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Identification of putative genetic modifying factors that influence the development of Papillon–Lefévre or Haim–Munk syndrome phenotypes

É. M. Pap,[1] K. Farkas,[2] L. Tóth,[2] B. Fábos,[3] M. Széll,[2,4] G. Németh[1] and N. Nagy[2,4]

[1]*Department of Obstetrics and Gynecology Szeged,* [2]*Department of Medical Genetics, and* [4]*Dermatological Research Group of the Hungarian Academy of Sciences, University of Szeged, Szeged, Hungary; and* [3]*Mór Kaposi Teaching Hospital, Kaposvár, Hungary*

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Summary

Background. Papillon–Lefévre syndrome (PLS; OMIM 245000) and Haim–Munk syndrome (HMS; OMIM 245010), which are both characterized by palmoplantar hyperkeratosis and periodontitis, are phenotypic variants of the same disease caused by mutations of the cathepsin C (*CTSC*) gene.

Aim. To identify putative genetic modifying factors responsible for the differential development of the PLS or HMS phenotypes, we investigated two Hungarian patients with different phenotypic variants (PLS and HMS) but carrying the same homozygous nonsense *CTSC* mutation (c.748C/T; p.Arg250X).

Methods. To gain insights into phenotype-modifying associations, whole exome sequencing (WES) was performed for both patients, and the results were compared to identify potentially relevant genetic modifying factors.

Results. WES revealed two putative phenotype-modifying variants: (i) a missense mutation (rs34608771) of the SH2 domain containing 4A (SH2D4A) gene encoding an adaptor protein involved in intracellular signalling of cystatin F, a known inhibitor of the cathepsin protein, and (ii) a missense variant (rs55695858) of the odorant binding protein 2A (OBP2A) gene, influencing the function of the cathepsin protein through the glycosyltransferase 6 domain containing 1 (GLT6D1) protein.

Conclusion. Our study contributes to the accumulating evidence supporting the clinical importance of phenotype-modifying genetic factors, which have high potential to aid the elucidation of genotype–phenotype correlations and disease prognosis.

Introduction

Papillon–Lefévre syndrome (PLS; OMIM 245000) and Haim–Munk syndrome (HMS; OMIM 245010) are both characterized by overlapping dermatological and dental symptoms, including hyperkeratosis of the palms and soles and severe periodontitis.[1,2] Patients with PLS can also develop mild mental retardation, calcification of the dura mater, hyperhidrosis and increased susceptibility to infections.[3–5] Specific features of HMS include pes planus, arachnodactyly, acro-osteolysis and onychogryphosis.[6–8] The prevalence of PLS is approximately four cases per million, and to date, approximately 300 cases have been reported worldwide. Parental consanguinity has been noted in > 50% of these cases.[4,9] The prevalence of HMS is approximately one case per million, and the majority of reported cases are descendants of a few consanguineous families from a religious isolate in Cochin, India. One unrelated Brazilian patient has also been reported. To date, < 100 HMS cases have been reported in the literature.[6–8] The ratio of affected males to females is 1 : 1 for both syndromes. PLS and

Correspondence: Dr Nikoletta Nagy, Department of Obstetrics and Gynecology, University of Szeged, Szeged, Semmelweis u. 1, 6725, Hungary
E-mail: nagy.nikoletta@med.u-szeged.hu

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HMS are both inherited in an autosomal recessive manner and develop as a consequence of mutations in the cathepsin C (*CTSC*) gene.[10,11] Currently, 89 *CTSC* gene mutations have been identified.[1,12] The majority of these mutations have been detected in patients with PLS, whereas only 4% have been associated with HMS.[1,2,7,8]

In light of the reported PLS and HMS phenotypes and the associated *CTSC* mutations, we hypothesized that PLS and HMS are the same entity with different phenotypic appearances.[1] Although it is difficult to establish genotype–phenotype correlations, the elucidation of these correlations is likely to have significant clinical relevance for the development of the different clinical variants (PLS and HMS), the disease mechanism and the development of future therapies.[1]

We recently investigated two Hungarian patients, one with PLS and one with HMS, who nonetheless carry the same homozygous nonsense mutation (c.748C/T; p.Arg250X) of the *CTSC* gene.[13] As there is currently no explanation for why one mutation can lead to these two different clinical variants (PLS and HMS), we were interested in the identification of phenotype-modifying genetic factors that could facilitate the understanding of the phenotypic differences between these patients. In this study, whole exome sequencing (WES) was used to identify putative phenotype-modifying genetic factors that could explain the observed clinical differences between these PLS and HMS patients carrying the same causative *CTSC* mutation.

Methods

Patients

The clinical phenotypes of the affected patients were reported in detail in a previous paper from our research group.[13] Briefly, Patient 1 was a Hungarian woman who presented with the typical HMS phenotype; mild hyperkeratotic plaques were observed symmetrically on her palms and soles, while onychogryphosis and arachnodactyly were noted on her fingers and pes planus on her soles. Patient 2 was a Hungarian man who presented with the classic PLS phenotype, i.e. moderate hyperkeratosis on his palms and soles. Both patients were missing all permanent teeth and using a permanent dental prosthesis. In our previous paper, we also reported the results of haplotype analysis, which raised the possibility that these patients are siblings.[13] It was not possible to genotype unaffected relatives.[13]

DNA samples

The two previously reported Hungarian patients, affected by PLS and HMS respectively, but carrying the same disease-causing mutation (c.748C/T; p.Arg250X) in the *CTSC* gene, were investigated.[12] DNA samples from both patients were used for WES (performed by UD-GenoMed Medical Genomic Technologies Ltd., Debrecen, Hungary; http://www.ud-genomed.hu/). The quality of the DNA samples was evaluated by agarose-gel electrophoresis.

Whole exome sequencing

In brief, 4 μg of DNA with a concentration of 100 ng/μL were used for library construction. A liquid chip capture system (Agilent Research Laboratories, Santa Clara, CA, USA) was used to efficiently enrich all human exon regions. High-throughput deep sequencing was subsequently performed on the Illumina (San Diego, CA, USA) platform. An exon kit (SureSelect Human All Exon V6 Kit; Agilent) was used for library construction and capture experiments, and a bioanalyser (Model 2100; Agilent) was subsequently used to verify the library insert size. The Illumina platform was used for sequencing according to the effective concentration of the library and the data output requirements. High-throughput paired-end sequencing (paired-end 150 bp; PE150; Agilent) was performed.

Bioinformatics analysis

After WES was completed, bioinformatics analysis was performed, including quality assessment of sequencing data, single-nucleotide polymorphism (SNP) detection and whole exome association analysis.

The sequencing data quality control requirements were as follows: sequencing error rate of each base position $< 1\%$, mean Q20 ratio $> 90\%$, mean Q30 ratio $> 80\%$, mean error rate $< 0.1\%$, alignment rate for sequencing reads $\leq 95\%$ and read depth of the base at one position ≥ 10 times.

Single nucleotide polymorphism

SNP testing was performed as follows: high-quality sequences were aligned with the human reference genome (GRCh37/hg19) to detect sequence variants, and the detected variations were analysed and annotated. Variants were filtered according to read depth, allele frequency and prevalence in genomic variant databases such as ExAc (v.0.3), ClinVar and Kaviar.

Variant prioritization tools (PolyPhen2, SIFT, LRT, Mutation Taster, Mutation Assessor) were used to predict the functional impact. All the identified candidate variants were confirmed by direct sequencing (Delta Bio 2000 Ltd., Szeged, Hungary; http://www.deltabio.hu/).

Results

A comparison of the WES data from these PLS and HMS patients carrying the same disease-causing mutation (c.748C/T; p.Arg250X) in the *CTSC* gene identified 34 variants, which were all present in the patient with HMS, but not in the patient with PLS, for whom no mutation or polymorphism was found. Two of the 34 variants were suggested as putative phenotype-modifying polymorphisms by variant prioritization tools: the rs34608771 SNP of the SH2 domain containing 4A (SH2D4A) gene and the rs55695858 SNP of the odorant binding protein 2A (OBP2A) gene. Both variants are common missense polymorphisms. Pathogenicity predictions for the identified phenotype-modifying factors are summarized in Table 1.

Discussion

Identification of the disease-causing mutations is extremely important for therapy or genetic counselling, but clinical genetics has already reached the limitations of the direct sequencing technology, as it is unable to answer clinically relevant questions such as genotype–phenotype correlations or disease prognosis, or explain the development of different clinical variants in patients carrying the same disease-causing mutation.[14] This is the case with the two PLS and HMS patients examined here and reported previously by our workgroup.[13] Although the same disease-causing *CTSC* mutation was identified in both patients, the causative mutation itself does not explain the striking phenotypic differences between them. To overcome this limitation, identification of the putative phenotype modifier genetic factors might be useful. Next-generation sequencing systems have become more popular and more widely available as their cost has decreased, and clinical genetics has now access to these high-throughput technologies.[14] In the field of monogenic skin diseases, ichthyosis is a good example of the clinical relevance of the phenotype modifier genetic factors, since the genetic modifiers identified to date have been found to contribute to the variable disease phenotype in this disease.[15]

Table 1 Pathogenicity predictions and clinical associations of the identified phenotype-modifying genetic factors.

SNP	rs34608771	rs55695858
Gene	*SH2D4A*	*OBP2A*
Location	Exonic	Exonic
Variant type	Missense	Missense
Analysis		
SIFT	Tolerated	Tolerated
Polyphen2	Benign	Possibly damaging
MutationTaster	Polymorphism	Polymorphism
Clinical associations	Development of the HMS phenotype	Development of the HMS phenotype
Reference	This study	This study

HMS, Haim–Munk syndrome; SNP, single nucleotide polymorphism.

The comparison of the WES data of our HMS and PLS patients identified a putative phenotype-modifying genetic variant (rs34608771 SNP) in the *SH2D4A* gene, which encodes a T-cell-expressed adapter protein that is expressed in T cells, B cells, macrophages and dendritic cells.[16] SH2D4A regulates T-cell receptor signal transduction in T cells, and in humans, its expression in T cells is increased in response to T-cell activation.[16] SH2D4A is linked to cathepsin C via cystatin F, a cysteine-protease inhibitor expressed selectively in immune cells, such as T cells, natural killer cells and dendritic cells.[17] The rs34608771 polymorphism of the *SH2D4A* gene has not been associated previously with any human disease; to our knowledge, this is the first study linking it to the development of the HMS clinical variant and raises its putative association with the phenotypic differences between PLS and HMS (Fig. 1).

The other putative phenotype-modifying genetic variant (rs55695858 SNP) is located within the *OBP2A* gene, which encodes an odorant-binding carrier protein that has a known environmental biosensor function. The OBP2A protein is expressed in the nasal structures, salivary and lachrymal glands, and lungs, and thus, has an oral sphere profile.[18] OBP2A interacts with the glycosyltransferase 6 domain containing 1 (GLT6D1) protein, encoded by the *GLT6D1* gene, which has been identified as a susceptibility locus for periodontitis by genome-wide association studies, and this association has been confirmed by several previous studies.[19] Although genetic variants of the *OBP2A* gene have been implicated in influencing the substrate-binding specificity of the encoded protein, none have previously been associated with the development of a human disease.[20,21] As periodontitis is a major feature of the PLS and HMS phenotypes, we suggest that the rs55695858 SNP of the *OBP2A* gene might

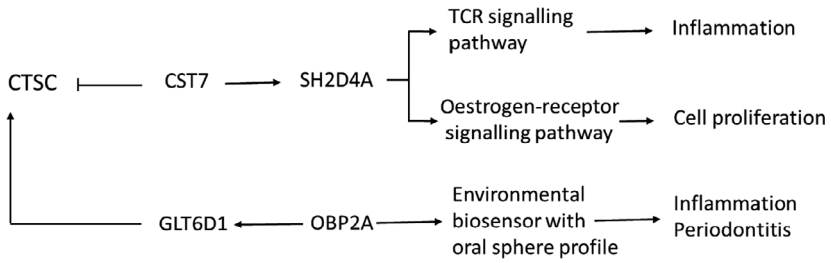

Figure 1 Schematic of the proposed mechanisms of the identified phenotype-modifying factors.

contribute to the phenotypic differences observed between PLS and HMS patients (Fig. 1).

Conclusion

Our study aimed to explain the phenotypic differences in PLS and HMS patients carrying the same disease-causing *CTSC* mutation by identifying phenotype-modifying genetic polymorphisms. It should be noted that, in addition to genetic factors, environmental or lifestyle factors might also contribute to the phenotypic differences between PLS and HMS. Further functional studies are needed to prove the clinical relevance of the identified phenotype-modifying genetic factors and to describe the underlying mechanism that explains their phenotype-modifying roles. Our study contributes to the accumulating evidence supporting the clinical importance of phenotype-modifying genetic factors and their potential to facilitate the elucidation of genotype–phenotype correlations or disease prognosis.[22]

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What's already known about this topic?

- PLS and HMS are caused by mutations of the *CTSC* gene.
- They are characterized by overlapping clinical features.
- They are phenotypic variants of the same disease.

What does this study add?

- Our study revealed two putative phenotype-modifying variants.
- The first was a missense mutation of the *SH2D4A* gene involved in the intracellular signalling of the cystatin F, a known inhibitor of CTSC.
- The second was a missense variant of the *OBP2A* gene influencing CTSC through GLT6D1.

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