INTRODUCTION

Pachyonychia Congenita is a very rare genetic disease. The global prevalence of the condition is estimated to vary between 1,000 and 10,000 cases [1]. The International Pachyonychia Congenita Research Registry (IPCRR) reported 1038 patients PC with 118 mutations, in 547 families in January 2021 [2]. PC disease is caused by mutations in 1 of 5 Keratin genes *KRT6A, KRT6B, KRT6C, KRT16, KRT17* [3]. This is an autosomal dominant inherited disease, about 70% of individuals diagnosed with PC have an affected parent, 30% cases caused by a de novo pathogenic variant [4]. Pachyonychia congenita is characterized by hypertrophic nail dystrophy, painful palmoplantar keratoderma and blistering, oral leukokeratosis, excessive sweating of the palms and soles, pilosebaceous cysts, palmoplantar hyperhydrosis, and follicular keratoses [5]. PC patients are observed in clinical and subclinical signs but nonspecific that can be mistaken for other nail disorders. The diagnosis is based on the identification of mutations in the Keratin gene [4]. There is no specific treatment available for PC [6], and gene therapy remains experimental. Some interventions aim to help patients cope with their psychosocial challenges and enhance their community integration [7], [8], [9]. Early diagnosis and treatment can prevent unnecessary complications and improve the patient's quality of life.

In the world, in 1904, Muller who was the first person described the clinical symptoms of PC. However, it was not until 2016 that the IPCRR provided a clear picture of PC disease and showed that overlapping clinical features may be associated with genotypes with specific mutations in patients PC [10].

In Vietnam, In Vietnam, no studies on PC have been published before. However, the first case of PC in Hai Phong was confirmed by Keratin gene analysis, which raised the awareness of health professionals and the public about this rare disease. Since then, efforts have been made to expand the screening, diagnosis and management of PC patients in Vietnam.

Therefore, how can PC patients be distinguished from those with nail thickening? What are the clinical, paraclinical and genetic features of PC in Vietnam? What are the treatment that can enhance the quality of life of these patients?.... To answer these questions, we conducted the project "Research on genotype, phenotype and outcomes of supportive care in children with PC" with the following three objectives:

1. To determine the prevalence of nail thickening in children at the National Hospital of Dermatology and Green International Hospital from August 1, 2019 to August 31, 2021.

2. To describe the phenotype and genotype of PC patients

3. To comment on the results of treatment and supportive care for these PC patients after 6 months.

We hope that the results of this study will contribute to early diagnosis and proper care for PC patients in Vietnam.

CHAPTER 1: LITERATURE

1.1. Pachyonychia Congenita

1.1.1. Definition

Pachyonychia Congenita (PC) is a rare autosomal dominant disease. The disease is caused by mutation in one of the five Keratin genes *KRT6A, KRT6B, KRT6C, KRT16, KRT17* on chromosomes 12q and 17q, which disrupts the synthesis of Keratin, a protein was found in nails and skin [4]. When Keratin deficiency, symptoms in the skin and nail system are appeared such as nail thickening, nail dystrophy, palmoplantar keratoderma and some other manifestations such as painful blistering, oral leukokeratosis, pilosebaceous cysts, increased sweating on the palms and soles, and keratosis pilaris on the trunk and extremities.

1.1.2. Epidemiology

- Prevalence: Recent global estimates found from 1,000 to 10,000 cases of PC [1]. As of January 2021, the IPCRR reported 118 mutations in 1038 patients from 547 families with genetically confirmed PC [2].
- Gender: there were no gender differences in PC [4].
- Age: The disease usually manifests at birth with characteristic nail thickening [4].

1.1.3. Genotype characteristics

As of January 2021, IPCRR contained genetic and survey data on 1038 PC patients with 118 different mutations, 412 PC-K6a patients, 93 PC-K6b patients, 34 PC-K6c patients, 338 PC-K16 patients, 161 PC-K17 patients.

Specific mutations have been reported by the international PC project [2]. Among them, most PC patients have mutations in keratin domain 1A or domain 2B, especially in the conserved boundary region. The mutations include substitutions and deletions, insertion, frameshift mutation [21], [26], [27]. PC patients in *KRT6B* and *KRT6C,* mutations are commonly found in the domain 2B. On the other hand, PC patients in *KRT6A, KRT16* and *KRT17* are commonly found in the 1A domain. Some patients had mutations in the head region of *KRT6A* (n = 9) and *KRT16* (n = 2), while mutations in the tail region (n = 18) were only seen in patients with *KRT6A*. Currently, there is no mutations in 1B or 2A domain.

Fifty-six unique variations were seen in patients with *KRT6A* mutations, 6 in *KRT6B*, 4 in *KRT6C*, 16 in *KRT16*, and 20 in *KRT17*. N172del (n=109) was the most common *KRT6A* mutation. This same mutation was the second most common KRT6B mutation seen (n=26) and third most common mutation *KRT6C* mutation seen (n=5). E472K also was a common mutation seen in *KRT6A* (n=19), *KRT6B* (n=52), and *KRT6C* (n=15) patients. N125S (n=17) was the most commonly seen mutation in *KRT16* patients, followed by R127C (n=13) [26].

1.1.4. Phenotype characteristics

In all types of pachyonychia congenita (PC) most characteristics are visible by age ten years.

1.1.4.1. Hypertrophic nail dystrophy: is typically noted within the first few months to years of life, though in rare instances it presents later in life. The nail dystrophy appears to fall into two phenotypes:

- Nails that grow to full length and have an upward slant caused by the prominent distal hyperkeratosis (often with an accentuated curvature of the nail)
- Nails that have a nail plate that terminates prematurely leaving a gently sloping distal region of hyperkeratosis and exposed distal finger tip

Nails may be discolored. The surface of the nail may be rough or smooth, the distal fingertip may appear slightly swollen [16].

1.1.4.2. Focal palmoplantar keratoderma usually presents during the first few years of life when a child starts bearing weight and walking. Blisters develop beneath the keratoderma resulting in intense pain. For many individuals, the blisters and constant foot pain are more severe in warmer weather than cooler weather. The pain associated with plantar focal blistering may require the use of crutches, canes, or wheelchairs. Rarely, keratosis palmoplantaris transgrediens (the contiguous extension of hyperkeratosis beyond the palmar and/or plantar skin) is present [4].

1.1.4.3. Oral leukokeratosis (thickened white patches on the tongue and cheek) is often present. In babies, oral leukokeratosis can be misdiagnosed as Candida albicans and may cause difficulty in sucking [4], [14].

1.1.4.4. Follicular keratosis, usually on the elbows, knees or trunk [2]. It is more prevalent in late childhood and teenage years and becomes less problematic in adults [4].

1.1.4.5. Pilosebaceous cysts including widespread steatocystomas/steatocysts (benign lesions) and vellus hair cysts. Cysts may increase in number at puberty. Pilosebaceous cysts have vary greatly in size and severity. These cysts often require surgery or removal when they rupture [4].

1.1.4.6. Natal teeth or prenatal teeth: natal teeth are usually associated with pathogenic variants in KRT17 [16]. Primary and secondary dentition is normal [4].

1.1.4.7. Other symptoms

Other findings that may occur: excessive sweating of the palms and soles (observed in approximately 50% of individuals), axillary and inguinal cyst formation, excessive production of waxy material in the ear, severe and unexplained ear pain, hoarseness (laryngeal involvement), reported primarily in young children. Although rare, laryngeal involvement may cause life-threatening respiratory distress requiring intervention, angular cheilitis, paronychia with pronounced edema [4].

1.1.5. Genotype-Phenotype Correlations

1.1.5.1. Phenotype Correlations by Gene

Table 1.2: Genotype-Phenotype Correlations

Phenotype	**Genotype**
Leukokeratosis with laryngeal, failure to thrive" and poor feeding in infancy	*KRT6A*
Focal non-epidermolytic palmoplantar keratoderma	*KRT6C, KRT16*
Steatocystoma multiplex, natal teeth	*KRT17*

1.1.5.2. Genotype-Phenotype Correlations

Based on data on 774 individuals with PC in the IPCRR, clear genotype-phenotype correlations are evident

In the following instances, the phenotype may vary among individuals with the same pathogenic variant:

- The same KRT17 pathogenic variant in the highly conserved helix initiation motif has been observed in classic PC and in a few individuals with the milder variant SM with few or no nail changes. The modifying factors responsible for this variable expressivity are not known.
- In a few reports of late-onset PC, pathogenic variants have been identified outside the helix boundary and some have questioned whether the location of the pathogenic variant affects the age at onset. However, the ages in these

cases are the expected ages at onset for the particular type of PC and should likely not be referred to as "late-onset."

1.1.6. Diangosis

1.1.6.1. Clinical diagnostic

Clinical diagnostic include 3 symptoms [4]:

- Nail thickening
- Plantar keratoderma
- Plantar pain

1.1.6.2. Diangosis

The diagnosis of PC is established in a proband with the triad of toenail thickening, plantar keratoderma, and plantar pain and/or by identification of a heterozygous pathogenic (or likely pathogenic) variant in one of the five genes keratin KRT6A, KRT6B, KRT6C, KRT16 and KRT17 [4], [26].

1.1.7. Treatment, suportive care

There are currently no specific treatments for PC, patients are mainly treated symptomatically [4], [6], [38]. The goals of treatment for patients with pachyonychia congenita (PC) are designed to address the four major manifestations of the disease: (1) excess keratin accumulation in the nail unit, the skin or the mucous membranes; (2) blisters; (3) the pain that is associated with blisters in some, but not in all, of the hyperkeratotic areas; (4) the keratin cysts in the dermis. Treatment options fall into four broad categories: non-invasive (mechanical), invasive (surgical), chemical, and pharmacological. However, the coordination between these measures is very important

Mechanical treatments

- Foot care: rest in bed, avoid excessive walking or standing, and keep the keratosis areas clean and avoid over-trimming them as this may worsen the pain. If blisters form, drain them with a sterile needle and apply moisturizers and creams with keratolytic agents. Remedies for sore feet: Reducing friction, injury, maintaining an ideal weight; wearing light socks, breathable shoes, and comfortable insoles that fit the foot shape; using crutches or a wheelchair if needed.
- Hand care: wear breathable and soft gloves, use an abrasive nail clipper, and avoid applying force on the nail plates.
- Tongue cleaning: oral hygiene, use a soft brush
- Skin care: Apply moisturizers or creams containing alpha-hydroxy acids or keratolytic agents to soften and exfoliate the skin.

Pharmacological treatment

-Emollients, such as Vaseline or lanolin-based products, are frequently used and have reported efficacy in moisturizing the skin.

-Creams with keratolytic agents (such as urea, lactic acid, salicylic acid, or propylene glycol) can help dissolve and remove the excess keratin.

-Retinoids are systemic medications that decrease keratinization, promote the differentiation and proliferation of epidermal cells, and have been prescribed for patients with PC.

- Hyperhidrosis is treated with aluminum chloride and this symptom is usually worse in the summer, better in the winter [2].
- If bacterial or fungal infection occurs, systemic antibiotics or antifungals are indicated. Culture and treatment with appropriate antifungal or antibacterial agents are a recurring feature of the regimen for many patients.
- Systemic antibiotics or antifungals are indicated for bacterial or fungal infection. Antibiotic therapy based on culture results is a frequent part of the management of PC. Some cases apply a topical anesthetic for painful blisters and fissures. Many patients disagree with the use of anti-inflammatory agents (especially nonsteroidal anti-inflammatory drugs) or anesthetic analgesics for pain control.
- The use of oral antihistamines or with local anesthetics or steroids for the patient's pruritus has been considered.

Surgical

Surgical approaches, including electrofulguration, deep curettage, and excision followed by grafting of autologous skin from an unaffected site, have been applied more successfully to nails than to palms or soles. Keratoses recur in transplanted skin on the soles; we could find no examples of successful surgery for the soles.

1.2. The research on PC in the word and Vietnam

1.2.1. In the world

Smith FJD and colleagues had a complete article about PC, the author described PC with clinical manifestations of nail hypertrophy, painful and blistering soles, oral leukokeratosis, cysts, horny cysts on trunk and extremities [4]. Forrest CE et al. also reported similar clinical features in PC patients [39].

According to IPCRR data, there are 118 different mutations in PC disease [2]. Every year, the authors report on newly discovered new genes. For example, Smith et al. reported a new homozygous mutation in the *KRT17* gene, this mutation was R94-98del (deleting the RLASY peptide chain) in 2001, and Cogulu.O discovered a mutation in the N92S gene of keratin 17 in 2009 [40], [42].

Treatment of PC is mainly symptomatic, such as foot pain relief, nail care, and some other mechanical therapy [4], [6], [43]. For pregnant women with PC, weight gain or changes in the hormonal environment during pregnancy may exacerbate painful keratosis symptoms, so this group also needs special attention during treatment. Currently, some researchers have evaluated various medications to enhance the quality of life for patients, such as botulinum toxin injection or topical sirolimus cream [7].

PC is an autosomal dominant disease; however, about 30% PC are self-mutation, and one case of mosaicism has been reported (1/774) [4]. In 2011, Pho et al. reported a case of a patient with PC and the parents were asymptomatic [46]. When the first child in an otherwise asymptomatic family is diagnosed as having PC, it is usually due to a spontaneous mutation that developed in utero.1 However, it is also possible that the diagnosis could be missed in a parent with exceptionally mild disease symptoms, or that germ cell mosaicism exists in an asymptomatic parent [46].

1.2.2. In Vietnam

Previously, there were no reports of congenital thick nail disease in our country. Recently, there are reports of authors Vu Van Quang and Chu Thi Ha on a case of congenital nail thickening which was confirmed by analysis of keratin gene mutations [47], [48]. This patient was misdiagnosed and mistreated for a long time. At present, children are in dire need of care to improve their quality of life, ensure their future study and work. Moreover,

there are still many children with congenital thickening of nails who have not been accurately diagnosed with the disease for timely management and treatment. Therefore, the research team hopes that after the above report, doctors will pay more attention to this rare disease to make a correct diagnosis and improve the quality of life of patients.

CHAPTER 2: OBJECTS AND METHODS OF RESEARCH

2.1. Subjects, location and duration

2.1.1. Subjects

- Subjects of research 1: Children had thickened nail and/or nail dystrophy who came to National Hospital of Dermatology and Venereology and Green International Hospital.

Selection criteria:

+ Patient under 16 years old [49].

+ The normal nail plate is regular along its length and is 0.5–0.7 mm for fingernails and 1–1,2 mm for toenails. So, nail thickening is the nail plate becomes abnormally thickened [50], [51]. Nail dystrophy is a term that covers various disorders that affect the shape, color, or texture of the nails [52].

- Subjects of research 2, 3: Children were diagnosed with PC and they received treatment and supportive care.

PC patients: who had mutation in one of five keratin genes *KRT6A, KRT6B, KRT6C, KRT16, KRT17*, specimens are the patient's blood or saliva [4], [10].

Children were diagnosed with PC and they received treatment and supportive care.

+ Patients with PC had mutations in one of five keratin genes: *KRT6A, KRT6B, KRT6C, KRT16, or KRT17*. The most common types of specimens that are used for genetic testing are blood and saliva [4], [10].

Exclusion criteria:

+ The children have a life-threatening condition that requires urgent intervention or necessitate emergency department care.

+ Children or their parents didn’t accept to participate in the study.

2.1.2. Duration: from 1/8/2019 to 31/8/2021.

2.1.3. Location: National Hospital of Dermatology and Venereology and Green International Hospital.

2.2. Methods

2.2.1. Research design

- Objective 1: a cross-sectional descriptive study
- Objective 2: case series study
- Objective 3: interventional study comparing before and after, no control group.

2.2.2. Sample size

- Objective 1: calculation formula

$$n = Z^2_{1-\alpha/2}\frac{p(1-p)}{\Delta^2}$$

p: the prevalence of diseases studied in the same community

∆: the desired deviation between the sampled disease rate (p) and the population proportion (P)

α: statistical significance level

$Z_{1-\alpha/2}$: Z value is obtained from Z table corresponding to selected α value.

We selected Δ= 0,05, α= 0,05, p= 0,286 (This rate is based on 6 months trial from 1/8/2019 to 31/1/2020, with 105 children nail thickening and/or nail dystrophy out of a total of 367 children with nail disease.).

We calculated a sample size of 314 patients. We obtained 374 patients who had the selection criteria

- Objectives 2, 3: all patients were diagnosed with PC, then they were treated and provided supportive care. We obtained 8 patients PC for inclusion in the study.

2.2.3. Study diagram

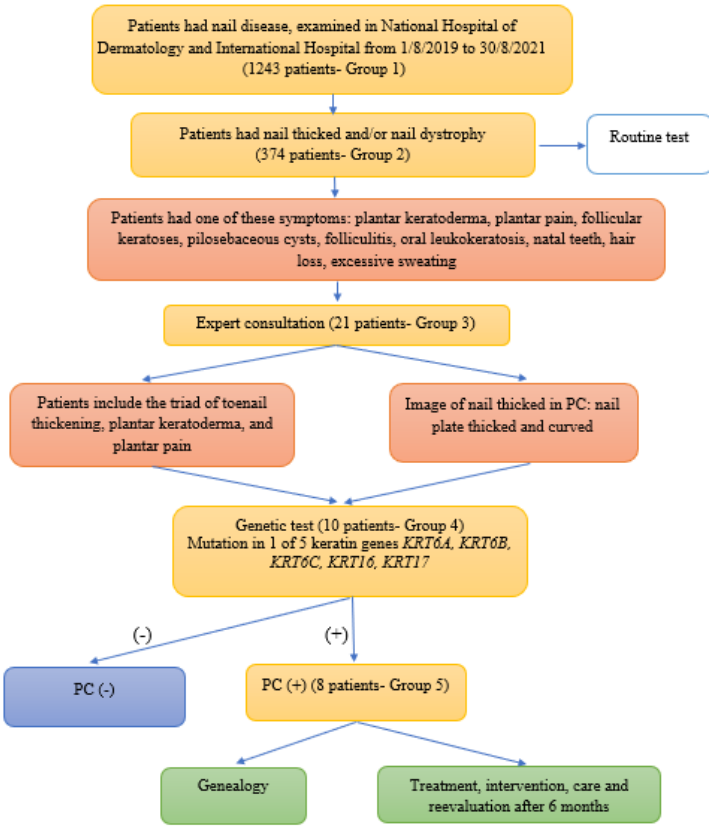


Figure 2.1: Study diagram

2.2.4. Indicators, variables in the study

Some criteria for evaluating indicators and variables:

- Diagnosis PC: mutation in one of five Keratin genes *KRT6A, KRT6B, KRT6C, KRT16, KRT17*, specimens are the patient's blood or saliva [4].

Table 2.4: Hemoglobin levels to diagnose anemia at sea level (g/l) WHO [59].

Age	Anemia Hb (g/l)	Mild	Moderate	Severe
0,5- <5	<110	100-109	70-90	<70
5- <12	<115	110-114	80-109	<80
12- 15	<120	110-119	80-109	<80

- Change symptoms:

+ No change: symptoms before and after treatment do not change

+ Relief: symptoms before and after treatment have improved a little or a lot

+ Cure: at the time after 6 months of care and support, the patient has no these symptoms.

- The questionnaire "A Quality of Life Assessment Measure for Pachyonychia Congenita", recognized by IPCRR [57].

- Children's pain score: using the Wong-Baker Faces Pain Rating Scale (WBFPRS) (0-10) [56], [61].

2.2.5. Techniques used in the study

- Blood tests: complete blood cell analysis and blood biochemistry

- Direct nail examination

- Genetic testing: Patients will be analyzed keratin gene by IPCRR that is located at the Laboratory of Molecular Genetics, Human Genetics Unit, Ninewells Hospital, University of Dundee, Scotland, UK. This result was independently confirmed in the United States by the laboratories of the private company GeneDx (Gaithersburg, Maryland) [30].

- Step 1: Take the patient's saliva sample, store it in the kit (Oragene DX OGD-500 kits) according to the manufacturer's procedure.

- Step 2: Extract genomic DNA from saliva and store it in the kit QIAamp DNA mini kit process.

- Step 3: Analyze each exon of the Keratin gene

Gene sequencing: DNA sequencing using an ABI 3700 automated machine (Applied Bio-systems, Foster City, CA, USA).

- Step 4: Compare gene banks online to find mutations

2.2.6. Data processing

Using SPSS 20.0 software to perform collected data.

2.2.7. Ethics in research

- The study was approved by the Scientific Research Council of Haiphong University of Medicine and Pharmacy

- Research information is strictly confidential and only serves research purposes.

CHAPTER 3: RESULTS

3.1. Determine the prevalence of nail thickening in children at the National Hospital of Dermatology and Venereology and Green International Hospital from 1/8/2019 to 31/8/2021.

3.1.1. General characteristics.

- The incidence of study subjects distributed evenly in age groups from 0- ≤ 5 years old, 5- ≤ 10 years old and 10- ≤ 15 years old: 27.5%, 38.2%, 34.3%
- The incidence of female patient was higher in male, 52.9% and 47.1%.

Table 3.2: Clinical characteristics (n=374)

Clinical characteristics	Number	Percent %
Nail pain	41	11.0
Itchy nail	79	21.1
Plantar keratoderma	32	8.6
Palmar keratoderma	36	9.6
Skin and mucosal damage	191	51.1
Other symptoms	35	9.4

Comment: 51,1% patients had skin and mucosal damage. Other symptoms accounted for a small percentage.

- There were 11,8% patients who had increased white blood cell, 3,4% patients with increased CRP, and 2,9% patients with decreased Hb.

Table 3.3: Features of direct microscopy nail (n=374)

Mycology results	Number	Percent %
Negative mycology results	249	66.6
Candida	98	26.2
Malassezia	6	1.6
Mycelium	12	3.2
Mycelium + *Candida*	8	2.1
Mycelium + *Malassezia*	1	0.3
Total	374	100.0

Comment: microscopy were positive results in 33,4% of cases and negative results in 66,6%. Major mycology is *Candida* (26,2%).

3.1.2. Incidence of patients with thickened nails

- The number of children who came to the National Hospital of Dermatology and Venereology and Green International Hospital from 1/8/2019 to 30/8/2021 was 191,499 patients.
- Patients with nail disease in 2 hospitals were 1243 patients.
- Patients with thicked nail and/or nail dystrophy in 2 hospitals were 374 patients.
- The rate of children with thicked nail compared to the total number of children came to 2 hospitals is 0.04%, while the number of children with nail disease is 30.0%.

Table 3.6: Distribution disease (n=374)

	Number	Percent %
Onychomycosis + Dermatophytosis	122	32,62
Psoriatica	120	32,08
Nail dystrophy	77	20,58
Palmoplantar keratoderma	21	5,61
alligator-skin disease	13	3,47
Pachyonychia	8	2,13
Other disease	13	3,74
Total	374	100,0

Comment: The proportion of patients with onychomycosis and dermatophytosis were the highest rate 32,62%, while the lowest prevalence rate (2,13%) was attributed to PC.

3.2. Describe genotypic and phenotypic of PC patients

3.2.1. General characteristics.

Table 3.9: General characteristics.

	Age of onset (years)	Age at diagnosis (years)	Age (as of 2022) (year)	Gender	Nation	History
Patient 1	< 1	1	5	Male	Kinh	-
Patient 2		4	9	Male	Kinh	-
Patient 3		5	11	Female	Kinh	-
Patient 4		1	5	Male	Kinh	+
Patient 5		3	9	Female	Kinh	-
Patient 6		2	6	Female	Kinh	-
Patient 7		7	11	Male	Kinh	+
Patient 8		1	1	Male	Kinh	-

Comments: the first clinical manifestations appeared very early, there were 3 patients diagnosed at 1-year-old, seventh- patient was diagnosed at 7-years-old. There were 3 females and 5 males. All the patients were Kinh people. Only fourth and seventh- patient were in the same family.

- Six patients had undergone operation, in which 4 for nails and 2 for toenails.
- Most of the patients showed symptoms of keratosis at the age of 1-4 years, accounted for 75%.

3.2.2. Genotype of PC patient

Table 3.13: Genotype

Patient	Gene mutation	Domain	cDNA change	Acid-amin change	Protein change
Patient 1	*KRT6A* NM_*005554.3*	1A	c.516_518delCAA	Del Asparagine	N172del
Patient 2	*KRT6A* NM_*005554.3*	1A	c.516_518delCAA	Del Asparagine	N172del
Patient 3	*KRT6A* NM_*005554.3*	1A	c.513C>A	Asparagine → Lysine	N171K
Patient 4	*KRT6A* NM_*005554.3*	1A	c.516_518delCAA	Del Asparagine	N172del
Patient 5	*KRT6A* NM_*005554.3*	2B	c.1397G>C	Arginine → Proline	R466P
Patient 6	*KRT6A* NM_*005554.3*	1A	c.516_518delCAA	Del Asparagine	N172del
Patient 7	*KRT6A* NM_*005554.3*	1A	c.516_518delCAA	Del Asparagine	N172del
Patient 8	*KRT17* NM_000422.2	1A	c.290_292delTCC	Del Serine	S97del

Comments: Among the patients with PC, 7/8 had mutations in *KRT6A* gene, 1/8 had mutations in *KRT17* gene; including 4 types: N172del, N171K, R466P, S97del. The dominant mutation domain is 1A, leading to the loss or replacement of the original amino acids.

3.2.3. Phenotype

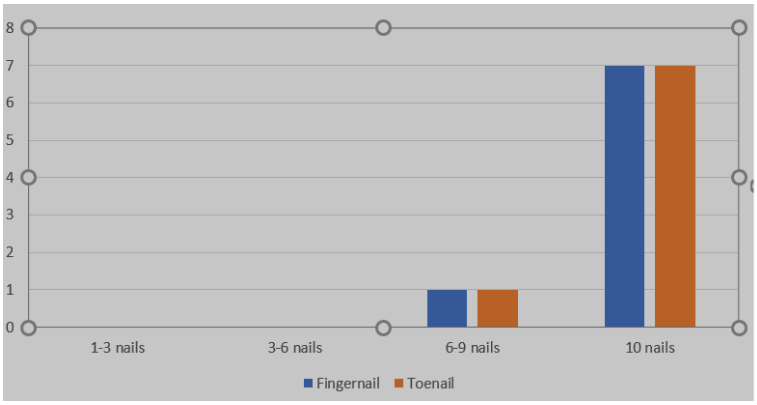


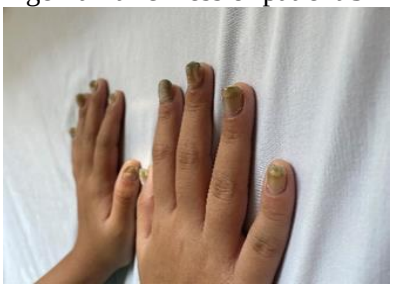
Figure 3.4: Phenotype of thicked nail in PC patient

Comment: Seven patients showed thickness of 10 fingernails and 10 toenails, only 1 patient had 7 fingernails and 7 toenails thick.

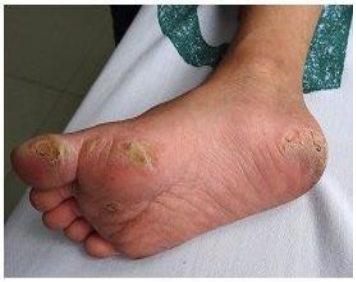
- All the patients presented with symptoms of plantar keratoderma, plantar pain, oral leukokeratosis, follicular keratosis. Additionally, one out of the eight patients had with teeth at birth.
- The majority of the patients reported experiencing cracked, painful, swollen and calluses of the feet. Currently, 3/8 of patients still exhibit these symptoms, while calloused feet are observed in all patients.
- 100% of patients had a history of nail infection, with most patients (7/8) had a history of toenail infection.

3.2.4. Images of patients

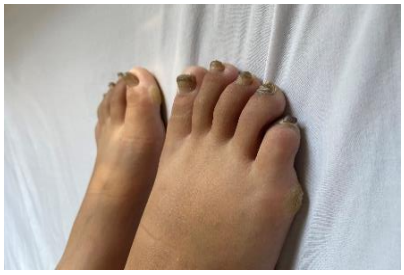
Fingernail thickness of patient 3



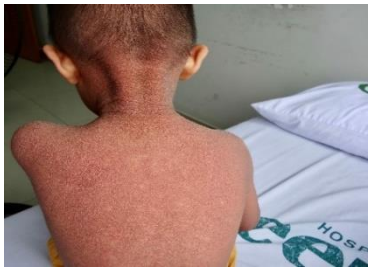
Plantar keratoderma of patient 2



Toenail thickness of patient 3

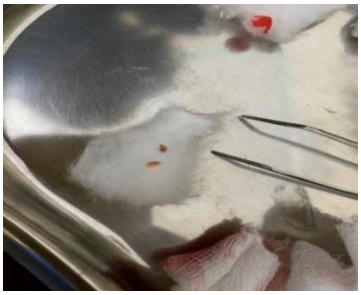


Skin damage of patient 2

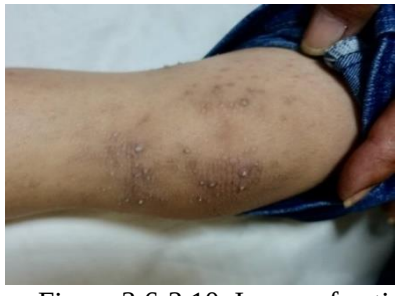


Oral leukokeratosis of patient 6

Natal teeth of patient 8



Follicular keratosis of patient 7



Nail infection of patient 8

Figure 3.6-3.10: Image of patient

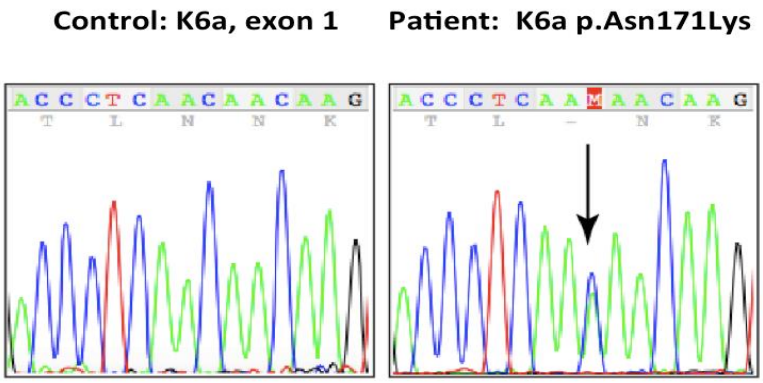


Figure 3.10: Mutation at exon 1 of *KRT6A*

Comment: Mutation at exon 1 of K6a gene produces an early termination triptych that stops K6a protein chain synthesis.

3.2.5. Genotype-Phenotype Correlations

Table 3.18: Phenotype Correlations by Gene

Phenotype	Genotype
Oral leukokeratosis, plantar keratoderma and pain, many thick nails, follicular keratosis	Mutation *KRT6A*
Less number of nails thickening	Mutation in 2B domain
Prenatal teeth	Mutation *KRT17*

Comment: Patients have oral leukokeratosis, plantar keratoderma and pain, many thicked nail, follicular keratosis that suggest mutation *KRT6A*, patients have less number of nails thickening that suggest mutation in 2B, patients have prenatal teeth that suggest mutation *KRT17*.

3.2.6. Genealogical chart

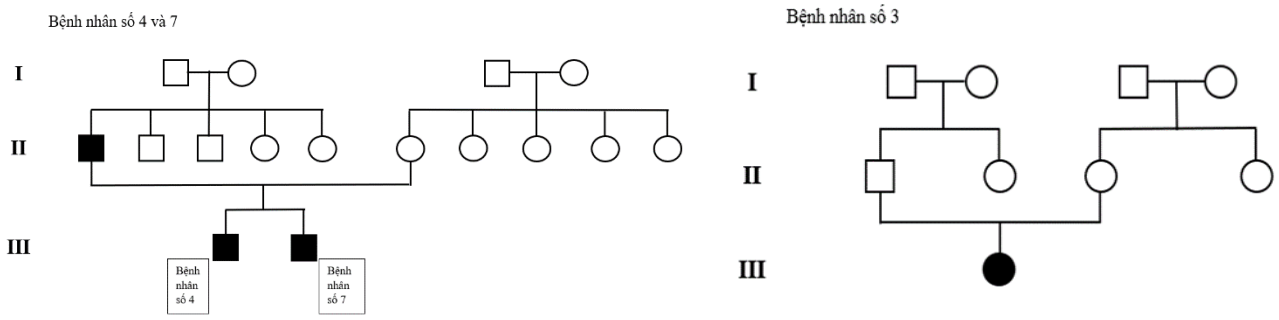


Figure 3.17: Genealogical chart of patient 4, 7 and 3

Comment: family pedigree number 3 suggests de novo mutation, family pedigree number 4 and 7 is inherited from the father.

3.3. Comment on the results of treatment and supportive care interventions for PC patients in 6 months.

- Most of the patients have problems such as bacterial infection, oral leukoplakia, foot pain and some other problems. In the last 3 months of the year, problems tend to decrease.

Table 3.19: Pain scale of patients before and after treatment and intervention

	Before treatment $\overline{X}$±SD	After treatment $\overline{X}$±SD	p
Score pain	6±1,69	4±1,51	<0,05

Comment: There is a difference in pain score scale before and after treatment with p<0.05.

- Before treatment, 100% of patients showed signs of nail thickness, oral leukoplakia, hair follicle hyperkeratosis, plantar keratosis, plantar pain and calluses of the feet; 37.5% of patients had foot blisters, plantar fissures and nail infections. After interventional supportive care, the patient's symptoms improved.

Table 3.21: Change in frequency of nail and plantar care before and after treatment (n=8)

Change in frequency care		Before treatment n (%)	After treatment n (%)
Plantar care	1 time/ week	2 (25,0)	0 (0,0)
	1 time/ month	5 (62,5)	8 (100,0)
	1 time/ 2 month	1 (12,5)	0 (0,0)
Palmar care	1 time/ 2 week	2 (50,0)	8 (100,0)
	1 time/ month	6 (50,0)	0 (0,0)
Nail care	1 time/ month	5 (62,5)	8 (100,0)
	> 1 time/ month	3 (37,5)	0 (0,0)
Average number of times of foot pain in a week $\overline{X}$ ± SD (min-max)		7,13±2,80 (1-10)	6,88±2,16 (2-9)

Comment: After guiding interventional care in six months, the patients learned the recommended frequency of nail care, toenail care and plantar care, maintaining monthly, and weekly for fingernails. The number of times of plantar pain per week in the group after the intervention was lower compared the group before the intervention, but the difference was not statistically significant.

Table 3.31: Comparison of change in mean score of quality of life of patients with PC

Score of quality of life of PC patient/ week	Before treatment	After treatment	p*
	Median (25^{TH}- 75^{TH})	Median (25^{TH}- 75^{TH})	
Number of time of plantar pain	3,0 (3,0-3,0)	3,0 (3,0-3,0)	>0,05

Number of time of nails interfered with your daily work	2,5 (1,25-3,0)	2,0 (1,0-2,0)	**<0,05**
Number of time of interfered with going shopping or looking after your home or garden	1,0 (1,0-1,75)	1,0 (0,25-1,0)	>0,05
Number of time of affected any social or leisure activity	1,5 (1,0-2,0)	1,0 (0,25-1,75)	>0,05
Number of time of difficult for doing any sport	2,0 (1,0-2,0)	1,0 (1,0-1,0)	**<0,05**
Number of time of prevented you from working or studying	1,0 (0,0-1,75)	1,0 (0,0-1,0)	>0,05
Number of time of skin created problems with your close friends or relatives	2,0 (1,25-2,75)	1,0 (0,25-1,0)	**<0,05**
Number of time of problem must be take care	1,0 (1,0-2,0)	1,0 (1,0-1,75)	>0,05
Total score of quality of life (11 questions)	16,5 (13,0-18,75)	12,0 (9,25-13,0)	<0,05

** Wilcoxon Signed Ranks Test*

Comment: the patient's overall quality of life improved after intervention and supportive care; Specifically, nails were less to interfere with daily work, change the number of times of plantar pain affected sports activities and reduce the influence of skin on the patient's relationship with people.

CHAPTER 4: DISCUSSION

4.1. The rate of pachyonychia congenita in children at the National Hospital of Dermatology and Venereology and Green International Hospital for the period of August 1, 2019 - August 31, 2021.

4.1.1 Characteristics of research subjects

By age: the number of patients between the groups 0- ≤ 5 years old, 5- ≤ 10 years old and 10- ≤ 15 years old were relatively equal, accounting for 27.5%; 38.2% and 34.3% respectively (Table 3.1). This shows that at any age, children can experience dermatological problems in general and nail pathology in particular. The rate of children with nail lesions in general ranges from 3 to 11% of children and there are about 75% of congenital and inherited syndromes with associated nail lesions [45].

By gender: female patients accounted for 52.9%, male patients were 47.1%. Thus, there is no difference between men and women who are suffered from diseases related to skin and nails. For example, pachyonychia congenita is an autosomal dominant genetic disease, the incidence of which is equal in men and women.

According to clinical characteristics: 51.1% of patients showed skin and mucosal lesions, followed by nail itching, nail pain and some other symptoms 9.4% (Table 3.2). Symptoms of nail pain and nail itching are common in patients with onychomycosis. Symptoms of nail pain and nail itching are sometimes not obvious in the group of young children, but we also carefully explored the clinical manifestations and examined carefully to identify

these two signs. According to author Nguyen Minh Huong, symptoms of nail pain and nail itching were only seen in 21.1% and 22.8% of patients, mainly in patients with Candida onychomycosis [68]. Thus, our results are quite similar to ones of the above two authors.

According to blood tests: the number of patients with leukocytosis accounted for 11.8%, CRP increasing accounted for 3.2%. Patients with leukocytosis and CRP increasing were mostly patients diagnosed with generalized pustular psoriasis. This demonstrates the concordance between clinical and subclinical. There is a few of anemic patients, accounting for 2.4%. Anemia patients are scattered in different age groups, with mild anemia, so patients have not been done further tests to find the cause of anemia.

According to the results of nail fungus examination: the results of Table 3.3 showed that 66.6% of patients did not find fungus on nail examination, and 33.4% of patients with nail examination showed fungus. Of the total study subjects, 26.2% of the patients with nail examination had Candida fungus, 3.2% of the patients had mycelium, 2.1% of the patients had mycelium and Candida, and 1.6% had Malassezia fungus, the lowest was in patients with mycelium and Malassezia fungus, accounting for 0.3%. The most of patients with a positive result of nail examination had a preliminary diagnosis of Onychomycosis by their doctor before microbiological testing. Some patients with other pathologies such as psoriasis, nail dystrophy... were found to have an accompanying fungal infection.

4.1.2 Percentage of children with pachyonychia congenita

Proportion of children with pachyonychia congenita and/or nail dystrophy: the results of the research in Table 3.6 showed that the rate of children with pachyonychia congenita and/or nail dystrophy compared to the number of children visiting the National Hospital of Dermatology and Green International Hospital is 0.19%, compared to 30.0% of the number of children with nail pathology. Although this rate does not fully reflect the prevalence of pachyonychia in Vietnam or the northern provinces, it also partially represents the prevalence of the disease in the two major northern cities. Pachyonychia congenita is a manifestation of the nail plate being thicker than normal. Nail dystrophy is a change in the complexion, color, and size of the nail. Nail disorders can arise at any age. About half of nail diseases are of infectious origin, 15% are due to inflammatory or metabolic conditions, and 5% are due to malignancy and pigmentation disorders. Therefore, the differential diagnosis of nail disorders is also much difficult [65].

Pathological distribution of research subjects: there are many diseases causing damage to nails, including symptoms of pachyonychia. Table 3.7 shows that the most common cause is nail fungus 32.62%, followed by psoriasis, the lowest is pachyonychia congenita with 2.13%. Onychomycosis is a nail infection caused by fungus, manifested by nail color change, thickened nail... The rate of nail infection in general in the world is about 5.5 % [31]; 0.2 to 2.6% [72] in children, about 90% of toenail fungus and 75% of nail fungus are caused by dermatophytes, especially Trichophyton mentagrophytes and Trichophyton rubru. The rarity of the PC makes it difficult to accurately gauge its prevalence [4]. According to some documents in the world, the number of patients with pachyonychia congenita is estimated from 7,000 to 10,000 cases, equivalent to about 0.9/1 million population [26].

4.2 Genotypes and phenotypes of patients with pachyonychia congenita

4.2.1 General characteristics of patients with pachyonychia congenita.

PC patients in the study had their first symptoms very early, all under 2 months old (Table 3.10). The first clinical manifestation is usually an abnormal and yellow nail. Thanks to IPCRR and clinical experience after the first case in Vietnam, our three patients were diagnosed quite early at 1 year of age. However, PC is a rare disease, so the diagnosis still has certain difficulties. Patient No. 7 was diagnosed at the latest at age of 7. At birth, 47.5% of PC patients had toenail changed, 40.6% of patients had nail changed and plantar keratosis in 6.9% of patients. By age of 5, these three major manifestations were found in 81.2%, 74.2% and 75.3% of genotyped PC patients, respectively. The correct diagnosis was made within the first year of life in 26.7% of patients although manifestations of toenail dystrophy were present in more than 65.3% of patients [5]. Currently, there are 2 PC patients in the study who are more than 10 years old. At this time, children enter the puberty stage when there are many psycho-physiological changes, so the clinical manifestations of the disease will be an obstacle to the patient's social relationships. In 8 PC patients of the study, there are 3 female patients and 5 male patients, reaching the ratio of 1/1.67. The patients are all Kinh people. Currently, according to IPCRR statistics, there are 53 countries and ethnic groups around the world recording patients diagnosed with PC. In which, the highest is the US with 484 cases, the United Kingdom of Great Britain and Ireland with 115 cases, a few countries have recorded the first cases such as Bulgaria, Chile .. . [2]. Patients No. 4 and 7 were born in the same family with a father whose mild clinical manifestations of PC disease. According to Smith, 70% of PC patients have a family history, ie, the disease is passed on to their children by one parent, and 30% is a self-mutation [4].

According to the results of Table 3.11, there are 4/8 patients who have ever undergone manicure surgery and 2/8 patients who have ever had pedicure surgery. In the process of taking the patient's medical history, we found that most of the patients were treated surgically without a definitive diagnosis. According to IPCRR 2018 statistics of PC patients, out of 91 toenails removed, 35 toenails never regrow, 28 partially regrow, 18 regrow completely, and 10 have a condition of unspecified regrowth. Of the 538 patients, 25 had their fingernails removed; 6 people remove both fingernails and toenails. Various chemical and surgical techniques have been used to prevent nail regrowth. The different results between patients may be explained by the lesions of the patients in different nail units. However, the majority of patients are satisfied because they find relief from pain, less trauma, less nail care, and less risk of nail infection [73].

The results of Table 3.12 show that 2/8 children showed signs of Palmoplantar keratodermas before 1 year of age. This manifestation in patients mainly occurs in stages under 4 years of age (6/8 patients). Thus, this symptom appears at the time when the child begins to learn to walk or has walked quickly. When the body's gravity along with the contact of the feet and the ground begins to increase, this symptom also increases. Although this symptom is present at birth in less than 10% of PC patients, according to the study results of Shah S et al., 24.8% of PC patients in general reported Palmoplantar keratodermas in 1-year-old children, 75.3% at age of 5 and 89.1% in the first decade of life [5]. In patients with PC-K6a, PC-K16, and PC-K17, the onset of vegetative keratosis usually occurs before the age of 5 years, while the onset of the patients with PC-K6b and PC-K6c is usually after the age of 5 years [5].

4.2.2 Genotypes of patients with pachyonychia congenita

After enrolling in the Pachyonychia Congenita Project (www.pachyonychia.org) (http://registry.pachyonychia.org/s3/ IPCRR), patients with suspected PC will receive genetic testing to confirm

the free clinical diagnosis. According to the results in Table 3.14, it is shown that 7/8 PC patients are due to *KRT6A* genetic mutation, 1/8 of patients are due to *KRT17* genetic mutation. In which, 5/8 PC patients are caused by CAA deletion mutation on the 1A helical domain causing loss of Asparagine (N172del), 1/8 of missense mutations 513C>A transform Asparagine into Lysine (N171K) and 1/8 of missense mutations 1397G>C on the 2B domain transforms Arginine into Proline (R466P), these mutations are on the *KRT6A* gene. There are 1/8 PC patients caused by a TCC deletion mutation in the 1A domain of the *KRT17* gene that causes loss of Serine (S97del). Currently, according to IPCRR statistics in 2021, there are 116 PC patients in 76 families caused by N172 del mutation, 18 PC patients in 13 families caused by N171K mutation, 3 PC patients in 2 families caused by R466P mutation and 2 PC patients in 2 families caused by S97del mutation [2].

In the past, by clinical examination, it is usually possible to determine the type of PC. Subsequently, molecular analysis can be performed to identify the exact gene defect (Smith, 2003). This involves DNA sequencing of the two appropriate candidate keratin genes; K6a and K16 for PC-1 and K6b and K17 for PC-2. Analysis is normally performed on genomic DNA extracted from blood samples as this is less invasive than obtaining biopsy material. In large families with several affected members it may be useful to perform linkage analysis before DNA sequencing (Smith et al, 2004). Using polymorphic genetic markers within the two keratin clusters on chromosome 12q (type II) and 17q (type I) it is often possible to exclude one locus and therefore halve the amount of DNA sequencing. Even in small families where statistically significant linkage cannot be obtained it may be possible to exclude one keratin locus (Smith et al, 2004). Linkage analysis can also be useful in families where there are several affected members but clinical distinction between PC-1 and PC-2 is unclear. Mutation screening normally focuses first on the two mutation ‘‘hot spots,’’ the highly conserved helix-boundary motifs at either end of the rod domain, and then if no mutation is found, analysis can be extended to other regions [74].

Smith et al had a report about genetic basis of PC in 2005 [74]. The early PC mutation studies were carried out on mRNA derived from skin biopsies to overcome the problem of pseudogenes. Mutation detection strategies based on PCR amplification of genomic DNA have been developed for all four keratin genes involved in PC and primers have been designed to specifically amplify full length K6a, K6b, K16, and K17. In 1994, the molecular basis of pachyonychia congenita (PC) was elucidated. Four keratin genes are associated with the major subtypes of PC: K6a or K16 defects cause PC-1; and mutations in K6b or K17 cause PC-2. Mutations in keratins, the epithelial-specific intermediate filament proteins, result in aberrant cytoskeletal networks which present clinically as a variety of epithelial fragility phenotypes. To date, mutations in 20 keratin genes are associated with human disorders. In the large research PC mutation in 2011, they report genetic analysis of 90 new families with PC, A total of 21 previously unreported and 22 known mutations were found, so it help to identifies mutation hotspot codons that may be useful in the development of personalized medicine for PC [27]. 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. 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) [27]. Approximately half of the families with *KRT6A* mutations identified have mutations in this locus. The most common PC mutation is the p.Asn172del [2].

Genomic DNA was extracted from saliva collected in an Oragene DNA sample collection kit (DNA Genotek, Kanata, ON, Canada) and extracted according to the manufacturer's protocol or from peripheral blood leucocytes using standard procedures. The coding regions of *KRT6A*, *KRT6*, *KRT6C*, *KRT16* and *KRT17* were amplified using primers specific to the respective functional genes and to avoid amplification of K16/K17 pseudogenes. Fourteen were previously unreported mutations, bringing the total number of different keratin mutations associated with PC to 105. The majority were missense mutations, with the remainder being small in-frame deletion, frameshift, nonsense or splice-site mutations in *KRT6A*, *KRT6B*, *KRT6C*, *KRT16* or *KRT17*. The most commonly reported mutation in PC is K6a p.Asn172del, found in 10 families here, and overall in ~30% of kindreds with mutations in K6a and in ~13% of all families with a PC mutation

Thus, PC mutations is caused by 1 of 5 genes *KRT6A*, *KRT6B*, *KRT6C*, *KRT16* and *KRT17*, medicine is increasingly to find more specific mutation and thereby opening a path to treat the disease.

4.2.3. Phenotype of patients with pachyonychia congenita.

The phenotype of patients with pachyonychia congenita: thicked nail and toenail dystrophy are the earliest and most common clinical features of PC. Among 8 patients with pachyonychia congenita, there are 7 patients with 10 fingernails and toenails that grow thicker, 1 patient with 7 thickened fingernails and toenails (Figure 3.4). This once again confirms that this is an important symptom of the disease and is one of the three symptoms that help in the clinical diagnosis of the disease [4]. Thickened nails in pachyonychia congenita has two forms: nails that are fully extended and curved like a bird's claw due to increased horny substance or nails with an early stop of growth, increased keratin at the tip of the nail. The common feature of PC is nail dystrophy; however, the clinical presentation of the nails can be variable, even in families, and is not always in the classic V-shape [77]. Due to the accumulation of keratin under the nail bed, nails develop abnormally at a very early age. This deformity can eventually lead to nail loss. Thickened fingernail symptoms in PC-K6a, PC-K6b groups can be seen in 98%, 97% of patients, respectively, toenail thickening symptoms can be seen in PC-K6a, K6b in 97%, 43% of patients, respectively. [2]. According to author Sonal Shah, thickening nail manifestation was observed in 98.2% of patients at the time of the report; The only exception was the absence of toenail dystrophy in 2 out of 8 PC-K6c patients (25.0%) [5]. PC-K6a patients were more likely to have all 10 toenails more affected at onset than PC-K16 or PC-K6b patients ($p < 0.001$) [41]. In general, the proportion of patients with thickened fingernails and toenails is quite high, in which the rate of thickening toenails (96.2%) is higher than that of fingernails (75.6%). Among PC mutations, patients with type K6a have the highest mean nail and toenail thickening, and often have fingernails thickened within the first year of life. The K6c mutation had the lowest average number of fingernails and toenails thickening [19]. Besides thickening of fingernails and toenails, changes in nail color were also mentioned in the reports. The overall color variation in the nail is most yellow, then brown and the rest [19].

Other common phenotypes of patients with pachyonychia congenita: besides thickened nails, pachyonychia congenita also has many other common clinical manifestations such as plantar keratosis, plantar fasciitis, leukoplakia, follicular hyperkeratosis (Table 3.16).

Plantar keratosis is an important sign of Pachyonychia congenita. Keratosis on the soles of the feet is usually more obvious than on the palms. Localized, non-erythema, hard keratosis, mainly in the pressure points of the feet or in areas of chronic use of the hands. According to the author Smith's data, Plantar keratosis in groups of PC-K6a, PC-K6b, PC-K6c, PC-K16 and PC-K17 is 84%, 94%, 86%, 97% and 66%, respectively. The rate of plantar keratosis is generally 86% [4]. The overlapping phenotypes of plantar keratosis, independent of genotype, can make the clinical diagnosis difficult [30].

Pain in the soles of the feet will appear after the symptoms of keratosis of the feet. During the first decade of life, 70/73 patients developed Plantar keratosis (95.9%), damaging their function; Pain appeared in children with PC-K6b later than in other types of PC ($p < 0.05$) [5].

Leukoplakia occurred in 70.3% of PC patients and was more closely related to PC-K6a than to any other type of PC ($p < 0.001$). Among those affected, the median age at onset was the first 3 weeks after birth, and 26/71 (36.6%) experienced their first occurrence within the first year of life. Leukoplakia is often confused with Candida disease but does not respond to antifungal treatment. Leukoplakia was most commonly noted on the tongue (68/71 (95.8%) [5].

Natal teeth almost confirmed the diagnosis of PC-K17 (86.0% with $p < 0.001$) but were recorded in 2 out of 46 children (4.3%) with PC-K6a. Teeth are described as soft or crumbly and rapidly falling out or described as normal in appearance and persist until the tooth falls out [5]. Duverger O et al. further demonstrated that keratins are produced by enamel-forming fibroblasts and are incorporated into adult human tooth enamel [78].

4.2.4 Genotypic and phenotypic relationship:

Table 3.17 shows that patients with leukoplakia, painful keratosis pilaris, follicular hyperkeratosis and a large number of nails thickened often suggest KRT6A mutation. Patients with a lower number of nails thickened may be associated with mutations on the 2B domain. Patients with thickened nails and neonatal teeth often suggest a KRT17 mutation. The presence of nail dystrophy at birth, particularly involving all nails, predicts PC-K6a or PC-K17 ($p < 0.001$); the concomitant development of oral leukocytosis and often hoarseness in the first year of life suggests the diagnosis of PC-K6a ($p < 0.001$); and the appearance of congenital teeth indicated PC-K17 ($p < 0.001$). In contrast, the early childhood development of palmar keratosis, especially when other features onsets later, may signal PC-K16. The presence of an isolated dystrophic fingernail or toenail was quite rare in PC (77/109 patients were associated with general pachyonychia congenita and 6/109 (5.5%) had only 1 fingernail thickened; 106/109 patients had thickened toenails and only 1/109 (0.9%) patients had 1 thickened toenail [81]. Follicular hyperkeratosis occurred most frequently in PC-K6a (80, 4%) and PC-K6b (42.9%) and was least common in PC-K16 (12.9%; $p < 0.001$ compared with PC-K6a) [5].

4.2.5 Patient's pedigree

The pedigree chart of the families in the study is clearly shown through 3 generations, including the family of patients number 4 and number 7. These two patients were born in a family with a father with a mild PC disease. We also took a sample of their father's saliva and sent it to IPCRR to support the diagnosis. As the results, the patient's father also has PC-K6a (N172del) type mutation, which coincides with the results of 2 children. The percentage of parentally affected offspring in PC pathology is about 70% [4]. Based on the pedigree chart of the remaining patients, which is suggested as self-mutation (new mutation). This rate in our research was 6/8 (75.0%)

patients. According to other studies such as Smith, the rate of self-mutation is about 30%, according to Sonal Shah, this rate is 40%, and in Wilson's study, this rate ranges from 58.3% to 60% [21], [27].

4.3. Results of supportive care interventions for patients with congenital pachyonychia congenita in 6 months.

4.3.1. Distribution of symptoms appearing during 6 months of follow-up

During the 6-month follow-up, supportive care for patients with pachyonychia congenita, the patients' common pathological problems were: infection, leukoplakia, foot pain and some other problems. During the process of taking the child's medical history and monitoring, we noticed that summer weather will cause children to have more problems in care, especially skin problems such as acne, this acne is prone to be pus or small blister-like lesions all over the body. The results of Table 3.20 also show that the number of children with infections, leukoplakia, foot pain or other problems in winter (October, November and December) is less than in autumn and summer months. Smith has judged that the environment with high temperature and high humidity will aggravate the disease [4].

4.3.2. Pain scale of patients before and after treatment and intervention

The results of Table 3.20 show that the average pain score of the group of patients with pachyonychia congenita is 6± 1.67, after treatment is 4± 1.51 (based on the Wong-Baker Faces Pain Rating Scale for children), with $p < 0.05$. Currently, there is no specific treatment for pachyonychia congenita, so supportive care to reduce the pain of Palmoplantar keratodermas is paid very much attention. With different types, recording pain scale is also different, in PC-K6a type pain score is usually from 6-10, with PC-K6b is 3-7, with PC-K16 is 4-9, with PC-K17 is 1-9. Thus, foot pain due to keratosis in the PC-K6a type has the most severe impact on PC patients.

4.3.3. Quality of life of patients before and after care and intervention

Tables 3.21 to 3.31 are descriptions of patients' quality of life before and after 6 months of supportive care. The quality-of-life questionnaire includes questions related to the past 1 week on issues such as frequency of foot pain, dose of pain medication, shame due to PC disease, fingernails interfering with daily routines. PC problems interfere with patient shopping, pain interferes with their sleep, pain interferes with social and recreational activities, pain makes it difficult to play sports. PC disease causes trouble for learning, skin problems create obstacles between patients and those around them, and problems occur when caring for PC disease. We asked and followed these questions in turn to assess the true quality of life of the patients. As a result, a number of problems have actually improved significantly such as less interfere with daily activities, pain interferes with social and recreational activities, pain makes it difficult for patients in playing sports and skin damage cause obstacles between the patient and the people around ($p<0.05$). The remaining issues have changed for the better but have not been significant ($p>0.05$). This positive is explained by the fact that we not only support direct care for older patients >6 years old, but we also spend time with the patient's parents and especially advise parents on the close relationship of the patient such as the patient's teachers, classmates, and close neighbors to avoid their alienation from the patient. Younger patients rarely participate in sports activities, while we recommend adult patients participate in sports that involve less impact on the feet, such as swimming or yoga. Patients with good nail care will help them improve their daily activities.

Studies have shown that PC is a disease that does not reduce life expectancy but seriously affects the quality of life of patients. Plantar pain in PC has a significant negative effect on quality of life. The cause of the pain is not fully understood but is thought to be related to the formation of deep blisters beneath thick scar tissue, which develop on pressure points of the surface of the foot. Blistering of the soles, along with associated pain, is a common feature of PC [80]. The most common initial sites of keratosis are in the 66/98 (67.3%) heel pressure points and the 63/98 (64.3%) bulge of the foot. During the first decade of life, 70/73 patients having keratosis (95.9%) with pain, which compromises their function [5]. More than 50% of children found that plantar keratoderma interfered with walking and playtime; however, less than half of the patients are also hindered with crawling, schoolwork, and housework. Effects on function usually begin after age 5 years, peak in adolescence, and occur most frequently with PC-K6a. PC affects the social life of patients at school-age and older. Most of the cases were met with restrictions related to dressing and playing sports, being teased and embarrassed about their nails, especially in adolescence. Of the 84 patients with nail-related PC, 66/84 (78.6%) concealed their fingernails, particularly by keeping the fingers curled 50/84 (59.5%) or crossed hand 46 (4.8%). Other acts include keeping hands in pockets 3/84 36.9%) , use nail polish 25/84 (29.8%), use fake nails 9/84 (10.7%) and wear gloves 7/84 (8,3%) [5].

So, adolescent patients are most affected by the psychosocial effects of PC, especially those with plantar keratoderma. Quality of life problems include limited sports, shyness in clothes, being teased and embarrassed about nails. Diagnosis allows for proactive management of psychosocial issues, including how a child can comfortably inform friends about his or her illness or how an adolescent can improve his or her appearance of nails and keratosis, thereby increasing

CONCLUSION

1. The rate of nail thickening in children at the National Hospital of Dermatology and Green International Hospital from 1/8/2019 to 31/8/2021

The rate of children with thicked nail compared with the number of children who visited the National Hospital of Dermatology and Venereology and Green International Hospital was 0.19%, compared with the number of children with nail disease was 30.0%.

There are 8 patients with PC who have been confirmed by analysis of Keratin gene.

2. Genotype and phenotype of patients with congenital thickening of nails

In 8 PC patients, there are 3 female patients, 5 male patients. The patients are all Kinh ethnicity. There are 2 patients with family history.

There are 7/8 patients with nail thickening due to *KRT6A* gene mutation, 1/8 patients due to *KRT17* mutation; including 4 types of mutations N172del, N171K, R466P, S97del. The dominant mutation domain is 1A. These mutations lead to loss of Asparagine or change Asparagine → Lysine or Arginine → Proline or loss of Serine.

All PC patients show nails thicked very early, under 2 months of age; most patients have 10 fingernails thicked and 10 toenails thicked (7/8 patients); Besides, all 8 patients have plantar keraroderma and pain plantar.

There is a relationship between phenotype and genotype in PC such as patients presenting with multiple nail thickening, oral leukokeratosis, follicular keratosis, and painful plantar which may suggest *KRT6A* mutation, patients with nail thicked and neonatal tooth that suggest a *KRT17* mutation. In addition, the low number of nails thicked may be related to mutations in the 2B domain.

3. Results of supportive care interventions for the above-mentioned congenital thick nail patients after 6 months.

All patients have improved clinical symptoms such as nail thickening, oral leukokeratosis or nail infection.

The patient's pain scale index was reduced after treatment, intervention, and support with $p<0.05$

The patient's quality of life is also changed in a positive direction, especially the nails are less hindered from daily work, pain less affects social activities, entertainment, sports activities. and skin lesions cause little interference between the patient and the people around ($p<0.05$).

RECOMMENDATIONS

1. Patients with early nail thickening should have genetic analysis to accurately diagnose the disease.
2. Greater care and genetic counseling is needed for patients with rare genetic diseases that improve their quality of life.
3. It is necessary to develop a system for analyzing keratin genes from genomic DNA at Hai Phong University of Medicine and Pharmacy in order to not be dependent on foreign countries and shorten the diagnostic time.

REFERENCES

1. Kaspar R.L. (2005). Challenges in developing therapies for rare diseases including pachyonychia congenita. J Investig Dermatol Symp Proc, 10(1), 62–66.
2. Janice Schwartz (2021). Pachyonychia Congenitia Project – Fighting for a cure. Connecting & helping patients. Empowering Research. <https://www.pachyonychia.org/>, accessed: 04/12/2021.
3. Pavlovsky M., Peled A., Sarig O., et al. (2022). Coexistence of pachyonychia congenita and hidradenitis suppurativa: more than a coincidence. Br J Dermatol, 187(3), 392–400.
4. Smith F.J., Hansen C.D., Hull P.R., et al. (2017). Pachyonychia Congenita. GeneReviews®. University of Washington, Seattle, Seattle (WA), 1–39.
5. Shah S., Boen M., Kenner-Bell B., et al. (2014). Pachyonychia congenita in pediatric patients: natural history, features, and impact. JAMA Dermatol, 150(2), 146–153.
6. Milstone L.M., Fleckman P., Leachman S.A., et al. (2005). Treatment of Pachyonychia Congenita. J Investig Dermatol Symp Proc, 10(1), 18–20.
7. Teng J.M.C., Bartholomew F.B., Patel V., et al. (2018). Novel treatment of painful plantar keratoderma in pachyonychia congenita using topical sirolimus. Clin Exp Dermatol, 43(8), 968–971.
8. Theocharopoulos I. and O'Toole E.A. (2019). Stopping pachyonychia congenita plantar pain with a statin?. British Journal of Dermatology, 181(3), 446–447.
9. González-Ramos J., Sendagorta-Cudós E., González-López G., et al. (2016). Efficacy of botulinum toxin in pachyonychia congenita type 1: report of two new cases. Dermatol Ther, 29(1), 32–36.
10. Kansal N.K. (2017). Pachyonychia Congenita: Brief Appraisal of History and Current Classification. Indian Dermatol Online J, 8(4), 287.
11. Chinazzo M., Lorette G., Baran R., et al. (2017). Nail features in healthy term newborns: a single-centre observational study of 52 cases. J Eur Acad Dermatol Venereol, 31(2), 371–375.
12. Chiaverini C., Bourrat E., Mazereeuw-Hautier J., et al. (2017). [Hereditary epidermolysis bullosa: French national guidelines (PNDS) for diagnosis and treatment]. Ann Dermatol Venereol, 144(1), 6–35.
13. Catalfo P., Musumeci M.L., Lacarrubba F., et al. (2018). Congenital Malalignment of the Great Toenails: A Review. SAD, 4(4), 230–235.
14. Hinkamp C.A., Shah N.H., Holland N., et al. (2018). Recurrent blistering distal dactylitis due to Staphylococcus aureus in an immunocompetent elderly woman. BMJ Case Rep, 2018.
15. Sravanthi A., Srivalli P., Gopal K.V.T., et al. (2016). Pachyonychia congenita with late onset (PC tarda). Indian Dermatol Online J, 7(4), 278–280.
16. Leachman S.A., Kaspar R.L., Fleckman P., et al. (2005). Clinical and Pathological Features of Pachyonychia Congenita. Journal of Investigative Dermatology Symposium Proceedings, 10(1), 3–17.
17. Jackson A.D.M. and Lawler S.D. (1951). Pachyonychia congenita; a report of six cases in one family, with a note on linkage data. Ann Eugen, 16(1), 142–146.
18. Smith F.J.D., Liao H., Cassidy A.J., et al. (2005). The Genetic Basis of Pachyonychia Congenita. Journal of Investigative Dermatology Symposium Proceedings, 10(1), 21–30.

19. Wu A.G. and Lipner S.R. (2021). Distinctions in the Management, Patient Impact, and Clinical Profiles of Pachyonychia Congenita Subtypes. SAD, 7(3), 194–202.
20. Wilson N.J., Messenger A.G., Leachman S.A., et al. (2010). Keratin K6c mutations cause focal palmoplantar keratoderma. J Invest Dermatol, 130(2), 425–429.
21. Wilson N., O'Toole E., Milstone L., et al. (2014). The molecular genetic analysis of the expanding pachyonychia congenita case collection. Br J Dermatol, 171(2), 343–355.
22. Fitzgerald D.M. and Rosenberg S.M. (2019). What is mutation? A chapter in the series: How microbes "jeopardize" the modern synthesis. PLoS Genet, 15(4), 1–14.
23. Durland J. and Ahmadian-Moghadam H. (2022). Genetics, Mutagenesis. StatPearls. StatPearls Publishing, Treasure Island (FL), 1–23.
24. Brown T.A. (2002). Chapter 14: Mutation, Repair and Recombination. Genomes. 2 edition. 2, Wiley-Liss.
25. Miles B. and Tadi P. (2022). Genetics, Somatic Mutation. StatPearls. StatPearls Publishing, Treasure Island (FL).
26. Wu T.T., Eldirany S.A., Bunick C.G., et al. (2021). Genotype-structurotype-phenotype correlations in pachyonychia congenita patients. J Invest Dermatol, 141(12), 2876-2884.e4.
27. Wilson N.J., Leachman S.A., Hansen C.D., et al. (2011). A large mutational study in pachyonychia congenita. J Invest Dermatol, 131(5), 1018–1024.
28. Craufurd D., MacLeod R., Frontali M., et al. (2015). Diagnostic genetic testing for Huntington's disease. Practical Neurology, 15(1), 80–84.
29. Zieman A.G. and Coulombe P.A. (2020). Pathophysiology of pachyonychia congenita-associated palmoplantar keratoderma: new insights into skin epithelial homeostasis and avenues for treatment. British Journal of Dermatology, 182(3), 564–573.
30. Liao H., Sayers J.M., Wilson N.J., et al. (2007). A spectrum of mutations in keratins K6a, K16 and K17 causing pachyonychia congenita. J Dermatol Sci, 48(3), 199–205.
31. Leung A.K.C., Lam J.M., Leong K.F., et al. (2020). Onychomycosis: An Updated Review. Inflamm Allergy Drug Targets, 14(1), 32–45.
32. Gupta A.K., Stec N., Summerbell R.C., et al. (2020). Onychomycosis: a review. J Eur Acad Dermatol Venereol, 34(9), 1972–1990.
33. Bardhan A., Bruckner-Tuderman L., Chapple I.L.C., et al. (2020). Epidermolysis bullosa. Nat Rev Dis Primers, 6(1), 78.
34. Zhan Y., Luo S., Pi Z., et al. (2020). A recurrent mutation of GJB6 in a big Chinese family with Hidrotic ectodermal dysplasia. Hereditas, 157(1), 1–4.
35. Sanches S., Rebellato P.R.O., Fabre A.B., et al. (2017). Do you know this syndrome? Clouston syndrome. An Bras Dermatol, 92(3), 417–418.
36. Ko D. and Lipner S.R. (2018). Onychogryphosis: Case Report and Review of the Literature. Skin Appendage Disord, 4(4), 326–330.
37. Duchatelet S. and Hovnanian A. (2015). Olmsted syndrome: clinical, molecular and therapeutic aspects. Orphanet J Rare Dis, 10, 33.

38. Pan B., Byrnes K., Schwartz M., et al. (2016). Peripheral neuropathic changes in pachyonychia congenita. Pain, 157(12), 2843–2853.

39. Forrest C.E., Casey G., Mordaunt D.A., et al. (2016). Pachyonychia Congenita: A Spectrum of KRT6a Mutations in Australian Patients. Pediatr Dermatol, 33(3), 337–342.

40. Smith F.J., Coleman C.M., Bayoumy N.M., et al. (2001). Novel keratin 17 mutations in pachyonychia congenita type 2. J Invest Dermatol, 116(5), 806–808.

41. Spaunhurst K.M., Hogendorf A.M., Smith F.J.D., et al. (2012). Pachyonychia congenita patients with mutations in KRT6A have more extensive disease compared with patients who have mutations in KRT16. Br J Dermatol, 166(4), 875–878.

42. Cogulu O., Onay H., Aykut A., et al. (2009). Pachyonychia congenita type 2, N92S mutation of keratin 17 gene: clinical features, mutation analysis and pathological view. Eur J Pediatr, 168(10), 1269–1272.

43. Goldberg I., Fruchter D., Meilick A., et al. (2014). Best treatment practices for pachyonychia congenita. Journal of the European Academy of Dermatology and Venereology, 28(3), 279–285.

44. Swartling C. and Vahlquist A. (2006). Treatment of pachyonychia congenita with plantar injections of botulinum toxin. Br J Dermatol, 154(4), 763–765.

45. Swartling C., Karlqvist M., Hymnelius K., et al. (2010). Botulinum toxin in the treatment of sweat-worsened foot problems in patients with epidermolysis bullosa simplex and pachyonychia congenita. Br J Dermatol, 163(5), 1072–1076.

46. Pho L.N., Smith F.J.D., Konecki D., et al. (2011). Paternal germ cell mosaicism in autosomal dominant pachyonychia congenita. Arch Dermatol, 147(9), 1077–1080.

47. Vũ Văn Quang, Chu Thị Hà (2018). Nhân hai trường hợp bị bệnh dày móng bẩm sinh tại khoa Nhi, Bệnh viện Quốc tế Green. Tạp chí Y học Thực hành, 8(1077), 16–20.

48. Chu H.T., Le D.H., Le T.V., et al. (2021). Pachyonychia Congenita Type PC-K6a: The first report in the Vietnamese population. Biomed Res Ther, 8(6), 4434–4438.

49. Quốc Hội (2016). Pháp luật Việt Nam. Luật trẻ em năm 2016 số 102/2016/QH13. Điều 1. 1–42.

50. Tosti A. and Piraccini B.M. (2014). Changes of the Nail Shape and Size. Nail Disorder. Springer, Italy, 12–14.

51. Lowell A. Goldsmith, Stephen I. Katz, Barbara A. Gilchrest, Amy S. Paller, David J. Leffell, Klaus Wolff A. and Piraccini B.M. (2012). Chapter 89. Biology of Nails and Nail Disorders. Fitzpatrick's Dermatology in General Medicine. 8, The McGraw-Hill Companies, New York, NY.

52. RichardK. Scher and C Ralph Daniel (2005). Nail signs and symptoms. Nails. third, Elsevier Sauders, USA, 1–6.

53. Shchagina O., Fedotov V., Markova T., et al. (2022). Palmoplantar Keratoderma: A Molecular Genetic Analysis of Family Cases. Int J Mol Sci, 23(17), 9576.

54. Garcia-Casal M.N., Pasricha S.-R., Sharma A.J., et al. (2019). Use and interpretation of hemoglobin concentrations for assessing anemia status in individuals and populations: results from a WHO technical meeting. Ann N Y Acad Sci, 1450(1), 5–14.

55. Lo S.F. (2011). Reference Intervals for Laboratory Tests and Procedures. Nelson Textbook of Pediatrics. Elsevier, 2466-2466.e16.

56. Tomlinson D., von Baeyer C.L., Stinson J.N., et al. (2010). A systematic review of faces scales for the self-report of pain intensity in children. Pediatrics, 126(5), e1168-1198.

57. Abbas M., Schwartz M.E., Smith F.J.D., et al. (2015). PCQoL: A Quality of Life Assessment Measure for Pachyonychia Congenita. J Cutan Med Surg, 19(1), 57–65.

58. Eliason M.J., Leachman S.A., Feng B., et al. (2012). A review of the clinical phenotype of 254 patients with genetically confirmed pachyonychia congenita. J Am Acad Dermatol, 67(4), 680–686.

59. World Health Organization (2011), Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System, .

60. Lê Nam Trà và cộng sự (2003). Các giá trị sinh học về hóa sinh. Các giá trị sinh học người Việt Nam bình thường thập kỉ 90-thế kỉ XX. Nhà xuất bản Y học, 79–89.

61. Garra G., Singer A.J., Domingo A., et al. (2013). The Wong-Baker pain FACES scale measures pain, not fear. Pediatr Emerg Care, 29(1), 17–20.

62. Starace M., Alessandrini A., and Piraccini B.M. (2018). Nail Disorders in Children. Skin Appendage Disord, 4(4), 217–229.

63. Anjaneyan G. and Kaliyadan F. (2020). Blistering Distal Dactylitis. StatPearls. StatPearls Publishing, Treasure Island (FL), 1–10.

64. Gupta A.K., Venkataraman M., Shear N.H., et al. (2022). Onychomycosis in children - review on treatment and management strategies. J Dermatolog Treat, 33(3), 1213–1224.

65. Wollina U., Nenoff P., Haroske G., et al. (2016). The Diagnosis and Treatment of Nail Disorders. Dtsch Arztebl Int, 113(29–30), 509–518.

66. Wagner G. and Sachse M.M. (2012). Congenital malalignment of the big toe nail. J Dtsch Dermatol Ges, 10(5), 326–330.

67. Võ Đông Xuân (2013). Nghiên cứu đặc điểm lâm sàng, một số yếu tố liên quan và giá trị của các xét nghiệm trực tiếp phát hiện nấm móng. Luận văn tốt nghiệp Bác sĩ chuyên khoa cấp 2, Trường Đại học Y Hà Nội.

68. Nguyễn Minh Hường (2017). Đặc điểm lâm sàng, chủng nấm gây bệnh và hiệu quả điều trị nấm móng bằng uống itraconazole liều xung kết hợp sơn ciclopirox 8%. Luận văn tốt nghiệp bác sĩ nội trú Trường Đại học Y Hà Nội.

69. Singal A. and Bisherwal K. (2019). Disorders of nail in infants and children. Indian Journal of Paediatric Dermatology, 20(2), 101.

70. Fletcher C.L., Hay R.J., and Smeeton N.C. (2004). Onychomycosis: the development of a clinical diagnostic aid for toenail disease. Part I. Establishing discriminating historical and clinical features. British Journal of Dermatology, 150(4), 701–705.

71. Vlahovic T.C., Joseph W.S., Scher R.K., et al. (2016). Diagnosis and Management of Onychomycosis: Perspectives from a Joint Podiatric Medicine–Dermatology Roundtable. Journal of the American Podiatric Medical Association, 106(2), 155–162.

72. Solís-Arias M.P. and García-Romero M.T. (2017). Onychomycosis in children. A review. Int J Dermatol, 56(2), 123–130.

73. DeKlotz C.M.C., Schwartz M.E., and Milstone L.M. (2017). Nail removal in pachyonychia congenita: Patient-reported survey outcomes. Journal of the American Academy of Dermatology, 76(5), 990–992.

74. Smith F.J.D., Liao H., Cassidy A.J., et al. (2005). The genetic basis of pachyonychia congenita. J Investig Dermatol Symp Proc, 10(1), 21–30.

75. Jiráková A., Rajská L., Rob F., et al. (2015). First case of pachyonychia congenita in the Czech Republic. Dermatol Ther, 28(1), 10–12.

76. Chiriac A., Rusu C., Murgu A., et al. (2017). First Report of Pachyonychia Congenita Type PC-K6a in the Romanian Population. Maedica (Bucur), 12(2), 123–126.

77. Ward K.M., Cook-Bolden F.E., Christiano A.M., et al. (2003). Identification of a recurrent mutation in keratin 6a in a patient with overlapping clinical features of pachyonychia congenita types 1 and 2. Clinical and Experimental Dermatology, 28(4), 434–436.

78. Duverger O., Carlson J.C., Karacz C.M., et al. (2018). Genetic variants in pachyonychia congenita-associated keratins increase susceptibility to tooth decay. PLoS Genet, 14(1), e1007168.

79. Steele L., Schwartz J., Hansen C.D., et al. (2021). Prevalence and Characterization of Itch in Pachyonychia Congenita. JAMA Dermatology, 157(11), 1378–1380.

80. McLean W.H.I., Hansen C.D., Eliason M.J., et al. (2011). The phenotypic and molecular genetic features of pachyonychia congenita. J Invest Dermatol, 131(5), 1015–1017.

81. Akasaka E., Nakano H., Nakano A., et al. (2011). Diffuse and focal palmoplantar keratoderma can be caused by a keratin 6c mutation. Br J Dermatol, 165(6), 1290–1292.

82. Li Y., Wang Y., Ming Y., et al. (2021). A KRT6A mutation p.Ile462Asn in a Chinese family with pachyonychia congenita, and identification of maternal mosaicism: a case report. BMC Med Genomics, 14, 259.

83. Mandelbaum M., Chao J.W., and Rogers G.F. (2017). Pachyonychia Congenita in a Toddler. Plast Reconstr Surg Glob Open, 5(5).

84. Abdollahimajd F., Rajabi F., Shahidi-Dadras M., et al. (2019). Pachyonychia congenita: a case report of a successful treatment with rosuvastatin in a patient with a KRT6A mutation. British Journal of Dermatology, 181(3), 584–586.

85. Lessard J.C. and Coulombe P.A. (2012). Keratin 16-null mice develop palmoplantar keratoderma, a hallmark feature of pachyonychia congenita and related disorders. J Invest Dermatol, 132(5), 1384–1391.

86. Cao Y.-A., Hickerson R.P., Seegmiller B.L., et al. (2015). Gene Expression Profiling in Pachyonychia Congenita Skin. J Dermatol Sci, 77(3), 156–165.

87. Samuelov L., Smith F.J.D., Hansen C.D., et al. (2020). Revisiting pachyonychia congenita: a case-cohort study of 815 patients. British Journal of Dermatology, 182(3), 738–746.