HYPOTRICHOSIS WITH JUVINILE MACULAR DYSTROPHY

Hypotrichosis with juvenile macular dystrophy (HJMD; MIM 601553), is an autosomal recessive human inherited disorder, caused by mutations in the P-cadherin encoding gene C*DH3*, positioned on human chromosome 16q22.1. Mutations in the gene *CDH3* also result in ectodermal dysplasia, ectrodactyly and macular dystrophy syndrome (EEM; MIM 225280). Affectees of both these disorders present thin hair and retinal macular dystrophy though individuals affected with EEM syndrome also show the distinctive characteristics of split hand/foot malformations (SHFM).

The P-cadherin encoding gene *CDH3* contains 16 exons encompassing 55 kb on human chromosome 16q22.1. It is a member of classical cadherins that produces the trans-membrane core of adheren junctions (Kremmidiotis *et al.,* 1998; Sprecher *et al.,* 2001; Indelman *et al.,* 2002, 2007). The CDH3 protein expresses in wide variety of tissues including hair follicle, pigmented epithelia of retina, and limb bud (Muller-Rover *et al.,* 1999). Numerous studies have shown the association of P-cadherin and E-cadherin, encoding by genes *CDH3* and *CDH1*, respectively, in biological mechanisms such as cell recognition, hair follicle morphogenesis, cell signaling and limb development (Goodwin and Yap, 2004; Shimomura *et al.,* 2008b). A transcription factor p63 is involved in regulation of the P-cadherin protein (Shimomura *et al.,* 2008b), which is a key regulator of broader cell adhesion gene expression programs (Carroll *et al.,* 2006). Interestingly mutations in the gene *p63* are also responsible for non-syndromic and syndromic forms of split hand/foot malformations (Ianakiev *et al.,* 2000). Similarities in phenotypic features of patients with mutations in the two genes *CDH3* and *p63* envisage that interaction between these two are playing vital role in the development of hair follicle and limb bud (Shimomura *et al.,* 2008b).

Clinical Features of Family M with HJMD

Family with an autosomal recessive hypotrichosis and juvenile macular dystrophy (HJMD), designated M for the study, has been ascertained from a remote area of Sindh province of Pakistan (Figure 5.1). As shown in the pedigree, the family consists of four generations containing six affected (II-2, II-3, III-1, III-2, IV-2, IV-3) and two unaffected (IV-1, IV-4) individuals.

Hair Abnormalities

Affected individuals of the family were born with thin scalp hair that had limited growth throughout life. In two affected females (IV-2, IV-3)**,** scalp hairs were completely absent at the time of birth (Figure 5.2 a-b). Thin and sparse eyebrows and eyelashes, and total absence of pelvic and pubic hair were observed in the affected members of the family. Skin, teeth, bones, fingernails and toenails showed normal growth in the affected members.

Fundus Examination

Affected members reported weakness and progressive loss of the eyesight at the age of 16-20 years. Marked deterioration of the epithelial macular pigment was observed during fundus examination of the two patients (II-3, III-1) of the family.

Electrophysiological and Electroretinography Tests

Electrophysiological and electroretinography tests exhibited abruption and reduction in wave amplitude in affected individuals (II-3, III-1) and revealed severe retinal dysfunction. The patients also reported time-dependent and gradual deficit in visual acuity.

Genetic Mapping and Sequencing *CDH3*

Considering the clinical features of affected individuals of the family M, it was tested for linkage to P-cadherin gene *CDH3* mapped on chromosome 16q22.1. Several polymorphic microsatellite markers (D16S3393, D16S3050, D16S3095, D16S2624, D16S3033), flanking the gene *CDH3*, were typed in six affected (II-2, II-3, III-1, III-2, IV-2, IV-3) and two unaffected individuals (IV-1, IV-4) of the family. The microsatellite markers used in genotyping were fully informative and all six affected individuals were homozygous with these markers, suggesting linkage of the family to *CDH3*. Disease-associated haplotypes generated using SIMWALK2 (Sobel and Lange, 1996) are presented in Figure 5.3. Haplotypes showed that linkage interval, flanked by markers D16S2620 (82.53 cM) and D16S3051 (92.75 cM), was 10.22 cM (13.15 Mb) according to Rutgers combined linkage-physical map of the human genome build 36.2 (Matise *et al*., 2007).

After establishing linkage of the family M to the gene *CDH3* mapped on chromosome 16q22.1, the gene was sequenced in all six affected and two unaffected individuals of the family. All sixteen exons and splice junctions sites of the gene *CDH3* were PCR amplified from genomic DNA of affected and unaffected individuals of the family using primers designed from intronic sequences of the gene (Table 2.11, Materials and Methods). The amplified PCR products were purified using the Rapid PCR Purification System (Marligen

Biosciences, Ijamsville, MD, USA) and sequenced in an ABI Prism 310 automated DNA sequencer, using Big Dye Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA, USA) following dideoxy chain termination method. Sequence variants were identified via BIOEDIT sequence alignment editor (BioEdit version 6.0.7).

Sequence analysis of the gene *CDH3* in the affected individuals of the family revealed transition of a nucleotide G to A at splice acceptor site of intron 10 (c.IVS10-1 G→A). The mutation was present in the heterozygous state in obligate carriers of the family (Figure 5.4) (Kamran-Ul-Hassan Naqvi *et al.,* 2010). In order to substantiate that the mutation does not represent a neutral polymorphism in this population, a panel of 100 controls unrelated unaffected individuals with same ethnicity, was analyzed for the mutation and it was not identified outside the family. The mutation identified in the family represents a novel splice site mutation.

Discussion

Cadherins are cell-cell adhesion transmembrane glycoproteins that form Ca^{+2} dependent intercellular junctions and play an essential role in the development and maintenance of adult tissues and organs (Conacci-Sorrell *et al.,* 2002). The cadherin family is divided into different subfamilies, including the classical E-cadherin encoded by gene *CDH1*, P-cadherin encoded by gene *CDH3*, N-cadherin encoded by gene *CDH2* and N-cadherin 2 encoded by gene *CDH12*, each signifying a specific tissue distribution (Takeichi, 1988; Offermanns and Rosenthal, 2008).

Anomalies in two classes of cadherins, E and P, have been shown to be linked to several isolated or syndromic forms of hair abnormalities. P-cadherin is a major constituent of the classical cadherins and cell-cell adhesion. During embryogenesis it expresses in the hair follicle placode and the matrix region in postnatal hair follicles, consistent with the hypotrichosis observed in the setting of HJMD and EEM (Jamora *et al.,* 2003; Bergman *et al.,* 2004; Shimomura *et al.,* 2010e). Hypotrichosis and macular dystrophy are common in both HJMD and EEM. Nevertheless, a striking difference is the presence of ectrodactyly/split hand and foot malformation in EEM. Furthermore, the identical mutations in different patients give rise to both phenotypes (Kjaer *et al.,* 2005), raising the possibility of modifier genes influencing disease expressivity and may be linked close to the gene *CDH3*. E-cadherin and P-cadherin exist close together on human chromosome 16 and are expected to inherit together except if rare recombination events occur. Being a classical cadherin, E-cadherin may be one of the modifier genes by means of compensation. Recently, it has been

shown that during limb development, both E- and P-cadherin are co-expressed in the apical epidermal ridge (Shimomura *et al.,* 2008b).

Shimomura *et al.* (2010e) suggested a modifier role for E-cadherin which might explain why some patients with P-cadherin mutations have normal limb development. In contrast, an overlapping of E- and P-cadherin expression is not seen in the retina or hair follicle. In the hair follicle, initially E-cadherin expression is elevated, but as the placode starts to form, the E-cadherin levels fall markedly and P-cadherin increases parallely (Jamora *et al.,* 2003). The downregulation of E-cadherin and the overexpression of P-cadherin persist throughout hair follicle development (Muller-Rover *et al.,* 1999). E-cadherin is expressed in the corneal epithelium whereas P-cadherin is involved in development of the eye, in particular the retinal pigmented epithelium (Xu *et al.,* 2002a). Hence, E-cadherin cannot substitute for P-cadherin in hair follicle and retina, which may explain why all patients with P-cadherin will have combined hypotrichosis and macular dystrophy while only some have ectrodactyly/split hand and foot malformation (Shimomura *et al.,* 2010e).

To date, 15 mutations including 4 deletions, 3 missense, 1 insertion, 3 nonsense and 4 splice sites have been reported in the gene *CDH3* in patients with HJMD (Figure 5.5; Table 5.1). In the present study, the mutation (c.IVS10-1 G →A) reported in the family M, is the third splice site mutation identified in the gene *CDH3* and is predicted to cause out of frame skipping of exon 11 and generates a pre-termination codon in exon-12. As a result it is possible that this mutation generates a truncated protein lacking most of the essential domains of CDH3 protein (Kamran-Ul-Hassan Naqvi *et al.,* 2010). Alternatively, *CDH3* mRNA containing a pre-termination codon might be abolished due to nonsense-mediated decay (Maquat, 1999). Previously two missense mutations (p.Arg503His, p.Glu504Lys) were found in exon 11 of *CDH3* in Arab Israeli and English families, providing indirect evidence that the deficiency of P-cadherin may lead to abnormal hair formation by interfering with β-catenin function. These mutations affect a highly conserved fourth extracellular domain of CDH3 protein (Indelman *et al.,* 2003; Indelman *et al.,* 2007).

The P-cadherin CDH3 protein consists of 5 extracellular domains (EC1-EC5), a transmembrane region and a short intracellular tail (Figure 5.5) (Yagi and Takeichi, 2000). The EC4 domain encoded by DNA sequence of exon-11 contains a DRE sequence motif, playing vital role in binding of P-cadherin to Ca^{+2} and establishing the folding conformation of the P-cadherin. The folding conformation in turn persuades cell-cell adhesion activity (Boggon *et al.,* 2002). The splice site mutation (c.IVS10-1 G →A), reported here in family

M, probably disturbs the mechanisms of cell-cell adhesion which are important for normal development of hair follicle, retinal pigment and other ectodermal appendages.

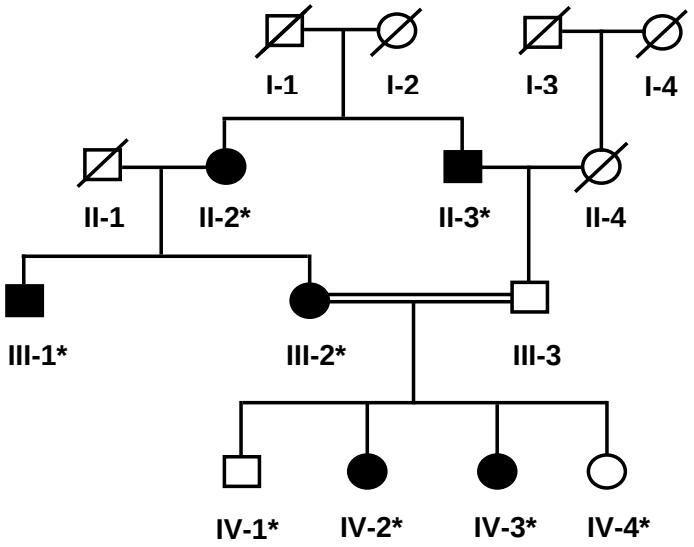

Figure 5.1: Pedigree drawing of the family M with autosomal recessive hypotrichosis with juvenile macular dystrophy (HJMD). Affected individuals are represented by filled symbols while unaffected family members by clear symbols. Symbols with crossed lines indicate the deceased individuals. Double lines between the individuals represent consanguineous union. The individual numbers labeled with asterisks indicate the samples which were available for the present study.

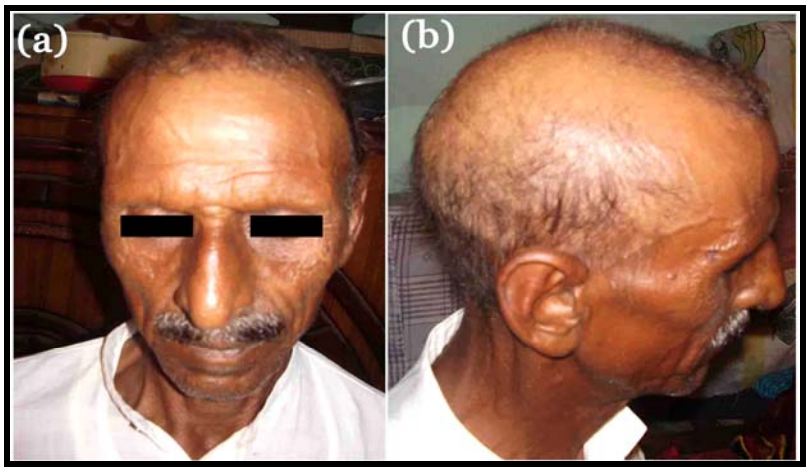

Figure 5.2 a-b: Clinical presentation of HJMD in an affected individual (III-1) of the family M. Note sparse hair on scalp, and sparse eyebrows and eyelashes.

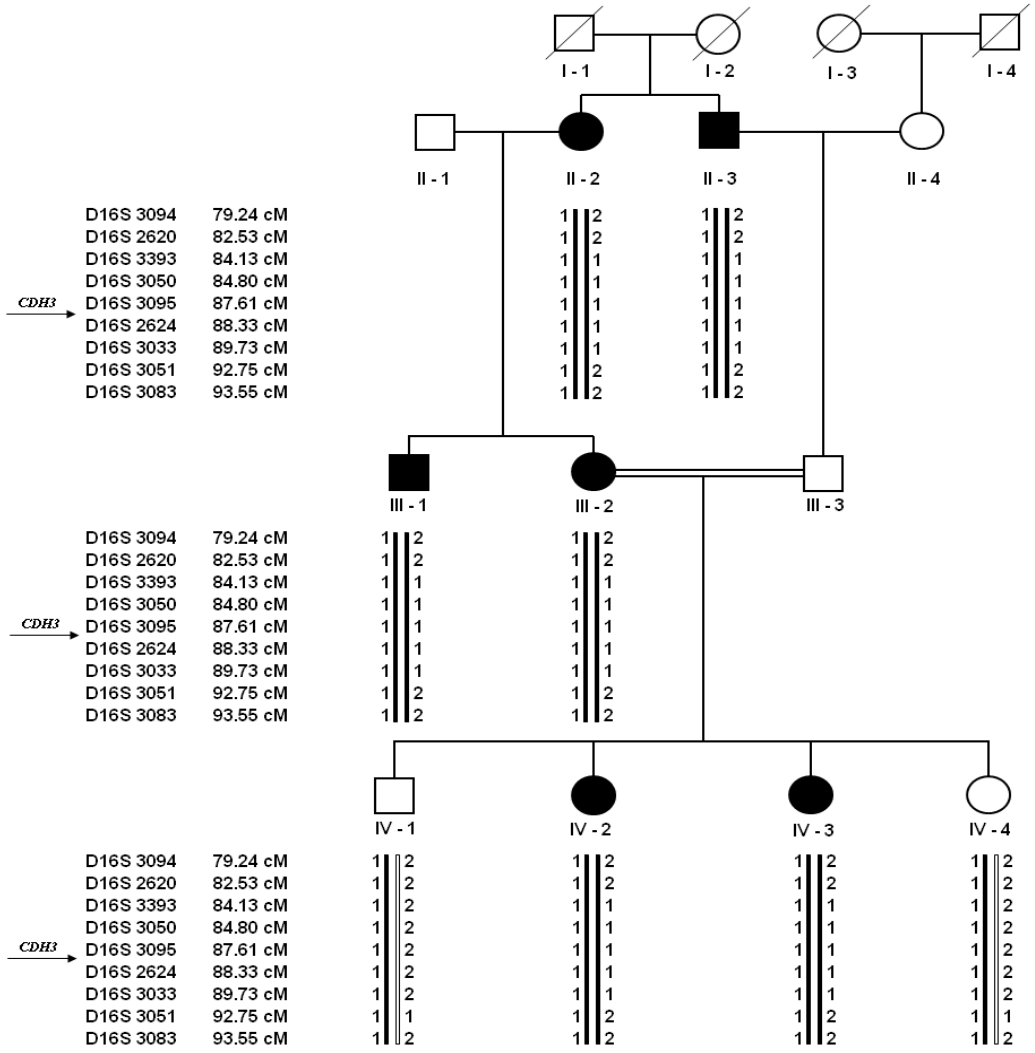

Figure 5.3: Haplotype analysis of the family M segregating autosomal recessive hypotrichosis with juvenile macular dystrophy (HJMD). The disease-associated haplotypes are shown below each genotyped individual. The region of homozygosity in affected individuals is flanked by microsatellite markers D16S2620 (centromere) and D16S3051 (telomere) on chromosome 16q22.1. Arrows indicate the position of the gene *CDH3* lies between markers D16S3095 and D16S2624. Genetic distance of the microsatellite markers depicted in centiMorgan is according to Rutgers combined linkage physical map of the human genome built 36.2 (Matise *et al*., 2007).

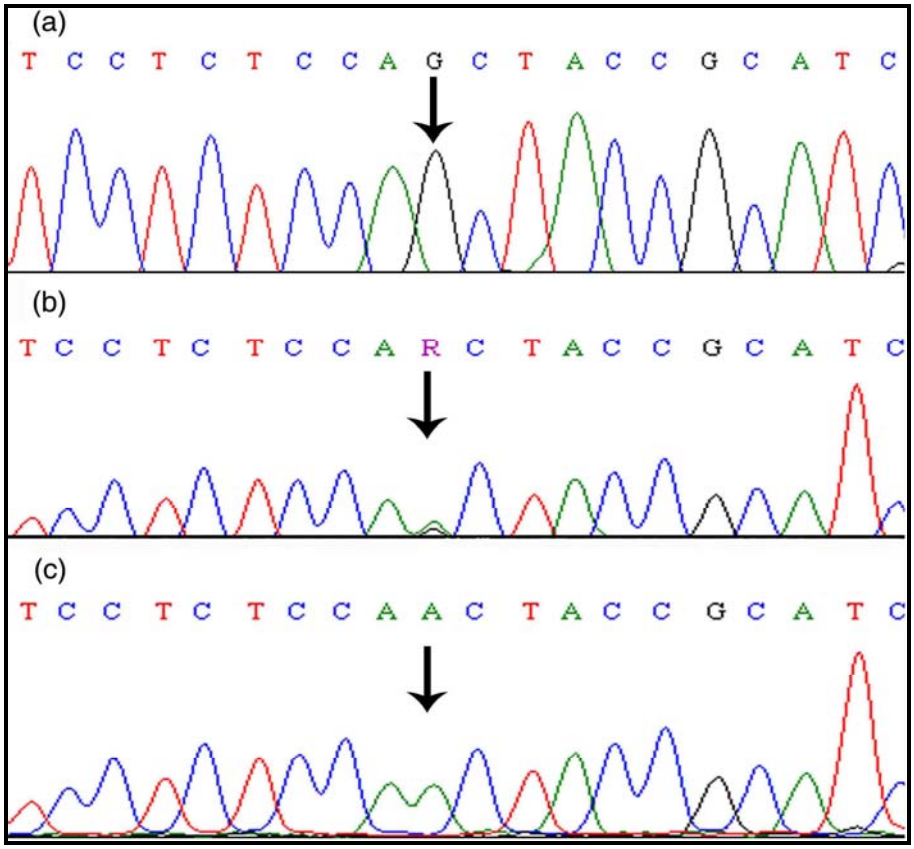

Figure 5.4 a-c: Automated DNA sequence analysis of exon 10 of the gene *CDH3* mutation in family M: **(a)** a control individual, **(b)** a heterozygous carrier individual (IV-1), **(c)** a homozygous affected individual (II-3). A single base pair G in control individual **(a)** is substituted into A in the affected individual **(c)**. Arrows indicate position of substitution of G nucleotide.

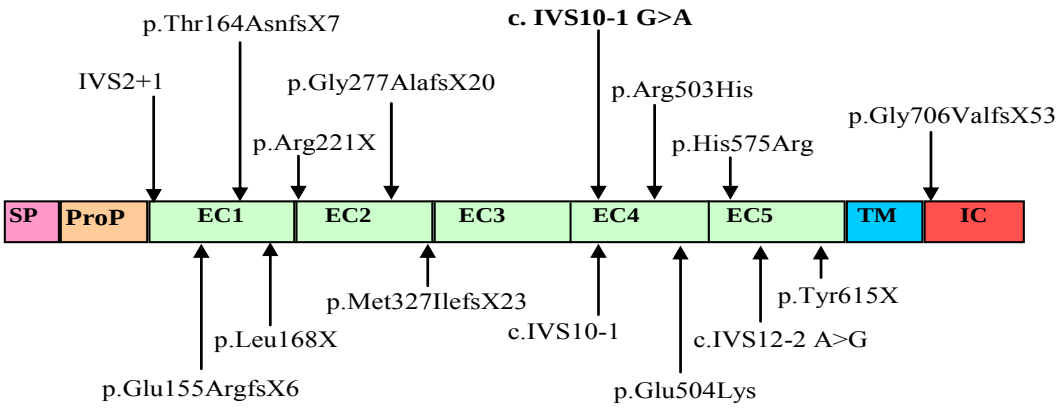

Figure 5.5: Schematic view of P-cadherin (CDH3) protein. Arrows indicating positions of the mutations reported in the gene *CDH3* so far. SP (rose color) denotes signal peptide whereas ProP (tan color) represents propeptide domain. Five extracellular domains (EC1-EC5) are boxed in lime. Transmembrane and intracellular domains are boxed in sky blue and red, respectively.

Table 5.1: Mutations in the gene *CDH3* demonstrating HJMD phenotype

Phenotypes	Mutation	cDNA Change	Protein Change	Change Effect	Reference
HJMD	Deletion	c.981delG	p.Met327IlefsX23	FS and PTC	Sprecher *et al*., 2001
HJMD	Missense	c.1508G>A	p.Arg503His	Amino acid substitution	Indelman *et al*., 2002
HJMD	Deletion	c.462delT	p.Glu155ArgfsX6	FS and PTC	Indelman *et al*., 2003
HJMD	Nonsense	c.503T>A	p.Leu168X	PTC	Indelman *et al*., 2003
HJMD	Deletion	c.829delG	p.Gly277AlafsX20	FS and PTC	Indelman *et al*., 2003
HJMD	Deletion	c.2112delG	p.Gly706ValfsX53	FS and PTC	Indelman *et al*., 2003
HJMD	Nonsense	c.1845T>A	p.Tyr615X	PTC	Indelman *et al*., 2005
HJMD	Nonsense	c.661C>T	p.Arg221X	PTC	Indelman *et al*., 2007
HJMD	Missense	c.1510G>A	p.Glu504Lys	Amino acid substitution	Indelman *et al*., 2007
HJMD	Missense	c.1724A>G	p.His575Arg	Amino acid substitution	Indelman *et al*., 2007
HJMD	Splice site	IVS2+1G>A	Exon 2 skipping	Protein truncation	Indelman *et al*., 2007
HJMD	Insertion	c.490insA	p.Thr164AsnfsX7	FS and PTC	Shimomura *et al*., 2008
HJMD	Splice site	IVS10-1G>T	Exon 11 skipping	Protein truncation	Jelani *et al*., 2009,
HJMD	**Splice site**	**IVS10-1G>A**	**Exon 11 skipping**	**Protein truncation**	**This study**
HJMD	Splice site	IVS12-2A>G	Exon 13 skipping	Protein truncation	Shimomura *et al*., 2010

HJMD: Hypotrichosis with juvenile macular dystrophy; *CDH3*: P-cadherin 3 gene; FS: frame shift; PTC: premature termination codon