Bibliography of Selected Articles


A review of the clinical phenotype of 254 patients with genetically confirmed pachyonychia congenita

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Background: Pachyonychia congenita (PC) is a group of autosomal dominant keratinizing disorders caused by a mutation in one of 4 keratin genes. Previous classification schemes have relied on data from case series and case reports. Most patients in these reports were not genetically tested for PC.

Objective: We sought to clarify the prevalence of clinical features associated with PC.

Methods: We surveyed 254 individuals with confirmed keratin mutations regarding their experience with clinical findings associated with PC. Statistical comparison of the groups by keratin mutation was performed using logistic regression analysis.

Results: Although the onset of clinical symptoms varied considerably among our patients, a diagnostic triad of toenail thickening, plantar keratoderma, and plantar pain was reported by 97% of patients with PC by age 10 years. Plantar pain had the most profound impact on quality of life. Other clinical findings reported by our patients included fingernail dystrophy, oral leukokeratosis, palmar keratoderma, follicular hyperkeratosis, hyperhidrosis, cysts, hoarseness, and natal teeth. We observed a higher likelihood of oral leukokeratosis in individuals harboring KRT6A mutations, and a strong association of natal teeth and cysts in carriers of a KRT17 mutation. Most keratin subgroups expressed a mixed constellation of findings historically reported as PC-1 and PC-2.

Limitations: Data were obtained through questionnaires, not by direct examination. Patients were self- or physician-referred.

Conclusions: We propose a new classification for PC based on the specific keratin gene affected to help clinicians improve their diagnostic and prognostic accuracy, correct spurious associations, and improve therapeutic development. (J Am Acad Dermatol 10.1016/j.jaad.2011.12.009.)

Key words: genodermatosis; hyperkeratosis; keratin; keratinizing disorder; keratoderma; pachyonychia congenita.

Pachyonychia congenita (PC) is a group of autosomal dominant disorders caused by a mutation in one of 4 keratin genes: KRT6A, KRT6B, KRT16, or KRT17.1-5 There are an estimated 5000 to 10,000 cases worldwide.6 The variable clinical findings affect a number of ectodermal structures, including nails, skin, teeth, and oral mucosa.2 Although Muller2 and Wilson and Cantar8 are credited with describing PC in 1904, Jadassohn and Lewandowski,9 whose names constitute the eponym for PC type 1, published the first case series of two siblings in 1906. Kumer and Loos10 proposed a
clinical classification scheme for PC variants based on their report of a 5-generation family with 23 affected family members. Classification criteria were developed and refined over subsequent years by authors who painstakingly reviewed and summarized the available literature.11-19 Two clinical subtypes ultimately emerged, the Jadassohn-Lewandowski PC type 1 and the Jackson-Lawler PC type 2.

In 1994, Munro et al20 studied a large Jackson-Lawler pedigree and linked the first PC gene to chromosome 17q12-q21. In 1995, McLean et al3 identified the first causative mutations in keratin genes KRT16 and KRT17. Additional mutations were subsequently identified in KRT6a and KRT6b—genes encoding the type II keratins that form heteropolymers with type I keratins K16 and K17.4,5 The identification of these mutations and the advent of clinical genetic testing allowed the classification of PC based on clinical and genetic criteria.

Erroneous reports of PC manifestations in patients who did not have PC have been clarified by investigators through genetic testing.21 Large, well-characterized and mutation-confirmed pedigrees offer the opportunity to draw valid conclusions regarding genotype-phenotype relationships.22 However, even these pedigrees are prone to bias because of shared modifier genes and environments that might influence the clinical presentation. This report summarizes data collected from 254 patients with mutation-verified PC (derived from 147 families) and, to our knowledge, represents the largest and most comprehensive genotype-phenotype study of PC to date.

METHODS

In 2004, the International Pachyonychia Congenita Research Registry (IPCRR) was established by the nonprofit organization Pachyonychia Congenita Project to collect clinical and genetic data on patients with PC worldwide. The registry was approved by the Western Institutional Review Board (study #20040468). All patients gave written informed consent and the study was conducted according to the Declaration of Helsinki Principles. Participant enrollment began in May 2004.

Participants in the registry were solicited through an Internet World Wide Web site designed to educate patients and physicians about PC (www.pachyonychia.org). Referral to the registry was permitted through patients, family, physicians, and family expansion. To be included in the registry, each patient completed a detailed questionnaire and provided information regarding whether and to what extent they were affected by the clinical features of PC. Patients were also asked about the age of onset and the impact each feature had on their quality of life. The completed questionnaire, along with photographs of visible skin and nail changes, was submitted to the IPCRR. A telephone consultation was arranged with a dermatologist on the Pachyonychia Congenita Project medical advisory board to: (1) clarify any confusing or missing information from the questionnaire; (2) confirm that the clinical features were consistent with PC; and (3) provide genetic counseling before mutation testing. Genetic testing was provided without charge and was performed in Dr Frances Smith's laboratory, University of Dundee, College of Life Sciences, Division of Molecular Medicine, Dundee, Scotland. Before being released to patients, the results were confirmed by independent testing of a buccal DNA sample by GeneDx (Gaithersburg, Maryland), a US Clinical Laboratory Improvement Amendments-certified laboratory. All participant data included in the analysis were from patients with a confirmed PC keratin mutation.

Statistical methods

We performed logistic regression analysis to compare how different PC keratin mutations influence the probability of developing a specific clinical finding. For outcomes such as age of onset and quality of life, ordinal logistic regression was used. Because there were more KRT6a carriers than other mutation carriers we used the frequency of a trait in the KRT6a group as a reference when calculating odds ratios (OR) for the same trait to occur in the other keratin groups. To increase the power of analysis, all family members having a PC phenotype were included in the test, with intrafamilial correlation adjusted. Software was used to perform the comparisons (STATA v9.2, StataCorp, College Station, TX).
RESULTS
An international case series of patients with mutation-verified PC

At the time the data were collected, 254 individuals had completed the necessary steps for inclusion in the IPCRR (Table I). Additional demographics (eg, country of residence) can be found at www.pachyonychia.org. In addition to the 254 patients harboring keratin mutations in one of the 4 “classic” PC genes (KRT6A, KRT6B, KRT16, and KRT17) 34 other individuals were found to have no detectable mutation, or mutations in other genes including connexin-30, KRT6C, and desmoglein-1. In this article we will focus on the results of those with mutations in the 4 classic PC keratin genes.

We present the most commonly reported clinical findings according to mutation status in Table II.

Major phenotypic features of PC

Three clinical features that were reported in more than 90% of patients across all mutation subtypes were thickened toenails, plantar keratoderma, and plantar pain (Table II).

Thickened toenails. Thickened toenails (Fig 1) were the most frequently reported clinical finding in the IPCRR with 249 of 254 (98%) patients reporting this phenotype (Table II). The average number of toenails affected was 8.8 (range 0-10, mode = 10). We performed logistic regression analysis to compare the relative likelihood of having all 10 toenails affected between the different PC keratin mutation carriers. Using the prevalence of 10 affected nails in participants with mutations in KRT6B, KRT16, and KRT17 as the reference, we found that KRT6A mutation carriers were 11.1 times as likely (P < .001) to have all 10 toenails affected. The average age of onset of toenail dystrophy across all keratin mutation types was 2.8 years with a median of 0.08 years (1 month). KRT6A mutation carriers had the earliest average onset at 0.35 years (about 4 months). The average age of onset for patients with a KRT6B, KRT16, and KRT17 mutations was 9.5, 6.8, and 0.9 years, respectively.

Plantar keratoderma. Plantar keratoderma (Fig 2) was the next most commonly reported finding, present in 241 of 254 (95%) patients (Table II). The registry includes individuals of ages younger than 1 year to older than 86 years (Table I). Of the 13 individuals who were not reported as having plantar keratoderma, 9 were younger than 1 year, and the oldest was 3 years of age (data not shown). The average age of onset of patients with a KRT6B, KRT16, and KRT17 mutations was 9.5, 6.8, and 0.9 years, respectively.

Plantar pain. Plantar pain was reported by 225 of 254 (89%) surveyed patients. The prevalence of pain was high across keratin subgroups (Table II). The age of the patient was found to have a dramatic effect on the reporting of plantar pain: only 3 patients older than 10 years did not report plantar pain (data not shown). Plantar pain was the most important feature of PC affecting quality of life (see below).

Other common clinical findings in PC

Fingernail involvement. As shown in Fig 3, thickened fingernails were reported in 220 of 254 (87%) patients (Table II). The prevalence was lower in the other mutation carriers, with 9 of 20 (45%) KRT6B and 56 of 76 (74%) KRT16 mutation carriers reporting at least one affected nail (Table II). Interestingly, when the number of fingernails affected was evaluated based on mutation type, patients with KRT6B mutations appeared to have far...
fewer nails affected on average compared with those with other keratin gene mutations. Comparison of the OR to develop fingernail involvement later than KRT6A mutation carriers revealed a statistically significantly elevated OR for KRT6B and KRT16 but not for KRT17 carriers.

Mucosal involvement. Oral leukokeratosis was reported in 177 of 254 individuals (70%) with the breakdown by mutation noted in Table II. Of the 177 individuals with self-identified oral leukokeratosis, 125 reported an average age of onset of 5.1 years with a median and mode of 0 years. Onset at birth was reported by 67 of 125 (54%), whereas 84 of 115 (73%) reported onset affected by 1 year of age and 119 of 125 (95%) by age 20 years (data not shown). KRT6A and KRT17 carriers had a significantly increased OR of earlier onset of oral leukokeratosis compared with KRT6B and KRT16 carriers.

Cysts. Pilosebaceous cysts and steatocysts have been reported in conjunction with PC types 1 and 2. Overall, 104 of 254 (41%) patients reported cysts of any type (Table II). KRT17 mutation carriers had a much higher likelihood of reporting cysts (OR of 23.2 [P = .003]) compared with KRT6A mutation carriers.

Natal teeth. The phenomenon of erupted teeth present at birth known as “natal teeth” has been reported in patients with PC. Of the 39 patients who reported the presence of teeth at birth, 36 were KRT17 mutation carriers. No KRT16 or KRT6B mutation carriers reported natal teeth and only 3 of 115 (3%) KRT6A carriers were affected (Table II).

PC reduces the quality of life
In Table III the severity and frequency of common PC symptoms are detailed.

Of the 240 patients who reported plantar pain, 214 indicated its frequency. A total of 138 (64%) indicated that their quality of life was affected at least weekly by plantar pain. 41 (20%) of respondents were affected “every month or two”; 26 (12%) reported being affected “seldom” (defined as “once a year or less”); and 9 (4%) indicated that they were never affected. Describing the pain, 99 of 240 (41%) reported it was “very painful, but do not use
medication”; 62 of 240 (26%) answered that they “often require medication for the pain”; and 79 of 240 (33%) reported that plantar pain was “somewhat to not painful.”

**DISCUSSION**

This cohort includes patients with the 4 most common PC keratin mutations in sufficient numbers to draw conclusions regarding the prevalence and penetrance of the most common PC clinical findings. To our knowledge, it includes the most diverse collection of patients of any cohort thus far evaluated and reduces the impact of genetic background or founder bias among the clinical features reported for different mutations.

Because of difficulties inherent in the study of a rare genodermatosis there are several limitations with our data. The patients were ascertained either by self- or physician-referral, not from a population-based assessment. Because most self-referrals were from individuals who became aware of PC through the Internet, our cohort probably represents a more affluent, better-educated population with access to health care. Because of the geographically dispersed nature of the cohort, our data were mostly gathered through telephone interviews and evaluation of photographs, rather than by direct examination, which makes the objective quantification of clinical severity more difficult. The quality-of-life measures and reporting of pain, although key to

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**Fig 2.** Clinical phenotype in genetically confirmed pachyonychia congenita. Plantar hyperkeratosis often follows pressure distribution, but can involve entirety of plantar surface. Environmental factors play significant role in development and persistence of plantar keratoderma.

**Fig 3.** Clinical phenotype in genetically confirmed pachyonychia congenita. Fingernail findings include dramatic elevation of fingernails because of subungual debris or premature termination of nail plate.
understanding the most important issues that affect patients with PC, were not performed using a validated metric.

The most common clinical findings in our PC cohort were toenail thickening, plantar keratoderma, and plantar pain. Only one patient, who was younger than 1 year, lacked all 3 findings. The impact of age on their prevalence is significant as children seldom develop plantar keratoderma or plantar pain before they start to walk. If children 3 years of age and younger were excluded from the analysis, 216 of 230 (94%) individuals would meet all 3 criteria and 227 of 230 (99%) of our participants would have met two criteria. For the clinician, these findings suggest that the presence of toenail dystrophy with plantar keratoderma and plantar pain in children older than 3 years is a much more sensitive means of clinically diagnosing PC than 20-nail dystrophy, which is often thought to be requisite to the diagnosis.

Additional diagnostic findings associated with PC included fingernail dystrophy, follicular hyperkeratosis, leukokeratosis, cysts, and natal teeth. Less common findings included ear pain, hoarseness, and hyperhidrosis. In the literature there are reports of corneal findings, deafness, skeletal abnormalities, and mental retardation associated with PC. We found no support for these findings among our cohort. A more detailed discussion of features spuriously associated with PC will be reported elsewhere.

Although a detailed discussion of the clinical findings linked to a specific mutation is beyond the scope of this article, we found that the KRT16 mutations, p.As125Ser and p.Arg127Cys, were strongly associated with lack of fingernail involvement whereas KRT16 mutation carriers with the p.Leu132Pro mutation frequently presented with 10-fingernail dystrophy. Our group has recently published a large study reviewing new and previously known mutations in patients with PC. As more data are gathered we hope to be able to provide phenotypic prognosis based on the specific mutation identified.

Plantar pain has the most profound effect on quality of life for most patients with PC as it can limit mobility and social interaction along with the ability to find and maintain work. The pain reported by patients with PC is often out of proportion to the extent or duration of callus, suggesting that the mechanism is not merely the result of pressure from callus formation. Clinicians will better meet the needs of patients with PC by inquiring about the extent of pain and helping them manage their calluses to facilitate pain reduction.

Historically, PC has been subdivided into two major phenotypic variants, PC-1 (Jadassohn-Lewandowski) and PC-2 (Jackson-Lawler). The PC-1/PC-2 classification was designed to improve the ability to predict phenotypic prognosis without genetic testing. The PC-1/PC-2 classification assumes that because certain keratin proteins predictably dimerize that a mutation in either protein gene will result in a similar clinical phenotype. Hence, a mutation in either of the PC-1 keratin proteins (K6a/K16) should cause similar features, whereas a mutation in K6b/K17 (PC-2 proteins) will present with a different, predictable phenotype.

Instead, our data demonstrated that clinical phenotypes overlapped substantially across genotypic categories and could not be used to predict genotype reliably. Specifically, we found a significant overlap of oral leukokeratosis, cysts, and natal teeth that purportedly distinguish PC-1 and PC-2. Overall, our data demonstrate that the PC-1/PC-2 nomenclature does not accurately reflect the molecular pathogenesis of PC and does not represent a rational or clinically useful classification at this time.

We recommend the elimination of the terms "PC-1" and "PC-2" and propose their replacement with notation of the specific keratin defect. In this classification scheme a diagnosis of PC-6a, PC-6b, PC-16, and PC-17 would correspond to mutations in the KRT6A, KRT6B, KRT16, and KRT17 genes, respectively. A designation of PC-U (unknown) may be applied when the classic clinical findings of PC are found in the absence of a known PC keratin gene mutation. Classification based on keratin mutation subtype will allow clinicians to provide more accurate prognoses for patients with PC. We recommend genetic testing for individuals with the triad of toenail dystrophy, plantar keratoderma, and plantar

<table>
<thead>
<tr>
<th>Impact on quality of life</th>
<th>Plantar keratoderma</th>
<th>Thickened toenails</th>
<th>Cysts</th>
<th>Thickened fingernails</th>
<th>Oral leukokeratosis</th>
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<td>No impact</td>
<td>19/216 (9%)</td>
<td>71/221 (32%)</td>
<td>63/154 (41%)</td>
<td>67/199 (34%)</td>
<td>40/64 (63%)</td>
</tr>
<tr>
<td>Sometimes a problem</td>
<td>56/216 (26%)</td>
<td>113/221 (51%)</td>
<td>65/154 (42%)</td>
<td>109/199 (55%)</td>
<td>23/64 (35%)</td>
</tr>
<tr>
<td>Always a problem, but</td>
<td>133/216 (62%)</td>
<td>36/221 (16%)</td>
<td>23/154 (15%)</td>
<td>22/199 (11%)</td>
<td>1/64 (2%)</td>
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<td>Unable to function</td>
<td>8/216 (4%)</td>
<td>1/221 (0.5%)</td>
<td>3/154 (2%)</td>
<td>1/199 (0.5%)</td>
<td>0/64 (0%)</td>
</tr>
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</table>

Table III. Impact of specific features of pachyonychia congenita on quality of life
pain as they have a high likelihood of carrying a PC mutation. Genetic testing is provided free of charge to all patients who enroll in the PC registry through the nonprofit patient advocacy group, Pachyonychia Congenita Project (www.pachyonychia.org).

We look forward to the development of specific genetic techniques to minimize or eliminate the clinical expression of this rare keratin disorder.25-27

REFERENCES
Best treatment practices for pachyonychia congenita

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Abstract
Background Numerous therapeutic modalities have been proposed to treat the manifestations of pachyonychia congenita (PC). While research hopes lie with molecular therapies, patients are in need of answers regarding the efficacy of conventional treatments.

Aim of the study To determine patients’ experience and preferences regarding conventional treatments for PC.

Methods The study population included 120 PC patients from 20 countries. The study was based on a patient survey developed by physicians and researchers from the International Pachyonychia Congenita Consortium and conducted via the internet. Using an effectiveness scale of 1 to 5, the patients were asked to grade treatments for different manifestations, including keratoderma, cysts, follicular hyperkeratosis, fingernail and toenail involvement.

Results Patients reported surgical treatments being most effective for cysts and mechanical treatments the most effective conventional therapeutic approach for all other investigated manifestations. The other conventional medical treatments were found to be non-effective to only slightly effective. Among patients with keratoderma, older people were more likely to report beneficial effect from mechanical treatments ($P = 0.04$), topical retinoids ($P = 0.04$) and topical steroids ($P = 0.02$). Likewise, females were more inclined to report filing and grinding beneficial than males ($P = 0.02$). Finally, carriers of KRT16 and KRT6a were more likely to benefit from keratolytics than carriers of mutations in KRT17 ($P = 0.04$).

Conclusions None of the currently available therapeutic options for PC are ideal, although they provide some relief, with mechanical/surgical options being preferred over medical therapies. These results emphasize the need for more efficient and targeted therapies.

Conflict of interest
None declared.

Funding sources
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Introduction
PC is a very rare keratinizing disorder estimated to affect between 5 and 10 thousand people worldwide.1 This disorder is transmitted as an autosomal dominant trait, and is caused by mutations in one of five keratin genes: KRT6A, KRT6B, KRT6C, KRT16, KRT17, which encode keratins K6a, K6b, K6c, K16 and K17 respectively.1,2 Most of the keratin mutations which cause PC are heterozygous missense mutations or small insertions/deletions which result in fragility of the epithelial cell cytoskeleton, leading to cell cytolysis and tissue blistering or hyperkeratosis.2–4

PC cardinal features were first reported by Muller5 and Wilson6 in 1904, and by Jadassohn and Lewandowski in 1906,7 and include painful and debilitating plantar keratoderma, hypertrophic toenail and fingernail dystrophy, follicular hyperkeratosis, palmar keratoderma, epidermal cysts, oral leukokeratosis, and occasionally hyperhidrosis, hoarseness and natal teeth.1,2

Clinical classification of PC variants was first suggested by Kumer in 1935.8 PC was eventually divided into two clinical subtypes: the Jadassohn-Lewandowski PC (type-1 PC) and the Jackson-Lawler PC (type-2 PC).1,2

This clinical classification was intended to assist estimation of the prognosis in the absence of genetic testing.1,2 After the discovery of the underlying cause of PC, genotype-phenotype analysis suggested initially that mutations in KRT6a/KRT16 and KRT6b/KRT17 were associated with type 1 and type 2 PC respectively.1 More recently, large-scale genetic analysis raised doubts regarding the clinical relevance of these correlations,1,2 leading to the establishment of a novel classification for PC.
based solely on molecular analysis resulting in PC-K6a, PC-K6b, PC-K6c, PC-K16 and PC-K17 as distinct types.1,2

Many therapeutic modalities have been proposed to treat the various clinical manifestations in PC, including retinoids,9 surgical and mechanical procedures, orthotics, keratolytics, pain medications10 and botulinum toxin.11 Recently, more targeted therapeutic strategies (including small interfering RNAs,12–14 rapamycin15 and simvastatin16) have been the focus of much attention. Unfortunately, as most of these advanced approaches still cannot be offered on a routine basis to patients because of expense and limited availability, patients are currently forced to rely upon the use of the available conventional strategies.

In this study, we used a patient survey approach to derive effectiveness data in PC. This methodology has been widely used over the past years to delineate guidelines for the treatment of disorders for which no controlled data are available.9

Methods

Patients

The study population included pachyonychia congenita patients from 20 different countries who were enrolled in the International Pachyonychia Congenita Research Registry (IPCRR) and recruited through Pachyonychia Congenita Project, a non-profit patient advocacy group established in 2004.1,2 All patients were diagnosed using a detailed clinical questionnaire and genetic testing results for a mutation in one of the PC-associated genes. The study was conducted according to the principles of the declaration of Helsinki and all patients gave their written informed consent.

Data collection

In addition to the extensive physician-validated data in the IPCRR, each patient completed an addendum survey via the internet providing information on treatments used for five categories of clinical manifestations of PC: keratoderma, cysts, follicular hyperkeratosis, fingernail and toenail involvement. In addition, all patients provided information on demographics, genetic status, the effect of the disease on their quality of life, the clinical manifestations of PC and the degree of effectiveness of the different treatments.

The patients were asked to grade each treatment they had used according to a treatment effectiveness scale of 1 to 5:

1- not effective at all
2- a little effective
3- somewhat effective
4- effective
5- very effective.

The patients were asked to grade different available treatments including mechanical treatments (such as filing, grinding, cutting, clipping or plucking the lesions), surgical removal of the lesions, surgical removal of the nail, incision and drainage of cysts, soaking of the nails to soften them before treatments, orthotics, custom made orthotics, topical and oral retinoids, pain medications, botulinum toxin, moisturizers, vaseline, keratolytic treatments, antibiotic ointments, antifungal ointments, topical and oral steroids, salicylic acid, treatments of the nails by medical professional and treatments of the nails in a nail salon.

Statistical analysis

All outcome variables are ordinal variables ranging from 1 to 5 – the higher the score, the higher the treatment effect. Univariate analysis was used to determine the relationships between each explanatory variable and the treatment outcome variables. The explanatory variables are the following: age, gender, quality of life score (QOL) and gene (categorical variable).

Pearson correlations were calculated between all continuous explanatory variables and the outcome variables. Wilcoxon Two-Sample or Kruskal–Wallis tests were used to compare between categorical explanatory variables and the outcome variables.

A P-value of 0.05 was considered significant. Statistical analysis was performed by SAS for windows version 9.2 (SAS Institute, Cary, NC, USA).

Results

The study included 120 PC patients, 67 females and 53 males, of all ages. The youngest patient was a 1-year-old baby and the oldest an 81-year-old patient. The average age was 38.5, and the median age was 39 years.

Keratoderma

Of the 120 patients who took part in the study, 113 patients reported having keratoderma. Forty-eight patients had clinical manifestations involving both palms and soles; 65 patients had only sole involvement.

Most conventional treatments were attributed mean scores of 2 to 3 (a little effective to somewhat effective) (Fig. 1). Patients reported mechanical treatments (such as filing, grinding, cutting, clipping), as the most effective conventional treatments corresponding to a mean score of 3.8. Pain control medications and orthotics ranked second in effectiveness.

Other medications such as antibiotic ointments, urea cream, oral and topical retinoids, oral and topical steroids, botulinum toxin injections, salicylic acid or antifungal treatments were reported as poorly effective.

Cysts

Of the 120 patients who took part in the study, 49 patients reported having cysts (Fig. 2). Surgical removal treatment was found to be effective to very effective, reaching a mean score of 4.32. The next most effective treatment reported was incision and drainage with a mean score of 4. Pain medications were
found to be of little effect. Other treatments, such as antibiotic ointments, intralesional and oral steroids and retinoids, were reported as not effective.

**Follicular hyperkeratosis**

Of the 120 patients who took part in the study, 63 patients reported being affected with follicular hyperkeratosis. The results (Fig. 3) showed that the available conventional treatments were slightly effective or not effective, with clipping or plucking the plugs leading with a mean score of 2.9.

**Toenail involvement**

Toenail involvement is very common in PC. Of the 120 patients in the study, 108 patients reported toenail involvement. The results for toenail involvement (Fig. 4) demonstrated that the most effective approach was mechanical treatment such as filing, grinding, cutting or clipping the toenails. Mechanical treatment received a mean score of 4. The patients reported that the second most effective treatment, with a mean score of 3.6, was soaking the nails to soften them before treatment. Of note, surgical avulsion of the nail was found to be ineffective, as were non-medical treatments provided in nail salons.
Fingernail involvement
Fingernail involvement was reported by 94 of the 120 patients in this study. Results pertaining to fingernail involvement resembled those obtained for toenail involvement (Fig. 5).

The most effective treatment was mechanical treatment such as grinding, filing, clipping or cutting the fingernails. Mechanical treatments received a mean score of 4 and the second most effective treatment was soaking and softening the nails. The other treatments ascertained were found to be not effective.

Univariate analysis
As multivariate analysis was found to be very unstable due to the wide range of available data, we used univariate analysis to

Figure 3 Mean scores of effectiveness of the different treatments used for follicular hyperkeratosis (reported by 63 patients). Treatment effectiveness colour scale is described in figure legend 1.

Figure 4 Mean scores of effectiveness of the different treatments used for toenail involvement (reported by 108 patients). Treatment effectiveness colour scale is described in figure legend 1.
further analyse the data and to determine the relationships between each explanatory variable and the treatment outcome variables. This analysis revealed three facts of clinical relevance for patients with keratoderma:

1. As patients grew older, they were more likely to report beneficial effect from mechanical interventions (such as filing, grinding) \((P = 0.04)\), topical retinoids \((P = 0.04)\) and topical steroids \((P = 0.02)\).
2. Females were more inclined than males \((P = 0.02)\) to describe mechanical interventions such as filing and grinding as beneficial.
3. Finally, carriers of mutations in \(KRT16\) and \(KRT6a\) were more likely to benefit from keratolytics than carriers of mutations in \(KRT17\) \((P = 0.04)\).

**Discussion**

Treating PC is challenging. PC is clinically multifaceted and the conventional treatments are directed at the different manifestations of the disorder. Currently, there are no specific treatments for PC.\(^{16,17}\) Each patient presents a unique constellation of conditions and a treatment plan must be individually tailored.\(^{17}\) Unfortunately, despite use of numerous approaches to relieve PC-associated symptoms, little is currently known regarding their relative efficacy.

Conventional treatment for nail disease in PC includes mechanical or surgical procedures, such as grooming or surgical removal of nails. The nails tend to re-grow unless complete ablation is performed. Follicular hyperkeratosis can be treated by oral and topical retinoids, keratolytic agents and alphahydroxy acid preparations. Cysts may be treated by incision, excision, drainage or by intralesional injection of steroids. In case of infection, oral antibiotics may be indicated.\(^{10}\)

Currently, retinoids are considered efficient drugs to treat hyperkeratotic disorders including PC.\(^{9,16}\) They act via retinoic acid response elements (RAREs) which are present in the keratin’s gene promoters, and inhibit gene expression.\(^{16}\) Retinoids, although reducing hyperkeratosis, may also cause thinning of the epidermis and blistering, leading to pain and possible infectious complications.\(^{10,16,18}\) Contradictory data regarding the efficacy of retinoids in PC have been published. Some case reports demonstrated an improvement of calluses with retinoid treatment.\(^{19-22}\) Other studies described patients with improvement of hyperkeratosis with retinoid treatment, but no change in pachyonychia.\(^{21,22}\) More recently, Gruber et al.\(^9\) analysed data collected in 30 PC patients who received systemic retinoid treatment. They found that 50% and 14% of their patients reported improvement in palmoplantar hyperkeratosis and pachyonychia respectively. The mean satisfaction score from the treatment was found to be 4.5 on a scale of 1–10. All patients reported suffering from adverse effects and 83% stopped using the drug.\(^{9}\) In contrast with these mixed results, others have reported no improvement with retinoids in PC\(^{23,24}\) and, in another series,\(^{25}\) four patients with palmoplantar keratoderma who received oral retinoids reported improvement in the appearance of their skin, but...
had to stop the treatment because of pain that restricted hand and foot function. Levels of evidence vary according to the methodology used in clinical studies, which in turn is often a function of patient population size. Randomized control studies are of course preferable; however, in diseases as infrequent as PC, such studies are not always possible. Alternative methodologies, also adapted to rare conditions in which controlled studies cannot be readily performed, rely upon the quantification of patient values and expectations.

Our study was based on a survey conducted among the largest group of PC patients ascertained to date for treatment efficacy. As all patients had been diagnosed with PC on the basis of both a careful physical examination and a full molecular analysis, we believe that the data collected faithfully reflect PC patients’ appreciation of conventional therapeutic modalities.

We found that the majority of conventional treatments were only marginally effective for keratoderma, with mechanical treatments being the most effective.

Palmoplantar keratoderma is a very common manifestation of PC, usually presenting as a child starts walking and bearing weight during the first few years of life. In a study by Eliason, et al., plantar keratoderma was present in 241 of 254 (95%) of patients. Of the 13 patients reported as not having plantar keratoderma, the oldest was 3-years old and nine were younger than 1 year. Among 241 patients who reported having plantar keratoderma, the age of onset ranged from birth to 30 years and the average age was 4.2 years. In our study, most of the patients who did not report plantar keratoderma were younger than 5 years of age.

Patients reported surgical treatments being most effective for cysts. Mechanical treatments were found to be the most effective conventional therapeutic approach for follicular hyperkeratosis, as well as for toenail and fingernail involvement. The other conventional medical treatments were found to be non-effective to only slightly effective.

In line with a previous study, keratolytics, which are widely used by the patients, were found to be of limited effectiveness for both palmoplantar keratoderma and nail problems. Response to treatment was highly individual, underscoring the need for clinical or molecular predictors of response to therapy. In this regard, the results of our univariate analysis, which remain to be independently confirmed, suggest a number of such predictive parameters. For example, our finding that carriers of mutations in KRT16 and KRT6a were more likely to benefit from keratolytics than carriers of mutations in KRT17 may be explained by the fact that the latter group has milder keratoderma than the former groups. This finding is consistent with phenotypic differences.

Overall, current available therapeutic approaches in PC are of borderline benefit. Therefore, the recent major advances in the search for PC-specific therapeutic strategies are of great importance. For example, a mutation-specific siRNA was found recently to lead to callus recession and pain control in a PC patient. However, these injections were found to be tremendously painful underscoring the need for new approaches for more efficient and practical ways of nucleic acid delivery to the skin. Other treatments under investigation for PC patients are rapamycin and simvastatin, but these therapies, although providing hope for patients, are not yet applied routinely.

In conclusion, although none of the currently available therapeutic approaches seem to be ideal, they do provide some relief to our patients. Mechanical/surgical options are preferred over medical therapies, such as retinoids, antibiotics or antifungal agents. These results emphasize the need for more efficient and targeted therapies.

Acknowledgements

We thank patients and their families in the International Pachyonychia Congenita Research Registry (IPCCR) for their support and members of the International Pachyonychia Congenita Consortium (IPCC) for development of the treatment survey.

References


An appraisal of oral retinoids in the treatment of pachyonychia congenita

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Background: Pachyonychia congenita (PC), a rare autosomal-dominant keratin disorder caused by mutations in keratin genes KRT6A/B, KRT16, or KRT17, is characterized by painful plantar keratoderma and hypertrophic nail dystrophy. Available studies assessing oral retinoid treatment for PC are limited to a few case reports.

Objective: We sought to assess overall effectiveness, adverse effects, and patient perspective in patients with PC receiving oral retinoids.

Methods: In a questionnaire-based retrospective cross-sectional survey of 30 patients with PC assessing oral retinoids (10-50 mg/d for 1-240 months), we determined the clinical score, satisfaction score, visual analog pain scale, and adverse effects.

Results: In 50% of patients there was thinning of hyperkeratoses (average improvement 1.6 on a scale from −3 to +3) (95% confidence interval 1.2-1.9, P < .001). In all, 14% observed amelioration of their pachyonychia; 79% did not experience any nail change. The self-reported overall satisfaction score with oral retinoid treatment was 2 or greater in 50% of the patients (mean 4.5 on a scale of 1-10). Although 33% reported decreased and 27% increased plantar pain with treatment, 40% did not notice any pain change. All patients experienced adverse effects, and 83% reported to have discontinued medication. Risk/benefit analysis favored lower retinoid doses (≤ 25 mg/d) over a longer time period (>5 months), compared with higher doses (>25 mg/d) for a shorter time (≤ 5 months).

Limitations: The retrospective, cross-sectional study design is prone to a recall bias.

Conclusion: Oral retinoids are effective in some patients with PC. However, many patients discontinued medication because adverse effects outweighed the benefits. Careful dose titration is warranted in patients informed about potential adverse effects. (J Am Acad Dermatol 10.1016/j.jaad.2011.02.003.)

Key words: keratins; keratoderma; oral retinoids; pachyonychia congenita.
PC is caused by dominant-negative mutations in keratin genes KRT6A, KRT6B, KRT16, and KRT17. As these keratins are expressed in differentiated epithelial structures such as the nail bed, palmoplantar epidermis, and the oral mucosa, these are the affected tissues in PC. The hitherto common division of PC into PC-1 and PC-2 subtypes according to the clinical presentation is being increasingly replaced with genotype-inclusive nomenclature (e.g., PC-K6a, PC-K6b, PC-K16, and PC-K17).

Treatment of PC is notoriously difficult. Because PC is rare (~1:500,000-1:1,000,000), available studies assessing therapeutic regimens are limited to a few case reports and case series. Basic measures include topical emollients, keratolytic agents, mechanical removal of excessive hyperkeratotic skin, and avoidance of physical activity. Among the systemic agents for treatment of PC, the best results have been reported with oral administration of vitamin A derivatives, i.e., retinoids. However, the evidence for their effectiveness is based on anecdotal reports and no systematic retrospective or prospective studies are available. For severe inherited disorders of cornification such as ichthyoses and psoriasis, oral retinoid therapy represents the treatment of choice. In PC, this therapeutic approach is particularly attractive, because in screening assays, retinoids have been noted to suppress mutant keratin expression (W.H. Irwin McLean, DSc, FRSE, oral communication, May 2010). We here present a questionnaire-based retrospective cross-sectional survey of 30 patients assessing effectiveness, adverse effects, and overall patient satisfaction of oral retinoid therapy for PC. Our goal was to establish the benefit/risk ratio, identify favorable dosing regimens, and determine if a future prospective trial for oral retinoid treatment of PC is justified.

METHODS

Patients

All individuals presenting with PC with known mutations in KRT6A, KRT16, or KRT17 who were enrolled in the International Pachyonychia Congenita Research Registry between 2004 and 2010 and previously treated with oral retinoids (acitretin, etretinate, isotretinoin, or vitamin A) at different doses and durations (Table I) were included in this questionnaire-based retrospective cross-sectional study. The study was conducted in accordance with the principles of the Declaration of Helsinki and written informed consent was obtained from all 30 patients before enrollment.

Questionnaire

Questionnaire-based patient scoring was used to evaluate clinical score, satisfaction score, visual analog pain scale, and adverse effects of treatment with oral retinoids by self-assessment. Patients were either interviewed in person or via telephone. The questionnaire items are summarized in Table II.

Statistics

All data were analyzed with software (SPSS, Version 17.0 for Windows, SPSS Inc, Chicago, IL). Statistical differences between groups were determined by using the Mann-Whitney U test with significance conferred when P less than .05. To assess predictors of the effectiveness of drug treatment we estimated odds ratios and 95% confidence intervals (CIs) with logistic regression modeling; this analysis was restricted to the retinoids acitretin and isotretinoin given the small number of cases on other retinoids.

RESULTS

Natural course of plantar hyperkeratoses in PC

As PC is a dynamic disease, 25 of the 30 patients (83%) (Table I) who received oral retinoids reported spontaneous changes in plantar hyperkeratoses while not using any medication. On a scale of −3 (much worse) to +3 (much better) plantar thickening was reported to range between −1.8 and 0.9, with an average change of −0.45, i.e., the majority of patients reported worsening of the disease while not taking any drugs. These results indicate that without medication plantar hyperkeratoses in PC varies over time with little spontaneous improvement.

Treatment effectiveness

Fifteen patients (50%) reported decreased plantar hyperkeratoses, i.e., thinning of calluses, when taking medication (Fig 1). On a scale from −3 (much worse) to +3 (complete improvement) the average

CAPSULE SUMMARY

- Available studies assessing oral retinoid treatment for pachyonychia congenita (PC), a rare keratin disorder characterized by painful plantar keratoderma and nail dystrophy, are limited to a few case reports.
- Our study shows that although treatment of PC with oral retinoids is effective in some individuals with PC, increased pain is a common adverse effect.
- The study presents the benefit/risk ratio of oral retinoids in PC and provides new insight in favorable dosing regimens.
improvement was 1.6 (95% CI 1.2-1.9, \( P < .001 \)).

Although 4 of the 28 patients (14%) for whom data on nail thickening were available reported amelioration of their pachyonychia (thinning of nails, lighter color) with an average improvement of 1.5 on a scale from 3 to 13, the majority of the individuals, ie, 22 (79%), did not experience any change in nail involvement and only two patients (7%) reported worsening of their pachyonychia. The self-reported overall satisfaction score with oral retinoid treatment was greater than or equal to 2 in 15 patients (50%) with a mean of 4.5 on a scale of 1 (lowest) to 10 (highest). Notably, only 7 patients (23%) recommend the use of oral retinoids to others and even fewer, 5 patients (17%), are still using the medication.

**Effects on pain**

Before treatment with oral retinoids, the overall pain when walking was quantified by a visual analog pain scale (0 meaning no pain and 10 the worst pain ever experienced) and reported as 6, which signified dreadful pain. Decreased plantar pain during therapy was reported in 10 patients (33%) whereas 8 patients (27%) experienced increased pain and the remaining 12 patients (40%) did not report any change in their pain.

In the majority of individuals with decreased pain, the improvement occurred within the first 3 to 4 weeks of taking oral retinoids. In the 10 patients with decreased plantar pain, the degree of pain amelioration ranged from 1 to 7 on a scale of 1 (minimal) to 10 (most), with a mean change of 3.4 (95% CI 1.8-5.1, \( P = .001 \)). In the 8 patients who experienced increased pain, the degree of pain worsening ranged from 3 to 10, with a mean change of 7.1 (95% CI 5.4-8.8, \( P < .001 \)).

**Retinoid dosing**

Among the participants of this study taking oral retinoids the dose ranged from 10 to 50 mg/d (Table I). Forty percent of patients who were treated with doses of more than 25 mg/d (higher doses),
reported overall effectiveness and a mean overall satisfaction score of 1.6 on a scale from 1 (lowest) to 10 (highest). In comparison, 73% of patients receiving oral retinoid doses of less than or equal to 25 mg/d (lower doses) reported overall effectiveness ($P = .14$) and a mean overall satisfaction score of 4.2 on a scale from 1 to 10 ($P = .02$). These results suggest that although lower doses are not significantly different in their effectiveness, the ratios among effectiveness, pain, and adverse effects were more favorable with lower doses. Decreased plantar pain during therapy with oral retinoids was reported in 50% treated with higher doses and 67% of patients treated with lower doses ($P = .53$), ie, higher retinoid doses were not superior in reducing pain.

**Treatment duration**

The duration of treatment with oral retinoids ranged from 1 to 240 months, with 50% of the patients receiving therapy for longer than 5 months (longer duration) and 50% less than or equal to 5 months (shorter duration) (Table I). Of patients who were treated for a longer duration, 67% reported overall effectiveness and a mean overall satisfaction score of 3.6 on a scale from 1 (minimal) to 10 (most).

**Table II. Questionnaire**

<table>
<thead>
<tr>
<th>Natural course of PC without treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Did your PC condition change at times when not using any drug treatment?</td>
</tr>
<tr>
<td>• If yes, indicate range of change on scale of −3 (much worse) to +3 (much better), with 0 being your normal condition</td>
</tr>
</tbody>
</table>

**Effectiveness/satisfaction**

<table>
<thead>
<tr>
<th>Was drug effective?</th>
</tr>
</thead>
<tbody>
<tr>
<td>What was your overall satisfaction on scale of 1-10 (1 = lowest, 10 = highest)?</td>
</tr>
<tr>
<td>Are you still using drug?</td>
</tr>
<tr>
<td>Would you recommend drug to others?</td>
</tr>
</tbody>
</table>

**Clinical treatment response**

| While taking drug did your PC calluses/nails change? |
| If yes, did calluses/nails get better or worse? |
| If calluses/nails got better, what was improvement? |
| What was clinical improvement/deterioration in your PC calluses/nails on scale of −3 (much worse) to +3 (complete improvement)? |

**Pain**

| On scale of 0 (no pain) to 10 (worst pain you have ever experienced), rate your overall pain before taking drug |
| While on drug, did you notice change in pain? |
| If yes, did change cause more/less pain? |
| If yes, indicate when change occurred |
| If yes, mark change in your pain using scale of 1 (minimal) to 10 (most) |

**Dose and duration**

| Which oral retinoid did you use (trade name)? |
| If you remember, what dose or strength of medication did you use? |
| How long did you take this medication? |
| Was dose of your retinoid medication changed during treatment? |
| If yes, to higher/lower dose? |
| If yes, why was dose changed? |
| Did change give more improvement? |
| Did change create more adverse effects? |

**Adverse effects**

| Did you experience any adverse effects? |
| Did you experience any dry eyes/dry lips/dry skin/peeling of skin/hair loss/headaches/bone or joint pain/other adverse effects while taking drug? |
| Did your doctor find any liver problems caused by use of retinoid treatment? |
| Did you think improvement you experienced while taking drug was sufficient that you wanted to continue in spite of any adverse effects? |
| Based on your experience, would you be interested in using oral retinoids again? |

PC, Pachyonychia congenita.
a scale from −3 (much worse) to +3 (complete improvement) was 0.7 for the longer duration group versus 0.3 for the shorter duration group ($P = .38$). A decrease in plantar pain was reported in 67% of patients treated for a longer period and 33% treated for a shorter duration, but this again was not statistically different ($P = .19$). Interestingly, the percentage of patients with increased plantar pain was lower in the group with longer treatment duration (33% vs 67%).

**Retinoid classes**

Only patients who had received acitretin (N = 12) or isotretinoin (N = 14) were further compared because of the small number of patients treated with etretinate or vitamin A (Table I). The overall effectiveness was 58% for acitretin and 36% for isotretinoin ($P = .26$), the overall satisfaction score was 3.5 for acitretin versus 2.1 for isotretinoin ($P = .14$). Ninety percent of patients in the acitretin group compared with 57% in the isotretinoin group reported thinning of calluses ($P = .13$) (Fig 1); the overall change on a scale from −3 (much worse) to +3 (complete improvement) was 0.9 versus −0.1 ($P = .05$). In all, 63% versus 50% experienced decreased plantar pain while on acitretin and isotretinoin, respectively ($P = .65$). Although not significantly different, these data indicate that acitretin may have a slight edge over isotretinoin in treating PC.

**Adverse effects**

All study patients experienced adverse effects. With the exception of one man all patients reported dry lips, 15 (50%) dry eyes, 27 (90%) dry skin, 11 (57%) peeling of the skin, 9 (30%) hair loss, 6 (20%) headaches, 5 (17%) bone or joint pain, 4 (13%) sun sensitivity, and one patient depression, fatigue, and developing of bone spurs, respectively. In one patient treatment with oral retinoids was stopped because of liver enzyme abnormalities.

Considering retinoid doses, ie, more than 25 mg/d versus less than or equal to 25 mg/d, the prevalence of adverse effects such as dry lips (90% vs 100%, $P = .29$), dry eyes (20% vs 64%, $P = .05$), dry skin (90% vs 91%, $P = .94$), skin peeling (50% vs 36%, $P = .54$), hair loss (20% vs 55%, $P = .11$), headaches (20% vs 18%, $P = .92$), and bone/joint pain (20% vs 36%, $P = .42$) was comparable. There was no difference in adverse effects relative to treatment duration and there was also no significant difference in adverse effects when comparing acitretin with isotretinoin.

Nine patients (30%) decided to continue treatment with oral retinoids despite adverse effects because they perceived PC improvement as sufficient. Based on their experience, 14 patients (47%) would be interested in using oral retinoids again, primarily because of the overall effectiveness they experienced. Sixteen patients (53%) would not use oral retinoids again. However, the question used for this assessment (Table II) did not address in detail...
why patients stated that they would or would not want to use oral retinoids again.

**Predictors of effectiveness**

To identify additional patient subsets that might be more likely to benefit from oral retinoid treatment, we used logistic regression modeling. The calculated odds ratios were 0.13 for female versus male (95% CI 0.02-0.89), 1.6 for acitretin versus isotretinoin (95% CI 0.3-9.6), and 0.7 for age, per 10 years (95% CI 0.4-1.5). These results indicate that neither patient age nor retinoid type were predictors of effectiveness. In contrast, a benefit from oral retinoid treatment was less likely in female than in male patients.

**DISCUSSION**

The few available case reports and case series assessing therapy of PC with oral retinoids yielded contradictory results with a tendency toward more favorable outcomes with treatment. Dupré et al8 asserted improvement of PC calluses and decreased pain in 3 patients treated with an aromatic retinoid, Hoting and Wäsilew9 reported a remission of palmo-plantar hyperkeratosis but no changes in pachyonychia in two patients treated with etretinate 75 mg/d and a relapse when reducing the drug to 30 mg/d after several months, Carabott et al10 described a patient who experienced reduced plantar hyperkeratosis after 3 months of etretinate therapy at a dose of 50 mg/d, and Lim et al11 reported amelioration of calluses but not of nail changes in a patient treated with 30 mg acitretin daily. In contrast, two additional case reports did not show any clinical benefit for oral retinoids in the treatment of PC. Thomas et al14 reported a father and his son who did not show improvement of plantar keratoderma despite therapy with high doses of isotretinoin. Similarly, Soyuer and Candan15 described failure of etretinate in a child with PC, but the treatment was only administered for 5 weeks, and the dose had to be progressively lowered because of hypertriglyceridemia. Consistent with the contradictory findings in these published reports, in the current study involving 30 patients from the PC registry, oral retinoids resulted in thinning of calluses (decrease in hyperkeratosis) in only a subset of study patients.

A very important aspect of PC treatment is that thinning of calluses does not necessarily imply decreased plantar pain when walking, as reported for other types of palmo-plantar keratoderma. In a family with keratoderma of the soles associated with blistering but lack of pachyonychia, treatment with isotretinoin resulted in callus reduction, but blistering worsened and pain increased.16 This was also observed by Fritsch et al,17 who described a dramatic improvement of hereditary epidermolytic palmo-plantar keratoderma in 4 patients treated with an oral aromatic retinoid for 5 months, resulting in normal-appearing skin. However, therapy had to be discontinued as the vulnerability and sensitivity restricted normal function of hands and feet. In the current study roughly only one third of patients experienced improvement of pain although 50% of the patients reported improved plantar hyperkeratosis, ie, in some cases even though calluses thinned, there was increased pain.

Recently, mutations in KRT6, KRT16, and KRT17 were correlated with characteristic clinical findings in patients with PC; ie, KRT6B was associated with increased pain intensity.18 When stratifying our patients by genotypes, because of the small subgroups, no further analysis regarding treatment effectiveness was possible.

This study has several limitations including its retrospective, cross-sectional study design, which is prone to a recall bias. Although the measurements were patient-based and subjective, ie, not assessed by a physician, the study end points are patient-centered in a positive sense, in that they should be highly relevant for reflecting patient perception of treatment. The lack of laboratory monitoring for potential adverse effects such as liver function testing may result in an overestimation of the benefit/risk ratio of the treatment modality, ie, our study design is biased to exclude patients with severe adverse effects. In the current study, discontinuation of oral retinoid therapy was only necessary in one patient because of elevated liver transaminases. This is in accordance with two previous studies, in which similar retinoid doses have been used for the treatment of various forms of ichthyoses and psoriasis, and no severe adverse effects were reported.12,13

This study demonstrates a potential advantage of treatment with lower doses of acitretin for a longer duration compared with therapy with higher doses, shorter duration, and isotretinoin. Recently it was shown that in the treatment of patients with psoriasis low-dose acitretin (25 mg/d) was associated with fewer common adverse effects than high-dose acitretin (50 mg/d).13 Because lower doses may have a better risk/benefit ratio, it might be beneficial to begin treatment at a lower dose (eg, acitretin 10-25 mg/d) with further dose adjustments based on patient’s response. Alternatively, treatment may be initiated at a higher dose and subsequently adjusted depending on pain and adverse effects. It is important that patients are fully informed about potential adverse effects before initiation of therapy including the possibility of increased pain when on oral retinoids.
In conclusion, the results of our study confirm that treatment of PC with oral retinoids is effective in some individuals with PC. Randomized, controlled, prospective clinical trials with both objective and patient-centered subjective end points are warranted to further define the patient subsets that most benefit from this treatment option.

We are indebted to the participating patients, and to Mary Schwartz and the members of the PC Project Medical Scientific Advisory Board for their valuable advice.

REFERENCES
The Phenotypic and Molecular Genetic Features of Pachyonychia Congenita

W.H. Irwin McLean¹, C. David Hansen², Mark J. Eliason² and Frances J.D. Smith¹

Pachyonychia congenita (PC) is an autosomal dominant genodermatosis caused by heterozygous mutations in any one of the genes encoding the differentiation-specific keratins K6a, K6b, K16, or K17. The main clinical features of the condition include painful and highly debilitating plantar keratoderma, hypertrophic nail dystrophy, oral leukokeratosis, and a variety of epidermal cysts. Although the condition has previously been subdivided into PC-1 and PC-2 subtypes, the phenotypic characterization of 1,000 mutation-verified PC patients enrolled in the International PC Research Registry, coordinated by the patient advocacy group PC Project, shows that there is considerable overlap between these subtypes. Thus, a new genotypic nomenclature is proposed, in which PC-6a represents a patient carrying a mutation in the K6a gene, etc. Although a rare disorder, PC represents a good model for therapy development, and international efforts are ongoing to develop and deliver siRNA, gene, correction, small molecule, and other strategies to treat this painful, disabling skin condition. The special relationship between PC Project and the PC research community has greatly accelerated the development pathway from gene identification to clinical trials in only a few years and represents a paradigm of hope for other orphan diseases.

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INTRODUCTION

Pachyonychia congenita (PC) is an uncommon autosomal dominant disorder of keratinization caused by mutations in any one of a number of keratin genes that are expressed in differentiated epithelial tissues. The condition was first described in the early twentieth century (Jadassohn and Lewandowski, 1906; Jackson and Lawler, 1951) but it was not until the early 1990s, with the emergence of molecular genetics technology, that the causative gene in a large Scottish PC family was mapped to one of the keratin gene clusters (Munro et al., 1994). Shortly thereafter, the causative mutations were identified in several PC patients in the KRT6A, KRT6B, KRT16, and KRT17 genes, encoding the keratin proteins K6a, K6b, K16, and K17, respectively (Bowden et al., 1995; McLean et al., 1995; Smith et al., 1998).

Keratins are the intermediate filament proteins specifically expressed by epithelial cells, in which they form a dense cytoplasmic network (Irvine and McLean, 1999; Omary et al., 2004). The primary function of the keratin cytoskeleton is to impart mechanical strength and resilience to epithelial cells and tissues. Disruption of this cytoskeletal system due to a genetic mutation in a keratin gene leads to extreme fragility of the epithelial cells and tissues in which the mutated keratin is expressed. Similar to several other keratin disorders, the vast majority of causative mutations in the PC-related keratins are heterozygous missense mutations or small insertion/deletion mutations that disrupt cytoskeletal function via dominant-negative interference and lead to epithelial cell fragility (McLean et al., 2005). In PC, this is manifest as cytolysis and hyperkeratosis in the subset of differentiated epithelial tissues in which K6a, K6b, K16, and K17 are predominantly expressed (Lane, 1993), specifically the palmpoplantar epidermis, nail bed, mucosae, and the pilosebaceous unit. Thus, the cardinal phenotypic features of PC are palmpoplantar (predominantly plantar) keratoderma; hypertrophic nail dystrophy; oral leukokeratosis; and a variety of cysts arising from hyperkeratosis of pilosebaceous apparatus (Figure 1).

A MOLECULAR CLASSIFICATION FOR PC SUBTYPES

In 2003, a patient advocacy group—Pachyonychia Congenita Project—was established to support those affected by PC and to both encourage and fund research into a cure for the condition (www.pachyonychia.org). To achieve this goal, the International PC Consortium (IPCC) was founded in early 2004. This is a group of clinicians and scientists actively researching the causes of PC and importantly, the development of new treatments for PC. The IPCC has met annually since 2004 and its membership is listed at www.pachyonychia.org.

An important part of the ongoing PC research program is the International PC Research Registry (IPCR), in which detailed phenotypic data are collected from patients and linked to genetic data. At the time of writing, close to 1000 PC patients have been identified by the PC Project. This has
led to the largest collection of linked clinical and genetic information yet assembled for a rare keratin disorder. Historically, PC has been split into two subtypes (PC-1, or Jadassohn–Lewandowski subtype; and PC-2, or the Jackson–Lawler subtype) on the basis of subtle differences in phenotype, primarily the presence or absence of pilosebaceous cysts (Jadassohn and Lewandowski, 1906; Jackson and Lawler, 1951). At present, with analysis of hundreds of patients in the IPCRR, limitations in the older classification, which was based on only a handful of non-genotyped cases, have become clear. In particular, many PC patients, regardless of genotype, have some form of epidermal cysts (see Wilson et al., 2011). Therefore, on the basis of the more comprehensive IPCRR data, a more rational and useful classification based on the mutated gene was proposed at the 2010 IPCC Symposium and has been adopted throughout the research papers in this issue of the *JID*. The new classification is PC-6a, PC-6b, PC-16, and PC-17, for a patient with a mutation in the gene encoding K6a and others proteins (the complete data set underlying this new nomenclature will be published elsewhere; Eliason et al., unpublished data). In cases in whom PC is suspected but no mutation has been found (or not looked for), the term PC-U (for unknown) will be used.

The predominant symptom in PC is plantar pain

Although hypertrophic nail dystrophy is the phenotypic feature that gave rise to the name of the condition, the most problematic symptom reported by PC patients is focal plantar keratoderma that is associated with severe pain. The plantar pain in PC is often highly debilitating and has considerable negative effect on quality of life. The reason for the pain is not fully understood but is thought to be related to blister formation deep underneath the thick callus that develops over the pressure points of the plantar surface (see Figure 1a). Plantar blistering, together with accompanying pain, is a common feature of PC that is under-reported in the literature (Eliason et al., unpublished data).

Nail dystrophy (Figure 1b), which can occur from a very early age, is variable in severity and in many cases not all 20 nails are affected. Toenails are more commonly affected than fingernails, which could be because of greater trauma exerted from shoes. Another feature of PC is oral leukokeratosis (Figure 1c). This is often one of the first signs of PC in babies and may lead to difficulty in feeding and is often mistaken for candidiasis in infants. Follicular keratoses are present in many cases of PC. Some individuals also develop cysts in the form of steatocysts (steatocystomas) and/or pilosebaceous cysts (Figure 1d). This feature is particularly associated with patients with a K17 mutation (see Wilson et al., this issue), in whom sometimes it is necessary to remove cysts surgically. The severity of the clinical features of PC can vary quite widely both among and within families. This may partly be because of individual lifestyle and care of PC and could also be because of the specific type of mutation, as well as other genetic and/or environmental factors.
Rapid therapy development in PC
Although it is a rare condition, PC is at the forefront of genetic therapy development in the dermatology field. In particular, the dominant-negative genetic mechanism in PC contributes to therapeutic strategies based on RNA interference (RNAi), especially in the form of short interfering RNA (siRNA). It has been demonstrated that mutant keratin alleles differing from wild type by a single-nucleotide point mutation can be potently and specifically silenced by carefully designed siRNA (Hickerson et al., 2008). This mutation-specific siRNA therapy approach has been progressed into the recently reported small-scale human clinical trial, in which efficacy was demonstrated (Leachman et al., 2010). This was the first time that siRNA had been used to treat a human skin disorder. The keratins involved in PC also show considerable functional redundancy, in particular when K6 is involved. Humans have three copies of a nearly identical KRT6 gene, encoding the K6a, K6b, and K6c proteins. Mouse knockout experiments strongly suggest that loss of one of these keratins may be tolerated (Wong et al., 2000; Wojcik et al., 2001) and therefore an alternative therapeutic approach would be to completely silence the defective keratin, regardless of mutation. To this end, highly potent gene-specific siRNA has been developed for PC (Smith et al., 2008). The major technical hurdle yet to overcome in both these therapeutic approaches is the development of a safe, effective, and patient-friendly method for routine delivery of siRNA into the epidermis. This is currently a major goal of the IPCC research groups, in addition to development of alternative therapies that include gene correction methodology, small molecule therapy, and other strategies (Kaspar et al., this issue).

CONCLUSION
Over the past 7 years, the PC research field represents a great example of how a small group of highly motivated patients and their families, together with a group of interested clinicians and scientists, can rapidly progress research from knowing only the identity of a gene defect to having new therapies that show efficacy in cells, in animal models, and in patients. Hopefully, this sustained, highly focused effort will shortly lead to a successful and widely applicable treatment for PC and the lessons learned along the way can be translated to other genetic skin disorders.

CONFLICT OF INTEREST
Dr McLean and Dr Smith filed a patent on therapeutic siRNA for PC. The other authors state no conflict of interest.

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Clinical and Genetic Features of PC
Pachyonychia Congenita Project
A Partnership of Patient and Medical Professional

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ABSTRACT: A rare disease like pachyonychia congenita (PC) poses barriers to the patient, medical professional, and scientist. The patient has challenges connecting to information, the medical professional has challenges connecting to patient experience, and the scientist has challenges connecting to a sufficient number of patients to do meaningful research. Recent collaboration between these groups has transformed our understanding of PC and its symptoms and method of diagnosis. PC Project is at the center of this collaboration and is providing new insights for the dermatologist and dermatology nurse, enabling better diagnosis of PC and counseling of a PC patient. The PC patient, medical professional, and scientist have an international advocate in PC Project, a patient-led, nonprofit project committed to connecting all these communities to the tools they need to improve the lives of those living with PC.

Key words: Blisters, Cysts, Keratoderma, Nail dystrophy, Pachyonychia, Partnership

“I was one proud mama! I watched my son hand PC brochures out to people (at our PC Awareness Day Event). When they asked questions, he answered them. If my grandmother was still alive she would be so proud to know how far we’ve come. No longer are we sitting on fingers at the dinner table, hiding our PC as she did…instead we are sharing with anyone information about PC, proud that we have an organization that stands by us. And, most importantly, we are not alone with PC. It may be rare…but we have met friends with it thanks to last year’s PC Patient Support Meeting. We are not alone!” (Jessica, pachyonychia congenita [PC] patient).

These words of joy reflect the power that can come from shared experience—knowing you are part of a larger community. This joy of knowing can be especially empowering when living with a rare disease like PC.

PC is an ultra-rare skin disorder that affects 2,000–10,000 people worldwide. The rarity of PC means that a person with PC usually never meets anyone else with PC other than affected family members. It means that researchers are challenged to find a sufficient number of PC patients to do meaningful research. It means that medical professionals do not have easy access to broad patient experience on which to base diagnosis or to learn if a treatment really works.

In 2004, PC Project [a patient-led 501(c)(3) charity based in Salt Lake City, Utah] set out to eliminate these barriers of isolation by creating an international collaborative network of patients, medical professionals, and scientists to be a catalyst to find effective treatments for the disease. Using high-technology tools, including custom software for the PC registry and database, an interactive Web site (www.pachyonychia.org), Webinars, and Web meetings (as well as E-mail, conference calls, and VoIP services or Skype), PC Project has connected researchers interested in keratin disorders, multidisciplinary specialists, and dermatologists to conduct basic and clinical research. This group of specialists is known as the International PC Consortium (IPCC). PC Project has also identified and recruited patient volunteers to provide personal histories and has in turn provided physician consultations and
genetic testing for these patients through the International PC Research Registry (IPCRR). PC Project continues to seek dermatologists and dermatology nurses to be partners in this international community.

Through this collaborative effort, researchers have made several discoveries that have transformed the understanding of PC and treatment for PC. These new insights have significance for the dermatologist and dermatology nurse in diagnosing and counseling a PC patient.

PC: WHAT WE HAVE LEARNED

The disease is characterized by accumulation of keratin in the skin and nails, which manifests as calluses, thickened nails, and cysts (see Figures 1–3). Under the calluses are extremely painful blisters on the soles and sometimes on the palms. Pain is one of the most consistent features of PC and a key in differential diagnosis (Eliason, Leahman, Feng, Schwartz, & Hansen, 2012). PC is caused by a single mutation in one of at least five keratin genes, KRT6A, KRT6B, KRT6C, KRT16, or KRT17 (Akasaka et al., 2011; Wilson et al., 2010, 2011). As PC is an autosomal-dominant disorder, there is a 50% chance of passing on the mutation with each conception. However, more than 45% of PC cases appear spontaneously where there is no family history of the disease.

The condition was first described in the early 20th century (Jadassohn & Lewandowski, 1906) as “pachyonychia,” which means “thickened nails.” However, PC refers to a spectrum of symptoms, which is determined by both the location and the nature of the causative mutation as well as by yet unknown additional genetic and environmental factors (which explain the fact that individuals carrying the very same mutation can display divergent clinical features). From detailed questionnaires gathered from nearly 500 patients participating in the IPCRR, those with genetically confirmed PC are consistently found to have a triad of features including nail dystrophy, palmar/plantar keratoderma (e.g., thickening of the skin), and pain.

PC shares symptoms with a number of disorders like epidermolysis bullosa simplex and some connexin disorders (e.g., Clouston syndrome). In PC, the unrelenting pain from blisters under the thick calluses on the soles of the feet is the principal life-altering feature for most with this disease. The callus usually begins when the child with PC first begins to walk, and the pain is usually constant by age of 10 years. It requires regular trimming of the callus and activity planning to limit time on one’s feet. Many must use canes, crutches, or wheelchairs or must crawl on their knees to manage the pain. In some forms of PC, the prevalent cysts are the most painful feature.

Documenting the variability of the symptoms across mutations and genes has led to a new classification system of PC to help clinicians improve their diagnostic and prognostic accuracy and improve therapeutic development. Rather than refer to PC-1 and PC-2, which inaccurately groups PC patients, the new nomenclature, a diagnosis of

![Figure 1](image-url)
PC-K6a, PC-K6b, PC-K6c, PC-K16, and PC-K17, corresponds to mutations in the KRT6A, KRT6B, KRT6C, KRT16, and KRT17 genes, respectively, and PC-U indicates those with an unconfirmed genetic mutation (McLean, Hansen, Eliason, & Smith, 2011).

THE ROLE OF THE DERMATOLOGY NURSE

Although there is no effective treatment for PC at this time, the dermatology nurse can be a very helpful partner to the PC patient by helping with diagnosis, care techniques, and provision of accurate information. Those with PC may require medical assistance when they experience an infection of the nails or cysts. A good relationship with a dermatology nurse can be extremely beneficial to patients with PC.

Also, the dermatology nurse can effectively assist the patient in participating in the IPCRR. Through the IPCRR, the patient can obtain genetic testing to determine whether the patient has a mutation in one of the genes associated with PC. Details regarding the gene and the specific mutation are provided with the test results, and genetic counseling is available. If the patient is found not to have PC, genetic testing is conducted for a number of other similar conditions with the overall goal to assist the patient and medical providers with a definitive answer regarding the condition. All PC Project services are provided at no cost to the patient and referring specialist.

The IPCC experts welcome the opportunity to support the nurse and/or doctor in diagnosis, treatment options, and care. The dermatologist or dermatology nurse may contact us directly or refer the patient to PC Project. Individual consultations for patients, physicians, and dermatology nurses treating PC patients may be scheduled with physicians serving on the PC Project Medical and Scientific Advisory Board.

Once the diagnosis of PC is confirmed, the PC patient’s de-identified data will be made available to researchers around the world (while preserving patient privacy and anonymity), and the patient will be eligible to participate in future studies and clinical trials.

Diagnosis

It is not uncommon for a person to reach his or her 40s or 50s before getting a correct diagnosis. Data from patients participating in the IPCRR have now revealed that plantar pain is a key diagnostic clue. In a recent study of 254 PC patients, plantar pain was reported by 225 of 254 (89%) surveyed patients. Only three patients older than 10 years old did not report plantar pain. The researchers concluded that plantar pain is the most important feature of PC affecting quality of life. The pain is related to the underlying blisters, and the thickness or extent of the visible callus may not reflect the degree of pain a patient experiences (Eliason et al., 2012).

Main Features of PC

Because of the IPCRR, the clinician who only rarely encounters these disorders now has better guidance and a more robust framework to make a clinical diagnosis (Irvine, 2012, p. 1758).
• Nail dystrophy (or thickening) often does not affect all 10 fingernails or all 10 toenails.
• Only toenails may be affected with fingernails remaining normal.
• Calluses on the soles of feet have underlying hidden blisters and are usually extremely painful with plantar pain being the number one concern for PC patients.
• Cysts are the second most common cause of pain in PC, and pain may also be from infected nails or follicular hyperkeratosis.
• Both steatocystoma and pilosebaceous cysts are found in nearly all types of PC, although those with PC-K17 have a greater number of steatocystomas.
• Nail infections may be initiated by nail trauma or excessive trimming.

Other Features of PC

• Follicular hyperkeratosis (most prominent in children and young teens)
• Oral leukokeratosis (often misdiagnosed as thrush or leukoplakia)
• Prenatal or natal teeth (usually PC-K17)
• Acute pain related to “first bite syndrome” (often misdiagnosed as “ear” pain)
• Laryngeal problems: thickening or nodules on the vocal chords causing hoarseness or, sometimes, difficulty in breathing (Treatment of the larynx may cause increased overgrowth but may sometimes be necessary to avoid obstruction.)

The rarity of the disease and its overlapping symptoms with other skin disorders make diagnosis a challenge. Commonly, people with PC may be misdiagnosed with onychomycosis or fungal infection of the nails or with other causes for palmar/plantar keratoderma. At the same time, people with only thickened or dystrophic nails may be told that they have PC. Furthermore, previously misattributed features such as deafness, mental retardation, diabetes, bony abnormalities, early menarche, corneal lesions, and cataracts can be safely excluded from the canon of PC manifestations (Irvine, 2012, p. 1758).

Genetic testing can confirm the clinical diagnosis of PC and is the only way to verify that the patient’s condition is in fact PC and not a related disorder.

Treatment

Review of the experience of PC patients in the IPCRR has shown that, although traditional therapeutics such as urea, salicylic acid, or oral retinoids may soften or reduce calluses, most patients abandon those therapies for lack of sufficient benefit (Elaison et al., 2012). When oral retinoids were recently assessed, findings show that, for most patients, there was no benefit and, instead, increased pain or the adverse side effects outweighed the benefits (Gruber et al., 2011). Because we cannot predict which patients may benefit from oral retinoids, an empirical trial with variable dosing is being evaluated as well as a topical retinoid application for PC.

Patients typically manage their own symptoms through careful mechanical trimming of the calluses to ensure a “not too thick and not too thin” result. Aggressive debridement may increase pain. Frequent filing and trimming of the nails is also necessary. Patients who experience PC with numerous cysts find that these must be drained or the cysts may need to be surgically removed because of pain or infection.

The results of a formal survey of patient experience with treatment and self-care will also be published soon. The survey collected detailed responses on treatment and care used by PC patients for specific conditions including keratoderma; cysts; follicular hyperkeratosis; and fingernails, toenails, and nail infections. The results will be published shortly and will provide a good reference for both the dermatologist and dermatology nurse.

Patients may need one or more medical services on a regular basis including quick access to prescriptions for antibiotics when infections arise, removal of cysts, pain medication, or other care related to the varied symptoms of PC. The dermatology nurse/dermatologist may need to help the PC patient find treatment for one or more of these issues.

PC Project: A Resource for the PC Patient and Medical Professional

Major benefits for PC patients and medical professionals are available through the PC Project, the IPCRR, and IPCC members. Participation in the IPCRR provides access to clinical trials, information on care techniques, publications from dermatology journals, annual patient support meetings, and Webinars. Through the IPCC, medical professionals have a team of support and information for their patients’ care.

Through the Web site (www.pachyonychia.org), both the patient and medical professional can stay informed about opportunities to participate in educational meetings, Webinars, and clinical trials. The Web site features the latest research news, extensive images, a complete bibliography of scientific articles, patient education brochures, a quarterly scientific newsletter, and a monthly news brief as well as an annual report. PC Project also maintains a Facebook page through which PC patients in the IPCRR can connect with each other.

Patient success with various care techniques does vary. To help PC patients find what may work for them, PC Project has catalogued patient reports into a PC Wiki section available on the Web site. Techniques used to manage the symptoms of PC are shown on a “Caring For PC” DVD, which is available upon request. The DVD and all printed materials are provided at no charge to patients and medical professionals.
The annual PC Patient Support Meeting (in Europe or the United States) is an especially important way a PC patient can meet, connect, and share with others who have PC. To help every PC patient who wants to attend, PC Project offers scholarships to patients in the IPCRR to attend the meeting.

To learn directly from physician experts about PC, interested dermatology nurses can sign up for one of the quarterly educational Webinar sessions sponsored by PC Project. There are also educational brochures specifically for medical professionals. In addition, for patients in the IPCRR, medical experts are available to provide counseling with a physician or nurse to individual patients.

We recognize that most medical professionals will rarely see a PC patient, so PC Project has set up a resource for those medical professionals wanting to confirm diagnosis or get the latest advice on management of the disease or clinical trials that may be ongoing.

WORKING TO CHANGE THE FUTURE: MAINTAINING RESEARCH MOMENTUM

PC Project is collaborating on a number of research strategies to develop an effective PC treatment. One of these research strategies is gene silencing. By inactivating the mutant keratin gene, researchers hope to eliminate PC symptoms. PC Project, through its biotech partner TransDerm, has completed a Phase Ib, FDA-approved clinical trial of a gene silencing strategy using siRNA therapy (Leachman et al., 2010). A second clinical trial of an improved siRNA developed by TransDerm will begin in 2013 (Hickerson et al., 2011). This second trial will involve use of dissolvable microneedles developed to deliver drugs in a more patient-friendly manner (Gonzalez et al., 2010). Two additional clinical studies (oral statins and topical rapamycin) will also enroll patients in 2013.

PC Project provides fellowship and grant funding to a variety of universities for the support of PC research. Since 2004, when the IPCC was formed with 23 physicians and researchers, PC Project has recruited 150 members of the consortium, each with a pledge to collaborate to improve treatment for PC. A number of ground-breaking basic and clinical research reports have been published in the last couple of years by members of the IPCC in leading dermatology journals. These reports are accessible to everyone through a searchable bibliography on the PC Project’s Web site or can be requested on a CD or in booklet format.

Topics of articles, which will be published shortly based on the results of current studies, include PC best practices, life history of PC, pathology of PC nails, pathology of PC cysts, and over 50 additional case studies including novel mutations as well as a revised PC summary article. The National Institutes of Health Office of Rare Disease Research and National Institutes of Health/National Institute of Arthritis and Musculoskeletal and Skin have been very helpful to PC Project in our research and outreach efforts.

RAISING RARE DISEASE AWARENESS

As PC is ultra-rare, the public and medical community are generally not aware of PC or the challenges it can present. This lack of awareness contributes to the isolation of many PC patients. The empathy and understanding of the general public will create a more supportive environment for those experiencing rare diseases like PC and generate more public support of rare disease research.

Raising funds for rare disease research and patient support has been challenging in terms of recruiting volunteers and supporters. However, because of the profound impact this disease can have on the life of both children and adults, parents, spouses, and family members are highly motivated to help improve their future. PC Project launched its first annual PC Awareness Day in 2012 to begin connecting and empowering the PC patient community in its support of public awareness. This first international effort resulted in dozens of local events in 10 countries and reached thousands around the world through print, TV, and radio media. PC Project just completed a 5- to 6-minute public awareness video on PC that can be found on YouTube. PC Awareness Day provided a new source of funds as well as an opportunity to increase awareness of PC. All 2012–2013 donations are matched $2-for-$1 by an anonymous individual sponsor, which triples each dollar received.

PC Project participates with other nonprofit groups, which specialize in areas important to PC research and patient support. These include Genetic Alliance (focuses on genetic disorders), National Organization for Rare Disorders (focuses on rare disorders), and the Coalition of Skin Diseases (focuses on skin disease research and patient advocacy). The American Academy of Dermatology and the Society of Investigative Dermatology are important partners in the effort to raise awareness and provide support for skin disease research. The best part of PC Project is the partnerships—helping people connect with each other and to connect the patients and scientists. PC Project was started to help one person, but now, it serves and unites more than 1,000 people with PC in over 50 countries. People with PC need to know that, by sharing information and working with others in the PC community, they can advance research and change the future. The growing number of patients, physicians, and scientists in our network of collaborators will move us more rapidly to an effective, patient-friendly treatment.

REFERENCES


Original Investigation

Pachyonychia Congenita in Pediatric Patients
Natural History, Features, and Impact

Sonal Shah, MD; Monica Boen, MD; Brandi Kenner-Bell, MD; Mary Schwartz, PhD; Alfred Rademaker, PhD; Amy S. Paller, MD

IMPORTANCE Nail dystrophy in early childhood often suggests a diagnosis of pachyonychia congenita (PC). No previous investigation has focused on the early signs of PC and the natural course of the disease.

OBJECTIVES To determine the course of pediatric PC, correlate the disease course with the clinical appearance and specific gene mutations, and assess the effect of pediatric PC on quality of life.

DESIGN, SETTING, AND PARTICIPANTS One hundred one patients or families with genetically confirmed PC from the International Pachyonychia Congenita Research Registry who completed a survey on the general clinical features of PC and an auxiliary questionnaire on the clinical presentation and quality-of-life issues related to pediatric PC.

EXPOSURE Individuals with pachyonychia congenita.

MAIN OUTCOMES AND MEASURES Completion of both surveys.

RESULTS At birth, toenail changes were present in 47.5% of patients; fingernail changes in 40.6%; and plantar keratoderma in 6.9%. By 5 years of age, these 3 key manifestations were found in 81.2%, 74.2%, and 75.3%, respectively, of individuals with genotype-confirmed PC. The correct diagnosis was made during the first year of life in 26.7% of patients despite the presence of toenail dystrophy in more than 65.3%. Clinical differences that distinguished PC subtypes included (1) later onset and less frequent occurrence of nail dystrophy and keratoderma in PC-K6b, PC-K6c, and PC-K16; (2) concurrent fingernail and toenail thickening in PC-K6a and PC-K17; (3) more palmar keratoderma in PC-K16; (4) cysts primarily in PC-K17 and follicular hyperkeratoses primarily in PC-K6a; (5) hoarseness and/or oral leukokeratoses in the first year of life most often in PC-K6a; and (6) natal teeth exclusively in PC-K17. Among pediatric patients, PC affected the social interactions and function of adolescents most profoundly.

CONCLUSIONS AND RELEVANCE Among patients with a detectable mutation, PC manifests with nail thickening and plantar keratoderma before school age in more than three-quarters of affected children, allowing early diagnosis. The highly visible nail changes and painful plantar thickening exert a psychosocial effect on most affected adolescents. Phenotype-genotype correlations in children with PC validate the new classification based on the affected gene.
Pachyonychia congenita (PC) constitutes a group of almost exclusively autosomal dominant disorders of paired keratins of the nails and skin. Since PC was initially reported in 1906 by Jadassohn and Lewandowsky,1 more than 500 cases have been registered or otherwise described. Pachyonychia congenita manifests as nail dystrophy, painful focal palmoplantar keratoderma, follicular keratoses, mucosal leukokeratoses, hoarse voice, cystic lesions, and, rarely, natal teeth.2,3

Underlying keratin gene mutations have been described in the 5 keratin genes, KRT6A (OMIM 148041), KRT6B (OMIM 148042), KRT6C (OMIM 612315), KRT16 (OMIM 148067), and KRT17 (OMIM 148069), which alter keratins 6a, 6b, 6c, 16, and 17, respectively.4-9 These genes are expressed in the nail bed, palmoplantar epidermis, and mucosa. Keratins play a key role in epidermal cell integrity and mechanical strength. Mutations in these 5 keratin genes cause epidermolysis and compensatory hyperkeratosis at these sites. Historically, 2 major subtypes were based on clinical characteristics. The Jadassohn-Lewandowsky PC type 1 (PC-1) often showed associated oral leukokeratoses,1 and the Jackson-Lawler PC type 2 (PC-2) often showed cysts and occasionally natal teeth.3,10 Pachyonychia congenita genotype 1 was originally linked to mutations in the type II keratin gene KRT6A and its type I expression partner KRT16 and PC-2 with mutations in KRT6B and KRT17.3,4,6,8,11

Pachyonychia congenita has been genotyped at no cost in individuals who enroll in an international registry, enabling comprehensive genotype-phenotype analysis.12-16 Phenotypic overlap among PC genotypes has now made obsolete the Jadassohn-Lewandowsky PC type 1 (PC-1) often showed associated oral leukokeratoses,1 and the Jackson-Lawler PC type 2 (PC-2) often showed cysts and occasionally natal teeth.3,10 Pachyonychia congenita genotype 1 was originally linked to mutations in the type II keratin gene KRT6A and its type I expression partner KRT16 and PC-2 with mutations in KRT6B and KRT17.3,4,6,8,11

Features of PC usually manifest during the first 3 years of life,17 allowing the diagnosis to be considered. However, little attention has been paid to the disease course, early diagnostic features, and effect on quality of life of PC in children. To facilitate early diagnosis and increase our understanding about the impact of PC in children, affected families and patients were polled about pediatric-specific issues.

Results

Of 254 patients enrolled in the IPCRR who completed the original questionnaire and had genetically confirmed PC, 101 responded to the addendum questionnaire (response rate, 39.8%). The demographic features of these 101 PC patients and the 8 PC-K6c patients who responded to the original questionnaire are described in Table 1. Of the returned questionnaires, 78.0% were completed by adult patients who were able to report the onset of features and impact of PC throughout the first 18 years of life, whereas 22.0% of the returned questionnaires reflected the experience to date of affected individuals currently in their first 2 decades of life. Among the respondents, 42.2% had mutations in KRT6A (PC-K6a), and an additional 28.4% had mutations in KRT16 (PC-K16). Almost 60% in the registry had a family history of PC, reflecting the high rate of spontaneous mutation in keratin genes. The diagnosis of PC by a physician was made before 1 year of age in 27 patients (26.7%, of whom 12 [44.4%] had a known family history) despite the presence of toenail and fingernail dystrophy in approximately 60% of the affected infants (see below). By 5 years of age, the correct diagnosis was made in 43 patients (42.6%, of whom 18 [41.9%] had a known family history) despite toenail dystrophy in 81% (82 of 101), fingernail dystrophy in 74% (75 of 101), and plantar keratoderma in 75% (76 of 101).

Toenail Dystrophy

The earliest and most common clinical characteristic of PC was toenail dystrophy (Figure 1A), noted to involve at least 2 digits in 98.2% of patients by the time of reporting; the only ex-
ception was absence of toenail dystrophy in 2 of the 8 PC-K6c patients (25.0%) (Table 1). Of the patients with toenail involvement, all 10 toenails were affected in 74.3%, 98.0% had involvement of toenails of the fifth digit, and 98.0% had changes of the hallucal nails by the time of reporting. Toenail abnormalities were present at birth in 39.0% of respondents overall, although they appeared most often in neonates with PC-K6a and PC-K17 (P < .001) (Supplement[eFigure, A]). By 1 and 5 years of age, nail dystrophy was noted in 65.3% and 80.2% of all respondents, respectively. By 5 years of age, all 46 children with PC-K6a, the most common subset, showed toenail changes. In contrast, toenail changes did not appear in children with PC-K6b until at least 1 year of age and progressively increased in occurrence thereafter. In 99.0% of patients with toenail dystrophy, more than 1 nail became dystrophic concurrently, and 70.3% had all 10 nails become dystrophic at the same time; patients with PC-K6a were more likely to have all 10 toenails affected at onset than were patients with PC-K16 or PC-K6b (P < .001). The hallucal nail was the most common of the toenails to first become affected (89.1% of PC patients with toenail dystrophy). The most common initial toenail change was thickening (77.2% of all patients), and 65.3% also showed toenail discoloration at onset. Toenail thickening progressed throughout the first decade of life in most of the patients. The nail dystrophy occurred before 6 years of age in 5 of the 6 PC-K6c patients with this feature (83.3%), but the dystrophy was mild, affecting only 1 toenail bilaterally (usually the fifth toenail) or, in 1 case, multiple toenails unilaterally. The 2 PC-K6c patients without nail involvement were adults.

Fingernail changes occurred overall in 76.1% of patients and, as with toenail changes, were most severe in PC-K6a. Overall, 40.6% of patients had fingernail changes at birth. Finger-
nail involvement occurred in most of the neonates with PC-K6a and PC-K17 but infrequently in neonates with PC-K16 and never in neonates with PC-K6b (P < .001) (Supplement eFigure, B). Fingernail changes never developed in 5 patients with PC-K6b (50.0%), 11 patients with PC-K16 (35.5%), 1 patient with PC-K17 (7.1%), and the 8 patients with PC-K6c (100.0%). By 5 years of age, all children with PC-K6a and 13 children with PC-K17 (92.9%) showed fingernail dystrophy, in addition to toenail dystrophy. Fingernail dystrophy developed simultaneously in all 10 nails in 68.7% of PC patients; only 6.0% reported involvement in only 1 nail, with no consistency as to the involved digit. The initial change in fingernails was nail thickening in 65 of the 86 patients with nail changes (75.6%), usually in combination with changes in color (41 [63.1%]). All patients with fingernail dystrophy, regardless of PC subtype, had toenail involvement. However, patients with PC-K6a and PC-K17 were more likely to develop dystrophy of their fingernails and toenails concurrently, whereas patients with PC-K6b and PC-K16 were more likely than other subtypes to develop toenail dystrophy first (P < .001).

The toenails and fingernail dystrophy varied in appearance from thickened, shortened, friable nails to nails with marked subungual thickening and a typical pinched, V-shaped curvature (Figure 1A and B). We found no correlation between the appearance of the nails and the PC subtype.

Periungual infections occurred in 76.6% of PC patients overall but more often in those with PC-K6a (43 of 46 [93.5%]) than those with other subtypes (P < .05). Most patients with
nail infections (52 of 73 [71.2%]) never sought treatment by a physician. Most of these patients treated nails by soaking them (38 of 61 [62.3%]), lancing purulent areas or trimming the nails (35 of 61 [57.4%]), or using topical medication (28 of 61 [45.9%]). Nail infection or its clearance did not lead to a change in appearance of the dystrophic nail.

**Painful Plantar Keratoderma**
The feature of PC that has the most profound effect on quality of life is painful plantar keratoderma (Figure 1C and D).12 Although present at birth in fewer than 10% of individuals with PC, 24.8% of PC patients overall noted plantar keratoderma by 1 year of age, 75.3% by 5 years of age, and 89.1% within the first decade of life (Supplement [eFigure, C]). Of the 27 patients diagnosed with PC before 1 year of age, 12 (44.4%) had plantar keratoderma by the time of diagnosis. In patients with PC-K6a, PC-K16, and PC-K17, the onset of plantar keratoderma usually occurred before age 5 years, whereas in patients with PC-K6b and PC-K6c, onset was usually after age 5 years. The most common initial locations of the plantar keratoderma were at pressure points on the heel (66 of 98 [67.3%]) and ball (63 of 98 [64.3%]) of the foot. During the first decade of life, 70 of the 73 patients with keratoderma (95.9%) had plantar pain, which compromised their function; pain occurred later in children with PC-K6b than the other subtypes (P < .05). Most patients with plantar keratoderma also had local skin infections (47 of 99 [47.5%]).

Palmar keratoderma occurred in only 45.5% of patients overall by the time of the response to the questionnaire. Of those who developed palmar keratoderma, 24 of 47 (51.1%) saw changes by 5 years of age, and 32 of 47 (68.1%) by 10 years (Supplement [eFigure, D]). Palmar keratoderma was more often reported in PC-K16 than in any other subtype (67.7%; P < .05) and was often complicated by painful erosions, bullae, or fissures of the palms, usually before 5 years of age.

**Other Clinical Manifestations**
Although PC-K6b and PC-K6c were often distinguishable from other forms because of their more limited manifestations, features other than nail dystrophy and keratoderma were helpful in differentiating among the PC-K6a, PC-16, and PC-17 subtypes in children. In particular, oral leukoplakia, cysts, and keratoses varied by PC subtype.

**Oral Leukokeratoses**
The oral leukokeratoses occurred in 70.3% of PC patients and were more strongly associated with PC-K6a than with any other subtype (P < .001). Of those affected, the median age at onset was 3 weeks, and 26 of 71 (36.6%) experienced a first occurrence during the first year of life. Oral leukokeratoses were often mistaken for thrush (Figure 1E) but failed to respond to antifungal therapy. Oral leukokeratoses were most commonly noted on the tongue (Figure 1E and F) (68 of 71 [95.8%]). Hoarseness was noted in 12.9% of patients overall, and PC-K6a patients were more likely to develop hoarseness than PC-K6b and PC-K16 patients (P < .05) but not PC-K17 patients. The onset of hoarseness was variable but always present before 3 years of age in the affected patients. Of the PC patients who developed hoarseness, 16 (93.3%) concurrently showed oral leukokeratoses. Natal teeth virtually clinched the diagnosis of PC-K17 (86.0% [P < .001]) but were noted in 2 of 46 infants (4.3%) of infants with PC-K6a. The teeth were described as soft or crumbly and were rapidly lost or were described as normal in appearance and persistent until the deciduous teeth erupted.

**Cysts**
Cysts were noted overall in 69.3% of patients with PC. Most patients who developed cysts had PC-K6a, PC-K6b, or PC-K17, without a statistically significant difference in the risk for developing cysts among these subgroups. Patients with the PC-K16 subtype were the least likely to develop cysts (P < .05), and cysts did not develop in PC-K6c patients. The onset of cysts occurred most often from 6 to 10 (31.6%) and 11 to 20 (36.8%) years of age. Patients primarily treated their cysts by lancing them (37 of 54 [68.5%]) or applying topical antibiotic ointment (26 of 54 [48.1%]).

**Follicular Hyperkeratoses and Hyperhidrosis**
Follicular hyperkeratoses were described in more than half of the PC patients, although only in 26 (25.7%) by preschool age (Figure 1G). They occurred most often in PC-K6a (80.4%) and PC-K6b (42.9%) and least commonly in PC-K16 (29.9%; P < .001 compared with PC-K6a). In patients with PC-K6a, 14 of 37 (37.8%) developed cysts at 1 to 5 years of age and 23 of 37 (62.1%) showed follicular hyperkeratoses by the second decade of life (Supplement [eFigure, E]). Only 1 PC-K16 patient developed follicular hyperkeratoses during childhood, and 27 of 31 patients with PC-K16 (87.1%) never developed follicular hyperkeratoses by the time of reporting. Hyperhidrosis was described in 51.5% of respondents of all ages, but in only 5 of 22 children (22.7%). Alopecia was not an issue in children with PC in the first decade of life, and its association with PC is questionable. In the original survey of 254 PC patients, 15 patients ranging in age from 15 to 81 years had hair loss. Of these patients, 10 were male and most had no other hair abnormality, raising the question of androgenic alopecia as the cause. All but 1 of these individuals had PC-K6a or PC-K16.

**Effect on Quality of Life in Adolescents**
Plantar keratoderma in PC was characteristically painful by the second decade of life and led to the greatest effect on quality of life. More than 50% of children found that the plantar dystrophy and keratoderma were helpful in differentiating among the PC-K6a, PC-16, and PC-17 subtypes in children. In particular, oral leukoplakia, cysts, and keratoses varied by PC subtype.
ets (31 [36.9%]), using nail polish (25 [29.8%]), using artificial nails (9 [10.7%]) and wearing gloves (7 [8.3%]).

PC-Like Early Nail Dystrophy and Connexin 30 Mutations

Only patients with gene-confirmed PC subsets completed the pediatric-specific questionnaire. However, among patients in the original registry questionnaire, 7 with nail dystrophy and clinically presumed PC had mutations in the connexin 30 gene (GJB6) (Table 3). Of these, 5 (71.4%) had abnormalities of the toenails and fingernails at birth, with the other 2 developing dystrophy of the fingernails and toenails concurrently at 2 and 4 years of age. Two additional features found commonly with connexin 30 mutations, but not PC, were hearing loss and alopecic patches or sparseness, with hair that was described as thin and sometimes brittle.

Discussion

Pachyonychia congenita constitutes a group of primarily autosomal dominant inherited disorders caused by mutations in paired keratins. Genotyping has been provided since 2004 as a service for families enrolled in the IPCRR and has yielded a wealth of information about PC characteristics and genotype-
The genetic disorder that is most commonly confused with PC is hidrotic ectodermal dysplasia (Clouston syndrome), which results from mutations in connexin 30. Five of the 7 individuals from 2 families with Clouston syndrome who were enrolled in the PC registry for genotyping also showed multiple toenails and fingernails affected at birth, and several individuals developed painful plantar keratoderma, providing an initial clue. By 5 years of age, plantar keratoderma is seen in 75.3% of children in addition to the nail dystrophy and is often painful. Thus, the diagnosis of PC should easily be suspected before kindergarten. Although genotyping should be performed through the registry to subclassify the disease, the combination of data about age at onset, concurrent dystrophy of fingernails and toenails, palmar keratoderma, and the presence of oral leukokeratosis and/or hoarseness, cysts, follicular keratoses, and natal teeth helps the practitioner to suspect a specific PC subtype and counsel families, even before genotyping is completed. For example, the presence of nail dystrophy at birth, especially involving all nails, predicts PC-K6a or PC-K17 (P < .001); the concomitant development of oral leukokeratosis and often hoarseness during the first year of life suggest the diagnosis of PC-K6a (P < .001); and the concurrence of natal teeth indicates PC-K17 (P < .001). In contrast, the development during childhood of palmar keratoderma, especially with later onset of other features, may signal PC-K16. The presence of an isolated dystrophic fingernail or toenail is quite uncommon in PC (77 patients with fingernail involvement overall and 6 [5.5%] with only 1 fingernail involved; 106 with toenail involvement and 1 with only 1 toenail involved [0.9%]); the exception is PC-K6c, in which localized nail involvement is common.18,19

These features, although helping to differentiate among the PC subtypes, also allow us to reject the old classifications of PC-1 and PC-2 in pediatric PC. For example, cysts and natal teeth are most common in PC-K17 (formerly classified as PC-2) but were both described in individuals with PC-K6a (formerly PC-1) in our pediatric cohort and other published series.12,17,18 Hair disorders, at one time attributed to PC-2 KRT6B and KRT17 mutations, do not seem to be associated with autosomal dominant PC more often than in the general population. Alopecia has recently been described in association with severe PC manifestations in a patient with homozygous dominant missense mutations (from each affected parent) in keratin 17.20 These data support restructuring of the classification of PC from 2 different subtypes to a system that categorizes the disease based on specific keratin mutations.12,16,21

As a keratinopathy, PC is associated with increased cellular fragility and compensatory epidermal thickening at the sites of gene expression (particularly palmar keratoderma and nail dystrophy). The greater dystrophy of toenails vs fingernails and of the hallux and fifth toenails likely reflect the propensity toward more pathological features with trauma. Natal teeth, which occurred in 13.9% of patients with PC overall and 86% of patients with PC-K17, have similarly been described in epidermolysis bullosa simplex, which results from mutations in keratins 5 or 14.22 The role of keratin abnormalities in tooth formation is poorly understood.

Our data confirm the frequent misdiagnosis of PC in pediatric patients, showing that most children manifest the key features in the first year of life, but the diagnosis is made in only about 25%. This delay in diagnosis may lead to inappropriate management (eg, topical or oral antifungals for presumed fungal infection, potent topical corticosteroids for presumed psoriasis) or incorrect information about prognosis and inheritance (eg, mistaking hidrotic ectodermal dysplasia with connexin 30 mutations for PC). The unifying feature of PC is nail dystrophy, although the clinical appearance of nails can be variable, even within families, and does not always show the classic V-shaped thickening. During the first year of life, dystrophy of the fingernails and toenails occurs in most of the affected infants, providing an initial clue.
derma. However, the presence in hidrotic ectodermal dysplasia of hearing loss (a common feature of connexin gene defects) and thin, sparse hair during childhood is not typical of PC. Another genetic disorder with which PC could be confused results from mutation of FZD6. The FZD6 gene encodes frizzled 6, a Wnt-signaling pathway receptor that is localized to the nail matrix; autosomal recessive mutations in FZD6 lead to hypertrophic nail dystrophy from birth without plantar or palmar keratoderma.²³,²⁴

Our study emphasizes the negative effect of the nail and skin changes of PC in preteenaged and adolescent patients. Most patients reported embarrassment, teasing, an effect on self-esteem, and an effect on their skin changes of PC in preteenaged and adolescent patients. Early diagnosis and discussion allow proactive management of psychosocial issues, including how a child can comfortably inform peers about the disorder or how a teenager can improve the appearance of nails and keratoderma, thus improving patient coping.

This study was limited by its retrospective nature, leading to the risk of recall bias, particularly when inquiring about disease characteristics at onset during the pediatric years given that most of the patients were well into their adult years when they responded to the questionnaire. Nevertheless, the data in this registry database show that PC can be diagnosed during early childhood based on the constellation of clinical features and genotyping. Genotyping is currently performed at no cost on a research basis (ie, not by a Clinical Laboratory Improvement Amendment–approved laboratory) by registering with the IPCRR (www.pachyonychia.org). The many genotype-phenotype and subtype-phenotype correlations allow for early classification, prediction of clinical features and their age at onset, and optimal management.

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Study concept and design: Kenner-Bell, Paller.

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REFERENCES


A Large Mutational Study in Pachyonychia Congenita

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Pachyonychia congenita (PC) is a rare autosomal dominant skin disorder characterized predominantly by nail dystrophy and painful palmpoplantar keratoderma. Additional clinical features include oral leukokeratosis, follicular keratosis, and cysts (steatocysts and pilosebaceous cysts). PC is due to heterozygous mutations in one of four keratin genes, namely, KRT6A, KRT6B, KRT16, or KRT17. Here, we report genetic analysis of 90 new families with PC in which we identified mutations in KRT6A, KRT6B, KRT16, or KRT17, thereby confirming their clinical diagnosis. A total of 21 previously unreported and 22 known mutations were found. Approximately half of the kindreds had mutations in KRT6A (52%), 28% had mutations in KRT16, 17% in KRT17, and 3% of families had mutations in KRT6B. Most of the mutations were heterozygous missense or small in-frame insertion/deletion mutations occurring within one of the helix boundary motif regions of the keratin polypeptide. More unusual mutations included heterozygous splice site mutations, nonsense mutations, and a 1-bp insertion mutation, leading to a frameshift and premature termination codon. This study, together with previously reported mutations, identifies mutation hotspot codons that may be useful in the development of personalized medicine for PC.

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INTRODUCTION

Pachyonychia congenita (PC) is a rare genetic skin disorder that is associated with mutations in one of four keratin genes, KRT6A, KRT6B, KRT16 or KRT17 (Bowden et al., 1995; McLean et al., 1995; Smith et al., 1998). The most striking feature of PC is the painful and debilitating plantar keratoderma (Figure 1). The mechanism underlying the plantar pain is poorly understood; however, the formation of blisters beneath the keratoderma is likely to be a major contributing factor. Palmar keratoderma is less frequent. Nail dystrophy presents in variable forms, from very minor or almost absent nail changes through to the classic hyper- trophic nail dystrophy that gives the condition its name (Leachman et al., 2005; Figure 1). Other epithelial structures can be affected, particularly the mucosae and the pilosebaceous unit. A more detailed description of the disorder is given in McLean et al. (2011).

Historically, PC has been subdivided into two subtypes, PC-1 (Jadassohn–Lewandowski type) or PC-2 (Jackson–Lawler type), on the basis of the clinical presentation alone (Jadassohn and Lewandowski, 1906; Jackson and Lawler, 1951). A combination of factors have led to the suggestion that PC should be reclassified. First, the advent of molecular genetics and the identification of the genes causing PC provide a rational means of classifying patients. Second, clinical analysis of the large case series collected by the International Pachyonychia Congenita Research Registry (IPCRR), fully linked to molecular genetic data, has shown that there is considerable phenotypic overlap between the historical PC-1 and PC-2 subtypes (Eliason et al., 2011). Thus, a new molecular genetic classification has been adopted, fully supported by members of the International Pachyonychia Congenita Consortium, whereby the subtypes of PC refer to the mutated keratin gene (PC-6a for a patient carrying a K6a mutation, PC-6b, PC-16, PC-17, and so on). The designation ‘PC-U’ is used for cases where the causative gene is unknown (McLean et al., 2011). The new classification will (a) help discourage publication of spurious case reports lacking molecular data (often with misdiagnosed cases or cases with coincidental findings unrelated to PC) and (b) allow better prognostic predictions and patient counseling, especially when referenced to the IPCRR clinical data set.

The 54 human keratins belong to the intermediate filament protein family that consists of at least six types; keratins make up the type I and type II intermediate filament proteins. A major function of keratins is to form structural cytoskeletal networks within epithelial cells that allow cells to withstand everyday stress and physical trauma. Keratins are expressed in pairs in tissue-specific and differentiation-specific patterns (Lane, 1993). The keratins associated with PC, K6a, and K16, K6b and K17 are predominantly expressed...
in basal/suprabasal layers of palmoplantar skin, as well as in epidermal appendages and oral mucosa. In PC, these epithelial cell compartments are rendered fragile by the expression of dominant-negative mutant keratins. All cases of PC with a confirmed molecular diagnosis, including those in the literature, http://www.interfil.org (Szeverenyi et al., 2008), and those in this study, are due to heterozygous dominant-negative mutations, inherited as an autosomal dominant trait. Although there are a small number of case reports of PC with recessive inheritance in the literature, there are no reports of recessive PC with a confirmed molecular diagnosis.

All keratins share a common protein structure consisting of a central \( \alpha \)-helical rod domain of 310 amino acids subdivided into the 1A, 1B, 2A, and 2B domains. These domains are connected by non-helical linker regions, L1, L12, and L2. The rod domain is flanked by short regions of sequence homology (H1 and H2 regions), followed by the variable, non-helical head (V1) and tail domains (V2). At either end of the rod domain are the helix boundary motifs (the helix initiation motif and the helix termination motif). These highly conserved motifs are thought to be important in mediating end-to-end interactions during filament assembly. The majority of mutations in PC occur in these helix boundary motifs, emphasizing the critical importance of these sequences for correct keratin filament formation and the mechanical resilience of epithelial cells.

The IPCRR was established in 2004 by the patient advocacy group, Pachyonychia Congenita Project (http://www.pachyonychia.org). At the time of writing, 478 families are registered (928 individuals), 223 families have completed the detailed questionnaire (Eliason et al., 2011) and 199 families have undergone genetic testing. Genetic testing results from some of these cases have been previously published (McLean et al., 1995; Smith et al., 1997, 2000, 2005; Liao et al., 2007a; Oh Adib et al., 2008; Cogulu et al., 2009; Gruber et al., 2009).

Here, we present the findings of 90 new families with mutations in \( KRT6A \), \( KRT6B \), \( KRT16 \), or \( KRT17 \). Within this case series, we identified 21 previously unreported mutations (22 families) and 22 known mutations (68 families). This mutation analysis study not only confirms the clinical diagnosis of these individuals but, together with previously reported mutations (http://www.interfil.org), also identifies mutation hotspot codons that may be useful in the development of future allele-specific therapies.

**RESULTS**

**Clinical details**

All individuals involved in this study were recruited through the IPCRR, an ongoing research program to identify PC patients worldwide. This research registry is approved by an institutional review board that complies with all principles of the Helsinki Accord (Western IRB Study no. 20040468). An
important part of this study was the detailed clinical consultations that were performed for all cases analyzed. This bank of data allows us now, and in the future as the number of cases analyzed increases, to identify any useful genotype-phenotype correlation for PC. The predominant clinical features of individuals involved in this study are summarized in Supplementary Table S1 online. Of the 90 families analyzed, 36 represent familial occurrence of PC, with many showing autosomal dominant inheritance through several generations; the remaining 54 cases represent spontaneous mutations.

Both common and rare dominant mutations cause PC

Within this large PC case series, pathogenic mutations were identified in the KRT6A gene in approximately half (52%) of the kindreds, whereas 28% had mutations in KRT16, 17% had defects in KRT17, and 3% had mutations in KRT6B (see Figure 2 and Supplementary Table S1 online). Mutations in KRT6A also account for ~50% of previously reported cases of PC (http://www.interfil.org), consistent with our finding here that this is the predominant PC gene. The majority of the mutations we identified in all four genes were heterozygous missense mutations occurring within one of the helix boundary motif regions. In addition, we found some small in-frame insertion/deletion mutations and, in particular, the common K6a p.N172del mutation was identified in 16 families.

An unusual V2 domain mutation in one PC family

A more unusual mutation identified was a 1-bp insertion in exon 9 of KRT6A, the last exon of this gene (K6a c.1511_1512insG). This insertion results in a frameshift and a premature stop codon just two amino acids upstream of the natural stop codon, whereby the last 60 amino acids of the K6a V2 domain are exchanged for a foreign peptide of 58 amino acids. Protein–protein BLAST (basic local alignment search tool) analysis showed that this mutant peptide sequence has no significant similarity to any human protein (data not shown; http://blast.ncbi.nlm.nih.gov/). Kyte–Doolittle hydrophilicity analysis revealed that the normal K6a V2 domain consists of alternating hydrophobic and hydrophilic sequences, followed by a short hydrophilic C terminus (Figure 3), consistent with the glycine-loop structure proposed by Steinert for keratin variable domains (Korge et al., 1992). In contrast, the mutant V2 domain is almost completely hydrophilic (Figure 3). In terms of protein

Figure 2. Mutational spectrum in pachyonychia congenita (PC). Pie chart showing percentage of families in this study with mutations in the four keratin genes, namely, KRT6A, KRT6B, KRT16, and KRT17.

Figure 3. Kyte-Doolittle hydrophilicity analysis of normal and mutant K6a V2 domain. (a) The normal K6a V2 domain consists of alternating hydrophobic and hydrophilic sequences, followed by a short hydrophilic C terminus, whereas (b) the mutant V2 domain is almost completely hydrophilic.
secondary structure predicted by Robson-Garnier analysis, the normal K6a V2 domain is predicted to adopt three large areas of sheet conformations, separated by short regions predicted to adopt turn conformations (Figure 3). This contrasts with the mutant V2 domain, which is predicted to consist largely of turn conformation with one helical region near the C terminus (Figure 3). This in silico analysis underscores the fact that the mutant polypeptide is very different in both sequence and predicted secondary structure from the wild-type K6a tail domain, consistent with a dominant-negative gain-of-function mutation, as seen in other keratinizing disorders due to C-terminal frameshift mutations in K1 (Sprecher et al., 2001, 2003; Richardson et al., 2006) or K5 (Sprecher et al., 2003). In the case of loricrin keratoderma, a similar C-terminal gain-of-function mutation has been shown to lead to creation of a new nuclear localization signal, which in turn leads to nuclear accumulation of mutant protein (Ishida-Yamamoto et al., 2000). The mutant K6a polypeptide sequence generated here was analyzed for this allele. In the case of decay is predicted to occur, leading to loss of expression of negative mutant protein, because nonsense-mediated mRNA termination codon. This is unlikely to create a dominant-exon, its deletion would lead to a frameshift and premature is skipping of exon 2; however, as this is an out-of-frame of the effects of these genomic mutations on RNA splicing.

Splice site mutations identified in five kindreds
Interestingly we also detected four previously unreported splice site mutations (in five families) at the intron 1/exon 2 boundary of KRT6A. These are clearly inherited in an autosomal dominant manner. Unfortunately, we were unable to obtain mRNA from lesional skin that would allow analysis of the effects of these genomic mutations on RNA splicing. One possible predicted consequence of this type of mutation is skipping of exon 2; however, as this is an out-of-frame exon, its deletion would lead to a frameshift and premature termination codon. This is unlikely to create a dominant-negative mutant protein, because nonsense-mediated mRNA decay is predicted to occur, leading to loss of expression of this allele. In the case of KRT5 and KRT1, both of which are type II keratin genes closely related to KRT6A, analogous mutations have been reported affecting the intron 1 splice sites (Rugg et al., 1999; Terron-Kwiatkowski et al., 2002). In both these genes, the mutation led to activation of an identical cryptic splice site in exon 1, producing a 66-nucleotide (22 amino acid) in-frame deletion. Given the strong sequence homology between these genes, it is probable that a similar mechanism will also occur with these KRT6A splice site mutations.

Nonsense mutations in a few PC families
In addition, two heterozygous nonsense mutations were also identified in PC cases. One of these was identified in the 2B domain of K6a, p.Gln435X, which is predicted to lead to expression of a truncated dominant-negative K6a protein, lacking the end of the rod domain and the tail domain. Because this mutation is close to the natural stop codon of K6a, it is likely to escape nonsense-mediated decay to some extent and, therefore, be expressed as mutant polypeptide (Frischmeyer and Dietz, 1999). Analogous mutations have been seen in K5 in dominantly inherited epidermolysis bullosa simplex (Muller et al., 1999; Livingston et al., 2001). The other nonsense mutation was found within the head domain of K16, p.Lys15X. Analogous premature termination codon mutations just downstream of the ATG codon have been reported in other dominant keratin disorders, including K5 in Dowling-Degos disease (Betzel et al., 2006; Liao et al., 2007b) and in K14 in Naegeli syndrome (Lugassy et al., 2006). Although it remains somewhat unclear whether these mutations act via haploinsufficiency or via expression of a dominant-negative mutant protein through use of an alternative initiation codon (McLean et al., 2003), it is however clear that they exhibit dominant inheritance (Betzel et al., 2006; Lugassy et al., 2006). Unfortunately, in the case of the two nonsense mutations identified here, it was not possible to obtain tissue to allow analysis of mRNA or protein.

A spectrum of keratin mutations cause PC
For each of the four genes associated with PC, it has been suggested that there are some codons that represent mutation hotspots, as well as several rare or even family-specific mutations. Our results confirm the previously identified mutation hotspots and also identify 21 previously unreported mutations. Table 1 summarizes the data from previous publications (http://www.interfil.org) together with the data from this large case series.

**DISCUSSION**
The mutation results from this PC case series of 90 families, together with those from at least 131 previously reported cases (http://www.interfil.org), provides a large data set for analysis in terms of where mutations occur within the keratin protein, the most common mutations, the types of mutations found, and allows for preliminary genotype-phenotype

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of different mutations</th>
<th>Number of recurrent mutations</th>
<th>Most common mutation site</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRT6A</td>
<td>39</td>
<td>13</td>
<td>K6a p.Asn171—as missense or deletion mutation (K6a p.Asn172del), this codon is mutated in 46% of those with KRT6A mutations, of which p.Asn172del accounts for 30% of all KRT6A mutations</td>
</tr>
<tr>
<td>KRT16</td>
<td>19</td>
<td>8</td>
<td>K16 p.Leu132Pro in 23% of families with KRT16 mutations</td>
</tr>
<tr>
<td>KRT17</td>
<td>22</td>
<td>8</td>
<td>K17 p.Asn92Ser in 36% of families with KRT17 mutations</td>
</tr>
<tr>
<td>KRT6B</td>
<td>4</td>
<td>1</td>
<td>K6b p.Glu472Lys in 71% of families with KRT6B mutations</td>
</tr>
</tbody>
</table>

Table 1. Summary of mutations identified in this study and previous publications
correlation. All cases with confirmed PC have a mutation in one of the four keratin genes associated with this disease, KRT6A, KRT6B, KRT16, and KRT17. In the course of running the international mutation screening service for PC, in concert with the IPCRR, we have received a small number of samples from isolated cases or families in which no mutation was found in these four keratin genes. In these cases, careful review of the clinical phenotype by the International Pachyonychia Congenita Consortium clinicians often has often led to correction of the diagnosis, confirmed by analysis of other keratin genes or non-keratin genes. For example, a few cases, in which there is alopecia in addition to nail dystrophy, have turned out to carry heterozygous connexin-30 mutations (Lamartine et al., 2000; Smith et al., 2002; van Steensel et al., 2003). Thus, Clouston syndrome should be considered in the differential diagnosis for PC. Similarly, a few families presenting with painful but very limited, circumscribed focal plantar keratoderma, with minimal or absent nail changes, have recently been shown to have mutations in the gene encoding K6c (KRT6C; Wilson et al., 2010). Taking these families into account, we have a small number of families (<5%) in which careful clinical evaluation is consistent with a diagnosis of PC and in whom we cannot detect a mutation in any exon or splice sites of the four PC keratin genes. Genetic linkage analysis in at least one such family has yielded statistically significant linkage to the vicinity of a keratin gene cluster (FJD Smith, unpublished data), and so we conclude that there are either intronic or genomic deletion/rearrangement mutations that are missed by conventional PCR analysis, or that at least one other keratin gene or a nearby related gene can lead to a PC-like phenotype in a minority of cases. Sequence analysis of these cases is ongoing in the laboratory.

All PC causative mutations found to date are heterozygous changes that exhibit autosomal dominant inheritance with a proven, or very probable, dominant-negative pathomechanism. It is important to note that no recessive cases of PC have been confirmed at the molecular level, despite a few case reports appearing in the literature, in which, for example, recessive inheritance may have been suggested by coincidental consanguinity. It is therefore important that case reports of already characterized genetic diseases be backed up by molecular analysis, otherwise the literature may become misleading. The recurrence risk of a sporadic case of a dominant disorder is very low (involving only the risk of gonadal mosaicism) which is difficult to estimate with certainty. In the dominant disorder achondroplasia OMIM no. 100800, this is of the order of one in a few hundred (Mettler and Fraser, 2000); however, in epidermolysis bullosa GJB6, this is of the order of one in a few hundred gonadal mosaicism) which is difficult to estimate with certainty. In the dominant disorder achondroplasia OMIM no. 100800, this is of the order of one in a few hundred (Mettler and Fraser, 2000); however, in epidermolysis bullosa simplex, this has been estimated at 2–5% (Pfendner et al., 2005). In contrast, the recurrence risk for a recessive condition is as high as 25%. Thus, there are important genetic counseling implications in the correct ascription of inheritance patterns. So far, we have confirmed gonadal mosaicism in only one PC family out of 199 analyzed from the IPCRR.

The majority of the mutations causing PC are located in one of the helix boundary motifs of the mutated protein, and most of the causative variants are missense or small in-frame insertion-deletion mutations. Less common types identified include splice site and premature termination codon mutations. There are a number of mutation ‘hotspot’ codons for each of the keratin genes associated with PC as well as mutations that appear to be family specific (Table 1). The most commonly mutated codon is K6a p.Asn171, either as a missense mutation (e.g. p.Asn171Lys and p.Asn171Ser) or as a deletion mutation (designated as p.Asn172del using the Human Genome Variation Society guidelines; http://www.hgvs.org). Approximately half of the families with identified KRT6A mutations have a mutation at this site; the most common PC mutation is the p.Asn172del mutation that, to date, has been found in 32 out of 221 PC families (14%) with known mutations (http://www.interfil.org and this study).

Despite the increased number of genotyped individuals and families, there is no apparent correlation between the clinical features observed and the protein domain harboring the mutation in PC. Nevertheless, there are two mutations in KRT16 in which the actual amino acid substitution appears to correlate with the severity of the clinical phenotype (see Fu et al., 2011). There are also some differences in clinical features depending on the gene involved, such as the presence of natal teeth in many, but not all, individuals with KRT17 mutations (Eliason et al., 2011). In PC, there can also be variation in clinical severity between mutations in the same gene and even between individuals with the same mutation. Polymorphisms, copy number variation, environmental factors, lifestyle, and patient care may account for some of this clinical variation. Therefore, an important conclusion of this large study of a keratin disorder is that PC can really be considered as a spectrum of phenotypes ranging from very mild to more severe, in which (a) the particular gene involved appears to have a moderate influence on phenotype and (b) the specific mutation generally appears to have little influence on phenotype.

The detailed clinical information that is obtained by the IPCRR also identified several cases, which were not included in this study, that presented with both typical and atypical features of PC and that were unlikely due to a mutation in any of the four keratin genes, KRT6A, KRT6B, KRT16, or KRT17. These were analyzed for mutations in other candidate genes. For example, several families presented with varying degrees of alopecia in addition to some features typical of PC, which was suggestive of Clouston syndrome (Lamartine et al., 2000; Smith et al., 2002; van Steensel et al., 2003) and mutations were subsequently identified in GJB6 (which encodes connexin 30). Another candidate gene considered for individuals presenting with palmoplantar keratoderma was the third K6 gene (KRT6C). We have identified mutations in KRT6C in several families presenting with palmoplantar keratoderma, but with only mild/no nail changes (Wilson et al., 2010).

Genetic analysis of individuals with PC not only confirms their clinical diagnosis but also aids in genetic counseling. The identification of mutations is especially important for the design of future mutation-specific and/or gene-specific therapies and, hence, the large, well-phenotyped, and fully genotyped case series we report here is an invaluable resource for future clinical trials.
MATERIALS AND METHODS

Clinical material
Genomic DNA was obtained with informed consent and ethical approval by an institutional review board that complies with all principles of the Helsinki Accords (Western IRB Study no. 20040468). Genomic DNA was extracted from peripheral blood lymphocytes using standard procedures or from saliva collected in an Oragene DNA sample collection kit (DNA Genotek, Ontario, Canada) and extracted according to the manufacturer’s protocol.

Mutation detection
The coding regions of KRT6A, KRT6B, KRT16, and KRT17 were amplified using primers specific to the respective functional genes to avoid amplification of KRT6C or pseudogenes (Supplementary Table S2 online). All primers were checked for single-nucleotide polymorphisms using Diagnostic SNPCheck (http://www.ngrl.org.uk/Manchester) and some were modified from our previous publications (Smith et al., 2005) to increase specificity. For each gene, there are two primer sets for the mutation hotspot exons to overcome the potential problem of very rare or as yet unidentified single-nucleotide polymorphisms in primers designed to amplify these regions. Previously unreported mutations were excluded from at least 90 control DNA samples (180 chromosomes) by sequencing or restriction enzyme digests. For full-length genomic PCR reactions, Takara buffer and LA Taq polymerase (Lonza Biologics PLC, Slough, UK) were used and for the smaller PCR reactions and HotStarTaq DNA Polymerase and buffer system (Qiagen, Crawley, UK) were used according to the manufacturer’s instructions. Specific PCR conditions for each primer set are available on request. PCR products were purified using QiiaQuick PCR spin columns (Qiagen) or ExoSAP (using Exonuclease 1 and Shrimp Antartic Phosphatase, New England Biolabs, Hitchin, Herts, UK) and sequenced using the manufacturer’s instructions. Specific PCR conditions for each primer set are available on request. PCR products were purified using QiiaQuick PCR spin columns (Qiagen) or ExoSAP (using Exonuclease 1 and Shrimp Antartic Phosphatase, New England Biolabs, Hitchin, Herts, UK) and sequenced using internal primers on an ABI 3100 Automated DNA sequencing machine (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions.

Protein bioinformatics
Hydropathicity (Kyte-Doolittle analysis) and protein secondary structure predictions (Robson-Garnier analysis) were performed using the Protein Analysis Toolkit function within the MacVector 9.0 software package (MacVector, Cary, NC).

CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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