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Revisiting pachyonychia congenita: a case-cohort study of 815 patients

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Summary

Background Pachyonychia congenita (PC) is a group of autosomal dominant disorders caused by mutations in one of five keratin genes (*KRT6A*, *KRT6B*, *KRT6C*, *KRT16*, *KRT17*). The establishment of an international registry containing clinical and molecular data led to the development of a disease classification based on the mutant gene and associated features.

Objectives To harness the same resource to clarify the prevalence of PC-associated clinical features, delineate phenotype–genotype correlations and identify prognostic features for disease severity.

Methods In total, 815 individuals with confirmed keratin mutations registered in the International Pachyonychia Congenita Research Registry were surveyed for clinical findings associated with PC. Data were analysed using various statistical methods, including the Student's t-test, χ^2-test and ANOVA tests for differences in means/proportions. Spearman correlation and logistic regression were used for phenotype–genotype correlations.

Results *KRT6A* mutations were associated with oral leucokeratosis, hoarseness, youngest age or highest number of fingernails/toenails involved, and use of walking aids. *KRT17* mutations were most commonly associated with cysts and natal teeth. Using logistic regression, we found that oral leucokeratosis was correlated with earlier toenail involvement, walking aids, nursing difficulties and hoarseness. Cysts were correlated with oral leucokeratosis, natal teeth and ear wax. Natal teeth predicted earlier toenail involvement, walking difficulties and cyst formation. Hoarseness was correlated with an increased number of involved fingernails.

Conclusions Here, we establish phenotype–genotype correlations in the largest cohort of patients with PC described to date and reveal novel and clinically useful predictors of disease course and manifestations.

What's already known about this topic?

- Pachyonychia congenita (PC) is a group of autosomal dominant disorders caused by mutations in one of five keratin genes (*KRT6A*, *KRT6B*, *KRT6C*, *KRT16*, *KRT17*).
- The main clinical features are nail dystrophy, palmoplantar keratoderma, oral leucokeratosis and cysts.
- The establishment of an international registry containing the clinical and molecular data of patients with PC led to the development of a disease classification based on the mutant gene and associated features.

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Conflicts of interest
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What does this study add?

- Data were collected via an international registry to clarify the prevalence of PC-associated clinical features, delineate phenotype–genotype correlations and identify prognostic features for disease severity.
- This is the largest cohort of patients with PC described to date.
- The earliest clinical manifestations of PC are nail dystrophy and palmoplantar keratoderma. Diagnosis can be suspected and confirmed in preschool years.
- Painful plantar keratoderma has the most profound and debilitating effect on quality of life and daily function.

Pachyonychia congenita (PC; MIM 167200, 167210, 615726, 615728, 615735) is a group of autosomal dominant disorders of keratinization caused by mutations in one of five keratin genes, including *KRT6A* (MIM 148041), *KRT6B* (MIM 148042), *KRT6C* (MIM 612315), *KRT16* (MIM 148067) or *KRT17* (MIM 148069),[1–6] which alter keratins 6a, 6b, 6c, 16 and 17, respectively.

The prevalence of PC in Western countries is 0·9 cases per million with a worldwide population of people with PC estimated to be between 5000 and 10 000.[2,7,8] PC was first described in 1904;[9] however, Jadassohn and Lewandowski published the first case series of the disease in 1906.[10] Based on these observations, PC was divided into two subtypes:[11–14] PC-1 (Jadassohn–Lewandowski type) featuring oral leucokeratosis and caused by mutations in *KRT6A* or *KRT16*; and PC-2 (Jackson–Lawler type) due to mutations in *KRT6B* or *KRT17*, featuring cysts and natal teeth.

This classification was based on the assumption that mutations in genes encoding keratin proteins that normally dimerize (K6a/K16 and K6b/K17) will result in a similar predictable clinical phenotype. However, subsequent studies showed considerable overlap between these clinical subtypes, leading to the reclassification of PC based on the mutant gene (PC-K6a, PC-K6b, PC-K6c, PC-K16 and PC-K17 resulting from mutations in *KRT6A*, *KRT6B*, *KRT6C*, *KRT16* and *KRT17*, respectively).[2,15]

The three most common clinical features associated with PC are thickened toenails (pachyonychia), plantar keratoderma (mostly focal) and plantar pain, which is the most important and debilitating feature affecting patients' quality of life (QoL).[2,15] Additional associated features are epidermal inclusion cysts (mostly steatocystomas and vellus hair cysts), follicular keratoses, mucosal leucokeratosis, hoarse voice and natal teeth (erupted teeth present at birth or by 1 month of age).[16]

Other possible features are ear pain and ear wax, angular cheilitis and abnormal sweating. Features reported in isolated cases with no established association with PC in larger cohorts are hearing loss, alopecia and corneal dystrophy.[17–22]

Here, we report clinical and genetic data collected from 815 patients worldwide with mutation-verified PC, registered in the International PC Research Registry (IPCRR). To our knowledge, this is the largest genotype–phenotype study of PC to date.

Patients and methods

Data collection

In 2004, the IPCRR was established by the nonprofit organization Pachyonychia Congenita Project with the aim of collecting clinical and genetic data on patients with PC around the world, as previously described.[2] Since its establishment, individuals with PC have been informed of and referred to the registry via a website (www.pachyonychia.org). All patients gave written informed consent. The IPCRR was approved by the WIRB (project number 20040468; study number 1057496) and the study was conducted according to the principles of the Declaration of Helsinki. Participant enrolment began in May 2004, as previously reported.[2] In order to be a participant in the registry, individuals were required to complete a comprehensive questionnaire providing detailed information regarding the clinical manifestations of the disease and whether (or to what extent) they affect their QoL. Patients were asked about thickened toenails/fingernails, palmoplantar keratoderma, plantar pain, oral leucokeratosis, follicular hyperkeratosis, hyperhidrosis, cysts, natal teeth, hoarseness, hair/eyes/ears involvement, hearing loss, psychosocial and learning disabilities. In addition, the patients answered questions addressing burden of disease with issues such as improving and worsening factors, pain, frequency of care, walking difficulties and cosmetic concerns. Age at presentation for several different clinical manifestations of PC was also recorded. The full questionnaire can be found at https://www.pachyonychia.org/patient-registry/.

After completion of the questionnaire, a case review or a telephone consultation was scheduled with one of the dermatologists on the Pachyonychia Congenita Project medical advisory board in order to (i) confirm that the individuals' clinical features are consistent with PC; (ii) obtain any missing data from the questionnaire and clarify any questions that participants may not have understood; and (iii) allow individuals to receive genetic counselling before mutation testing. The

analysis includes data from 815 participants enrolled in the registry between May 2004 and June 2018.

Molecular analysis

Once officially registered in the IPCRR, genetic testing was performed free of charge. Saliva samples were collected in Oragene Dx OGD-500 kits (DNA Genotek, Ottawa, ON, Canada) and genomic DNA was extracted using a QiaAmp DNA mini kit (Qiagen, Germantown, MD, U.S.A.). In most cases, genetic testing, by polymerase chain reaction (PCR) and Sanger sequencing of the coding regions of the five PC-associated keratin genes, using primers specific to the respective genes, was completed at the University of Dundee (School of Life Sciences, Dundee, U.K.). In cases where no mutation was identified in one of these keratin genes, clinical details were re-examined and other candidate genes, including *GJB6*, *TRPV3*, *DSG1* and *AAGAB*, were screened by Sanger sequencing. The results for all cases were confirmed by independent genetic testing by a certified U.S. clinical laboratory (GeneDx, Gaithersburg, MD, U.S.A.).[2] In more recent cases (since mid-2016), genetic testing was performed by a certified U.S. clinical laboratory (myGenomics, Alpharetta, GA, U.S.A.) using a next-generation sequencing approach. As other mutated genes can manifest with overlapping features of PC, a nine-gene panel, including four of these possible genes (*GJB6*, *TRPV3*, *DSG1*, *AAGAB*) and the five PC-associated keratin genes, was designed using Agilent Sure Select (Agilent Technologies, Santa Clara, CA, U.S.A.). Samples were first run on the gene panel and pathogenic mutations were confirmed by PCR and Sanger sequencing. All 815 individuals included and analysed in this study were genetically confirmed to have PC.

In total, 112 cases were referred to the IPCRR between 2014 and 2018 but were, in the end, not diagnosed with PC and were not included in this study.

Statistical analysis

Continuous variables are expressed as mean ± SD or as median (interquartile range) where appropriate. Categorical variables are presented as percentages.

Differences in means and proportions of factors/parameters across the phenotype–genotype categories were statistically tested by the Student's *t*-test, one-way ANOVA and the χ^2-test, respectively. Phenotype–genotype correlations were described with the Spearman correlation coefficient.

Univariate logistic regression analyses were performed for each clinical feature in order to identify the most significant predictors of each outcome. Covariates that were found to be associated with each outcome were candidates in the stepwise multivariate logistic regression analysis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated in the final models. All tests of significance were two tailed. A P-value < 0·05 was considered to be statistically significant. All analyses were performed using SAS version 9·3 (SAS Institute, Cary, NC, U.S.A.).

Results

Mutation analysis

In total, 815 patients with PC were included in the registry following completion of the required steps mentioned above. The demographic data are given in Table 1. Mutations in *KRT6A* and *KRT16* were the most common (41% and 31%, respectively) followed by mutations in *KRT17*, *KRT6B* and *KRT6C* (Table 1; Fig. 1). Spontaneous mutations were documented in 249 patients (30·6%), with the remainder of mutations being inherited (Table 1). A list of previously unpublished mutations in PC-associated genes is available in Table S1 (see Supporting Information).

Phenotypic features of pachyonychia congenita

Thickened nails

The earliest and most common clinical feature of PC is nail dystrophy (Fig. 2a). The median number of affected fingernails (mean 6·4) and toenails was 10 (mean 8). In 65% and 55% of patients, respectively, fingernail and toenail involvement appeared during the first year of life. Seventy-five per cent of patients had at least one involved fingernail (n = 614), while 96·1% had at least one involved toenail (n = 783). Fifty-three and 65% had all 10 fingernails and toenails affected, respectively (Fig. 3). Altogether, 415 patients (50·9%) had all 20 nails affected. Of the patients displaying only two affected toenails, 77·4% (n = 41/53) had involvement of the fifth toe, which is prone to repetitive trauma (data not shown). In most patients, fingernail/toenail involvement influenced normal function and was mostly associated with cosmetic disturbances, pain and time required for nail care; however,

Table 1 Epidemiological and molecular characteristics of the pachyonychia congenita cohort (n = 815)

Median (range) age at evaluation (years)	30 (0–87)
Sex	
Male	388 (47·6)
Female	427 (52·4)
Mutated gene	
KRT6A	332 (40·7)
KRT16	251 (30·8)
KRT17	134 (16·4)
KRT6B	74 (9·1)
KRT6C	23 (2·8)
Mutation type	
Spontaneous	249 (30·5)
Inherited	566 (69·4)
Worldwide distribution	
North America	398 (48·8)
Europe	284 (34·8)
Asia	62 (7·6)
Central and South America	47 (5·8)
Australia	19 (2·3)
Africa	5 (0·6)

Data are n (%) unless otherwise indicated.

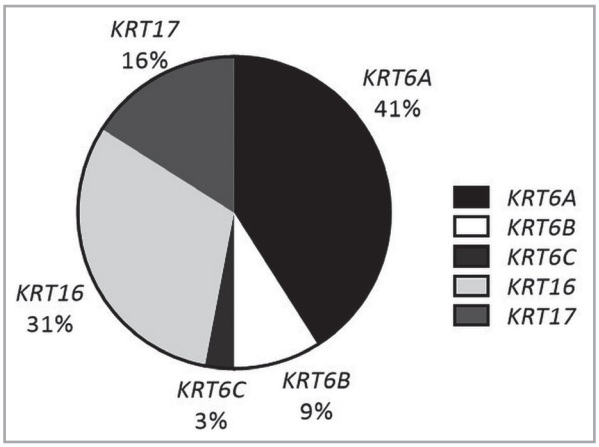

Fig 1. Mutation distribution in 815 patients with pachyonychia congenita (PC). The prevalence of mutations in PC-associated genes in our cohort.

a persistent effect was reported in approximately 20% of patients. Approximately 70% and 50% of patients experienced episodes of secondary fingernail and toenail infections, respectively (Table S2; see Supporting Information).

Palmoplantar keratoderma and plantar pain

Painful plantar keratoderma is the feature of PC that has the most profound and debilitating effect on QoL (Fig. 2b, c). Characteristics of palmoplantar involvement are detailed in Table S2. In most patients, palmoplantar involvement was first observed between 1 and 9 years of age.

In total, 69·6% of patients in our cohort reported palmar involvement with palmar keratoderma (diffuse thickened skin or calluses) being the most common finding. Of the 24·7% ($n = 201$) reporting no palmar involvement, 33 patients were < 3 years of age. The most commonly affected areas were the palms, the palmar aspect of fingers and the fingertips. Palmar keratoderma resulted mainly in cosmetic disturbances and pain (reported by 50% of patients); however, significant pain was evident in only 10% of patients. Fifty per cent of patients reported no impact of palmar involvement and in only 1% of patients did it result in an inability to function.

In contrast to palmar keratoderma, plantar involvement was identified in 94·2% of patients in our cohort. Of these, 30 patients were < 3 years of age, suggesting that pain had not yet developed at the time of completing the questionnaire. Significant pain was reported in > 60% of cases. A persistent effect on daily function was noticed in > 60% of cases with daily foot care required by 26% of patients. Most patients reported significant walking difficulties requiring special foot products and shoes; 19% of the cases used walking aids, but only 2% reported complete inability to walk.

Mucosal involvement

Oral leucokeratosis (Fig. 2d) was reported in 440 of 812 individuals (54·2%) with the most frequent involved area being

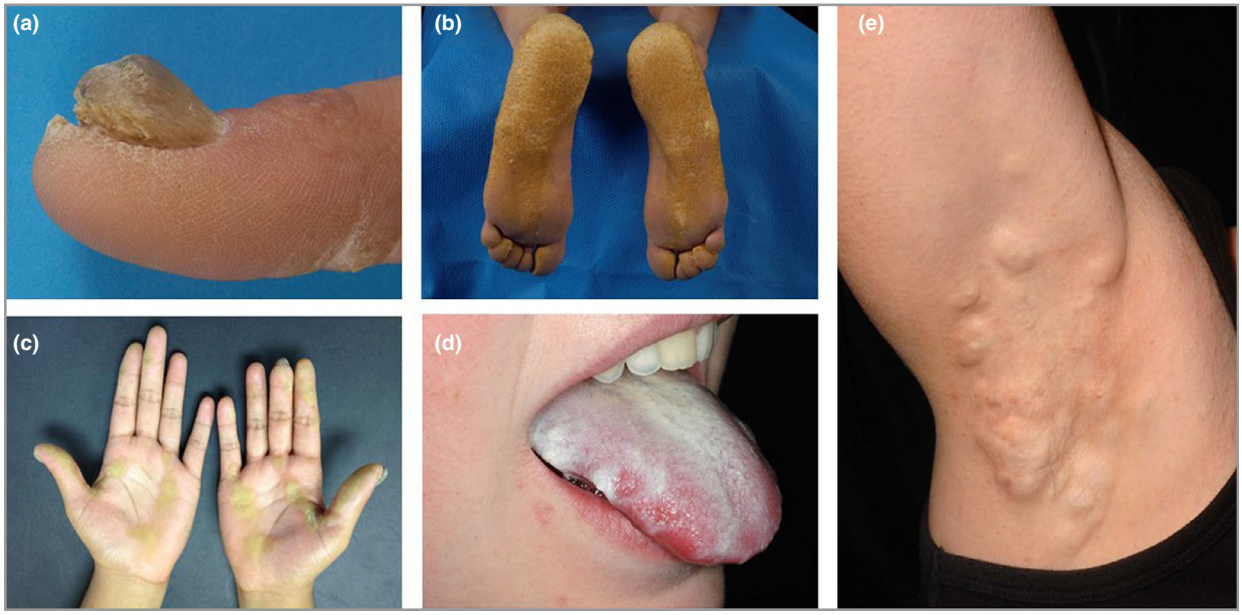

Fig 2. Clinical phenotypes in patients with genetically confirmed pachyonychia congenita. (a) Fingernail involvement in a patient carrying the heterozygous mutation p.Asn172del in *KRT6A*. Note the significant subungual hyperkeratosis with fingernail elevation and premature termination of the nail plate. (b) Diffuse yellowish hyperkeratotic plantar plaques in the same patient as (a). (c) Focal palmar keratoderma, which follows pressure distribution in a patient carrying a *KRT16* mutation. Environmental factors and mechanical pressure play a role in the development and persistence of these findings. (d) Oral leucokeratosis over the tongue is a patient carrying heterozygous p.Asn172del mutation in *KRT6A*. (e) Steatocystoma multiplex lesions over the right axilla in a patient carrying the p.Asn172del mutation in *KRT6A*.

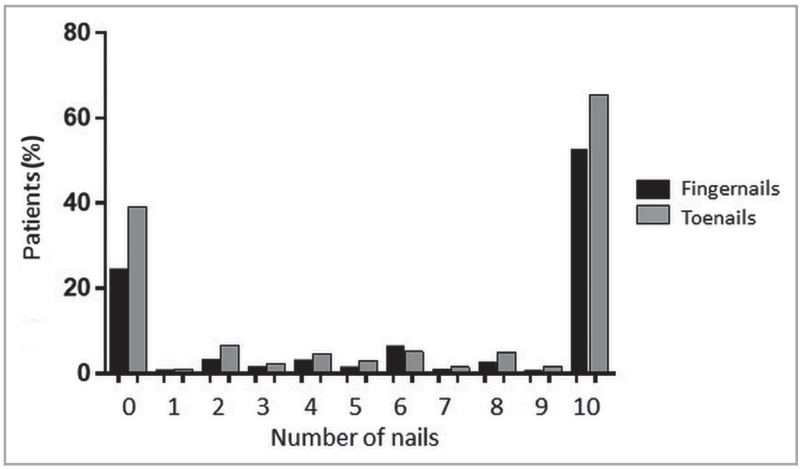

Fig 3. Nail involvement in patients with pachyonychia congenita.

the buccal mucosa (Table S3; see Supporting Information). Of those affected, 60% experienced a first occurrence during the first year of life. Thirty-three per cent reported the need for daily oral care, while in most patients oral leucokeratosis had no effect on function and was not associated with pain. Eighty-eight of 384 (22·9%) reported nursing difficulties, with 'natal teeth' present in 114 of 806 (14·1%). Hoarseness was reported by 191 of 794 patients (24·1%). Other reported dental anomalies included premature decay (n = 17), enamel loss (n = 5), abnormal growth/structure (n = 5) and supernumerary/double tooth row (n = 2).

Cysts

Overall, 439 of 810 (54·2%) patients reported cysts of any type (Table S4; see Supporting Information). Follicular hyperkeratosis (39%), pilosebaceous cysts (29%) and steatocystomas (24%) were the most frequent findings (Fig. 2e). Approximately 20% of patients reported daily cyst care, while no need for cyst care was reported in 30–40% of patients. Association with pain was evident in 60% of patients. However, in most cases no pain medication was required.

Other clinical features

Approximately 50% of patients reported impaired sweating (reduced or excessive amount of sweat), and ear wax or pain were evident in 27·8% (n = 222/799) and 14·3% (n = 97/680), respectively. In total, 207 of 791 patients (26·2%) reported emotional impairments, with depression/mood changes (20%), anger-management difficulties (10%), suicidal feelings (7%) and anxiety (3%) being most common. These can be expected given the effect of this chronic debilitating disease on QoL. Other nonspecific clinical features that had been previously observed in the context of PC were relatively rare in our cohort and, accordingly, are probably not directly related to the disease. In general, hair abnormalities were reported in 92 of 788 patients (11·7%), including thin, coarse/dry, brittle or excessively thick hair in most cases. Hair loss was reported in only 76 of 702 patients (10·8%). Hair abnormalities have a high prevalence in the general population (e.g. male pattern alopecia reaching a prevalence of 50% by the age of 50 years) and increased hair shedding in female patients may be related to various aetiologies, including dermatological, endocrinological, nutritional disorders, stress, inflammatory and infectious disorders, or drug administration.[23–25] Although hair abnormalities have previously been attributed to PC, especially PC-2 associated with *KRT17* mutations, based on the results of the current study and other previous reports,[26] it is impossible to draw any conclusions regarding a direct association between PC and hair anomalies. Corneal findings and hearing loss were previously attributed to PC in isolated reports;[11,14,22,27] however, ocular anomalies were rare in patients with PC (4%). Hearing abnormalities were reported in 117 of 787 patients (14·9%); however, in most patients these were partial hearing impairment or tinnitus-related hearing complaints with reported associations with ear wax, work, ear infections, Ménière disease and even oral medications. In addition, 34 patients who reported hearing loss were 60 years of age or older, suggesting that decreased hearing acuity may be age related. Accordingly, we cannot conclude that either ocular or hearing anomalies are directly associated with PC.

Moreover, we did not observe an increased incidence of mental retardation in our cohort, which is in contrast to previous isolated reports.[11,27]

Impact of pachyonychia congenita-associated clinical manifestations on quality of life

The effect of the most characteristic features of PC (palmar and plantar keratoderma, fingernail and toenail dystrophy, oral leucokeratosis and cysts) on QoL is summarized in Table 2. Plantar keratoderma is the clinical feature associated with the most significant effect on daily function. Of the 628 patients who reported plantar involvement, 391 (62·3%) emphasized

Table 2 Impact of major pachyonychia congenita clinical features on quality of life (QoL) and pain

	Plantar keratoderma	Palmar keratoderma	Fingernail dystrophy	Toenail dystrophy	Oral leucokeratosis	Cysts
Impact on QoL						
No impact	55/628 (8·8)	268/514 (52·1)	168/539 (31·2)	187/668 (28·0)	182/287 (63·4)	146/386 (37·8)
Sometimes a problem	148/628 (23·6)	175/514 (34·0)	269/539 (49·9)	320/668 (47·9)	88/287 (30·7)	180/386 (46·6)
Always a problem, able to function	391/628 (62·3)	66/514 (12·8)	95/539 (17·6)	150/668 (22·5)	17/287 (5·9)	52/386 (13·5)
Impossible to function	34/628 (5·4)	5/514 (1·0)	7/539 (1·3)	9/668 (1·3)	0/287 (0)	8/386 (2·1)
Associated pain						
Not painful	42/739 (5·7)	260/515 (50·5)	NA	NA	197/269 (73·2)	155/384 (40·4)
Sometimes painful	201/739 (27·2)	202/515 (39·2)	NA	NA	69/269 (25·7)	149/384 (38·8)
Very painful but no need for medication	314/739 (42·5)	25/515 (4·9)	NA	NA	2/269 (0·7)	45/384 (11·7)
Often requires medication to handle pain	182/739 (24·6)	28/515 (5·4)	NA	NA	1/269 (0·4)	35/384 (9·1)

Data are n (%). NA, not available.

Table 3 Genotype–phenotype correlation

Parameter	*KRT16*	*KRT17*	*KRT6A*	*KRT6B*	*KRT6C*	P-value
Male sex	52·2	38·1	46·6	54·1	50	NS
Mutation type (spontaneous)	23·1	28·4	42·5	14·9	4·2	< 0·001
Fingernail dystrophy (at least one nail)	56·6	85·1	97·6	45·9	0	< 0·001
Mean ± SD age of fingernail dystrophy onset (years)[a]	3·0 ± 1·8	1·4 ± 0·9	1·1 ± 0·4	3·5 ± 1·7	NR	< 0·001
Mean ± SD no. of affected fingernails (median)	4·1 ± 4·3 (2)	6·9 ± 3·7 (9)	9·3 ± 2·1 (10)	2·4 ± 3·2 (0)	0·0 ± 0·0 (0)	< 0·001
Toenail dystrophy (at least one nail)	96·4	95·5	97·9	98·6	62·5	< 0·001
Mean ± SD age of toenail dystrophy onset (years)[a]	2·9 ± 1·6	1·4 ± 0·8	1·2 ± 0·4	2·8 ± 1·28	3·2 ± 1·6	< 0·001
Mean ± SD no. of affected toenails (median)	6·7 ± 3·3 (8)	8·6 ± 2·8 (10)	9·6 ± 1·8 (10)	7·0 ± 3·0 (8)	1·6 ± 1·6 (2)	< 0·001
Palmar keratoderma	86·6	61·8	66·2	50	41·2	< 0·001
Plantar keratoderma	99·6	82·8	92·9	98·6	100	< 0·001
Use of walking aids	22	4·5	23·8	21·4	4·8	< 0·001
Cysts	25·6	91·8	60·2	67·6	17·4	< 0·001
Oral leucokeratosis	35·6	26·3	88·3	24·3	21·7	< 0·001
Natal teeth	0·0	77·3	3·3	0·0	4·2	< 0·001
Nursing difficulties	3·0	21·0	39·1	2·9	10·0	< 0·001
Hoarseness	8·1	22	38·9	18·9	18·2	< 0·001
Ear wax	18·4	27·8	37·3	23·9	8·7	< 0·001
Ear pain	9·3	11·6	20·2	9·3	18·2	< 0·01

Data are percentage unless otherwise indicated. NS, not significant; NR, not reported. [a]Age was divided into six separate categories: (i) birth –1 year; (ii) 1–4 years; (iii) 5–10 years; (iv) 10–14 years; (v) 15–19 years; (vi) ≥ 20 years.

the persistent and negative effect of this finding on their QoL. In a further 34 patients (5·4%), plantar involvement resulted in inability to function. This correlated with > 60% of patients reporting that their plantar keratoderma resulted in significant pain. In contrast to plantar keratoderma, other clinical features of PC were not associated with a significant effect on QoL or pain, in most patients.

Phenotype–genotype correlation

Phenotype–genotype correlations are summarized in Table 3. Young age at diagnosis or involvement of a high number of fingernails/toenails was significantly associated with mutations in *KRT6A*. Plantar keratoderma was common among all patients with PC, regardless of the causative mutation; however, palmar

Table 4 Pachyonychia congenita-associated clinical phenotypes correlation

		Fingernail dystrophy			Logistic regression	
	All patients	Yes	No	Univariate P-value	OR (95% CI)	P-value
No. of affected toenails	8·1 ± 3·1	9·3 ± 1·7	4·2 ± 3·2	< 0·001	1·73 (1·59–1·88)	< 0·001
Age of toenail dystrophy onset[a]	8·1 ± 3·1	9·3 ± 1·7	4·2 ± 3·2	< 0·001	1·73 (1·59–1·88)	< 0·001
		Toenail dystrophy				
		Yes	No			
Number of affected fingernails	6·4 ± 4·3	6·6 ± 4·2	0·5 ± 2·0	< 0·001	1·65 (1·32–2·05)	< 0·001
		Palmar keratoderma				
		Yes	No			
Age of fingernail dystrophy onset[a]	1·8 ± 1·4	1·9 ± 1·5	1·4 ± 1·0	< 0·001	1·3 (1·06–1·59)	0·01
Use of walking aids	21·5	24	14·2	0·01	2·12 (1·09–4·13)	0·02
		Plantar keratoderma				
		Yes	No			
Age of toenail dystrophy onset	1·9 ± 1·3	1·3 ± 0·7	2·0 ± 1·4	< 0·001	4·33 (1·45–12·93)	0·008
Natal teeth	13·1	11·7	36·4	< 0·001	0·37 (0·18–0·76)	0·007
		Oral leucokeratosis				
		Yes	No			
Age of toenail dystrophy onset[a]	1·9 ± 1·3	1·5 ± 1·0	2·4 ± 1·5	< 0·001	0·36 (0·22–0·58)	< 0·001
Walking aids	19	25·1	11·7	< 0·001	3·4 (1·17–9·9)	0·02
Nursing difficulties	22·9	34·1	6·5	< 0·001	3·51 (1·1–11·16)	0·03
Natal teeth	14·2	8	21·6	< 0·001	0·19 (0·08–0·46)	< 0·001
Hoarseness	24	34	12·3	< 0·001	2·54 (1·02–6·33)	0·04
		Natal teeth				
		Yes	No			
Age of toenail dystrophy onset[a]	1·9 ± 1·3	1·3 ± 0·7	2·0 ± 1·4	< 0·001	0·33 (0·20–0·57)	< 0·001
Unable to walk	2	6·7	1·2	< 0·001	21·94 (3·83–125·42)	< 0·001
Oral leucokeratosis	54·5	30·7	58·5	< 0·001	0·063 (0·031–0·12)	< 0·001
Cyts	54·3	88·4	48·8	< 0·001	7·64 (3·02–19·34)	< 0·001
		Hoarseness				
		Yes	No			
Number of affected fingernails	6·4 ± 4·3	8·1 ± 3·4	5·8 ± 4·4	< 0·001	1·27 (1·04–1·55)	0·02
Oral leucokeratosis	53·8	76·3	46·7	< 0·001	2·7 (1·13–6·48)	0·03
Nursing difficulties	21·5	42·2	13·1	< 0·001	2·47 (1·21–5·03)	0·01
		Cysts				
		Yes	No			
Oral leucokeratosis	54·2	62	44·9	< 0·001	2·35 (1·29–4·27)	0·004
Natal teeth	14	22·8	3·6	< 0·001	10·61 (3·47–32·39)	< 0·001
Ear wax	27·9	38·2	15·5	< 0·001	4·19 (2·07–8·50)	< 0·001

Data are mean ± SD or percentage. OR, odds ratio; CI, confidence interval. [a]Age was divided into six separate categories: (i) birth–1 year; (ii) 1–4 years; (iii) 5–10 years; (iv) 10–14 years; (v) 15–19 years; (vi) ≥ 20 years.

keratoderma was most common with *KRT16* mutations and less common in patients with *KRT6C* mutations. The use of walking aids was associated with *KRT6A* mutations, while specialist foot products were most commonly associated with *KRT6B* mutations. *KRT6A* mutations were also significantly associated with oral leucokeratosis, nursing difficulties, hoarseness and ear wax/pain. Cysts and natal teeth were most commonly associated with *KRT17* mutations. Lack of fingernail involvement was most commonly associated with mutations in *KRT16* (57%) and *KRT6B* (19%) (Fig. S1; online Supporting Information), while this finding was very rare in patients with *KRT6A* mutations (3%). The *KRT16* mutations p.Asn125Ser and p.Arg127Cys, and the *KRT6B* mutation p.Glu472Lys, were strongly associated with lack of fingernail involvement, which is in line with previous reports.[2,28] Hair loss, hearing abnormalities, ocular involvement and emotional impairments did not correlate with any specific genetic defect (data not shown).

Predictors of clinical outcomes

We aimed to decipher the clinical predictors of disease course and manifestations. Logistic regression models were run for each clinical feature, in order to identify the most significant predictor(s) for each clinical outcome (Table 4). Based on these models, as expected, increased number of affected toenails and earlier age of toenail involvement predicted fingernail dystrophy, while an increased number of affected fingernails was correlated with toenail involvement.

Interestingly, the earlier onset of toenail involvement was correlated with the development of plantar keratoderma, while age of fingernail dystrophy onset and use of walking aids predicted palmar keratoderma. In addition, the presence of natal teeth predicted the absence of plantar keratoderma. Oral leucokeratosis was significantly correlated with earlier toenail involvement (1·5 years vs. 2·4 years), nursing difficulties, hoarseness and use of walking aids. However, natal teeth and oral leucokeratosis were inversely correlated, whereas natal teeth and cysts significantly predicted each other's development. Oral leucokeratosis and ear wax were additional predictors for the development of pilosebaceous cysts.

Discussion

Epidemiological data on rare diseases are seldom available. The present study highlights the importance and usefulness of global registries for orphan conditions. Indeed, it identified an unprecedented large number of correlations between clinical manifestations and causative genetic defects, as well as a number of clinical predictors, which together are likely to improve the counselling of families with PC or at risk for the disease. Moreover, the fact that the PC cohort studied here is the largest reported to date indicates that the features that emerged from the study are likely to be of general relevance to patients with PC.

Further reinforcing the validity of our findings, the relationships that we identified between clinical manifestations and causative genetic defects seem to correlate with basic aspects of the disease's pathobiology (for further details see Appendix S1, Supporting Information). The expression of K6/16 in normal skin is restricted to nail bed epithelium, palmoplantar skin, suprabasal orogenital mucosa and follicular upper outer root sheath,[29] while K17 is mainly present in the epithelial appendages (including sebaceous glands), orogenital mucosa, nail bed epithelium and nail matrix.[30–32] This may explain the fact that palmoplantar involvement is more prominent in patients carrying *KRT6/16* mutations, while cysts are most commonly observed with *KRT17* mutations. K6 is expressed in both cornified and noncornified oral mucosa,[33] explaining its significant association with oral leucokeratosis.

Recent studies attributed nonstructural function, including innate immune regulation (K16), differentiation and cell growth through mammalian target of rapamycin signalling (K17), to PC-associated proteins, which may influence the resultant phenotype displayed by patients.[31,34,35] Among novel functions of PC-associated keratins, it has been shown that K6 and K17 are both incorporated into the enamel matrix,[36] which may provide an explanation for the dental anomalies seen in patients with PC. The association of natal teeth with *KRT17* mutations is probably related to the fact K17 is expressed in the early stages of epidermal appendage development.[37]

This study confirms the PC-associated features and phenotype–genotype correlations previously reported in smaller series.[2,26] A previous study reported that although most patients manifest key features of PC in the first years of life, diagnosis is usually delayed and only 25% of the cases are diagnosed by 1 year of age.[26] Here we show that nail dystrophy is the initial clue, appearing in the first year of life in most cases, while by 4 years of age plantar keratoderma is evident in approximately 60% of cases.[26] Accordingly, diagnosis can easily be confirmed in the preschool years and a combination of clinical manifestations (nails dystrophy, keratoderma, oral leucokeratosis, cysts and natal teeth) should raise the suspicion for specific PC subtypes prior to genotyping. Nail dystrophy involving most nails predicts PC-K6a or PC-K17. The additional presence of oral leucokeratosis should strongly suggest PC-K6a, while natal teeth and cysts would point to PC-K17.

In conclusion, individuals displaying nail dystrophy, plantar keratoderma and plantar pain, the three most common clinical features of PC, should be referred for genetic testing given the high likelihood of a PC-related mutation in the face of this constellation of clinical signs. Early diagnosis is tremendously significant for patients and families, allows for appropriate care and genetic counselling, and may, in some cases, suggest therapeutic solutions.[38,39]

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig S1. Correlation between mutations in pachyonychia congenita-associated keratin genes and lack of fingernail dystrophy.

Table S1 Pachyonychia congenita (PC)-associated keratin gene mutations in the International PC Research Registry.

Table S2 Clinical features of nails and palmoplantar involvement.

Table S3 Clinical characteristics of mucosal involvement.

Table S4 Characteristics of pachyonychia congenita-associated cysts.

Appendix S1 Supplementary discussion.