Mutation in *WNT10A* Is Associated with an Autosomal Recessive Ectodermal Dysplasia: The Odonto-onycho-dermal Dysplasia

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Odonto-onycho-dermal dysplasia is a rare autosomal recessive syndrome in which the presenting phenotype is dry hair, severe hypodontia, smooth tongue with marked reduction of fungiform and filiform papillae, onychodysplasia, keratoderma and hyperhidrosis of palms and soles, and hyperkeratosis of the skin. We studied three consanguineous Lebanese Muslim Shiite families that included six individuals affected with odonto-onycho-dermal dysplasia. Using a homozygosity-mapping strategy, we assigned the disease locus to an ~9-cM region at chromosome 2q35-q36.2, located between markers *rs16853834* and *D2S353,* with a maximum multipoint LOD score of 5.7. Screening of candidate genes in this region led us to identify the same c.697G→T (p.Glu233X) homozygous nonsense mutation in exon 3 of the *WNT10A* gene in all patients. At the protein level, the mutation is predicted to result in a premature truncated protein of 232 aa instead of 417 aa. This is the first report to our knowledge of a human phenotype resulting from a mutation in *WNT10A,* and it is the first demonstration of an ectodermal dysplasia caused by an altered WNT signaling pathway, expanding the list of WNT-related diseases.

The ectodermal dysplasias (EDs) are a large and complex nosological group of diseases that have in common anomalies of the hair, teeth, nails, and sweat glands, with or without anomalies in other organs and systems. The incidence is estimated to be ~7 in 10,000 births. So far, >200 different pathological clinical conditions have been recognized and defined as EDs, all with Mendelian modes of inheritance.[1] Classification of EDs was first initiated by Freire-Maia and Pinheiro and was based on clinical descriptions and the ectodermal structures involved.[2–4] This classification was followed by another one that introduced biological mechanisms involved in the pathogenesis of EDs.[5] Two different groups were thus delineated, each likely to result from mutations in genes with similar functions and possibly involved in the same regulation of development mechanisms and/or in pathogenesis. Characterization of the genes' functions and of the different signaling pathways involved in ~30 different types of EDs led to expansion of the classification of EDs into four major functional subgroups that depend on molecular and biochemical criteria: cell-cell communication and signaling, adhesion, transcription regulation, and development.[6]

In the past few years, many publications indicated the importance of the Wnt pathway in hair-follicle morphogenesis, skin and teeth embryogenesis,[7–10] and tooth renewal.[11] The *Wnt* gene family comprises genes showing strong sequence similarities to mouse *Wnt-1* and *Drosophila wingless* genes (Wnt Homepage and Laboratory of Randall Moon). The *Wnt* genes encode small secreted proteins, ~350–400 residues in length, that are members of a large family of secreted cysteine-rich proteins that regulate cell-to-cell interactions during embryogenesis. They are found in almost all animal genomes (especially vertebrates). At least four Wnt signaling pathways have been elucidated, of which the canonical Wnt/β-catenin signaling pathway,[12] which activates target genes in the nucleus, is the best understood. In humans, 19 WNT proteins have been identified that share 27%–83% amino acid sequence identity and a conserved pattern of 23 or 24 cysteine residues (reviewed by Cadigan and Nusse[13] and Wodarz and Nusse[14]).

Since mutations in Wnt pathway genes in mice and zebrafish generally lead to developmental defects,[15] severe abnormalities were expected to be due to mutations in *WNT* pathway genes in humans. However, despite considerable progress in understanding the mechanisms involved in WNT signaling pathways and their roles in development, studies implicating WNT signaling in human diseases and clinical conditions other than cancer have only recently emerged.[16–22]

In this report, we show that *WNT10A* is the causative gene for a rare ED, odonto-onycho-dermal dysplasia (MIM 257980),[23–27] expanding the list of WNT-related diseases.

Clinical findings.—Three consanguineous Lebanese Shiite Muslim families were included in this study (fig. 1): two of them (families 1 and 3) have already been described elsewhere,[26,27] and one (family 2) was a branch of the first

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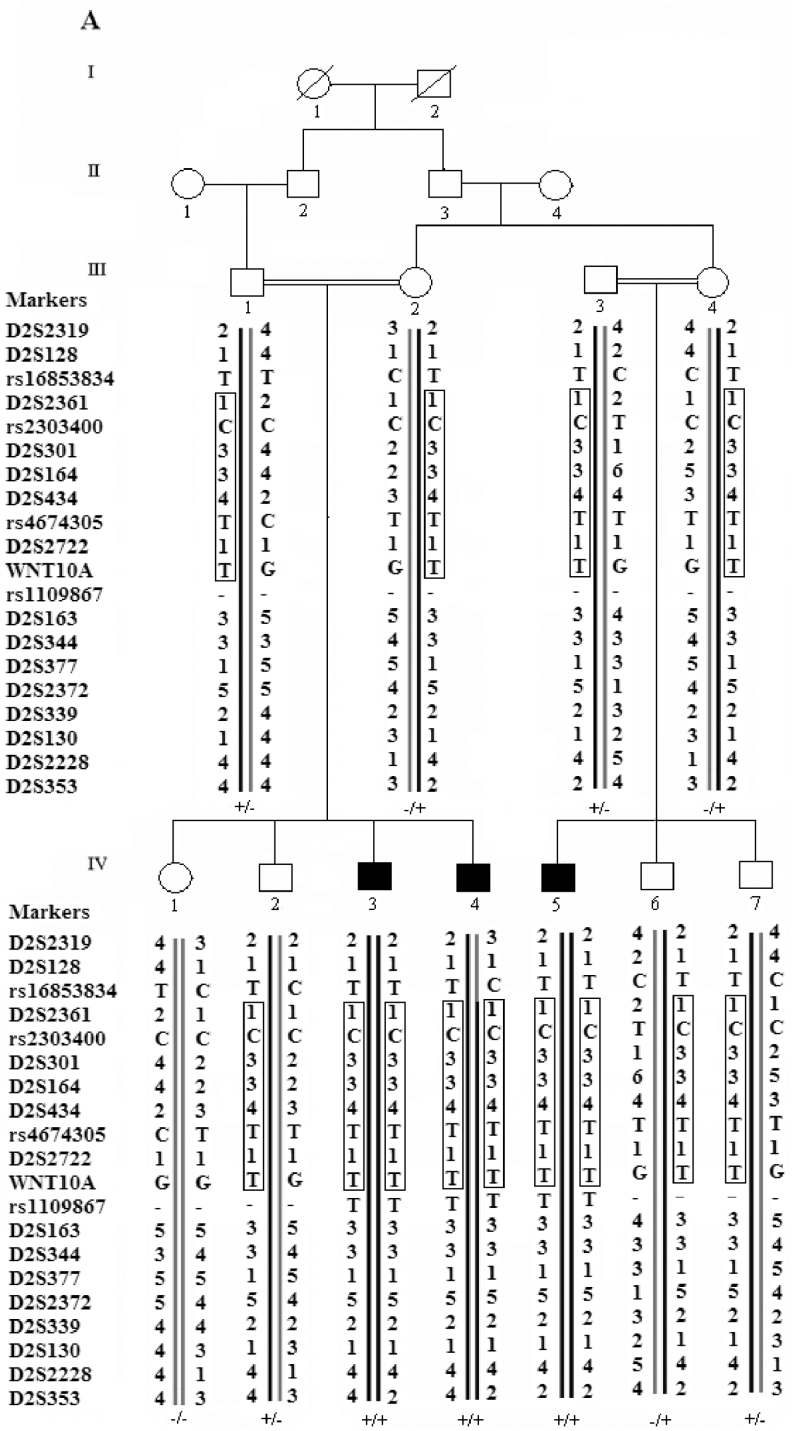
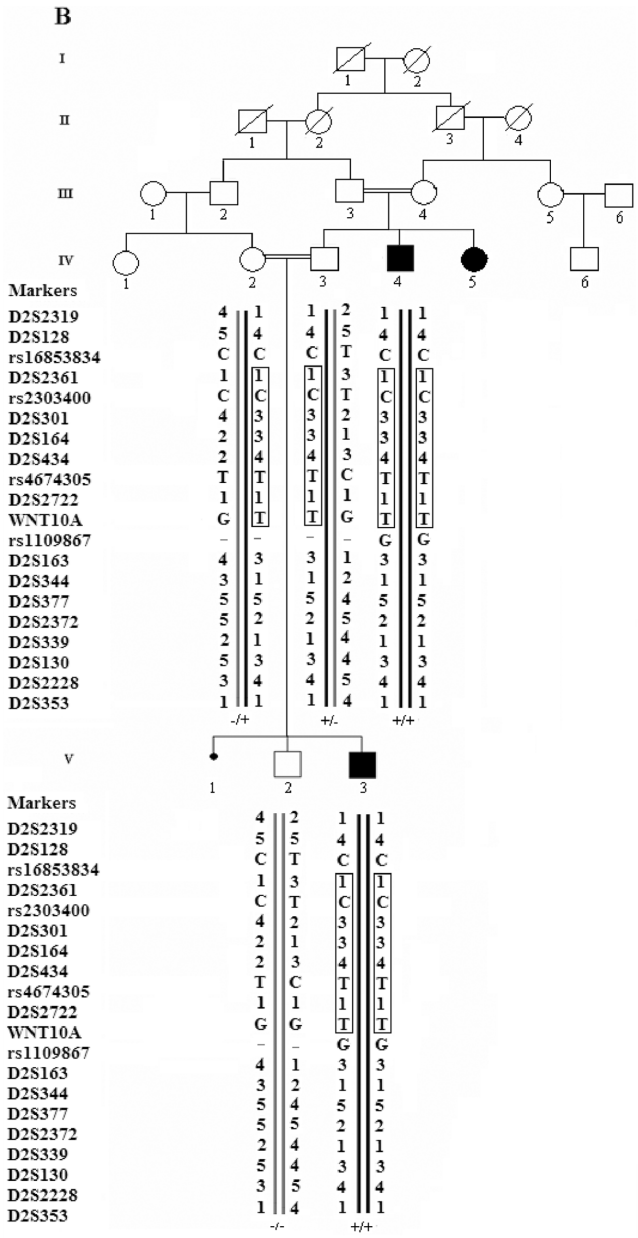
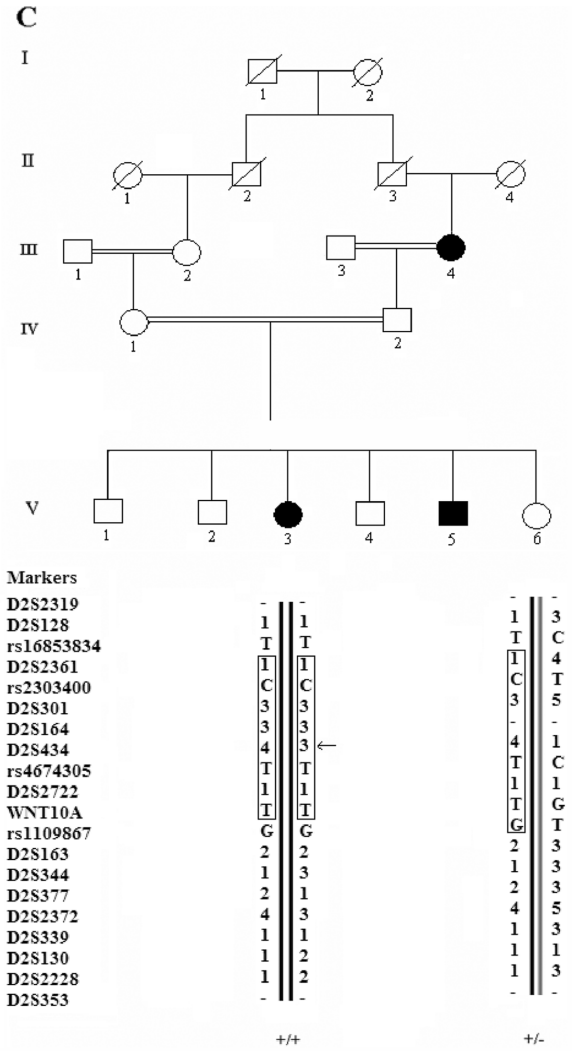


Figure 1. Pedigrees, haplotypes, and *WNT10A* mutation status of family 1 (*A*), family 2 (*B*), and family 3 (*C*). Individuals' numbers are listed below the pedigree symbols. Genotyped markers from the chromosome 2q34-q36.2 region are shown to the left, and individuals' allele numbers for each marker are given next to the bar. Black bars represent the haplotype segregating with the odonto-onycho-dermal gene. *WNT10A* is located between markers *D2S2722* and *rs1109867*. The mutation genotypes of *WNT10A* are shown below haplotypes. + indicates mutation Glu233X; − indicates wild-type allele. A border indicates the common haplotype 1-C-3-3-4-T-1-T between the three families, and the arrow shows the neomutation in patient V:3 of family 3.



of the two families described by Fadhil et al.[23] Clinical findings are summarized in table 1. In brief, patients in family 1 presented with dry hair, pilar keratosis, severe hypodontia, smooth tongue, onychodysplasia, palmar erythema, and keratoderma and hyperhidrosis of palms and soles. Histology of the skin showed orthokeratotic, hyperkeratosis, hypergranulosis, and mild acanthosis in the epidermis. Scanning electron microscopy of the hair showed longitudinal depressions in some hairs.[27]

In family 2, two patients were examined, a boy and his paternal uncle. The boy (V:3 in fig. 1*B* and case 1 in family 2 in table 1) was the second son of consanguineous Lebanese parents. Pregnancy was uneventful, and delivery occurred at term. At birth, scalp hair was almost absent, and fingernails were barely visible. Nails progressively started to grow, but very slowly. According to the boy's father, the deciduous teeth erupted normally but were widely spaced and abnormal in shape. Hyperkeratosis of palms and soles appeared at around age 3 years. He never complained of unusual discomfort from heat. Growth and mental development were normal. At age 5 years, his physical measurements were within the normal limits. He had dry skin all over his body, with a diffuse follicular hyperkeratosis, dry and thin hair, sparse eyebrows, widely spaced and malformed deciduous teeth, a smooth tongue with very few fungiform and filiform papillae, dystrophic



fingernails and toenails, and recurrent folliculitis. Palmoplantar erythema with mild hyperkeratosis and hyperhidrosis of palms and soles was found. His uncle (IV: 4 in fig. 1*B* and case 2 in family 2 in table 1) was examined at age 46 years. He also presented with dry hair, severe hypodontia, smooth tongue, onychodysplasia, palmar erythema, and mild keratoderma and hyperhidrosis of palms and soles.

In the third family,[26] one patient was reinvestigated (V: 3 in fig. 1*C*). She presented with dry skin, thin and dry hair that never grew normally, almost absent permanent teeth, a smooth tongue with very few fungiform and filiform papillae, thin and concave fingernails, severely dystrophic toenails, and palmar erythema. She complains from time to time of an excessive palmoplantar sudation. Electronic microscopic examination of the hair, which had already been performed and was reported elsewhere,[26] showed thin hair with longitudinal depressions. At the bottom of these depressions, cuticular cells presenting a longitudinally striated aspect were found, as if the keratin fibrils were observable under the cell membrane.

Molecular genetics.—After informed consent was obtained from the adult subjects and the parents of the children, peripheral blood samples from 18 subjects were collected, and genomic DNA was isolated from lymphocytes by use of standard methods. Because all the patients included in the study were born to consanguineous marriages, we assumed identity by descent and performed homozygosity mapping[28] on pooled DNA by a first genomewide screen of family 1.

A set of 382 highly polymorphic fluorescently labeled markers on chromosomes 1–22 (ABI PRISM Linkage Map Set, version 2.0 [PE Biosystems]) were chosen from the Généthon linkage map,[29] as described elsewhere.[30] The first set of markers was not sufficiently informative, since several loci were homozygous in all affected and unaffected individuals. Genotyping of additional markers around these loci allowed the identification of a locus ho-

Table 1. Review of Clinical Features of Our Patients

Clinical Feature	Family 1			Family 2		Family 3
	Case 1	Case 2	Case 3	Case 1	Case 2	Case 1
Sex	M	M	M	M	M	F
Absence of hair at birth	±	−	+	+	?	−
Congenital absence of nails	±	−	+	+	?	−
Dystrophic fingernails	+	+	+	+	−	+
Dystrophic toenails	+	±	+	+	−	+
Erythematous atrophic patches on the face	−	−	−	−	+	−
Palmoplantar keratoderma	+	+	+	±	+	−
Hyperhidrosis	+	+	+	+	+	+
Oligodontia	+	+	+	+	+	+
Reduced papillae of the tongue and/or smooth tongue	+	+	+	+	+	+
Partial alopecia (male pattern)	−	−	−	−	+	−
Sparse eyelashes and/or eyebrows	−	−	−	+	−	−
Sparse axillary and/or pubic hair	−	−	−	?	−	?
Abnormal hair pattern	±	±	±	−	+	+
Diffuse follicular hyperkeratosis	+	−	+	+	−	±
Dry skin	+	+	+	+	+	+

NOTE.— ± means "more or less."

mozygous by descent at marker *D2S301* (University of California–Santa Cruz [UCSC] accession number AFM214YE1) at chromosome 2q35. To refine the size of the putative shared homozygous region, 14 additional microsatellite markers (*D2S2319, D2S128, D2S2361, D2S164, D2S434, D2S2722, D2S163, D2S344, D2S377, D2S2372, D2S339, D2S130, D2S2228,* and *D2S353*) chosen from databases (UCSC and Ensembl) and four highly heterozygous SNPs (*rs16853834, rs2303400, rs4674305,* and *rs1109867*) chosen from dbSNP were genotyped. Haplotype analyses and recombination events allowed the restriction of the homozygous candidate region to a 9-Mb interval between markers *rs16853834* and *D2S353* (UCSC Genome Browser accession number AFM296VH9) at chromosome 2q35-2q36.2 (fig. 1*A*). In family 2, we found linkage to the same region (fig. 1*B*). Thus, we performed parametric multipoint linkage analysis with Easylinkage version 5.0, using a recessive model and complete penetrance. Cumulative linkage analyses of the two families showed a significant positive LOD score of 5.74 on chromosome 2q35-36.1 at $\theta = 0.001$.

There were ~70 genes in the homozygous candidate region, several of which were known to be expressed in skin and teguments and could therefore be implicated in the disease (Genatlas and UCSC Genome Browser). The coding region, as well as exon-intron boundaries and 5′ and 3′ UTRs of six of those genes (*EPHA4* [GenBank accession number NM_004438], *INHA* [GenBank accession number NM_002191], *DNAJB2* [GenBank accession number NM_006736], *VIL1* [GenBank accession number NM_007127], *WNT6* [GenBank accession number NM_006522], and *WNT10A* [GenBank accession number NM_025216.2]), were amplified by PCR from genomic DNA from one patient per family (families 1 and 2) with the use of intronic primers (sequence of primers and amplification conditions available on request). Primers were designed using Primer3 and OLIGOS v.9.3 and were checked for specificity by use of BLAST, and DNA sequences were obtained from UCSC or GenBank databases. Mutation screening was performed by fluorescent bidirectional sequencing of genomic amplified PCR products on an ABI3130 automated sequencer (Applied Biosystems).

We identified a homozygous c697G→T transversion in exon 3 of *WNT10A* in each affected patient tested from the two families (fig. 2). The mutation cosegregates with the phenotype in families 1 and 2, comprising a total of five patients, and was not found in 200 control chro-

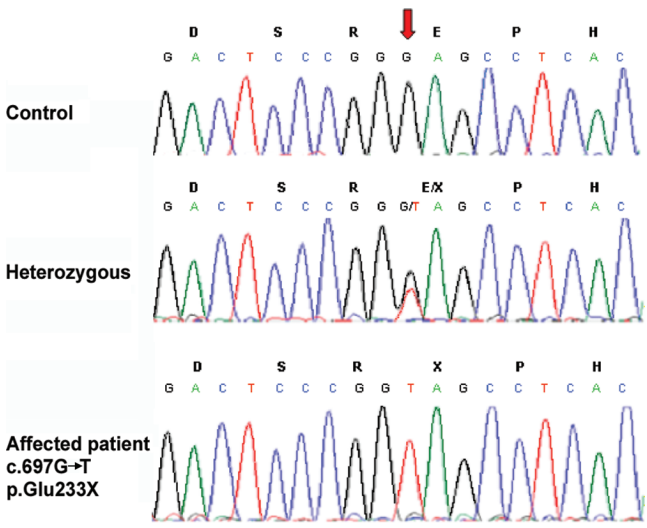


Figure 2. *WNT10A* mutation associated with odonto-onycho-dermal dysplasia. Electropherograms of *WNT10A* exon 3 in a control individual (top), one parent (middle), and an affected patient (bottom). DNA sequence analysis revealed a homozygous G→T substitution at nucleotide 697 (from the translation start site) in the affected patient, causing the Glu233X mutation. The parent is heterozygous with respect to the Glu233X mutation.

mosomes tested by endonuclease-restriction digestion with the use of *Ava*I (BioLabs), thus making a polymorphism unlikely. At the protein level, the c.697G→T mutation causes the substitution of a negatively charged glutamic acid residue for a stop codon (p.Glu233X), which results in a premature, truncated protein of 232 aa, instead of 417 aa, that lacks the cysteine-rich prosite pattern PS50311, which is highly conserved in the WNT superfamily. Screening of the *WNT10A* gene for mutations in one patient of the third family revealed the same mutation. The three families investigated here denied any relationship with one another. Nevertheless, all affected individuals of the three families showed the identical haplotype of 1-C-3-3-4-T-1 for markers *D2S2361, rs2303400, D2S301, D2S164, D2S434, rs4674305,* and *D2S2722,* respectively, with a probable neomutation at *D2S434* for patient V:3 of family 3, who is heterozygous 3/4 instead of 4/4 for this marker. These data, in addition to the fact that the three families belong to the same religious community, suggest that there is an ancestral haplotype from marker *D2S2361* to *WNT10A* included, and that the mutation found in *WNT10A* in the patients of the three families comes from a common ancestor.

Studies on mice showed that *Wnt10a* is up-regulated in skin of developing embryos,[7] in placodes at the onset of follicle morphogenesis, and in postnatal hair follicles beginning a new cycle of hair growth.[8] Also, *Wnt10a* is expressed in the epithelium when tooth morphogenesis begins.[31] The involvement of Wnt10a through the Wnt/β-catenin signaling pathway has previously been suggested in the apical ectodermal ridge in chick limb formation[32] and in tooth development in mice.[15] In the absence of WNT signaling, β-catenin is phosphorylated and degraded. Since β-catenin is required for localized expression of regulatory genes, initiation of hair- and tooth-follicle placode formation,[10] and tooth renewal as well as tooth aspect,[11] its absence results in the disruption of different ectodermal organs. In humans, the present observation shows that *WNT10A* plays a crucial role in the development and/or regulation of the ectodermal derivatives. The mutation found in *WNT10A* might affect β-catenin in the same way as the absence of WNT signaling does, given the fact that the resulting truncated protein might be nonfunctional.

Interestingly, Millar et al.[33] demonstrated that *Wnt3* is expressed in developing and mature hair follicles and that its overexpression in transgenic mouse skin causes a short-hair phenotype, by altered differentiation of hair-shaft precursor cells, and cyclical balding, by hair-shaft structural defects, and is associated with an abnormal profile of protein expression in the hair shaft. Our patients had hair that grew very slowly and never became very long (except for individual IV:4 in fig. 1*A*). It is likely that the abnormal structure of the hair observed by light and scanning electron microscopy[27,26] and the short-hair phenotype are caused by defects in the proliferation or differentiation of hair-shaft precursor cells. But, in contrast to *Wnt3,* where overexpression led to this phenotype, truncated or loss of functional WNT10A altered normal hair growth, suggesting that the two *WNT* genes might use different pathways.

Recently, Liu et al.[34] demonstrated that Wnt-β-catenin signaling is activated in developing fungiform placodes and taste-bud cells and plays a central role in the formation of tongue papillae. These authors found that Wnt10b, another subtype of the Wnt10 protein that shares 59% sequence identity with Wnt10a,[7,35] was specifically elevated in developing fungiform placodes. They also found that any alteration of β-catenin leads to an absence or marked reduction in fungiform papilla morphogenesis. In the families we studied, fungiform and filiform papillae were very few. Thus, although there are at present no in vivo data confirming a role of *WNT10A* in the tongue, it is tempting to speculate about the role of the latter in tongue papillae, which could explain the presence of a smooth tongue in our patients.

On the other hand, other studies found that mutations in the genes encoding R-spondin 4 and R-spondin 1, characterized as a small family of growth factors, are responsible for anonychia and palmoplantar hyperkeratosis, respectively.[36,37] The furin-like repeats encoded by exons 2 and 3 of the R-spondin family of protein genes are believed to be required for activation and stabilization of β-catenin. Thus, it is not surprising that a mutation in the *WNT10A* gene leads to abnormal nails and palmoplantar keratoderma by perturbing keratinocyte proliferation and differentiation, as seen in our patients.

Overall, the phenotypic expression of the *WNT10A* mutation described in the three families was mild when compared with the malformations caused by mutations in other members of WNT family.[16,18] Moreover, clinical inter- and intrafamilial heterogeneity was noted. Indeed, the patients from family 3 were not given diagnoses of having odonto-onycho-dermal dysplasia because they showed an absence of keratoderma and had normal facial skin without erythema, atrophy, or telangiectasia.[26] Nevertheless, the overlapping features with families 1 and 2 led us to sequence *WNT10A*. The same mutation was found. A clinical follow-up with one patient of family 3 showed that, in addition to the details described elsewhere,[26] she had a smooth tongue and short hair that she rarely cut. Still, neither follicular hyperkeratosis nor keratoderma was noted. The mild phenotype and the clinical heterogeneity between patients suggest that there are alternate pathways and/or environmental factors that may contribute to compensate for the abnormal WNT10A protein.

In conclusion, we report, for the first time to our knowledge, a gene of the WNT family that when mutated causes ED, confirming the importance of the Wnt pathway for the normal formation and regeneration of hair, teeth, skin, and nails in humans. Although the name of the present pathology is odonto-onycho-dermal dysplasia, the abnormal hair indicates that it is more of a tricho-odonto-onycho-dermal dysplasia. The latter could fit in the cell-

cell communication and signaling group of Lamartine's classification,[6] showing that an altered epithelial-mesenchymal interaction seems to be one of the most important mechanisms in the pathogenesis of EDs. *WNT10A,* but also other *WNT* genes such as *WNT6* that are coexpressed with *WNT10A*[35] or *WNT10B* and that are known to be expressed in teeth,[31,38] could be considered good candidates for conditions similar to the one we report here, such as that described by Fried,[39] tricho-odonto-onycho-dermal dysplasia with cysts of the eyelids syndrome (called "Schopf-Schulz-Passarge syndrome" [MIM 224750])[40]; the entity reported by Salamon et al.,[41] characterized by hypodontia, sparse and dry hair, dystrophic nails, dry skin, and hyperkeratotic papules on the hands; and the one described by Pinheiro et al.,[42] characterized by hypodontia, onychodysplasia, mild palmoplantar keratosis, and sparse hair, eyelashes, and eyebrows. Reports of further families with ED with *WNT* mutations will allow better characterization of the molecular pathways controlling differentiation and proliferation of ectodermal organs.

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Web Resources

Accession numbers and URLs for data presented herein are as follows:

BLAST, http://www.ncbi.nlm.nih.gov/BLAST/
dbSNP, http://www.ncbi.nlm.nih.gov/SNP
Ensembl, http://www.ensembl.org/index.html
Genatlas, http://www.genatlas.org
GenBank, http://www.ncbi.nih.gov/Genbank/ (for *EPHA4* [accession number NM_004438], *INHA* [accession number NM_002191], *DNAJB2* [accession number NM_006736], *VIL1* [accession number NM_007127], *WNT6* [accession number NM_006522], and *WNT10A* [accession number NM_025216.2])
Laboratory of Randall Moon, http://faculty.washington.edu/rtmoon
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for odonto-onycho-dermal dysplasia and Schopf-Schulz-Passarge syndrome)
Primer3, http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi
UCSC Genome Browser, http://www.genome.ucsc.edu (for *D2S301* [accession number AFM214YE1] and *D2S353* [accession number AFM296VH9])
Wnt Homepage, http://www-leland.stanford.edu/~rnusse/wntwindow.html

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