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We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.
Clouston Syndrome Can Mimic Pachyonychia Congenita

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We studied three families suffering from nail abnormalities who had previously been diagnosed as pachyonychia congenita. No keratin gene mutations were detected. Sequencing of connexin 30 (GJB6 gene) in these patients identified heterozygous missense mutations G11R and A88V that are known to be associated with Clouston syndrome. This unexpected finding expands the Clouston syndrome phenotype and suggests that some patients diagnosed with pachyonychia may in fact be suffering from Clouston syndrome. Key words: connexin 30/genodermatosis/GJB6/nail dystrophy/pachyonychia congenita. J Invest Dermatol 121:1035–1038, 2003

Original Article

Clouston syndrome (hidrotic ectodermal dysplasia, HED, OMIM 129500) is an autosomal dominant ectodermal dysplasia characterized by hypotrichosis, severe nail dystrophy, and often palmoplantar hyperkeratosis as well as hyperpigmentation of the skin over large joints (Rajagopalan and Tay, 1977; Fraser and Der Kaloustian, 2001). Teeth and eccrine gland function are normal. Sensorineural deafness can be part of the phenotype; mental retardation has also been described (Copeland et al, 1977). The degree of the alopecia and the hyperkeratosis are apparently variable, with lack of the latter having been reported in one family. Mutations in the gap junction protein connexin 30 (GJB6 gene) have been found in several families of different ethnic origins. Almost all families described so far have inherited one of two recurrent missense mutations, G11R or A88V (Lamartine et al, 2000). Recently, we have described a novel connexin 30 mutation, V37E, in a sporadic case of Clouston syndrome (Smith et al, 2002).

Pachyonychia congenita (PC) is a group of ectodermal dysplasias whose most obvious phenotypic characteristic is hypertrophic nail dystrophy. Some forms of PC are known to be caused by mutations in differentiation-specific keratins. There are two main types of the disease: type 1 (PC-1) where nail dystrophy is often accompanied by focal keratoderma and sometimes oral leukokeratosis (Jadassohn and Lewandowsky, 1906); and type 2 (PC-2) where there are a variable number of additional features including pilosebaceous cysts, natal teeth, and angular cheilosis (Jackson and Lawler, 1951). The PC-1 phenotype is associated with mutations in keratins 6a and 16 (McLean et al, 1995; McLean et al, 1995), whereas the PC-2 phenotype has been linked to mutations in keratins 6b and 17 (McLean et al, 1995; Smith et al, 1998).

We here describe three patients, all originally diagnosed with variant forms of PC. The oldest patient was suffering from what was originally thought to be a new type of PC consisting of mild thickening of the nails associated with hypotrichosis universals. The other two patients were suffering from pachyonychia only. Hearing was normal in all three patients. An unexpected finding of a connexin 30 mutation in these patients indicates that the diagnosis should be Clouston syndrome, warranting evaluation of previously described “new” hair—nail dysplasias. All patients were seen with informed consent and all investigations with the approval of the appropriate local Medical Ethics Committees.

Materials and Methods

Mutation analysis Genomic DNA was extracted from whole blood by standard procedures. A 1104 bp fragment spanning the full length of the GJB6 gene (connexin 30) was amplified with primers Cx30P1 (forward) 5′ GGC AGG GAG TTG AAG TTG T AA 3′ and Cx30P2 (reverse) 5′ ACG TTG TGT ATG AAT GGA GCA 3′ as previously described (Smith et al, 2002). PCR products were purified using the Qiaquick PCR purification kit (Qagen, Crawley, UK) and sequenced on an ABI 3100 automated DNA sequencer (ABI, Foster City, CA) using primers Cx30P1, Cx30P2 (above), and Cx30P7 (reverse) 5′ GAC CCC TCT ATC GTA GCA ACC TT 3′.

Mutation G11R does not create or destroy any restriction site so a primer was designed with a mismatch to create a new BstI site in combination with the mutation. Genomic DNA was amplified using primers Cx30BstI (forward) 5′ TGG ATT GGG CAC GTA CAT TGT TG TTA CGC TGC 3′ (mismatched base underlined) and Cx30BstI (reverse) 5′ CAC TTT CTC GTG GGC AGC CAC CAT CAT CAT CAT 3′ in standard PCR buffer containing 1.5 mM MgCl2 and 4% dimethylsulfoxide. PCR conditions were 94°C 5 min × 1; 94°C 30 s, 55°C 45 s, 72°C 1 min × 35; and 72°C 5 min × 1. The resultant 128 bp fragment was digested with 10 U BstI (New England Biolabs) at 50°C for at least 4 h. Digests were analyzed on 3% agarose minigels.

Mutation A88V abolishes an HaeII site. Genomic DNA was amplified with Cx30P1 and Cx30P2 (as above) and PCR products were digested with 8 U HaeII (New England Biolabs) at 37°C for a minimum of 4 h. Digests were analyzed on 1.5% agarose minigels.

Results

Clinical findings Two of the families in this study have been reported previously. Briefly, the proband in family 1 (van Steensel et al, 2000) is a 63-year-old Dutch male, who was originally diagnosed with a new variant of PC, consisting of thickening of the nails (Fig 1a) and almost universal...
hypothenosis (Fig 1b). The alopecia and nail phenotype was entirely consistent throughout affected members of the family. No tooth abnormalities were noted and sweating was normal. Notably, hearing was normal and deafness did not occur elsewhere in the family. Several family members were affected in a manner consistent with autosomal dominant inheritance. DNA was available for study only from the proband.

The proband in family 2, a 13- y-old male of Moroccan descent, was diagnosed with PC without other abnormalities (Fig 1c). His parents were not consanguineous. The case was previously described in Chang et al (1994). Several members of his family were also affected with a combination of nail thickening, yellow discoloration of nails, and subungual hyperkeratosis. The abnormalities appeared during the first few months of life. The nail changes were identical to those seen in PC associated with keratin mutations; hence the disorder was considered as a possible allelic variant. Due to problems of patient consent and access, DNA was only available from the proband.

The proband in family 3 was a 25-y-old Dutch woman, who was also first diagnosed with a variant of PC due to nail deformities (pincer nails, subungual hyperkeratosis, and chronic paronychia) (Fig 1d, e). Her fingernails were all divergent with thickening of the nail plate, distal onycholysis, subungual hyperkeratosis, and paronychia. The toenails were thickened and curved and she suffered from very mild focal keratoderma of the soles, mainly on the heels (not shown). There was no alopecia, the eyebrows were sparse laterally, and the eyelashes were both thin and sparse. Hearing was normal. Since 1996, the proband has suffered from relapsing infections of the fingernails, which began as green discoloration with pus formation underneath the nail folds. She has been treated with ciprofloxacin and itroconazol and uses latex gloves for contact with water. As a child she was admitted to hospital because of patchy alopecia of the scalp, which had been interpreted as alopecia areata. Her mother had thickened toenails and excessive callus formation on the soles, suggesting dominant inheritance. No other family members were affected. DNA was available only from the proband.

Identification of connexin 30 mutations. PC has previously been shown to be caused by dominant-acting mutations in differentiation-specific keratin genes, K6a, K6b, K10, and K17, that are expressed in the nail bed and other ectodermal structures (Bowden et al, 1995; McLean et al, 1995; Smith et al, 1998). We screened these keratin genes as previously described (Smith et al, 1999a; 1999b; Terrinoni et al, 2001); however, we found no mutations in any of the patients (data not shown). The GJB6 gene (connexin 30) was chosen for screening because of the pachyonychia-like nail changes we observed in our recently reported Clouston syndrome patient, who had a novel connexin 30 mutation (Smith et al, 2002).

In both families 1 and 2 we found a heterozygous missense transition mutation 31G→A by direct sequencing of PCR products. The mutations predict the substitution of an arginine for a glycine at codon 11 of the connexin 30 polypeptide (G11R), as shown in Fig 2. This mutation, in combination with a mismatch primer, creates a novel BclI restriction enzyme site, which was used to confirm the mutation in connexin 30 PCR fragments by restriction digestion. This mutation has been previously reported in nine out of 12 classical Clouston syndrome kindreds of various ethnic backgrounds (Lamartine et al, 2000).

In family 3, we identified a heterozygous missense mutation A88V in the connexin 30 gene. (Fig 2). This mutation has also been reported previously in three cases of Clouston syndrome (Lamartine et al, 2000). Mutation A88V deletes an HaeIII restriction enzyme site, which was used to confirm the mutation by restriction digestion (data not shown). Both mutations were excluded from 50 control genomic DNA samples by the restriction digests described above (data not shown).

Figure 1. Clinical findings. (a) Fingernail of the proband in family 1, showing subungual hyperkeratosis and severe curvature of the nail. These features strongly resemble the type of nail dystrophy observed in PC due to keratin mutations; however, in this patient the causative mutation is G11R in the connexin 30 gene. (b) The proband of family 1 (shown here) and other affected individuals in this kindred had nearly complete alopecia. Only a few remaining hairs in the temporal region can be seen. (c) Severe hypertrophic nail dystrophy of the toenails in the proband from family 2. This patient carries the heterozygous missense mutation G11R in the connexin 30 gene. (d) Fingernails of the proband in family 3 showing some thickening of the nail plate, distal onycholysis, subungual hyperkeratosis, and paronychia. (e) Toenails of the proband in family 3 showing thickening and abnormal curvature of the nails. This patient carries heterozygous missense mutation A88V in the connexin 30 gene.
Here, we describe three families where the original diagnosis was that of variant types of PC. Upon mutation analysis, these patients all have genetic defects in connexin 30 that have been shown previously to cause hidrotic ectodermal dysplasia, also known as Clouston syndrome (Lamartine et al., 2000). Apparently, Clouston syndrome can have many guises. The phenotype is usually described as consisting of hypotrichosis; thick, ridged, and extremely short nails; palmoplantar hyperkeratosis; and hyperpigmentation of skin over large joints. There is one report of a family with Clouston syndrome lacking the palmoplantar hyperkeratosis but this symptom is not generally regarded as part of the phenotype (Hassel et al., 1996). Palmoplantar hyperkeratosis with hypotrichosis is also seen in a few cases of PC (Templeton and Wiegand, 1997) and other ectodermal dysplasias without pachyonychia (van Steensel et al., 2002). In all the pachyonychia patients whom we have studied where mutations in keratin genes have been identified, however, there have been no instances of hypotrichosis or alopecia. Thus, the presence of alopecia in addition to hypertrophic nail dystrophy may be indicative of a connexin defect, rather than a keratin mutation. Thickening of the nails, shown here to be clinically very similar to the nail changes seen in PC, can apparently also be a manifestation of Clouston syndrome. Phenotypic variation has been reported previously in relation to connexin mutations in both skin disease and hearing loss, as reviewed recently (Kelsell et al., 2001; Richard, 2001a; 2001b). Specifically, in GJB2 missense mutations D66H and R75W have been shown to have variable effects on the skin and frameshift mutation 35delG gives rise to variable degrees of hearing loss. Thus, the phenotypic variability reported here, i.e., nail dystrophy with or without alopecia, is likely to be part of a general phenomenon in the human connexin disorders.

In two patients, both of whom had been previously reported as having variant types of PC (Chang et al., 1994; van Steensel et al., 2001), we demonstrated the presence of mutation G11R in connexin 30. Family 1 also had associated universal hypotrichosis. Complete hypotrichosis has been described in Clouston syndrome (Smith et al., 2002) and in hindsight the diagnosis should have been entertained in this patient. In families 2 and 3, however, affected family members had isolated pachyonychia in the absence of other ectodermal abnormalities. These families were more readily confused with pachyonychia, particularly the PC-1 (Jadassohn–Lewandowsky) variant, due to K6a/K6f mutations, where the other ectodermal features, leukoplakia and focal keratoderma, may be mild or absent in some cases. Connexin 30 mutations G11R and A88V were identified in families 2 and 3, respectively.

It is of interest to note that the same mutation can have disparate effects on hair, nails, and palmoplantar skin. At present, we have no explanation for this observation; however, the genetic background of different families and individual patients is likely to play a role. It is possible that polymorphisms in keratins, connexins, or other genes encoding epithelial structural molecules could influence the phenotype. From the clinical phenotype alone, it is not possible to distinguish the Clouston syndrome nail dystrophy from the ones associated with keratin mutations. No nail biopsies have been performed in our patients; it is therefore not possible to establish a histologic correlation between genotypic and phenotype.

Given our findings, it can be expected that some disorders characterized by thick or brittle nails with or without hair abnormalities may turn out to be phenotypic variants of Clouston syndrome. There exist several reports of patients suffering from hair and/or nail dysplasias that have been classified as new disorders (Calzavara-Pinton et al., 1991; Pinheiro and Freire-Maia, 1992; Christianson and Fourie, 1996). We suggest that some of these patients could in fact be suffering from Clouston syndrome and that they should be examined for connexin 30 mutations. As connexin 30 is encoded by a small single exon gene, mutation screening is both straightforward and inexpensive and should perhaps be considered as part of the mutation screening protocols for pachyonychia. Similarly, Clouston syndrome should be considered as part of the differential diagnosis for pachyonychia and vice versa. It should be noted that we have in fact sequenced a few patients diagnosed as having a form of PC for all four keratins involved in PC, i.e., K6a, K6b, K16, and K17, and also connexins 26 and 30. These patients did not have mutations in any of these genes. Therefore, there are other as yet unknown genes that can cause hypertrophic nail dystrophy that is clinically indistinguishable from PC.

In conclusion, Clouston syndrome can have a variable presentation and can mimic other forms of hair and/or nail dysplasia. We recommend that any patient diagnosed with a type of PC with or without hair abnormalities be tested for the presence of connexin 30 mutations. This will help expand the spectrum of phenotypes associated with these mutations and may give some clues as to the cause of the phenotypic variability.

REFERENCES

