Whole-exome sequencing reveals a recurrent mutation in the cathepsin C gene that causes Papillon–Lefevre syndrome in a Saudi family

Yaser Mohammad Alkhiary a,1, Musharraf Jelani b,c,*,1, Mona Mohammad Almramhi b, Hussein Sheikh Ali Mohamoud b,d, Rayan Al-Rehaili a, Hams Saeed Al-Zahrani b, Rehab Serafi e, Huanming Yang a,f, Jumana Yousuf Al-Aama b,g

a Oral and Maxillofacial Prosthodontics Department, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia
b Princess Al-Jawhara Albrahim Center of Excellence in Research of Hereditary Disorders, King Abdulaziz University, Jeddah, Saudi Arabia
c Medical Genetics and Molecular Biology Unit, Biochemistry Department, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan
d Human Genetics Research Centre, Division of Biomedical Sciences (BMS), St. George’s University of London (SGUL), London SW17 0RE, United Kingdom
e Department of Dermatology, King Abdulaziz University Hospital, Jeddah, Saudi Arabia
f Department of Genetic Medicine, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
g BGI-Shenzhen, Shenzhen 518083, China

Received 29 March 2015; revised 27 May 2015; accepted 10 June 2015
Available online 16 June 2015

Abstract Papillon–Lefevre syndrome (PALS) is a rare, autosomal recessive disorder characterized by periodontitis and hyperkeratosis over the palms and soles. Mutations in the cathepsin C gene (CTSC) have been recognized as the cause of PALS since the late 1990s. More than 75 mutations in CTSC have been identified, and phenotypic variability between different mutations has been described. Next generation sequencing is widely used for efficient molecular diagnostics in various clinical practices. Here we investigated a large consanguineous Saudi family with four affected and four unaffected individuals. All of the affected individuals suffered from hyperkeratosis over the...
1. Introduction

Papillon–Lefèvre syndrome (PALS, MIM 245000) is a rare, autosomal recessive genodermatosis. The disease was first described by two French scientists, Papillon and Lefèvre, in 1924 (Papillon and Lefèvre, 1924). It mainly affects skin and teeth, leading to hyperkeratoderma over the palms and soles, also known as palmoplantar hyperkeratosis, and premature loss of primary or secondary dentition (Haneke, 1979; Papillon and Lefèvre, 1924). Hyperkeratosis first appears in the early stages of childhood (prior to age 3–4); however, late-onset alterations have also been reported (Pilger et al., 2003). In general, hyperkeratotic features are not severe in PALS, and as reviewed recently, the diffuse type is more common than the punctuate type in most cases (Nagy et al., 2014). Lesions over the elbow, knee, and knuckles resembling psoriasis may also develop in some cases (Toomes et al., 1999). Recurrent mild pus-generating skin infections with self-healing are also observed (Gorlin et al., 1964; Haneke, 1979).

Periodontitis and gingivitis are associated with both primary and secondary dental anomalies, and appear at the time of the two episodes of tooth eruptions: one at ~3 years of age and the second at ~15 years of age (Fardal et al., 1998; Lundgren and Renvert, 2004; Toomes et al., 1999). Hyperkeratosis first appears in the early stages of childhood (prior to age 3–4); however, late-onset alterations have also been reported (Pilger et al., 2003). In general, hyperkeratotic features are not severe in PALS, and as reviewed recently, the diffuse type is more common than the punctuate type in most cases (Nagy et al., 2014). Lesions over the elbow, knee, and knuckles resembling psoriasis may also develop in some cases (Toomes et al., 1999). To date, more than 75 pathogenic variants from various ethnic groups have been identified as causing PALS and overlapping phenotypes (Nagy et al., 2014). Haim–Munk syndrome (HMS, MIM 245010) is characterized by palmoplantar keratoderma, periodontitis, arachnodactyly, acroosteolysis, pesplanus, and onychogryposis, and is caused by recessive mutations in CTSC (Hart et al., 2000b). Similarly, aggressive periodontitis, which is characterized by severe periodontal inflammation, leading to tooth loss, without the involvement of skin abnormalities, is another overlapping phenotype of PALS and is also caused by mutations in CTSC (Hart et al., 2000c; Hewitt et al., 2004).

Here we present clinical and molecular analysis of a consanguineous five-generation family from Saudi Arabia segregating an autosomal recessive PALS phenotype.

2. Materials and methods

2.1. Ethical approval

Ethical approval for this study was obtained from the King Abdulaziz University (KAU), Jeddah, Saudi Arabia (ref # 24-14), according to the Helsinki Declaration. The parents of the affected children signed informed written consent for their willingness to participate and to publish the results for academic research purposes.

2.2. Study subjects

The five-generation family (Fig. 1) resided in a remote southwestern region of Saudi Arabia. Family information for the pedigree was obtained by interviewing the parents of the affected children. All family members, including the four affected siblings, were thoroughly examined in the Department of Oral and Maxillofacial Prosthodontics, Faculty of Dentistry, KAU, Jeddah Saudi Arabia. Venous blood samples from four affected (V-1, V-2, V-4, V-6) and four unaffected (III-1, IV-1, V-3, V-5) family members were collected in EDTA tubes, and genomic DNA was extracted and quantified using standard methods (Ahmed et al., 2015).

2.3. Homozygosity mapping

Genomic DNA of three affected (V-1, V-2, V-6) and one unaffected (V-3) individual was subjected to 300 K HumanCytoSNPs12 microarray analysis using an iScan platform (Ilumina, USA) following the manufacturer’s protocols. The common regions of homozygosity were identified using GenomeStudio Genotyping Module v1.0 (Ilumina).

2.4. Whole-exome sequencing

Two micrograms of genomic DNA from the index patient (V-1) were used for human whole-exome analysis with paired-end-sequencing at 100x resolution. Libraries were constructed using a 51-Mb SureSelect library kit (Agilent Technologies, USA). The target regions with average throughput depths of more than 120 and 100-bp paired-ends reads were sequenced using a HiSeq2000 platform (Ilumina). BWA (http://bio-bwa.sourceforge.net/) and SAMTOOLS (http://samtools.sourceforge.net/) were used for alignment of sequences and copy number variants, and small indel detection, respectively. The obtained reads were mapped to the UCSC human genome database hg19 (http://genome.ucsc.edu/), and were compared with 1000 genomes (http://www.1000genomes.org/data) and dbSNP (http://www.ncbi.nlm.nih.gov/snp) databases. Pathogenicity of the obtained variants was predicted using LRT (http://www.genetics.wustl.edu/jflab/lrt_query.html), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/), MutationTaster
2.5. Sanger sequencing

The potential candidate variant was validated by Sanger sequencing in all eight family members. The CTSC reference sequence (ENSG00000109861) was obtained from the Ensembl genome browser (http://www.ensembl.org/Homo_sapiens/). Primer3Plus (http://www.bioinformatics.nl/) was used to design the upstream (5'-TCAGGGTAACATGAAGA-3') and downstream (5'-TTTGCATGGAGAATCTGC-3') primers for PCR amplification of the c.899G>A region from genomic DNA of each subject. PCR products were sequenced using a Big Dye Terminator v3.1 Cycle Sequencing Kit and an ABI 3500 Genetic Analyzer (Life Technologies, USA). Sequence variants were identified using BioEdit sequence alignment editor version 6.0.7 (www.mbio.ncsu.edu/bioedit.html).

3. Results

3.1. Clinical features

All four affected individuals presented with classical PALS symptoms, including psoriasiform lesions over knuckles, hyphidrosis, palmar-plantar hyperkeratosis, and periodontal inflammation (Fig. 2). Detailed dental examinations revealed mild differences among the affected siblings. The index patient (V-1), a 17-year-old boy, had extensive loss of alveolar bone in the lower jaw, leading to loss of the lower anterior teeth. The patient also had generalized severe periodontitis, leading to multiple instances of furcation involvement and tooth mobility. Likewise, the periodontal health of patient V-2, a 15-year-old boy, was affected, with several instances of grade III furcation involvement and tooth mobility. The third affected individual (V-4), an 11-year-old boy, had generalized mild to moderate bone loss that included furcational involvement of the first molars, and again contributed to the loss of multiple teeth. The youngest affected patient (V-6), a 6-year-old girl, had generalized mild bone loss, spacing, and crowding in the lower anterior teeth. She had also mobility in the primary dentition. The hair and nails appeared normal in all affected individuals, and all were otherwise healthy without involvement of any of the vital organs. The unaffected siblings (V-3, V-5) had normal dentition with no phenotypic indications of PALS, and were clinically indistinguishable from healthy individuals.

3.2. Genetic analysis

We combined the results of microarray SNP genotyping with the whole-exome sequencing data. We found that the three affected individuals (V-1, V-2, and V-6) shared a common region of homozygosity, at chromosome 11q13.2–11q23.2 (chr11: 69,953,729–112,031,624 bp); however, it was not shared by the unaffected individual (V-3). This region corresponded to a 42-Mb region on human genome MapViewer (annotation release 106; http://www.ncbi.nlm.nih.gov/mapview/), which contained 518 labeled genes and 670 variants. As the affected individuals were the products of a consanguineous union, the possibility of homozygous mutation was more likely. We identified 55 variants for further investigation after screening all 670 variants using the criteria: only homozygous single-nucleotide variations (SNV) with altered nucleotide depth of more than 24, present in the exonic or splice-site regions, and having non-synonymous, frameshift, or stop-gain effects. We further narrowed the list to seven variants by removing minor allele frequency greater than 0.05. Of these, three SNVs (XRRA1: NM_182969, c.622G>A, p.Val208Ile; CTSC: NM_001814, c.899G>A, p.Gly300Asp; and ELMO1: NM_001130037, c.952G>A, p.Ala318Thr) were not reported in the 1000 genome database (Oct. 2011 release) (Supplementary Table 1). Prediction software analyses
and Sanger sequencing validation of all family members confirmed the mutation within CTSC as a pathogenic variant. The mutation was a single nucleotide transition from guanine to adenine in exon 7 of CTSC at cDNA position 899, leading to a single amino acid substitution from glycine to aspartate at amino acid position 300. The obligate carriers were heterozygous (Fig. 3). This variant was not detected in 212 ethnically matched control chromosomes, and the possibility of neutral polymorphism was excluded. A second homozygous missense variant (c.458T>C, p.Ile153Thr) in exon 3 of CTSC was also identified; however, it corresponded to a single nucleotide polymorphism (rs217086) with a minor allele frequency of >90% in the 1000 genome database, and was therefore not likely to be pathogenic.

4. Discussion

With the recent advances in next-generation sequencing technologies, whole-exome analysis has significantly improved pathogenic variant identification, especially in hereditary skin disorders with inter- and intra-familial phenotypic variability (Lai-Cheong and McGrath, 2011; Salam et al., 2014). In the current study, we combined genome-wide homozygosity mapping with whole-exome analysis for a successful and efficient molecular diagnosis.

The PALS disease locus was first mapped to 11q14 in 1997, and 2 years later, the causative mutations in CTSC were identified (Fischer et al., 1997; Hart et al., 1999; Toomes et al., 1999). Interestingly, CTSC involvement has been ruled out in several PALS patients through traditional DNA sequencing, despite establishing linkage to 11q14 (Hart et al., 2000a; Khan et al., 2014). More recently, the mutation spectrum of PALS, HMS, and AP1 phenotypes has been widely studied; however, a clear genotype–phenotype correlation could not be established. In an attempt to summarize the genotype–phenotype correlation, a recent review outlined all the known mutations in CTSC (Nagy et al., 2014). Only one is listed for the HMS phenotype, while seven cause more than one phenotype, including PALS (Nagy et al., 2014). Several polymorphisms were associated with the PALS phenotype; however, in our family, one of these polymorphisms (rs217086) was also detected in unaffected healthy individuals.

Two missense mutations, including c.899G>A in an affected sibling from one family and c.815G>C in affected

Figure 2  Clinical presentation of the affected individuals. The index patient (V-1) showed psoriasiform lesions over the knuckles (A), hyphidrosis and hyperkeratosis over the palm (B) and sole (C), and periodontal inflammation (D and E). Radiological examination of patients V-1 and V-4 showing extensive loss of the alveolar bone in the lower jaw leading to loss of the lower anterior teeth.
siblings from four unrelated families, have previously been
associated with the PALS phenotype in patients from Saudi
Arabia (Zhang et al., 2001). The mutation c.815G>C was
identified as possible evidence of the founder effect being pre-
sent in the four families, whose distant relationships were not
known (Zhang et al., 2001). Here we report further evidence of
the founder effect in $CTSC$ from the same population. As the
clinical features of the c.899G>A mutation have not previ-
ously been clearly described, the current study included a
detailed clinical description of our patients who carried the
same mutation as previously reported (Zhang et al., 2001).
Our findings expand the knowledge on $CTSC$ pathogenicity
in PALS, and will provide a basis for genotype–phenotype
correlations in this rare disorder.

5. Conclusions

This study describes the complete clinical and molecular
assessment of PALS in a consanguineous family of Saudi ori-
 gin. Next generation sequencing has been widely adopted for
efficient molecular diagnosis because of its precision and cost
effectiveness. The Saudi population is unique, and to date
there is no publically available reference genome database
for this population. Whole-exome sequencing may provide
better data for constructing reference databases in Saudi
Arabia, and may replace traditional methods of variant
detection.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This project was funded by the Deanship of Scientific Research
(DSR), King Abdulaziz University, Jeddah, under Grant No.
(4/165/1435/HiCi). The authors, therefore, acknowledge with
thanks DSR for technical and financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found,
in the online version, at http://dx.doi.org/10.1016/j.sjbs.2015.06.007.

References

Ahmed, S., Jelani, M., Alrayes, N., Mohamoud, H.S., Almramhi,
M.M., Anshasi, W., Ahmed, N.A., Wang, J., Nasir, J., Al-Aama,
J.Y., 2015. Exome analysis identified a novel missense mutation in
the CLPP gene in a consanguineous Saudi family expanding the
clinical spectrum of Perrault Syndrome type-3. J. Neurol. Sci. 353, 149–154.
Fardal, O., Drangsholt, E., Olsen, I., 1998. Palmar plantar keratosis
and unusual periodontal findings. Observations from a family of 4
members. J. Clin. Periodontol. 25, 181–184.
Fischer, J., Blanchet-Bardon, C., Prud’homme, J.F., Pavek, S.,
Steijlen, P.M., Dubertret, L., Weissenbach, J., 1997. Mapping of
Papillon–Lefevre syndrome to the chromosome 11q14 region. Eur.
J. Hum. Genet. 5, 156–160.
Gorlin, R.J., Sedano, H., Anderson, V.E., 1964. The syndrome of
palmar-plantar hyperkeratosis and premature periodontal
destruction of the teeth. A clinical and genetic analysis of the
Papillon-Lefevre syndrome. J. Pediatr. 65, 895–908.
Haneke, E., 1979. The Papillon–Lefevre syndrome: keratosis palmo-
plantaris with periodontopathy. Report of a case and review of the
cases in the literature. Hum. Genet. 51, 1–35.
Hart, T.C., Hart, P.S., Bowden, D.W., Michalec, M.D., Callison, S.A.,
Walker, S.J., Zhang, Y., Firatli, E., 1999. Mutations of the
cathepsin C gene are responsible for Papillon–Lefevre syndrome. J.
Med. Genet. 36, 881–887.
Hart, P.S., Zhang, Y., Firatli, E., Uygur, C., Lotfazar, M., Michalec,
M.D., Marks, J.I., Lu, X., Coates, B.J., Scow, W.K., Marshall, R.,
Williams, D., et al, 2000a. Identification of cathepsin C mutations
in ethnically diverse Papillon–Lefevre syndrome patients. J. Med.
Genet. 37, 927–932.
Hart, T.C., Hart, P.S., Michalec, M.D., Zhang, Y., Firatli, E., Van
Dyke, T.E., Stabholz, A., Zlotogorski, A., Shapiro, L., Sokolne,
W.A., 2000b. Haim-Munk syndrome and Papillon-Lefevre syn-
drome are allelic mutations in cathepsin C. J. Med. Genet. 37, 88– 94.
Hart, T.C., Hart, P.S., Michalec, M.D., Zhang, Y., Marazita, M.L.,
Cooper, M., Yassin, O.M., Nusier, M., Walker, S., 2000c. Localisation
of a gene for prepubertal periodontitis to chromosome
11q14 and identification of a cathepsin C gene mutation. J. Med.
Genet. 37, 95–101.
Hewitt, C., McCormick, D., Linden, G., Turk, D., Stern, I., Wallace,
L., Southern, L., Zhang, L., Howard, R., Bullon, P., Wong, M.,
Widmer, R., et al, 2004. The role of cathepsin C in Papillon–
Lefevre syndrome, prepubertal periodontitis, and aggressive peri-
donitis. Hum. Mutat. 23, 222–228.
Khan, F.Y., Jan, S.M., Mushtaq, M., 2014. Papillon–Lefevre syndrome (PLS) without cathepsin C mutation: a rare early onset partially penetrant variant of PLS. Saudi Dent. J. 26, 25–28.

Lai-Cheong, J.E., McGrath, J.A., 2011. Next-generation diagnostics for inherited skin disorders. J. Invest. Dermatol. 131, 1971–1973.

Lundgren, T., Renvert, S., 2004. Periodontal treatment of patients with Papillon–Lefevre syndrome: a 3-year follow-up. J. Clin. Periodontol. 31, 933–938.

Nagy, N., Valyi, P., Csoma, Z., Sulak, A., Tripolszki, K., Farkas, K., Paschali, E., Papp, F., Toth, L., Fabos, B., Kemeny, L., Nagy, K., et al., 2014. CTSC and Papillon–Lefevre syndrome: detection of recurrent mutations in Hungarian patients, a review of published variants and database update. Mol. Genet. Genomic Med. 2, 217–228.

Papillon, P.H., Lefevre, P., 1924. Two cases of symmetrically familial palmar and plantar hyperkeratosis (Meleda disease) within brother and sister combined with severe dental alterations in both cases. Bull. Soc. Fr. Dermatol. Syphiligr., 82–87.

Pilger, U., Hennies, H.C., Truschnegg, A., Aberer, E., 2003. Late-onset Papillon–Lefevre syndrome without alteration of the cathepsin C gene. J. Am. Acad. Dermatol. 49, S240–3.

Salam, A., Simpson, M.A., Stone, K.L., Takeichi, T., Nanda, A., Akiyama, M., McGrath, J.A., 2014. Next generation diagnostics of heritable connective tissue disorders. Matri. Biol. 33, 35–40.

Toomes, C., James, J., Wood, A.J., Wu, C.L., McCormick, D., Lench, N., Hewitt, C., Moynihan, L., Roberts, E., Woods, C.G., Markham, A., Wong, M., et al. 1999. Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. Nat. Genet. 23, 421–424.

Zhang, Y., Lundgren, T., Renvert, S., Tatakas, D.N., Firatlı, E., Uygur, C., Hart, P.S., Gorry, M.C., Marks, J.J., Hart, T.C., 2001. Evidence of a founder effect for four cathepsin C gene mutations in Papillon–Lefevre syndrome patients. J. Med. Genet. 38, 96–101.