Phenotypic Diversity and Mutation Spectrum in Hypotrichosis with Juvenile Macular Dystrophy

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Hypotrichosis with juvenile macular dystrophy is a rare autosomal recessive disorder characterized by abnormal growth of scalp hair during infancy, and by the later occurrence of macular degeneration leading to blindness during the first to third decade of life. Hypotrichosis with juvenile macular dystrophy was recently shown to result from mutations in *CDH3* encoding P-cadherin. In this study, we assessed 27 individuals, including nine patients, belonging to five families in an attempt to characterize further the *CDH3* mutation spectrum and delineate possible phenotype–genotype correlations. Deleterious biallelic mutations, predicted to lead to the translation of a dysfunctional protein, were found in all affected individuals. Four of these mutations are novel. Affected individuals of two large separate apparently unrelated families of Arab Israeli origin were found to carry the same homozygous mis-sense mutation (R503H) in exon 11 of the *CDH3* gene. This mutation, which alters a Ca^{2+}-binding site in the fourth extracellular domain of P-cadherin, was previously described in a third unrelated Arab Israeli family. Using haplotype analysis for a series of polymorphic markers encompassing the *CDH3* gene, we obtained evidence suggesting a founder effect for R503H in the Arab Israeli population. We also compared the dermatologic and ophthalmologic features of 22 hypotrichosis with juvenile macular dystrophy patients with known recessive mutations in *CDH3*. Whereas hair paucity and macular degeneration were found in all patients, we noticed significant interfamilial and intrafamilial differences in hair morphology, associated skin findings as well as severity and age of onset of visual disability. Altogether, our results obtained in a series of families of various ethnic origins firmly establish mutations in *CDH3* as the proximal cause of hypotrichosis with juvenile macular dystrophy and demonstrate genetic homogeneity as well as phenotypic heterogeneity in this disorder.

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Hypotrichosis with juvenile macular dystrophy (HJMD; MIM601553) is a rare autosomal recessive disorder characterized by early hair loss followed by progressive degeneration of the central retina, culminating in blindness. The syndrome was recently shown to result from mutations in *CDH3* on 16q21 (Sprecher *et al*, 2001; Indelman *et al*, 2002). *CDH3* encodes P-cadherin (Shimoyama *et al*, 1989), a classical cadherin, which is strongly expressed in both the hair follicle and the retinal pigment epithelium (Muller-Rover *et al*, 1999; Xu *et al*, 2002). Indirect evidence suggests that P-cadherin deficiency may lead to abnormal hair development by interfering with β-catenin function (Indelman *et al*, 2002). Also, retinal pigment epithelial cell development has been shown to depend on a number of components of the *wnt*/β-catenin signaling system (Olschwang *et al*, 1993; Liou *et al*, 2002). Alternatively, as classical cadherins are responsible for ensuring cell–cell adhesion at the adherens junction, aberrant hair and retinal differentiation may be the consequence of weak or absent cell–cell contact within the upper hair matrix zone or between retinal pigment epithelial cells (Indelman *et al*, 2002).

In this study, we assessed a series of affected individuals with the clinical features of HJMD (**Table I**). We obtained informed and written consent from each participant according to a protocol reviewed and approved by the institutional board (Helsinki Committee) and by the Committee for Genetic Studies of Israeli Ministry of Health. Family 1 is French origin and has been described elsewhere (Raison-Peyron *et al*, 2000). Family 2 originates from the Turkish mainland and family 3 lives in Turkish Cyprus. Families 4 and 5 are of Arab Israeli extraction, unrelated and deny any relationship with another Arab Israeli HJMD family previously described (Indelman *et al*, 2002). All affected individuals were found to display abnormally sparse hair at birth that never grew thereafter. Whereas scalp hair of most individuals was short and firmly rooted in the scalp, the hair of individuals 5–7 and

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Abbreviations: HJMD, hypotrichosis with juvenile macular distrophy.

Table I. Mutation spectrum and phenotype diversity in HJMD

Family	Origin	Scalp hypotrichosis	Hair changes	Decreased visual acuity[a]	Macular pigment degeneration	Abnormal electrophysiologic studies[d]	Associated clinical findings	Mutation	Exon	Reference
1	French	+	None	+ (35)	+	+	Atopic dermatitis	L168X/2112delG	5/14	This study
2[f]	Turkish	+	NA[e]	+ (2)	+	+	–	462delT/462delT	5	This study
3[f]	Turkish	+	NA	+ (5)	+	+	Keratosis pilaris	829delG/829delG	7	This study
4[f]	Arab Israeli	+	Pili torti	+ (1)	+	+	–	R503H/ R503H	11	This study
5[f]	Arab Israeli	+	Telogen effluvium[b]	–[c]	+	+	Centro- facial lentigines[b,g]	R503H/ R503H	11	This study
6[f]	Arab Israeli	+	Pili torti	+ (4)	+	+	–	R503H/ R503H	11	Indelman *et al* (2002)
7[f]	Druze	+	Pseudomonilethrix	+ (15)	+	+	–	981delG/981delG	8	Sprecher *et al* (2001)

[a]Earliest age of onset in each family is given in parentheses.
[b]Observed in two of three affected children.
[c]Eldest child was 14 y of age at the time of examination.
[d]Included flash-electroretinogram, flash-visual evoked potentials and electrooculogram.
[e]NA, not available (Permission to obtain hair samples or skin biopsy was denied).
[f]Consanguineous family.
[g]Partial syndactyly in individual 7–12.

5–12 (**Fig 1*c***) was curly and could easily be plucked out. The latter two patients displayed centrofacial lentiginosis (**Fig 1*a***). All individuals except from the three affected children of family 5 displayed decreased visual acuity. Microscopic examination of hair revealed a variety of anomalies (**Table I**). In all affected families, eye fundus examination disclosed degenerative changes of the macula and electrophysiologic studies confirmed abnormal function of the posterior pole.

We screened the entire coding sequence of *CDH3* as previously described (Indelman *et al*, 2002) and identified biallelic mutations in all affected individuals of families 1 to 5 (**Table I**). Interestingly, the proband of family 1 was found to carry two heterozygous pathogenic sequence alterations, and thus represents the first case of compound heterozygosity for mutations in *CDH3* (**Fig 1*b***). Patients in families 1 to 3 were found to carry mutations predicted to introduce premature termination codons and to result either in mRNA decay or in the translation of a truncated cadherin molecule (**Fig 1*b***). All affected members of families 4 and 5 were homozygous for a G→A transition at *CDH3* cDNA position 1508. This mutation results in the substitution of a histidine residue for an arginine residue at position 503 of the amino acid sequence (R503H). We confirmed segregation of the mutation in both families using a polymerase chain reaction–restriction fragment length polymorphism assay previously described (Indelman *et al*, 2002) (**Fig 1*c***). R503H has previously been reported in another apparently unrelated kindred of Arab Israeli origin (Indelman *et al*, 2002) (family 6, **Table I**), suggesting the possibility that the mutation originated from a common founder. To test this hypothesis, we genotyped the members of all three families for seven polymorphic markers spanning the *CDH3* locus over 19 cM as previously described (Sprecher *et al*, 2001). Haplotype analysis revealed that all affected individuals shared a homozygous 0.5 Mb chromosomal segment between marker D16S3025 and marker D16S3067 (**Fig 1*d***). These data support a founder effect for R503H in the Arab Israeli population.

We compared dermatologic features (e.g., hair morphology and associated skin findings) as well as ophthalmologic features (e.g., age of onset of visual disability and visual acuity) with the results of the mutation analysis (**Table I**). No systematic correlation could be established between phenotypic characteristics and the type (mis-sense *vs* non-sense/frameshift) or location of the *CDH3* mutations among 22 HJMD patients. Phenotypic heterogeneity and lack of phenotype–genotype correlation were not only observed across various ethnic backgrounds or between families carrying the same mutation on an identical genetic background but also within single families. Patients carrying the same mutation (R503H) on the same haplotype background (families 4–6) exhibited divergent cutaneous features: two first-degree affected cousins demonstrated centrofacial lentiginosis and telogen effluvium (individuals 5–7 and 5–12), whereas other patients, such as the brother of patient 5–7, did not. Patients carrying other mutations in *CDH3* demonstrated hair morphologic abnormalities or had normal hair microscopic examination (**Table I**). Wide variations in age of onset and degree of visual disability were observed among patients, even when carrying identical mutations. Finally, no correlation could be established between the type of hair anomaly and the degree of macular damage.

Since the initial identification of a deleterious *CDH3* mutation in several consanguineous families, a total of six mutations in 10 separate families of various ethnic origins have been reported to cause HJMD (**Table I**). Two of these mutations, 981delG and R503H, were observed repeatedly, reflecting founder effects in the Druze and Arab Israeli populations, respectively. All six mutations are expected to impair markedly the function of P-cadherin. Five of these mutations (981delG, L168X, 2112delG, 462delT, 829delG) are predicted to result in the synthesis of a truncated form of P-cadherin or in the absence of P-cadherin. The sixth mutation, R503H, alters a highly conserved motif located within the fourth extracellular domain of P-cadherin (Indelman *et al*, 2002). This motif plays an important part in Ca^{2+} binding and determination of the final P-cadherin conformation (Troyanovsky, 1999). Mutations affecting the DRE motif have also been recently described in *CDH23*, a gene mutated in Usher syndrome type 1D and coding for a protocadherin involved in stereocilia organization in hair cells (Astuto *et al*, 2002).

In addition, based on the examination of the largest series of HJMD-affected individuals described to date, we observed substantial interfamilial phenotypic variation (**Table I**). Family 5 demonstrated an intriguing case of intrafamilial phenotypic heterogeneity. Two of three affected individuals in this family displayed centrofacial lentiginosis. We could not find in the literature any report of an association between congenital hypotrichosis, telogen effluvium and centrofacial lentiginosis as observed in family 5. Although it is unlikely that centrofacial lentiginosis represents a direct manifestation of a *CDH3* mutation in family 5, it is striking to note that the two children with centrofacial lentiginosis also presented with curly hair and massive telogen effluvium, which were not observed in other HJMD patients, including another affected sibling. Taken together, these observations may suggest the effect of a recessively inherited and nonlinked modifier trait, modulating the phenotypic expression of HJMD in family 5. The association of hair abnormalities with aberrant pigmentation is particularly interesting in view of mounting evidence suggesting interdependence between hair

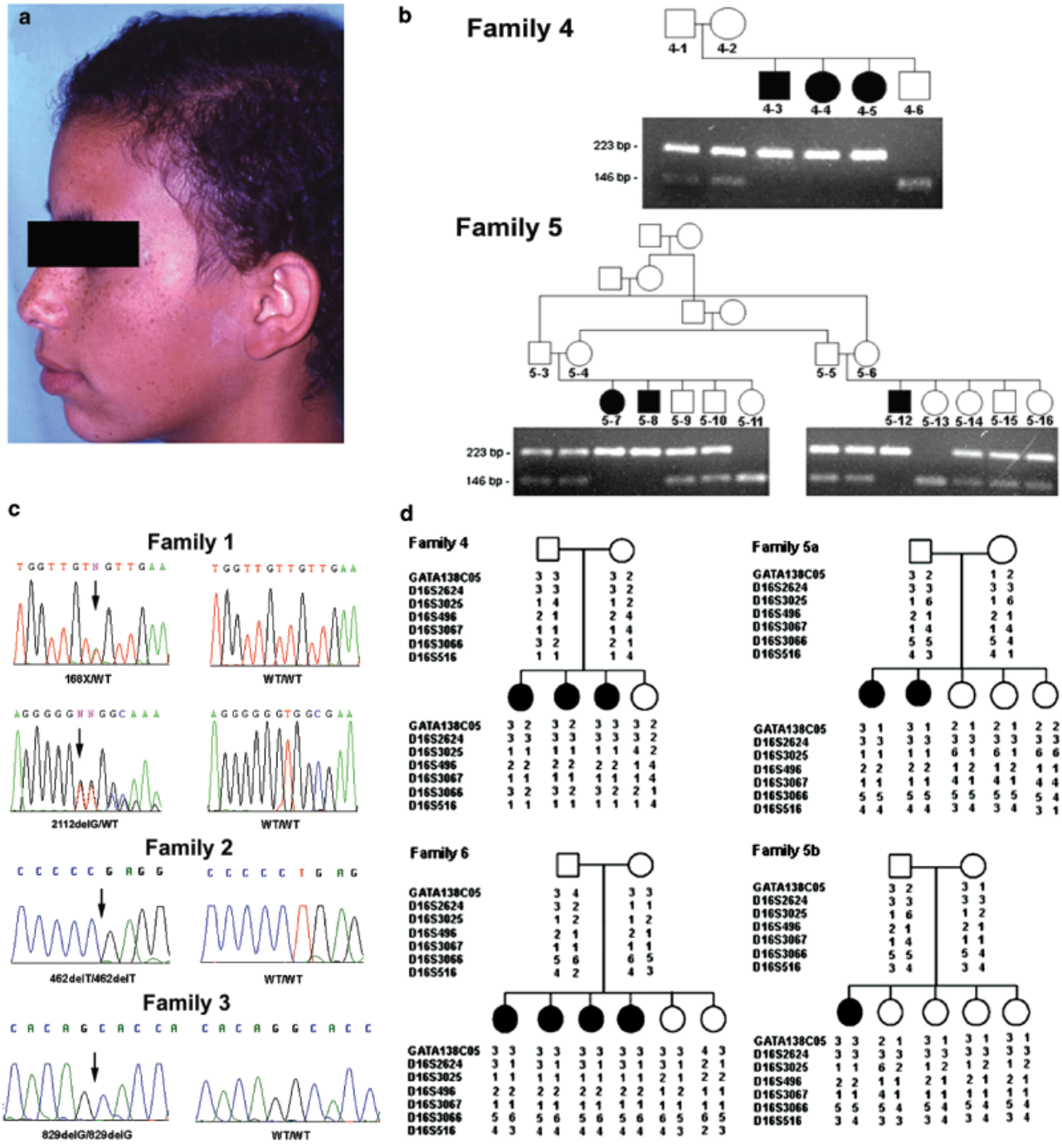

Figure 1. A feature analysis of HJMD family. (*a*) Curly and short hair as well as numerous lentigines on both sides of the nose and on the cheeks are present in affected individual 5–7 of family 5. (*b*) Mutation analysis. Analysis of the *CDH3* coding sequence revealed an heterozygous non-sense mutation in exon 5 (L168X) and an heterozygous deletion in exon 14 (2112delG) in family 1 patient; an homozygous 462delT mutation in exon 5 in family 2 patient; an homozygous 829delG mutation in exon 7 in family 3 patient (*left panels*). The right panels give the corresponding wild-type sequences. (*c*) Segregation of R503H in family 4 and family 5 demonstrated by polymerase chain reaction–restriction fragment length polymorphism. Exon 11 was polymerase chain reaction-amplified from each member of the two families. A 223 bp long polymerase chain reaction fragment was digested with *Bsi*EI. R503H abolishes a recognition site for *Bsi*EI: thus affected individuals display a 223 bp fragment, healthy homozygous individuals display a 146 bp fragment and carriers of the mutation display both fragments. The lower band of 77 bp is not shown. (*d*) Haplotype analysis in three HJMD families. Family 6 has previously been characterized (Indelman *et al*, 2002). All family members were genotyped using seven microsatellite markers encompassing the *CDH3* gene.

development, hair cycling, and melanocyte lineage ontogenesis (Ideta *et al*, 2002; Nishimura *et al*, 2002).

In summary, these results obtained in a series of families of various ethnic origins firmly establish mutations in *CDH3* as the proximal cause of HJMD and demonstrate genetic homogeneity as well as phenotypic heterogeneity in this disorder. The identification of asymptomatic macular damage in family 5 patients suggests that ophthalmologic examination should become an integral part of the diagnostic work-up of patients presenting with recessive hypotrichosis.

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CD34 in Human Hair Follicle

To the Editor:

CD34, also known as human hematopoietic progenitor cell antigen, is a heavily glycosylated transmembrane protein expressed on developmentally early lymphohematopoietic stem and progenitor cells, and on a significant number of acute leukemias (Krause *et al*, 1996). In human skin CD34 has been described in vascular endothelial cells, in a subset of dendritic/spindle-shaped cells (Nickoloff, 1991), and in some cutaneous tumors (Cohen *et al*, 1997).

In a previously published study we could demonstrate the expression of CD34 in cells from the outer root sheath of hair follicles (Poblet *et al*, 1994). In that study, sections from normal human skin of formalin-fixed, paraffin-embedded biopsies were immunostained with a monoclonal antiCD34 antibody (clone QBEND/10). A clear membranous staining of outer root sheath cells could be observed. This was the first report to demonstrate the CD34 expression in a certain type of keratinocytes. We showed that the CD34 staining was very specific for epithelial cells of the external root sheath, and that the staining was limited to cells located below the attachment of the arrector pili muscle and above the matrix cells. Because CD34 is a progenitor cell antigen we suggested a possible relation of these cells with stem cells of hair follicles.

We read with interest the excellent article by Trempus *et al* that has recently appeared in this Journal (Trempus *et al*, 2003). These authors demonstrate CD34 immunohistochemical staining of murine hair follicle bulge keratinocytes. In addition they confirmed the presence of CD34 mRNA in that cells. In general, the immunohistochemical staining pattern of murine keratinocytes concords with the staining pattern that we described in human hair follicle keratinocytes, although the bulge region in human hair follicles can not be as readily identified as it is in mice hair follicles.

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Response

To the Editor:

We are writing in response to the Letter to the Editor submitted by Poblet and Jiminez entitled "CD34 in the Human Hair Follicle." In this letter, they point out that we neglected to refer to their 1994 paper "QBEND/10 (Anti-CD34 Antibody) in External Root Sheath Cells and Follicular Tumors" (Poblet