Short Report

First \textit{HPSE2} missense mutation in urofacial syndrome


Urofacial syndrome (UFS) describes the combination of urological problems and an inverted facial expression upon attempts to smile. Seventeen independent familial cases from different ethnicities have been described so far. Some of these have been linked to chromosome 10q. Very recently, homozygous loss-of-function mutations affecting the gene \textit{HPSE2} were identified in nine cases. Here, we describe a consanguineous UFS family from Pakistan with three of six siblings affected. We establish linkage to the chromosome 10q critical region and identify two non-synonymous \textit{HPSE2} variants. \textit{In silico} analysis and screening of controls defines c.631T>C (p.Y211H) as a novel benign SNP and c.1628A>T (p.N543I) as the disease-causing mutation. Our study exemplifies the challenges in proper clinical diagnosis of UFS and, thereby, supports the hypothesis of the disease being under diagnosed. By identifying the first \textit{HPSE2} missense mutation it also provides a starting point for studies aimed at functionally understanding the unusual combination of symptoms as characterizing UFS.

Conflict of interest

The authors declare no conflict of interest.

The combination of urological symptoms and abnormal facial expression was first described in 1979 (1) and subsequently termed urofacial syndrome (UFS) (2). Initially, this recessive phenotype was considered to be locally restricted to a highly inbred population in Columbia (2), but several case reports published since showed UFS to occur worldwide (3–8). Patients typically present in childhood with nocturnal/diurnal enuresis and recurrent urinary tract infections. The additional presence of an ‘inverse’ facial expression when attempting to smile excludes common neurogenic bladder syndromes and defines UFS. If left untreated, the disease may be fatal because of renal failure (2).

UFS has been linked to 10q23-q24 in several unrelated families (4, 9, 10). Very recently, two concomitantly published studies reported
homozygous HPSE2 mutations in a total of nine UFS families (11, 12). The mutational spectrum comprising nonsense \( (n = 3) \) and frameshift alterations \( (n = 4) \) as well as large exonic deletions \( (n = 2) \) suggests a loss-of-function mechanism. The protein product of HPSE2 shows 44% homology to heparanase1, an enzyme cleaving heparin sulphate glycosaminoglycans in the extracellular matrix (12), and was therefore named heparanase2. The physiological and pathological roles of heparanase2, however, have not been addressed so far.

The present study reports on a Pakistani UFS family with three affected siblings. It establishes linkage to the UFS critical region and identifies the first HPSE2 missense mutation underlying this disease. It thereby offers a starting point for studies aimed at a deeper understanding the pathophysiology underlying UFS.

**Patients and methods**

Patients and controls

The family consisting of the parents (first degree cousins) and their six children presented for genetic counselling at the Department of Human Genetics and Molecular Biology at the University of Health Sciences in Lahore. After informed consent was obtained, a 5 ml EDTA blood sample was taken from every family member and DNA prepared according to a standard salting out procedure. A total of 113 anonymized DNAs of Pakistani volunteers served as an ethnically matched control sample.

Linkage analysis

DNA samples were genotyped using the Affymetrix GeneChip® Human Mapping 250K Sty Array. Linkage analysis was performed assuming autosomal recessive inheritance, full penetrance, consanguinity and a disease gene frequency of 0.0001. Multipoint LOD scores were calculated using the program Allegro (13). Linkage to the UFS critical region was specifically investigated by applying a set of eight microsatellite markers (Table S1, Supporting information).

Sequencing of HPSE2

Appropriate primers were designed (Table S2) such as to amplify the 12 coding exons of HPSE2 (NM_021828) together with >30 bp of neighbouring intronic and untranslated region (UTR) sequences. DNA from the index patient was used as the primary template. Exons found to harbour alterations were subsequently sequenced in all family members.

Screening for HPSE2 variants in controls

The single base alteration c.631T>C abolishes a PsiI restriction site thereby allowing straightforward design of an appropriate restriction fragment length polymorphism (RFLP) assay. No restriction site is created or lost by alteration c.1628A>T. We therefore designed a forward primer introducing a mismatch such that an AgsI site is created in the normal but not the variant c.1628 allele. Both RFLPs (see Fig. S1 for details) were first validated on the UFS family and subsequently applied to screen the above-described control cohort.

**Results**

All patients show a typical UFS phenotype that is recessively inherited

The index patient had initially been referred to a tertiary care facility because of recurrent fever associated with lower urinary tract infections. The family history suggested that two sisters have suffered from similar but less severe symptoms since early childhood. Detailed clinical investigation as carried out subsequently on all three patients confirmed a familial form of urological disease the severity of which highly varies. Table 1 summarizes the relevant clinical findings. In addition to the urological problems all three patients displayed an inverse facial expression when attempting to smile (Fig. 1a). Neither the parents, being first degree cousins, nor the three other siblings (two girls aged 16 and 18 years and one boy aged 10 years, respectively) had urinary complaints or evidence for an abnormal facial expression (see Fig. 1b for complete pedigree). Taken together, these observations led to the diagnosis of autosomal recessive UFS.

The UFS critical region is homozygous in affected but not in unaffected family members

On the basis of consanguinity of the family and the apparent recessive mode of inheritance we performed SNP-based homozygosity mapping. Using Allegro and a reduced marker panel of approximately 20,000 SNPs we identified three regions that are homozygous in all patients but not in their unaffected siblings. The corresponding LOD score of 2.78 for all three regions is the maximum score expected in this family (Fig. S2). By considering all SNPs from the 250K array these regions were precisely mapped to chromosomes 1q21.1-1q23.2 (size 10.6 Mb), 2p24.2-2p22.3 (size 15.8 Mb) and 10q23.3-10q25.1 (size 17.1 Mb). The latter region centres around the UFS critical
Table 1. Clinical findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>III-3</th>
<th>III-5&lt;sup&gt;a&lt;/sup&gt;</th>
<th>III-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current age (years)</td>
<td>15</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Presence of ‘inverted smile’</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Presenting complaints</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary urgency/incontinence</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Constipation</td>
<td>Chronic</td>
<td>Chronic</td>
<td>Off and on</td>
</tr>
<tr>
<td>B/L flank and suprapubic pain</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>H/O recurrent high-grade fever</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kidney and urinary bladder examination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/L hydro/-ureteronephrosis</td>
<td>Mild</td>
<td>Moderate</td>
<td>Mild</td>
</tr>
<tr>
<td>Decreased GFR right kidney</td>
<td>++</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Decreased GFR left kidney</td>
<td>−</td>
<td>+++</td>
<td>−</td>
</tr>
<tr>
<td>PMRV</td>
<td>150 ml</td>
<td>60 ml</td>
<td>40 ml</td>
</tr>
<tr>
<td>Vesicoureteric reflux</td>
<td>−</td>
<td>B/L (grade IV)</td>
<td>−</td>
</tr>
<tr>
<td>Trabeculated bladder</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Laboratory parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine (pH, sugar, protein, WBC and RBC)</td>
<td>Normal</td>
<td>WBC increased</td>
<td>Normal</td>
</tr>
<tr>
<td>Blood haemoglobin (g/dl)</td>
<td>13.3</td>
<td>7.6</td>
<td>12.0</td>
</tr>
<tr>
<td>ESR (mm/1st hour)</td>
<td>38</td>
<td>110</td>
<td>40</td>
</tr>
<tr>
<td>BUN</td>
<td>Normal</td>
<td>Increased</td>
<td>Normal</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>Normal</td>
<td>Increased</td>
<td>Normal</td>
</tr>
</tbody>
</table>

B/L, bilateral; BUN, blood urea nitrogen; ESR, erythrocyte sedimentation rate; GFR, glomerular filtration rate; H/O, history of; PMRV, post-mictional residual volume; RBC, red blood cells; WBC, white blood cells.

<sup>a</sup>Index patient, additional findings specific to this individual: occasional episodes of headaches, frequently develops periorbital and pedal oedema, generalized pallor.

Fig. 1. Novel cases of urofacial syndrome in a Pakistani family. (a) Three sisters show an inverted facial expression when attempting to smile. They also suffer from a variety of childhood onset urological problems. (b) The unaffected parents of the patients are first degree cousins. DNA was available from family members II-2, II-3 and III-1 to III-6 (current ages for generation III are provided in parenthesis; arrow denotes the index patient).

HPSE2 alteration c.1628A>T, but not c.631T>C, affects a conserved residue, is predicted to be damaging, and absent in controls

Sequencing of all HPSE2 exons in the index patient revealed the presence of two homozygous missense variants not annotated as SNPs in public databases (Fig. 2a). Variant c.631T>C (p.Y211H) localizes to exon 4 whereas c.1628A>T (p.N543I) falls into the terminal exon 12. As expected, both variants were present in the homozygous and heterozygous state in the other patients and the parents, respectively. Consistent with the allele distribution that had been observed by SNP-based array analysis (data not shown) none of the healthy siblings carried the variants. Residue 211 is not conserved amongst vertebrates; birds even carry a histidine, i.e. the residue resulting from the c.631T>C change of human HPSE2 (Fig. 2b). Moreover, p.Y211H is classified as a benign alteration by PolyPhen [Position-Specific Independent Counts (PSIC) score difference: 0.212]. In contrast, position 543 is asparagine in all vertebrates (Fig. 2b) and p.N543I is predicted to be probably damaging (PSIC score difference: 2.861). To further elucidate a pathogenic potential of the alterations in question we designed appropriate RFLPs (Figs 2c and S1) and screened a cohort of 113 ethnically matched controls. The c.631T>C variant was found nine times in the heterozygous state while one control individual was even homozygous. No additional c.1628A>T alleles were identified.
First HPSE2 missense mutation in urofacial syndrome

(a) Sequence traces showing presence/absence of two single base alterations in the family members as specified to the left (for identities of individuals III-1, II-3 and III-3, see labels on pedigree in Fig. 1b). (b) Conservation of the HPSE2 protein regions affected by the missense variants (centred at position 10 of 19-residue stretches; marked by arrow heads). Shown below are the residues that are also found in human HPSE1 (cons1) and in HPSE2 of all other vertebrates (cons2). The Y211 region is generally less conserved than the N543 region. (c) Restriction fragment length polymorphism (RFLP) data allowing detection of heterozygous and homozygous presence of the variants in question. An arrow denotes the normal allele, an arrowhead the variant allele (detailed descriptions of the RFLPs are provided in Fig. S1) (L, size standard).

Discussion

The present study reports on a family in which three siblings are affected by classical UFS. To our knowledge this brings the total number of published independent cases with familial UFS to 18 (1, 3–8, 11, 12). UFS could thus be considered an extremely rare disease. However, neurogenic bladder, vesicourethral reflux and urinary infections in childhood are rather frequent clinical findings. The additional presence of an abnormality in facial expression is probably not reported/recognized in the context of a routine urological setting. Therefore, clinical diagnosis of UFS has to be regarded very challenging. This is also exemplified by our family in which diagnosis of a genetic disorder in general and UFS in particular was only made upon hospitalization of the most severely affected index case. In line with speculations put forward previously (5, 12, 14) we suggest that UFS may be far more frequent than the sparse literature suggests.

In our family, a total of three genomic regions link to the UFS phenotype with the maximum expected LOD score of 2.78 (Fig. S2). One of these is the 10q region which contains the HPSE2 gene mutated in the majority of UFS families (11, 12). Sequencing identified two homozygous missense variants in all three patients but not in healthy siblings; both parents were heterozygous for these variants (Fig. 2a). In silico analysis and screening of ethnically matched controls indicated the p.Y211H exchange in exon 4 to be a benign SNP amongst Pakistanis. The p.N543I variant, in contrast, is highly probable to represent the causative mutation having occurred on a p.Y211H allele background.

Two of the nine HPSE2 mutations reported to date are in frame exon deletions. Modelling of the resulting three-dimensional structures suggested that they result in an unstable or inactive protein (12). A loss-of-function mechanism is also predicted for the remaining nonsense and frameshift alterations (11, 12) as they should entail nonsense-mediated decay. In our family, a missense mutation causes a UFS phenotype that appears clinically undistinguishable from the forms associated with the above mutations. Unfortunately, we were unable to investigate the impact of this mutation on mRNA stability as HPSE2 is not expressed in peripheral blood [data not shown; see also Ref. (12)]. With c.1628A>T falling into the terminal exon 12 and with no in silico evidence for an effect on splicing we would still predict unaltered protein levels. The alteration of residue 543 is therefore probable to completely abolish proper heparanase2 function. On the basis of homology to heparanase1, the highly conserved residue 543 is predicted to reside outside of both the catalytic domain and the C-terminus required for secretion and activation (15). Instead, it is part of a highly conserved but as yet uncharacterized amino acid stretch (Fig. 2b). Future investigations into the physiological and pathological function(s) of heparanase2 will need to define domains mediating cell surface trafficking, substrate specificity and protein–protein interactions. Our finding of the first pathogenic HPSE2 missense alteration provides a starting point for pertinent studies which might, eventually, lead to a better understanding of the pathophysiology underlying the remarkable
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combination of phenotypes as observed in UFS patients.

Supporting Information

The following Supporting information is available for this article:

Fig. S1. Restriction fragment length polymorphisms to detect variants c.631T>C and c.1628A>T. Polymerase chain reaction (PCR) primers are depicted in bold and italic; recognition sites of the restriction enzymes applied in bold and underlined. (a) Upon restriction with PsI a 321-bp PCR product from the normal allele yields two fragments of sizes 92 and 229 bp, respectively. Variant c.631T>C (nucleotide c.631 in red) abolishes the PsI recognition sequence. PCR product from the altered allele, thus, remains undigested. (b) The forward primer introduces a C>T alteration at nucleotide c.1624 (in green) such as to create a second AgsI restriction site in the 395-bp product from the normal allele. Variant c.1628A>T (nucleotide c.1628 in red) abolishes this artificially created site. Restriction patterns for normal and altered allele, thus, differ (45 + 48 + 302 vs 93 + 302 bp, respectively).

Fig. S2. Genome-wide linkage analysis for the urofacial syndrome phenotype in the family presented. A total of three genomic regions (arrows) are homozygous in all patients but neither in their parents nor their healthy siblings. The corresponding LOD score of 2.78 is the maximum score expected considering the family structure (see Fig. 1b for pedigree).

Fig. S3. Haplotype analysis of the urofacial syndrome chromosome 10 critical region using microsatellites (see Table S2 for physical position of markers and of HPSE2 gene). (a) The disease haplotype (boxed) is present in heterozygosity in the parents. The affected children, but not unaffected siblings, are homozygous. (b) Exemplary raw data for the most terminal markers for all six children (order of individuals as in above pedigree).

Table S1. Localization of SNPs defining the chromosome 10 region linked to the phenotype, microsatellites applied to confirm this linkage and the HPSE2 gene

Table S2. Primers used to amplify and sequence the 12 exons of HPSE2

Additional Supporting information may be found in the online version of this article.

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Acknowledgements

We thank the family for agreeing to participate in the study. We also thank H. Kiesewetter and K. Stein for technical assistance. This work was funded by grants of the Deutsche Forschungsgemeinschaft to C. B. and C. A. H.

References