Cellular and Molecular Biology [TM] **55**, N°2, 111-117
DOI 10.1170/T860

ISSN 1165-158X
2009 Cell. Mol. Biol.[TM]

EXCLUSION OF *FERROCHELATASE* GENE MUTATIONS IN PATIENTS WITH SEASONAL PALMOPLANTAR KERATODERMA

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Received, April 28th 2009; Accepted May 20th, 2009; Published July 1st, 2009

Abstract – Erythropoietic protoporphyria (EPP) is an autosomal dominant disorder that results from a deficiency of ferrochelatase (FECH), the last enzyme in the heme biosynthetic pathway. The characteristic clinical symptoms usually manifest in early childhood on the sun-exposed areas of the body. They are due to protoporphyrin-induced photosensitivity and include pain, burning and stinging of the skin, followed by erythema and edema. Recently, the occurrence of predominantly seasonal palmar and palmoplantar keratoderma in patients with homozygous mutations in the *FECH* gene has been reported. These data suggested that palmoplantar keratoderma might be a clinical sign of EPP. Palmoplantar keratodermas (PPKs) are a heterogeneous group of genetic skin diseases and include a seasonal variant, erythrokeratolysis hiemalis et estivalis (EH), also known as keratolytic winter erythema. Because the skin symptoms in the latter disorder are similar to those reported for recessive EPP we examined the *FECH* gene in three unrelated Dutch Caucasian patients with a previous diagnosis of EH in whom mutations in several other genes had been excluded. However, sequencing analysis of the entire coding regions and the adjacent splice sites of the *FECH* gene in these individuals revealed absence of mutations. Hence, our data largely exclude the possibility that *FECH* mutations might be responsible for the palmoplantar skin phenotype observed in EH.

Key words: Porphyria, erythropoietic protoporphyria, ferrochelatase, erythrokeratolysis hiemalis et estivalis, keratoderma, palmoplantar keratoderma

INTRODUCTION

Erythropoietic protoporphyria (EPP) (OMIM 177000) is a cutaneous porphyria that results from a hereditary deficiency of ferrochelatase (FECH) (E.C. 4.99.1.1), the last enzyme along the pathway of heme biosynthesis, which catalyzes the insertion of iron into protoporphyrin IX to form heme [1, 2]. The disease has an estimated prevalence of one in 130.000 and commonly manifests in early childhood [3].

Clinical symptoms in EPP are due to accumulation and deposition in the skin of protoporphyrin, a potent photosensitizer. Upon sun exposure, signs of cutaneous photosensitivity can develop, including pain, burning, itching, erythema, and swelling. Repeated and chronic sun exposure may lead to lichenification, subtle scarring, and hyperpigmentation, particularly over the knuckles and back of the nose [2, 4]. Up to 5% of EPP patients are at risk of developing severe hepatic complications such as progressive hepatic dysfunction and liver failure [2, 5].

EPP is inherited as an autosomal dominant trait with incomplete penetrance. Recently, it has been shown that co-inheritance of a common hypomorphic allele, IVS3-48C, in *trans* to a deleterious heterozygous *FECH* gene mutation

Abbreviations: EH, erythrokeratolysis hiemalis et estivalis; **EPP**, erythropoietic protoporphyria; **FECH**, ferrochelatase; **PCR**, polymerase chain reaction; **PPK**, palmoplantar keratoderma

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leads to reduction of the residual FECH activity below a critical threshold and clinically overt disease [6, 7]. In exceptional cases, homozygous inheritance of *FECH* mutations is associated with strongly decreased FECH activity levels of less than 10% of normal [3].

Previous studies by other groups and our selves indicated that in recessive EPP a possibly characteristic cutaneous sign can develop, that is palmar or, in rarer instances, palmoplantar keratoderma [3, 8]. Affected homozygous EPP patients may reveal both a seasonal and a perennial palmar keratoderma [3, 8]. To date, both acquired and inherited variants of palmoplantar keratodermas (PPK) have been described, the latter showing considerable clinical and genetic heterogeneity [9-12]. Interestingly, there exists a seasonal variant of PPK, erythrokeratolysis hiemalis et estivalis (EH) (OMIM 148370), also referred to as keratolytic winter erythema or Oudtshoorn disease [13]. This rare disorder is characterized by symmetric, transgredient erythema and peeling of the palms and, to a lesser extent, the soles. Occurrence is typically provoked by either cold or warm weather [14].

Because the seasonal PPK reported in patients with EPP is strongly reminiscent of Oudtshoorn disease, we speculated that the latter disorder might be associated with *FECH* mutations as well. Therefore, we sequenced the *FECH* gene in three unrelated individuals whom we had recently diagnosed with EH.

CASE REPORTS, METHODS, AND RESULTS

Patient 1

The first patient was a 7-year-old healthy Caucasian Dutch female. During winter, about three months prior to her first presentation in our outpatient clinic, she had developed skin lesions on her palms and soles. Her further medical history was unremarkable and triggering or aggravating factors could not be identified. The family history revealed an uncle and grandfather with eczema but nobody else had skin symptoms similar to hers. Topical ointments containing corticosteroids and calcipotriol had no effect. Upon physical examination we saw symmetric erythematosquamous and slightly hyperkeratotic plaques with fissures and rhagades on her palms and soles. The erythema was clearly transgredient. Further, unsharply demarcated erythematous macules were noted on her knees and elbows.

Patient 2

The second patient was an 8-year-old Caucasian Dutch male with a medical history of clubfeet, urinary incontinence due to a small bladder, and complaints of chronic constipation. During infancy he had developed treatment-refractory palmoplantar cutaneous lesions. Other family members were not affected. Clinical examination showed moderately demarcated symmetric, transgredient erythema with scaling and lichenification on palms and soles.

Patient 3

The third patient was a 16-year-old healthy Dutch Caucasian female who shortly after birth had developed non-itching skin lesions on her palms and soles, which relapsed frequently, several times a year. Both her medical and family history were unremarkable and she did not use regular medication. On physical examination we saw symmetric, sharply demarcated erythema with mild hyperkeratosis and centrifugal peeling on palms and soles (Figure 1).

In all three patients we made a presumptive diagnosis of EH.

Mutation analysis in the FECH gene

Prior to the study we obtained informed consent from the parents in accordance with local institutional guidelines. DNA was isolated from peripheral blood leucocytes using a simple salt-precipitation method described elsewhere.

Polymerase chain reaction (PCR) amplification of all coding regions and adjacent splice sites of the *FECH* gene, including the intronic polymorphism IVS3-48T/C, was performed with specific intronic primers tailed with universal M13(-21) or M13-R sequences (primer sequences available from the authors upon request). PCR was performed with an initial denaturation at 95°C for 5 min; followed by 40 cycles of denaturation at 95°C for 1 min; annealing at primer specific temperature for 30 s; and extension at 72°C for 1 min; ending with 72°C for 10 min, in a Biometra® TGradient thermal cycler (Whatman Biometra®, Göttingen, Germany). Regular Taq DNA polymerase was used for PCR according to the suppliers protocol (Invitrogen). PCR products were purified with ExoSap-it (USB Europe). Sequencing was performed on the PCR product with an ABI 3730 DNA genetic analyzer from Applied Biosystems

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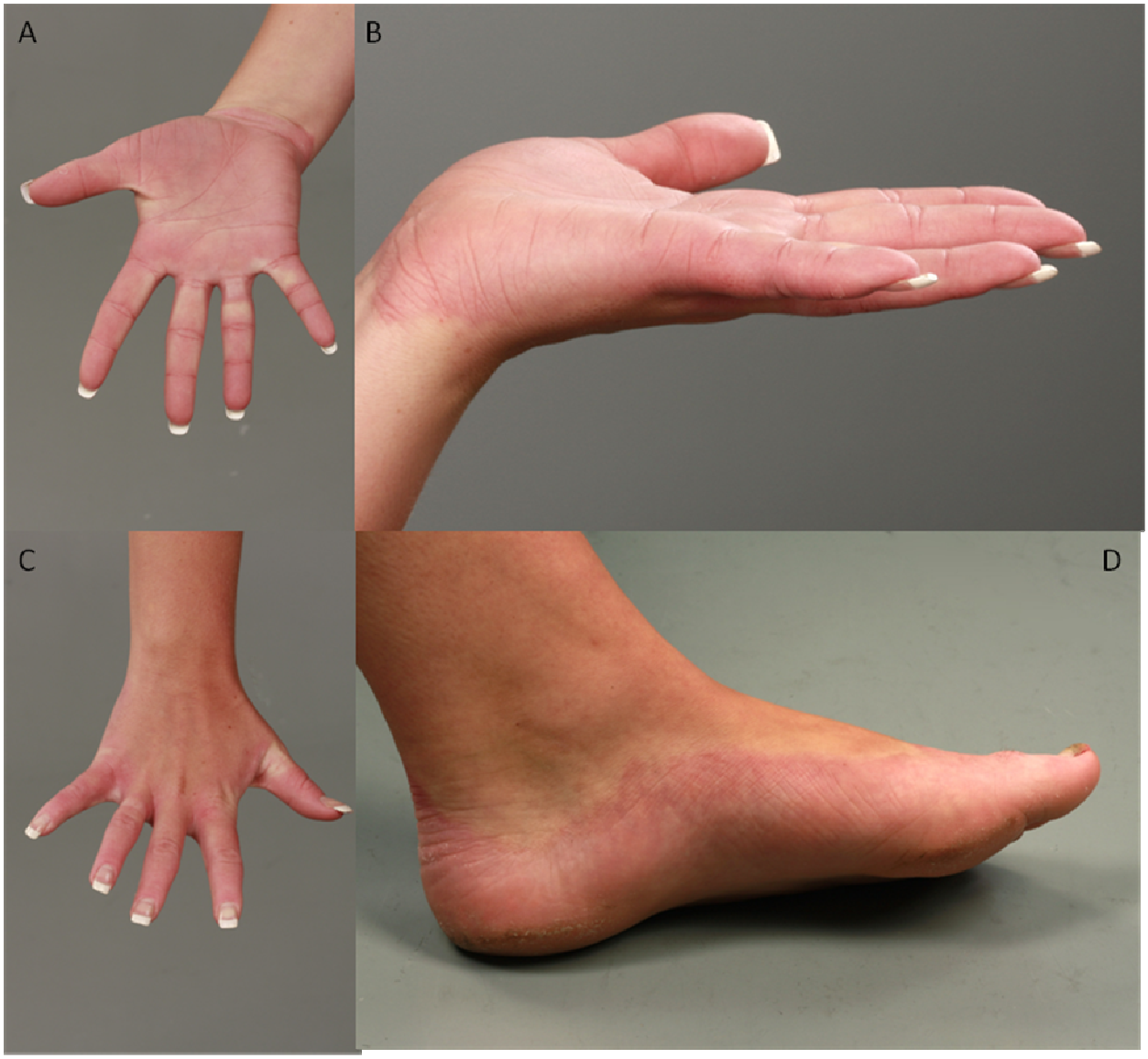

Figure 1. Symmetric, sharply demarcated erythema with mild hyperkeratosis and centrifugal peeling on palms and soles of a 16-year-old female with erythrokeratolysis hiemalis et estivalis.

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Table 1. Clinical data of British and Spanish EPP patients with homozygous *FECH* mutations reported previously and the three Dutch patients with erythrokeratolysis hiemalis et estivalis in this study.

Patient	Origin	Sex	Onset of keratoderma	Family history of keratoderma	Clinic of keratoderma	Reference
1	**British**	f	1.4 years	yes	Confluent waxy palmar hyperkeratosis with fine peeling; sharp cutoff at wrists	[8]
2		m	1.0 years	yes	Hyperkeratosis and peeling of first interdigital web; patchy keratosis and peeling over palmar surface of digit joints and pulps	[8]
3[1]		f	0.5 years	yes	Palmar hyperkeratosis with sharp cutoff at wrist; transgredience to involve first interdigital web; focal plantar hyperkeratosis	[8]
4[1]		m	0.4 years	yes	Mild hyperkeratosis of first interdigital web	[8]
5		m	0.3 years	no	Confluent palmar hyperkeratosis with peeling; lateral aspects dry, white, and cracked; slight onycholysis; hyperkeratosis on medial aspect of forefoot and great toes	[8]
6		m	<1 years	no	Marked keratosis of first interdigital web and radial border of index fingers with fissuring at index finger joints.	[8]
7		f	0.25 years	no	Waxy keratoderma over the whole palm; mild fine peeling; more obvious in summer, regresses in winter; sharp cutoff at wrist. Nail dystrophy.	[8]

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8		m	0.25 years	yes	Mild hyperkeratosis of first interdigital web.	[8]
9		f	2.0 years	yes	Mild hyperkeratosis of first interdigital web.	[8]
10	**Spanish**	m	60 years	no	Sharply demarcated, symmetrical palmar erythema with diffuse hyperkeratosis and fine scaling, that spread to the dorsal aspects of the hands.	[3]
11	**Dutch**	f	7 years	no	Erythematosquamous plaques with rhagades on palms and soles; erythematous lesions on knees and elbows; occurrence in winter	Present study
12		m	unknown (young)	no	Erythema, squamae, and lichenification on palms and soles	Present study
13		f	0.1 years	no	Sharply demarcated erythema on palms and soles; hyperkeratosis on palms; several times a year skin peeling of palms and soles	Present study

Clinical data of patients with homozygous *FECH* mutations and palmoplantar keratoderma reported previously and the three Dutch patients with erythrokeratolysis hiemalis in this study. f: female; m: male. [1]Consanguineous parents

Inc. (Foster City, CA, USA) using the BigDye deoxy terminator V1.1 cycle sequencing kit (Applied Biosystems) with the universal M13 primers. Sequence-analysis was performed with the software tools Phred, Phrap, and Consed [15-17].

Exclusion of mutations and IVS3-48C in the FECH gene

Sequencing of the *FECH* gene did not show a mutation in any of the patients studied. Furthermore, the hypomorphic allele IVS3-48C was absent in all individuals.

DISCUSSION

In this study we performed mutation analysis of the *FECH* gene in Dutch patients with PPK and the diagnosis of EH. Recently, others and we have shown that homozygous inheritance of *FECH* mutations can be associated with the manifestation of palmar and palmoplantar keratoderma in recessive EPP [3, 8]. Until then, beside the well-known cutaneous signs of acute and chronic photosensitivity the only other skin symptom described in EPP was porokeratosis, which, interestingly, was observed in a homozygous 17-year-old male [18].

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Interestingly, there were interindividual differences in clinical presentation of PPK in recessive EPP. The majority of patients showed keratoderma on the palms and/or soles in a seasonal and intermittent pattern and usually before photosensitivity commenced [8]. However, one patient developed late onset and persistent keratoderma that was exclusively confined to the hands, in particular to the back of the hands [3]. In support of these findings from the United Kingdom and Spain, researchers from the Swiss porphyria center in Zürich have recently presented at the European Conference on Porphyrias in Madrid, Spain, their unpublished data on homozygosity of *FECH* mutations due to mosaicism underlying overt EPP with seasonal keratoderma.

Hence, these reports suggest that PPK might be a clinical indicator of recessive EPP [3, 8]. Furthermore, the question is raised if sequence alterations in the *FECH* gene could also give rise to other associated cutaneous symptoms, in particular on the palms and soles, that may have been overlooked in the past. Interestingly, EH, a rare autosomal dominant cutaneous disorder, strongly resembles the PPK associated with EPP. It shows a seasonal and variable clinical expression pattern that is characterized by cyclic erythema, hyperkeratosis and recurrent, centrifugal peeling on the palms and soles [13].

Thus, we reasoned that EH might be caused by *FECH* mutations and sought to elucidate if sequence deviations in the *FECH* gene are responsible for the phenotype in three patients with this diagnosis. It should be emphasized that we were well aware that none of the patients studied here exhibited photosensitivity and that biochemical studies could not be performed because we did only have access to DNA samples and were therefore not able to measure e.g., protoporphyrin levels in erythrocytes. Still, we considered our approach straightforward, for the following reason. Sequencing of intron 3 of the *FECH* gene in all three individuals revealed absence of the hypomorphic allele IVS3-48C. Presence of this intronic polymorphism in *trans* to a heterozygous *FECH* mutation is an apparent prerequisite for the development of photosensitivity in EPP patients [6, 7]. Therefore, we should expect our patients not to be photosensitive. This is in line with previous results, in which we showed that heterozygous carriers of a missense mutation in the *FECH* gene, p.Q285R, who do not carry the hypomorphic allele IVS3-48C in *trans*, do not develop photosensitivity, although homozygous inheritance of the same mutation in one family member resulted in clinically overt EPP associated with palmar keratoderma [3].

Here, we did not detect *FECH* mutations in any of our EH patients. Thus, sequence alterations in this gene are probably not responsible for the palmoplantar skin phenotype in these individuals and the etiology of EH still remains elusive. Because the PPKs are a clinically and genetically heterogeneous group of disorders and clinical signs of these diseases can be found in a syndromic and non-syndromic context, including homozygous EPP, we consider it worth the effort to exclude *FECH* mutations, in particular if patients also have a medical history of photosensitivity.

ACKNOWLEDGEMENTS

JF is a board member of the European Porphyria Initiative (EPI) and was, in part, supported by grant number A04155HS, GIS-Institut des Maladies rares: Network on rare diseases to the European Porphyria Initiative (EPI), and a grant from the European Union to the European Porphyria Network (EPNET), Program of Community Action in the Field of Public Health, project no. 2006107.

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