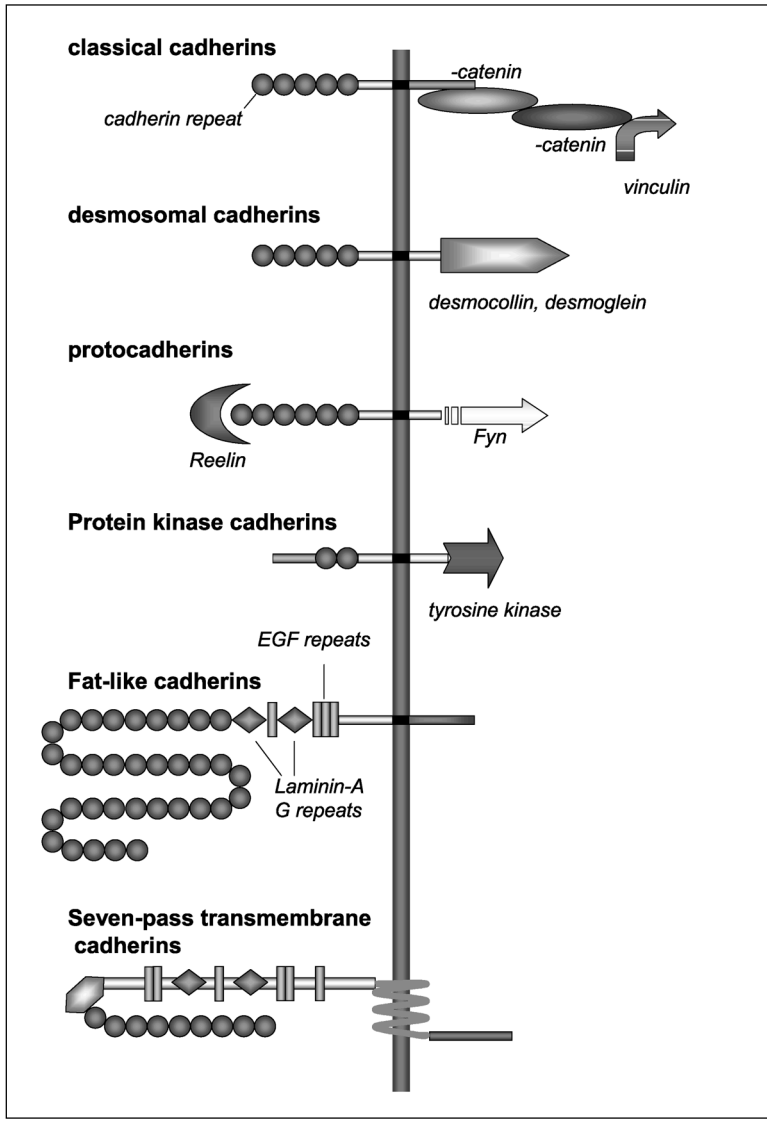

Figure 2. The cadherin superfamily. Schematic overview of representative members of the cadherin superfamily. See text for details.

by classical cadherins (and strengthened by their link to cytoskeleton), that appear in high density in adherens junctions as this contact broadens across neighbouring membranes. Moreover, cadherins seem to play a role in recruiting sec6/8, a multiprotein complex that targets exocytic vesicles with specific molecular components to selected docking sites on the (basal lateral) plasma membrane, thereby establishing epithelial apical-basal polarity.[12] The importance of cadherin-mediated cell adhesion for polarity and movement during embryonic morphogenesis is highlighted by the observation of left-right asymmetry after disruption of N-cadherin function during chick gastrulation.[13]

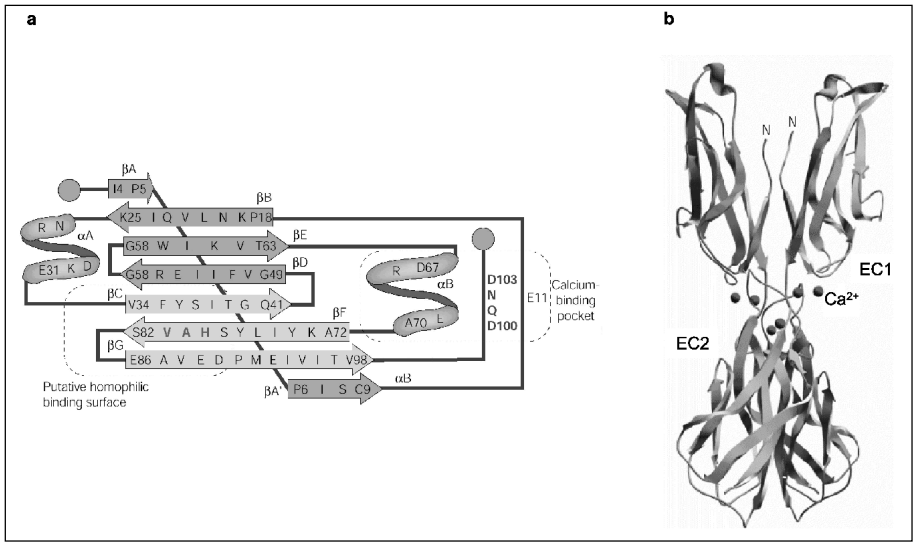

Figure 3. Structure of the cadherin domain and *cis*-dimer formation (based on analysis of EC1 of mouse E-cadherin). (a) Schematic topology of the amino-terminal EC1. βA, βA', βB, βE and βD (green/dark grey), and βC, βF and βG (yellow/light grey) form β-sheets. α-helices are on left and right. Dotted lines indicate the putative homophilic binding surface and the Ca^{2+}-binding pocket. (b) Each cadherin *cis*-dimer associates with six Ca^{2+} ions primarily via the residues of the linker region between EC1 and EC2 (Reprinted by permission from Tepass et al.: Cadherins in embryonic and neural morphogenesis. Fig. 1a, Nat Rev Mol Cell Biol 2000; 1(2):91-100; copyright 2001 Macmillan Magazines Ltd.).

Many cadherins show strong expression in neural tissues. Protocadherins of the diverse Pcdhα/CNR group show specific expression in selected brain areas/tissues and function as receptors for the extracellular matrix molecule reelin, an interaction that is assumed to be crucial for positioning of neuronal sub-populations in the cortex.[14] Particularly noteworthy in view of the focus of this book is the physiological role of N-cadherin that was shown to be required for axonal outgrowth and guidance in the retina.[15] The finding that numerous cadherins localize to synapses in structures resembling epithelial adherens junctions suggests that cadherins may be important in building complex neural networks. Cadherin-mediated adhesion seems, in turn, to be influenced by synaptic activity, suggesting a role of cadherin both in synapse plasticity and activity modulation,[16] hence making cadherins excellent candidates for being involved in long term potentiation (LTP) at synapses.[6]

A remarkable feature of protocadherin genes is their genomic organization in clusters, strikingly resembling clustering of the immunoglobulin genes in the mammalian genome, and giving rise to a large variety of similar but distinct transcripts from each cluster that could be involved in formation and reorganization of synaptic connections in nervous tissues (overview in Ref. 17). A recent review about the role of cadherins in both embryonic and neural morphogenesis was published by Tepass et al.[4]

Role of Cadherins in Human Disease

Prior to the elucidation of the role of cadherins in sensory disorders, members of this protein family were mainly known to be implicated in malignancies. E-cadherin mutations were found in invasive gastric cancer and various other neoplasms, mainly of the digestive system. The role of cadherins in cancerogenesis is thought to be related to impaired cell adhesion, which, in turn, leads to a higher degree of invasiveness.

Role of Cadherins in Human Retina

The expression (and its variability during development) as well as the localization of various cadherins was documented in several publications (see Ref. 3 and references therein). The particular importance of three cadherins, cadherin-23, protocadherin-15, and cadherin-3, in retinal function has been revealed only recently by the identification of cadherin gene mutations in autosomal recessive disorders, that all show retinal pathology as a common feature.

Previous studies in mouse and human showed that cadherin-23 is expressed in a variety of tissues, including the neurosensory epithelia of the inner ear and the retina.[18-20] Indirect immunofluorescence on the murine retina with an antiserum generated against the cytoplasmic domain at the C-terminus of the human protein indicates that cadherin-23 is localized primarily in two distinct compartments of the photoreceptor cells, at the synapse and the inner segment (Fig. 4). Anti-cadherin-23 staining in the inner segment is rather diffuse and not restricted to membranes. This may be due to staining of de novo synthesized protein in diverse cellular compartments. The bright staining of the outer plexiform layer of the retina suggests that cadherin-23 is a prominent component of the ribbon synapse of rod photoreceptor cells. In the nervous system, both sides of synaptic junctions contain highly specialized structures that promote rapid and efficient signal transmission from pre-synaptic terminal to post-synaptic membrane (for review see Refs. 21 and 22). While the complex cytomatrix of post-synaptic density is thought to be important for clustering of post-synaptic receptors, the numerous structural elements at the pre-synaptic button may be necessary for exocytosis of synaptic vesicles at the pre-synaptic active zone. Molecular analysis of ribbon synapses demonstrated that these specialized synapses, that transmit signals both from auditory hair cells and photoreceptor cells, exhibited an even higher complexity in their composition.[23] The detection of cadherin-23 at the ribbon synapse of the photoreceptor cells by immunofluorescence suggests that cadherin-23 is required for the proper function of ribbon synapses, perhaps by forming adhesive contacts. It is commonly accepted that cell-cell adhesion molecules of the two synaptic sides may interact with each other via their extracellular domains protruding into the extracellular space of the synaptic cleft keeping components of the cytomatrix of both synaptic membranes well-organized.[21]

Cadherin-23 *Mutations in Usher Syndrome Type 1D*

Usher syndrome (USH) is an autosomal recessive disorder characterized by sensorineural hearing loss and early onset visual impairment due to retinitis pigmentosa (RP), a degenerative disease of photoreceptors (overview in Ref. 24). Three USH subtypes are distinguished according to the degree of clinical symptoms. Usher syndrome type 1 (USH1) is the most severe form with profound congenital deafness, vestibular dysfunction, and early onset RP, and is a common cause of deaf-blindness in developed countries.[25] In addition to the clinical differences between the different subtypes, USH is also heterogeneous genetically. To date, six loci have been mapped for USH1 (*USH1A-USH1F*), whereas four of the underlying gene defects have been identified (overview in Ref. 26).

Using a positional candidate approach to identify the USH1D gene mapped previously to the long arm of chromosome 10, a novel member of the cadherin gene superfamily, *CDH23*, was identified. *CDH23* encodes a protein of 3,354 amino acids with a single transmembrane domain and 27 cadherin repeats, and is expressed in a wide range of tissues, including the cochlea and retina. Mutations in *CDH23* were shown to underlie both USH1D[18] and an autosomal recessive non-syndromic form of deafness (*DFNB12*, Ref. 19).

All but one of the *CDH23* mutations identified in USH1D patients occurred in portions of the gene that encode the extracellular part of the protein. The only mutation affecting the cytoplasmic domain reported so far was found in an atypical case of USH1 with mild retinal phenotype. As shown in Figure 5, for all but one of the USH1D mutations, a truncated gene product is predicted. Missense mutations have been found only in USH1 patients with more 'severe' mutations on the other allele (compound heterozygosity) or, if homozygous, in patients

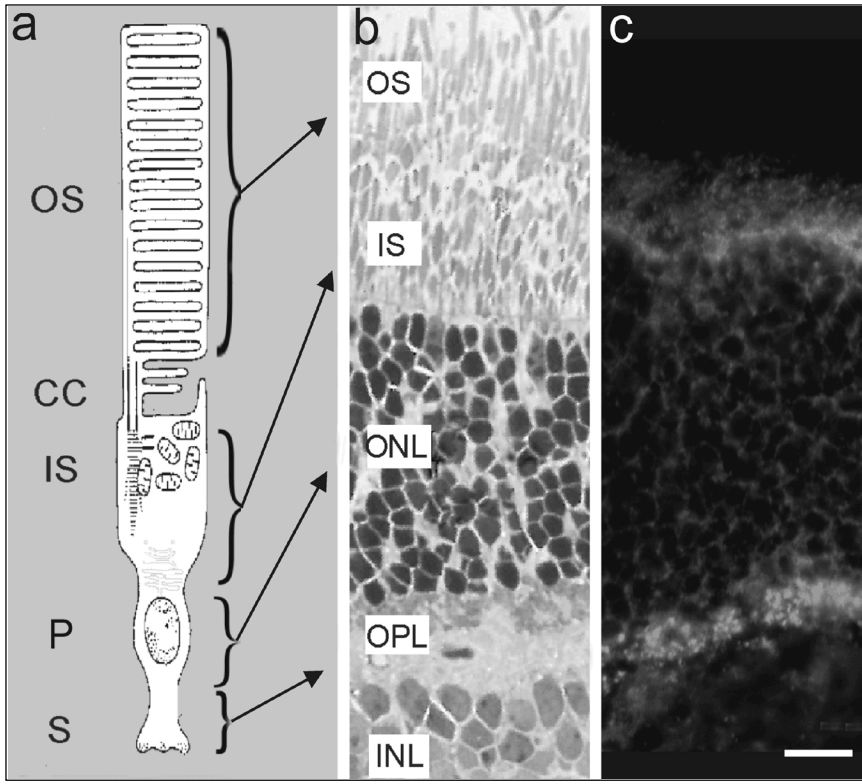

Figure 4. Localization of cadherin-23 in the retina by immunofluorescence. (a) Diagram of a vertebrate rod photoreceptor cell. Outer segment (OS), connecting cilium (CC), inner segment (IS), perikaryon with nucleus (P), synaptic terminal (S). (b) Light microscopic image of a semi-thin section through the mouse retina. In the outer plexiform layer (OPL), rod photoreceptor cells are connecting to the 2nd order retinal neurons (bipolar cells, horizontal cells) via ribbon synapses. Outer nuclear layer (ONL), Inner plexiform layer (IPL). (c) Indirect immunofluorescence in a longitudinal cryosection through the mouse retina. Note prominent anti-cadherin-23 immunofluorescence (Alexa488, Molecular Probes) present in the ONL at the synapses of photoreceptor cells. Additional staining is found in the IS of photoreceptor cells. Bar: 8 μm

with atypically mild retinal phenotype.[18] In contrast, all disease relevant changes identified in patients with non-syndromic deafness (*DFNB12*) were missense mutations.[19] These observations suggest that the inner ear function is already sensitive to 'minor' changes caused by missense mutations, whereas heavily reduced or absent protein function result, in addition, in retinal impairment. A summary of *CDH23* mutations in patients with DFNB12 and USH1D is shown in Figure 5. Of note, 83.5% of the 24 disease alleles identified to date predict protein truncation, with a high proportion (58.5%) of mutations that lead to aberrant splicing. In a panel of 52 USH1 patients, *CDH23* mutations accounted for about 10% of cases.[27]

The orthologous murine gene, *cdh23,* is mutated in *waltzer* (*v*), a mouse model for USH1D.[18] All *waltzer* mutants analysed to date have *cdh23* mutations, for which a loss of function of the gene product is predicted.[28-30] As the *v* mouse presents no obvious retinal pathology, only the inner ear morphology was investigated in greater detail. Stereocilia of hair cells in *v* mutants are heavily disorganized suggesting that cdh23 may have a "cross-linking" function for stereocilia in hair bundle formation.[20] Clearly, it is unknown at present whether the reported defects in

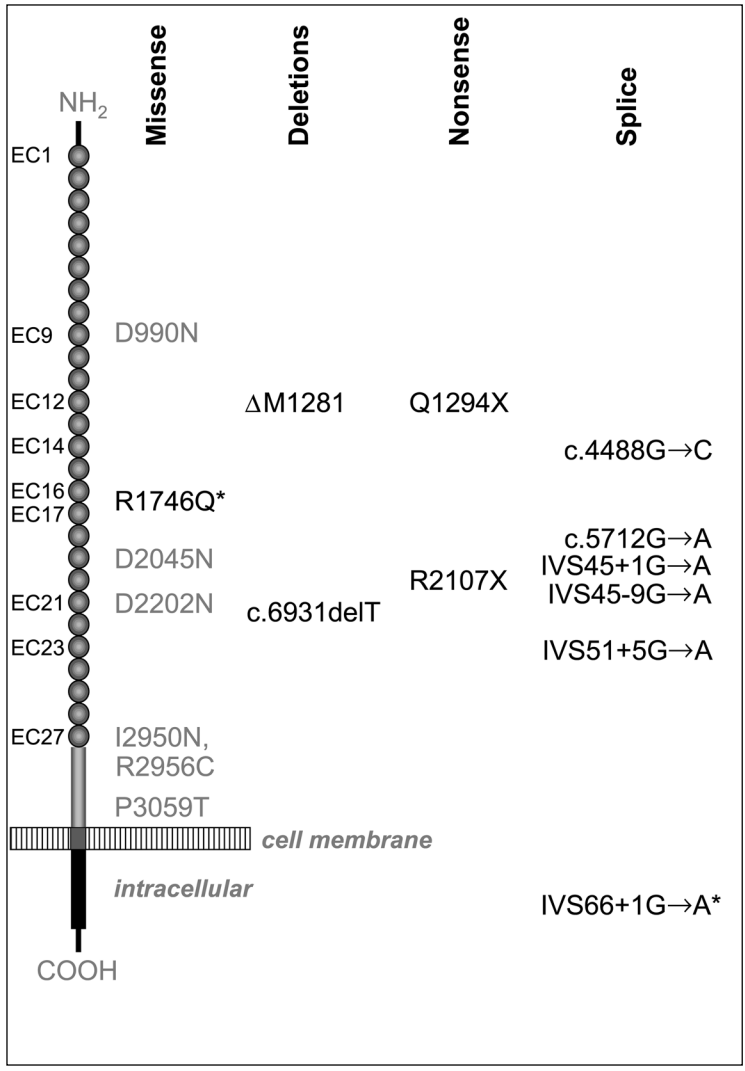

Figure 5. Cartoon of the molecular structure of human *CDH23*, with the position of disease-relevant mutations (on the right) and ECs (on the left). *CDH23* mutations causing non-syndromic deafness (DFNB12) are in grey letters whereas mutations that lead to additional retinal affection (USH1D) are given in black letters. A star indicates mutations associated with mild retinal phenotype. Note that autosomal-recessive DFNB12 is caused by missense mutations only, whereas in USH1D, all mutations except R1746Q (that was found in homozygous state only in cases of atypical USH1), predict a truncated gene product.

development and morphogenesis result from impaired Ca^{2+}-dependent adhesion or from loss of other yet unknown functions of cdh23, or both.

The retina is a complex system of neuronal cells connected with each other and arranged in a well-defined order. Defects in cell adhesion may impair both development and maintenance of this architecture. In contrast to the inner ear symptoms, that seem to be due to developmental defects impairing the structure of stereociliae, retinitis pigmentosa in USH1D patients (the

morphological basis of which has not been yet investigated) is unlikely to result from a comparable mechanism. It is possible that *CDH23* mutations affect rather the maintenance of the retinal structure than its development. Yet, the precise roles of CDH23 in the human retina still await to be determined.Based on our preliminary results on histological localization of cadherin-23 in the mature mammalian retina, it is tempting to speculate that retinitis pigmentosa in patients with Usher syndrome type 1D may in part result from a functional impairment of the ribbon synapse of photoreceptor cells, and hence a defect in signal transmission. It will be interesting to see whether the cadherin-23 deficient *waltzer* mice exhibit defects in synaptic function and whether cadherin-23 and the products of the other Usher 1 genes are assembled at the photoreceptor synapse to a protein complex as recently suggested by Petit (2001) for the stereovilli of the mechanosensitive hair cells.

Protocadherin-15 *Mutations in Usher Syndrome Type 1F*

USH1F, the disease locus being near the *USH1D* locus on chromosome 10, represents a condition clinically indistinguishable from the other USH1 syndromes. In a positional cloning approach, mutations in a novel cadherin gene, *PCDH15*, were identified both in USH1F families[31,32] and the corresponding mouse model *ames waltzer* (*av*).[33] For all mutations identified in human studies, a truncated PCDH15 protein, and therefore a loss of function is predicted. *PCDH15* is expressed in retina, brain, cochlea, lung, and kidney. As in the mouse models for USH1B and USH1D (*shaker1* and *waltzer*, respectively), *av* mice have inner ear defects but no retinal degeneration. Nonetheless, electroretinography in different *shaker1* mice showed, compared to unaffected mice, a weaker response to light, with reduced a- and b-waves documenting an abnormal physiological situation.[34] Clearly, the same could also be true for the cadherin mutants.

Cadherin-3 *Mutations in Hypotrichosis with Juvenile Macular Dystrophy*

Sprecher et al[35] reported the identification of a protein-truncating 1 bp deletion in the *CDH3* gene encoding P-cadherin in patients with hair loss and progressive macular degeneration leading to early onset visual handicap. CDH3 belongs to classical cadherins and the gene has previously been shown to be expressed in the retinal pigment epithelium. The 981delG mutation predicts a protein lacking three of the five extracellular domains, the transmembrane domain, and the cytoplasmic tail. The mutation may therefore define a functional null allele. Of note, loss of CDH3 in mice does not cause hair or retinal abnormalities, which might be due to expression of other cadherins in the affected tissues and/or functional redundancy.

Conclusion

To date, a number of mutant proteins of the cadherin superfamily has been discovered to be causal for various retinal diseases. Three cadherin molecules, though expressed in several tissues throughout the body, are implicated in distinct retinal (and, as in USH, cochlear) phenotypes: CDH23, PCDH15, and CDH3. Most likely, the list is far from being complete. The process, by which mutations lead to photoreceptor degeneration is still not fully understood. Data from the inner ear phenotype of USH1 mouse models suggest that loss of cell adhesion is a crucial event. More experimental work is needed to investigate the functions of cadherins involved in retinopathies, also with respect to other putative functions than adhesion, such as signal transduction.

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