A Tunisian family with a novel mutation in the gene CYP4F22 for lamellar ichthyosis and co-occurrence of hearing loss in a child due to mutation in the SLC26A4 gene

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Abstract

Background: Co-occurrence of two genetic diseases is challenging for accurate diagnosis and genetic counselling. The recent availability of whole exome sequencing (WES) has dramatically improved molecular diagnosis of rare genetic diseases in particular in consanguineous populations.

Methods: We report here on a consanguineous family from Southern Tunisia including three members affected with congenital ichthyosis. The index case had a hearing loss (HL) and ichthyosis and was primarily suspected as suffering from Keratitis-Ichthyosis-Deafness (KID) syndrome. WES was performed for the index case and all members of the nuclear family were sequenced (Sanger method).

Results: The WES approach allowed the identification of two strong candidate variants in two different genes: a missense mutation c.1334T>G (p.Leu445Trp) in exon 11 of SLC26A4 gene, associated with isolated HL and a novel missense mutation c.728G>T (p.Arg243Leu) in exon 8 of CYP4F22 gene likely responsible for ichthyosis. These two mutations were predicted to be pathogenic by three pathogenicity prediction softwares (SIFT, PolyPhen, Mutation Taster) to underlie the HL and ichthyosis respectively.

Conclusions: The present study raises awareness about the importance of familial history for accurate diagnosis of syndromic genetic diseases and differential diagnosis with co-occurrence of two distinct clinical entities. In addition, in countries with limited resources WES
sequencing for a single individual provide a cost effective tool for molecular diagnosis confirmation and genetic counselling.

1. Introduction

Consanguinity refers to marriages between members who share at least one common ancestor, it is a deeply rooted social trend in the Arab world (1). A recent work conducted in different localities of Tunisia indicated an overall rate of 29.8% of consanguineous marriages (2). These marriages are practically the norm in some regions in particular in the Southern part of the country (3). It has been estimated that autosomal recessive diseases risk is increased six folds as a consequence of consanguineous marriage (4). In addition, the co-occurrence of two or more diseases although rare might be encountered in endogamous populations and are challenging for appropriate diagnosis (4, 5). Autosomal recessive congenital ichthyosis (ARCI) includes a wide range of different clinical subtypes: congenital ichthyosis form erythroderma (CIE; OMIM 242100); lamellar ichthyosis (LI; OMIM 242300); and harlequin ichthyosis (HI; OMIM 242500) (6). ARCI is a genetically heterogeneous disease with more than 115 mutations in TGM1 gene which is responsible for the majority of cases of LI and some cases of CIE (7). Rare mutations in ALOX12B, ALOXE3, NIPAL4, CYP4F22 and ABCA12 have also been detected in patients with LI/CIE phenotypes (8). Hereditary hearing loss (HL) corresponds also to a classic example of genetic heterogeneity with more than 45 genes and 69 loci for autosomal recessive non-syndromic deafness (DFNB) (http://hereditaryhearingloss.org/). Despite the broad heterogeneity of DFNB, GJB2 mutations encoding connexin-26 account for more than 30% of the cases in most populations around the Mediterranean sea (9). In the literature the co-occurrence between ichthyosis form erythroderma and HL is known as KID syndrome caused by mutations in GJB2 (10). In the present study, we report on the co-occurrence of ichthyosis and HL in a Tunisian consanguineous family in which the index case was primarily suspected as suffering from Keratitis-Ichthyosis-Deafness (KID) syndrome.

2. Materials and methods

2.1. Patients and clinical evaluation

The index case individual TN-V-7 (Fig. 1) was first referred to the Otorhinolaryngology Department at La Rabta Hospital in Tunis, at the age of 4-years, for the exploration of congenital deafness. He is born to a consanguineous marriage between two paternal first cousins originating from Southern Tunisia. The parents suspected the HL at the age of six
months. The otoscopic, head and neck examination were normal. Audiological evaluation (auditory brainstem response) confirmed the bilateral profound sensorineural HL. Computed tomography of the temporal bones, magnetic resonance imaging of the inner ear, cardiac and renal ultrasonography were unremarkable. The fundus of the eye was normal. The examination of the thyroid lodge was normal and there was no biological dysfunction (absence of goiter). Inner ear MRI ruled out the presence of enlarged vestibular aqueduct (EVA).

According to the familial information, the index case was born as a collodion baby with a membrane covering the entire surface of his body associated with ectropion and eclabion. It was complicated by an acute respiratory distress syndrome. In addition, he has joint contractures due to the tightened skin of his extremities. Hence, the patient was hospitalized to manage his life threatening skin condition. Within the first week of life the parchment-like membranes disintegrated with fragments peeling off, leaving transient erosions on the abdomen. Later on, he progressively developed a generalized lamellar ichthyosis covering the entire surface of his body with thick brown plate-like lamellar scales without sparing of the folds. At the age of 8-year old, another dermatological examination revealed lamellar scales involving his face located mainly on his frontotemporal regions (Fig. 2). He also had a mild palmoplantar keratoderma with hyperlinearity. The brown lamellar scales also involved his scalp with temporal scaring alopecia and a decline of the hair implantation line. His nails were normal. He did not show ectropion. He was treated with moisturizing and keratolytic creams. The co-occurrence of ichthyosis and congenital hearing loss was suggestive of KID syndrome. After informed consent, the parents were interviewed for familial history. Four other cases of ichthyosis were recorded but no other case of HL was identified in this family (Fig. 1). The older sister of index case, individual TN-V-5 had xerosis at birth without a history of collodion baby but unfortunately she died two months later. A second brother, individual TN-V-6 (six years old) was affected by ichthyosis; he was also born as collodion baby and developed at the age of one-month a generalized fine scaling phenotype consistent with lamellar ichthyosis (LI). Two parental cousins were also affected by ichthyosis, individual TN-V-3 died at the age of two months due to ichthyosis complications and her sister TN-V-4 (eleven years old) was born as colloidion baby, she is still alive with a moderate phenotype of LI . Based on familial history, the phenotype of the index case, individual TN-V-7 could correspond either to KID syndrome or to the co-occurrence of two different clinical entities ichthyosis and HL. Molecular diagnosis is needed to confirm one of these hypotheses.

2.2. Genetic analysis

This study was conducted according to the declaration of Helsinki and to the ethical standards of the authors Institutional Review Board (Registration number IRB00005445, FWA00010074).

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After signing an informed consent, EDTA-blood samples were collected and genomic DNA was extracted using the standard salting-out method (11).

Individual TN-V-7 was first prescreened for GJB2 because mutations in this gene have been observed in several KID patients (12) and account respectively for 30% of HL (13) and for TGM1 gene mutations that have been also reported for several cases of ichthyosis in Tunisian population (unpublished data). Molecular investigation of TGM1 gene for ichthyosis and GJB2 for KID syndrome and HL was performed as previously described (13, 14).

After the exclusion of the possible involvement of GJB2 and TGM1 mutations, we used WES to identify the loci responsible for ichthyosis and HL. To confirm the familial segregation all the family members were sequenced (TN-IV-3/ TN-IV-7/ TN-IV-8/ TN-V-4/ TN-V-6/ TN-V-7) with Sanger method using specific primers designated with Primer3 software (http://primer3.ut.ee/) (Table 1).

For the WES data analysis, based on familial history and pedigree, we hypothesized an autosomal recessive mode of disease transmission and the presence of the causative mutations at the homozygous state. To identify pathogenic variants, we filtered out polymorphisms using the Single Nucleotide Polymorphism Database dbSNP132. We removed all the variants reported in 1000 genomes, Hapmap, and Exome variant server databases. We identified the variants present in the coding exons and in the flanking splice sites. From the SNP and indels files, we selected nonsense, frame-shifting (indels), missense, and splice-site mutations, as they were more likely to be pathogenic. Only the variants with a read depth greater than 5 were retained. After application of these 5 filtering steps, three variants passed the frequency criterion (MAF <0.01).

3. Results

WES data analysis allowed the identification of one biallelic sequence variant c.1334T>G (p.Leu445Trp) located in exon 11 of SLC26A4 gene that is already known to be involved in HL. In addition, we identified a missense mutation c.728G>T (p.Arg243Leu) and a synonymous variant c.729C>A (p.Arg244Arg) occurring in exon 8 of CYP4F22 gene responsible for LI phenotype (LI type 3) (OMIM 604777). The novel missense mutation in the CYP4F22 (Var 19:15651317 G/T) was not found in ExAC (15) and it was predicted to be pathogenic by three pathogenicity prediction programs SIFT (16), PolyPhen2 (17) and Mutation Taster (18), the values predicted were 0.00, 0.796 and 0.999, respectively. In addition, the region flanking the variant is well conserved throughout the species. The other novel synonymous variant c.729C>A (p.Arg244Arg) changes the nucleotide sequence without
directly altering the sequence of the encoded protein. The presence of the three variants in the individuals affected by ichthyosis as well as the familial segregation were confirmed by Sanger sequencing. (Fig. 1).

4. Discussion

We report here on a co-occurrence of ichthyosis and HL in a patient primarily suspected as suffering from KID syndrome. In the absence of GJB2 gene mutations, WES analysis was conducted and allowed the identification of the underlying mutations in two different genes CYP4F22 and SLC26A4.

Analysis of variants in these two genes extracted from WES data allowed the identification of two novel variants in the CYP4F22 gene: a silent synonymous variant c.729C>A (p.Arg244Arg) changing the nucleotide sequence of the gene without altering the sequence of the encoded protein and a novel missense mutation c.728G>T (p.Arg243Leu) in CYP4F22 gene, causing LI phenotype. Mutations in CYP4F22 gene are responsible for the LI (19), it encodes a protein of the cytochrome-P450 family 4 which is an epidermal ω-hydroxylase decisive for the formation of acylceramides. So far, LI due to CYP4F22 mutations is extremely rare and only seven reports are available in the literature (20). In the present study, TN-V-7, TN-V-6 and TN-V-4 were born with a collodion membrane and had xerosis. However, their siblings died at the age of two months due to ichthyosis complications even if they were not born as collodion babies. This shows an intra-familial clinical variability of ichthyosis due to CYP4F22 gene mutations. It has been reported that patients bearing homozygous missense mutations in this gene were not born as colloidion babies while patients harbouring one or two truncating mutations affecting a substrate-binding region were born with a collodion membrane (21). Sugiura et al have reported a Japanese girl belonging to a non-consanguineous family, born as a collodion baby and carrying compound heterozygous missense mutations in CYP4F22 gene (22). In a recent study, Gruber et al have also shown intra-familial clinical variability in a family with two affected siblings baring a homozygous splice site mutation in CYP4F22 gene, predicted to lead to loss of the protein. This mutation was associated with a collodion membrane in one child and dry skin without collodion at birth in her sister (23). This shows again that the phenotype at birth does not correlate with the type of mutation; genetic or environmental modifiers could influence phenotypic expression of the disease. Consequently, further studies are required to elucidate the pathophysiology associated with the CYP4F22 gene.

For HL, we identified a biallelic missense mutation c.1334T>G: p. (Leu445Trp) in exon 11 of SLC26A4 (solute carrier family 26, member 4, nm 000441). SLC26A4 encodes pendrin, a
transmembrane ion transporter exchanging chloride for other anions, such as iodide in the thyroid gland or bicarbonate in the inner ear (24). In the literature, nearly 200 SLC26A4 mutations are responsible for non-syndromic HL (DFNB4) (24) and Pendred syndrome (PS) (OMIM*605646) (25). PS is a genetic disorder that causes early HL and affects the thyroid gland (26). The mutational spectrum varies among ethnic groups suggesting the existence of founder effects (27, 28). The p.Leu445Trp mutation was previously identified in several Tunisian families with HL and intrafamilial variability of thyroid manifestations (29). For patient TN-V-7 reported here, the examination of the thyroid lodge was normal and there was no biological dysfunction. Goiter manifestations might depend on the age of patients, as younger patients may develop them after puberty (29).

5. Conclusions

Co-occurrence of two or more genetic conditions mimicking syndromic hereditary diseases or phenocopies are more and more encountered in consanguineous populations.

This study shows that WES could be used as a reliable and time-efficient technique for molecular diagnosis of heterogeneous and phenocopy disorders in consanguineous populations. The association of two diseases in the same individual should be considered in order to establish appropriate diagnosis and should be taken into account for genetic counselling, in particular in countries where consanguineous and endogamous marriages are still culturally favoured.

Abbreviations

ARCI: Autosomal recessive congenital ichthyosis  
HL: Hearing loss  
DFNB: Autosomal recessive non-syndromic deafness  
KID: Keratitis-Ichthyosis-Deafness  
SIFT: Scale-invariant feature transform  
PolyPhen: Polymorphism Phenotyping  
WES: Whole exome sequencing  
CIE: Congenital ichthyosis form erythroderma  
LI: Lamellar ichthyosis  
HI: Harlequin ichthyosis  
OMIM: Online Mendelian inheritance in man  
PS: Pendred syndrome  
EVA: Enlarged vestibular aqueduct

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Contributors

Conception and design of the study: SA CP. performed the experiments: MS ZR NL CB LR RM OB NC. Analyzed the data: MS ZR CB LR. Contributed reagents/materials/analysis tools: SA CP. Drafted the paper: MS. Contributed in the critical revision of the manuscript for important intellectual content: ZR NL GA OM HY CP SA. Clinical evaluations of patients: AZ JM MM GB. All authors approved the final manuscript.

References


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Table 1 Sequences of the primers used to validate the mutations by Sanger sequencing.
<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>CYP4F22-8F</td>
<td>AGACCTGTAACCCAGCACTT</td>
</tr>
<tr>
<td>CYP4F22-8R</td>
<td>AGGCTGATCTCCAATTCCAG</td>
</tr>
<tr>
<td>SLC26A4-EX11_12F</td>
<td>TGAGCTGGAAGACACAAGGG</td>
</tr>
<tr>
<td>SLC26A4-EX11_12R</td>
<td>GGGAATATAGTGATATGGCAGG</td>
</tr>
</tbody>
</table>

**Legends:**

**Fig. 1** Pedigree of the Tunisian family TN-V-7 analyzed using the whole exome sequencing (WES) strategy, the black arrow indicates the patient with the co-occurrence of ichthyosis and hearing loss. The arrows with the number indicate the two mutations in the *CYP4F22* gene: 1: the novel missense mutation c.728G>T (p.Arg243Leu)/ 2: the silent synonymous variant c.729C>A (p.Arg244Arg) and the missense mutation in the *SLC26A4* gene: 3: c.1334T>G (p.Leu445Trp).

**Fig. 2** Phenotypical features of patient TN-V-7 (8 years old) affected by the co-occurrence of hearing loss and ichthyosis. **A** Brown lamellar scales involving his scalp located on the temporal regions. **B** Brown, plate-like adherent lamellar scales involving the trunk.
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Fig. 2 Phenotypical features of patient TN-V-7 (8 years old) affected by the co-occurrence of hearing loss and ichthyosis. A Brown lamellar scales involving his scalp located on the temporal regions. B Brown, plate-like adherent lamellar scales involving the trunk.