Loss-of-Function Mutations in HPSE2 Cause the Autosomal Recessive Urofacial Syndrome

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Previously, we localized the defective gene for the urofacial syndrome (UFS) to a region on chromosome 10q24 by homozygosity mapping. We now report evidence that *Heparanase 2* (*HPSE2*) is the culprit gene for the syndrome. Mutations with a loss of function in the *Heparanase 2* (*HPSE2*) gene were identified in all UFS patients originating from Colombia, the United States, and France. HPSE2 encodes a 592 aa protein that contains a domain showing sequence homology to the glycosyl hydrolase motif in the *heparanase* (*HPSE*) gene, but its exact biological function has not yet been characterized. Complete loss of HPSE2 function in UFS patients suggests that HPSE2 may be important for the synergic action of muscles implicated in facial expression and urine voiding.

The urofacial syndrome (UFS [MIM 236730]) is an autosomal recessive disease characterized by urological and facial abnormalities.[1,2] The typical symptom for UFS patients is a distorted face, as if in pain or sadness, when they smile or laugh. However, dysfunctional voiding, such as repeated episodes of urinary tract infection, dysuria or incontinence, frequency, urgency, or enuresis, is the life-threatening component of the syndrome. This gradually progresses to severe upper tract damage and the development of bladder trabeculation, vesicoureteral reflux (VUR), hydroureternephrosis, and subsequent renal failure.[1–3] Nevertheless, no apparent neurological or urinary obstructive pathology has been noticed in any UFS patients.

Previous studies in patients originating from Colombia localized the disease gene to a region on chromosome 10q24 by homozygosity mapping.[4,5] Subsequent fine mapping provided preliminary evidence that the disease gene could be located in a genomic interval of approximately 250 kb DNA between markers *D10S2500* and *D10S2511*.[6] Mutation screening in UFS patients was also carried out in two candidate genes, *GOT1* (MIM 138180) and *CNNM1* (ancient conserved domain protein 1, *ACDP1*) (MIM 607802).[5–7] *GOT1* contains 9 exons and spans 33.7 kb of genomic DNA. It is a ubiquitous pyridoxal phosphate-dependent enzyme, and it plays an important role in amino acid metabolism and in the urea/tricarboxylic acid cycles. *CNNM1* contains 11 exons and spans a genomic region of 67.56 kb DNA sequence. *CNNM1* is a novel gene belonging to a new gene family that could be implicated in divalent ion transport.[8–10] Unexpectedly, no path-

ological mutation was discovered in these two plausible candidate genes, raising the possibility that the disease gene may be outside of the refined interval. This was a distinct possibility, because it is sometimes difficult to distinguish between recombination events and point mutations in homozygosity mapping.

To accurately redefine the disease interval, we selected multiple families with recombination events on both ends of the disease interval, which includes patient II.1 of UFS-30 collected in the United States and patients II.1 of UFS-2, II.1 of UFS-12, II.1 of UFS-13, IV.1 of UFS-14, V.1 of UFS-15, and II.1 of UFS-18, recruited from Colombia (Figure 1). Because these patients carry two identical disease chromosomes inherited from their parents, they were homozygous for the markers in the region without recombination. Haplotype analysis was then carried out to determine the disease interval. As shown in Figure 2, chromosomal regions for each patient without recombination (markers with homozygous genotypes) were boxed. Heterozygous genotypes were characterized in patients II.1 of UFS-2, II.1 of UFS-13, II.1 of UFS-18, and II.1 of UFS-30 at marker *D10S1433* and its adjacent telomeric markers, indicating that *D10S1433* defines the telomeric boundary for the disease interval. Similarly, patients II.1 of UFS-3, II.1 of UFS-12, IV.1 of UFS-14, and V.1 of UFS-15 showed heterozygous genotypes at marker *D10S603* and other centromeric markers, demonstrating that *D10S603* could be the breakpoint for centromeric recombination. Therefore, the disease region was placed at an expanded interval between markers *D10S1433* and *D10S603*.

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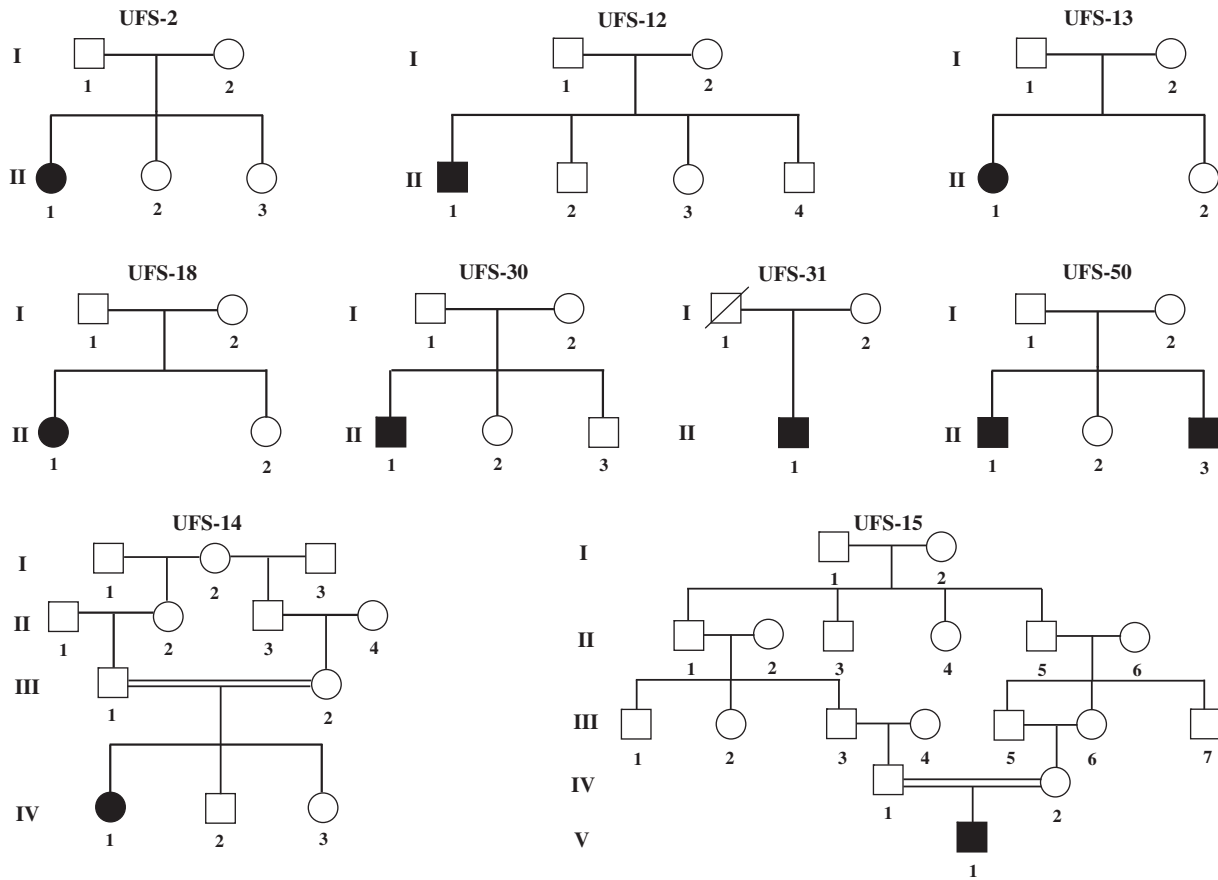
Figure 1. Pedigrees Used for Redefining UFS Interval and Candidate Gene Mutation Screenings
Pedigrees of UFS-2, -12, -13, -14, -15, and -18 were collected from Colombia, pedigrees UFS-30 and UFS-31 were recruited from the United States, and pedigree UFS-50 was obtained from France. Patients of UFS-30 and UFS-31 share a common Irish heritage, and patients in pedigree UFS-50 are of European descent. Of note, patients in pedigrees UFS-2, -12, -13, -18, -30, -31, and -50 were from unrelated marriages, whereas patients in pedigrees UFS-14 and -15 were from consanguineous marriages.

Seventeen known genes and two unnamed transcripts (*LOC100289312* and *NCRNA00093*) were characterized within this newly defined disease interval (Figure 3).

Mutation screening was then carried out for the 14 new genes and the two above indicated unnamed transcripts located in the centromeric region of the interval between *D10S198* and *D10S603*. We selected three patients, originating from Colombia (II.1 of UFS-2), the United States (II.1 of UFS-30), and France (II.1 of UFS-50), with each representing a disease haplotype (mutation), except for II.1 of UFS-50, who inherited two different disease haplotypes from his parents. We also included a pooled control DNA (equal amount of DNA pooled from eight normal individuals) to evaluate normal polymorphisms. We designed a PCR walking strategy, with each PCR flanking a 600–800 bp of genomic region. The screenings have

Figure 2. Redefined UFS Interval Based on Haplotype Analysis of Patients with Recombination Events
Patients II.1 of UFS-2, II.1 of UFS-13, II.1 of UFS-18, II.1 of UFS-12, IV.1 of UFS-14, and V.1 of UFS-15 were Colombian, whereas II.1 of UFS-30 was a United States patient. Markers with the homozygous genotype were boxed to define the region without recombination. Alleles for markers *D10S1443* and *D10S603* in patients with recombination events are in bold to show the telomeric and centromeric breakpoints, respectively. The disease interval was placed between markers *D10S1433* and *D10S603*.
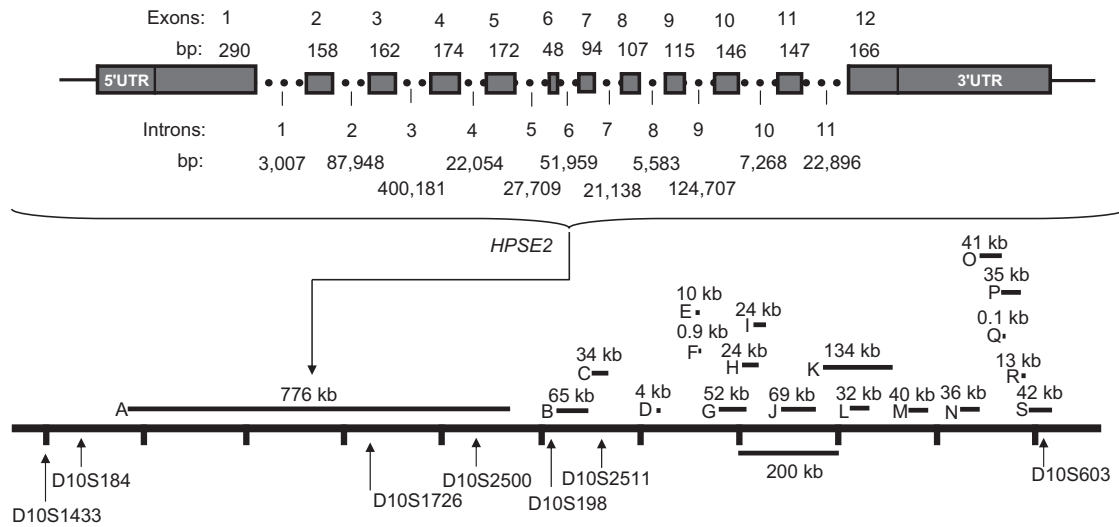
Figure 3. A Transcriptional Map for the Newly Defined UFS Disease Interval

Top: a detailed genomic structure for the *HPSE2* gene. Bottom: all candidate genes located between *D10S1433* and *D10S603*. The size in genomic DNA (kb) for each transcript is shown within the figure. The order and genomic location of each microsatellite marker within the map was defined based on the genomic sequence information from the Ensemble database. The letters represent the following: A, HPSE2 (NM_021828); B, CNNM1 (NM_020348); C, GOT1 (NM_002079); D, NKX2-3 (NM_145285); E, SLC25A28 (NM_031212); F, LOC100289312 (NM_002343029); G, ENTPD7 (NM_020354); H, COX15 (NM-078470); I, CUTC (NM-015960); J, ABCC2 (NM_000392); K, DNMBP (NM_015221); L, NCRNA00093 (NR_024130); M, CPN1 (NM_001308); N, ERLIN1 (NM_006459); O, CHUK (NM_001278); P, CWF19L1 (NM_018294); Q, SNORA12 (NR_002954); R, BLOC1S2 (NM_0173809); S, PKD2L1 (NM_016112).

covered 4 kb of genomic DNA flanking the promoter region and 5′ UTR region, entire exons, 120 bp of exon/intron junctions, and the 3′ UTR region of each candidate gene. Unfortunately, we were not able to identify any apparent pathological mutation in these genes (data not shown).

Finally, we turned our attention to the region telomeric to *CNNM1* (*ACDP1*), which contains a single gene (*HPSE2*) that spans 776 kb of genomic DNA (Figure 3). A homozygous nonsense mutation (c.1516C>T) was first identified in exon 11 of the *HPSE2* gene in the Colombian patient (Figure 4A). This mutation, designated R506X (c.1516C>T), produces a truncated protein that misses 86 amino acids at the C terminus of the HPSE2 protein. The mutation abolishes the TaqI restriction site that is present in the wild-type DNA. PCR amplification followed by TaqI digestion was used for rapid genotyping of the 31 other UFS patients in our collection. As expected from the haplotype data on these patients, all UFS patients from Colombia are homozygous for the R506X (c.1516C>T) mutation (data not shown).

We then screened mutations for the rest of the UFS patients. Patients from two United States pedigrees (II.1 of UFS-30 and II.1 of UFS-31) with a common Irish heritage share one same disease haplotype. Unlike the Colombian patients, these two patients carry a homozygous 2 bp deletion (c.1465_1466delAA) in exon 10 at nucleotide positions 1465 and 1466 (start codon as position 1) (Figure 4B), resulting in a larger protein of 613 aa, with a completely different sequence for the last 125 aa because of reading frame shift. Of note, these patients carry a point mutation

at marker *D10S2499* that was previously used to define the centromeric boundary.[6]

Unlike the homozygous mutations characterized in the above patients, two French patients (II.1 and II.3 of UFS-50) from the same family of European decent carry two different disease haplotypes originating from their nonconsanguineous parents (Figure 1): the first is identical to the United States haplotype, and the second is unique to the French patients. As expected, compound heterozygous loss-of-function mutations were characterized in these two patients. One is the same 2 bp deletion mutation found in the United States patients (c.1465_1466delAA), whereas the other is a 2 bp deletion mutation (c.241_242delCT) in exon 1 at nucleotide positions 241 and 242 (start codon as position 1) (Figure 4C).

The full-length *HPSE2* gene consists of 2353 bp of nucleotides encoding a protein of 592 aa that contains a region showing sequence homology to the glycosyl hydrolase motif in the *heparanase* (*HPSE*) gene,[11] but its exact biological function has not yet been characterized. Interestingly, phylogenetic analysis revealed that no *HPSE2* orthologs exist in species of nonvertebrates such as *Drosophila melanogaster* (see Figure S1 available online). HPSE2 is evolutionarily conserved among diverse vertebrates. For example, the similarity between mouse and human is 93% for the DNA coding sequence and 97% at the protein sequence level (Figure S2).

Because of the involvement of both the urinary system and the facial muscles for the UFS phenotype, it has been thought that UFS may result from defects in a region of the brain that controls micturation and facial muscle.[1,3,12]
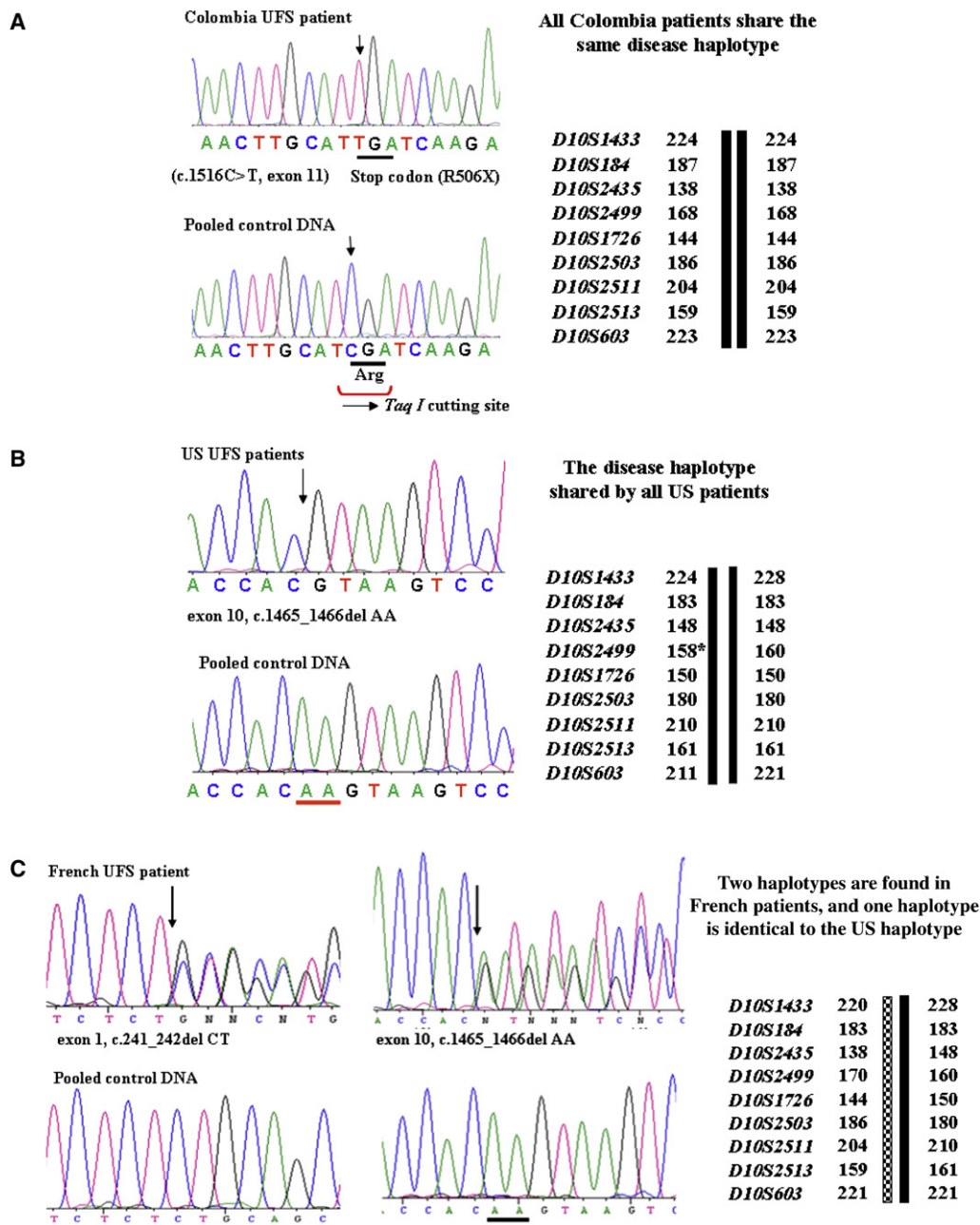
Figure 4. Mutations Identified in UFS Patients
(A) All Colombian patients share one identical disease haplotype and carry a homozygous nonsense mutation c.1516C>T (R506X) in exon 11.
(B) The United States patients possess one identical disease haplotype and carry a homozygous AA deletion (c.1465_1466delAA) in exon 10 at nucleotide positions 1465 and 1466 (start codon as position 1).
(C) The French patients carry two different disease haplotypes: the first is identical to the United States patients, and the second is different from other patients. They carry a 2 bp AA deletion (c.1465_1466delAA) in exon 10, like the United States patients, and a 2 bp CT deletion (c.241_242delCT) in exon 1 at positions 241 and 242 (start codon as position 1).

Contrary to this hypothesis, cranial MRI analysis of UFS patients showed negative results for lesions in the brain, and MRI analysis further demonstrated a normal spine and conus medularis in the UFS patients, suggesting a non-neurological origin for the disease pathogenesis.[3,13] Furthermore, RT-PCR analysis failed to detect *HPSE2* expression in the brain and spinal cord. On the contrary, both the facial muscle and urinary bladder showed high levels of *HPSE2* expression. However, unlike urinary bladder, very low levels of *HPSE2* mRNA were detected in the stomach and intestine, and, unlike facial muscle, *HPSE2* was almost completely absent in the skeletal muscle (data not shown). Given the phenotypic characteristics of UFS patients (they show distorted facial expression and detrusor-sphincter dyssynergia), our results suggest that HPSE2 could regulate the coordinated action of

muscles implicated in facial expression and urine voiding in the periphery. Moreover, the characterization of the *HPSE2* gene for the syndrome has now paved the way to fully dissect the underlying mechanisms for the puzzling observations on the clinical phenotype of patients with this devastating disease.

In summary, this study identified three loss-of-function mutations in the *HPSE2* gene in UFS patients from multiple countries, which provides evidence for the conclusion that *HPSE2* is the culprit gene responsible for the syndrome. Voiding disorders such as frequency, urgency, enuresis, dysuria or incontinence without apparent neurological abnormality, and urinary obstruction are highly prevalent in the general population but are greatly underrecognized and underreported.[12,14–19] Because UFS patients share clinical, radiological, and urodynamic features with those patients with dysfunctional voiding in the general population,[3,13,20–22] it will be interesting to determine whether altered HPSE2 function is implicated in the pathogenesis of general voiding disorders.

Supplemental Data

Supplemental Data include two figures and one table and can be found with this article online at http://www.ajhg.org.

Web Resources

The URLs for data presented herein are as follows:

NCBI Protein Database, http://www.ncbi.nlm.nih.gov/protein/
Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/Omim/
The Ensembl Project genome database, http://uswest.ensembl.org/

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