Urinary tract effects of *HPSE2* mutations in humans and mice

SUPPLEMENTAL TEXT, TABLE AND FIGURES

Family histories and investigations

Family 1. The male proband has first cousin Saudi Arabian parents. Third trimester US detected left-sided hydronephrosis; it progressed in the first year and he received prophylactic antibiotics. Micturating cystourethrography (MCUG) showed bladder trabeculation without other anomalies, and plasma creatinine concentration (pCr) was normal. He grimaced from birth. Sequencing *HPSE2* revealed a homozygous frameshift in exon 1, c.57dupC (p.Ala20Argfs*45) [NM_021828.4], generating a premature stop codon at position 64. His asymptomatic parents were heterozygous.

Family 2. The female proband (V:3) is from a consanguineous Romani family living in Portugal (Supplemental Figure 1A and B). Antenatal ultrasound scans (USs) were normal but she grimaced from infancy. Aged three years, she experienced abdominal pain and urinary frequency. US revealed residual urine in a thick-walled bladder but no hydronephrosis. She was administered an α1 adrenergic antagonist to reduce outflow resistance and anticholinergics to reduce detrusor contractility. Aged five, she had nocturnal enuresis and normal pCr. Her paternal male cousin (V:1) also grimaces. He experienced lumbar pain and dysuria when nine years. US revealed a large thick-walled bladder and bilateral hydronephrosis; urodynamics showed dyssynergia. He self-catheterized and received anticholinergics. US at 11 years showed no hydronephrosis but the bladder remained abnormal. Polymerase chain reaction (PCR) failed to amplify *HPSE2* exon 3 in V:3 and V:1. Multiplex ligation-dependent probe amplification (MLPA) and gap PCR showed both had a homozygous in-frame deletion of 11.2 kb encompassing exon 3. IV:1 (mother of V:1),

IV:3 (father of V:3) and III:4 (paternal grandmother of V:1 and V:3) were heterozygous and had no urinary symptoms. III:4, however, and two of her paternal cousins (III:1 and III:2) grimace. III:4 carries a second *HPSE2* mutation, c.457C>T (p.Arg153*) [NM_021828.4] which would cause nonsense-mediated decay; DNA was not analysed in III:1 and III:2.

Family 3. The female proband has white British/American parents. Second trimester US detected megacystis and postnatal US revealed bladder wall thickening. MCUG showed a fir tree-shaped, trabeculated bladder; VUR was absent. At six years she was enuretic and urodynamics showed a normal capacity dyssynergic bladder. Dimercaptosuccinic acid isotope scanning (DMSA) identified a left kidney focal defect. Continence was established at nine years following α1 adrenergic antagonist administration. pCr was normal at 14 years. Examination by a Neurologist reported grimacing upon smiling, diminished central forehead and lateral eyebrow movements, and an inability to seal the lips. Sequencing *HPSE2* revealed novel compound heterozygous mutations, c.724delC (p.Leu242*) and c.1099-1G>A [NM_021828.4]. The former introduces a premature stop codon after a frameshift and the latter leads to loss of a splice acceptor and introduces a premature stop codon (see ***Splicing Experiment*** in **Methods**). The asymptomatic father was heterozygous for c.1099-1G>A.

Family 4. The male proband (Supplemental Figure 1C and D) from a consanguineous Turkish family presented with urosepsis aged two years when: US revealed bilateral hydroureteronephrosis; MCUG showed a large, incompletely-emptying trabeculated bladder but no VUR; and DMSA showed bilateral focal defects. Diurnal enuresis was managed by intermittent urethral catheterization. He had recurrent urosepsis. pCr was elevated at three (1.7mg/dl; normal <1.0mg/dl) and 12 (3.7mg/dl) years. *HPSE2* sequencing revealed a homozygous nonsense mutation, c.457C>T (p.Arg153*) [NM_021828.4], with heterozygous asymptomatic parents. Only then was it realized that he had a typical UFS grimace. His

younger brother (Supplemental Figure 1E and F) was homozygous and also grimaces. Aged eight years, he had primary nocturnal enuresis and MCUG revealed bladder pseudodiverticulae and unilateral VUR; pCr was normal.

Family 5. The male proband from a consanguineous Turkish family presented at four years with diurnal enuresis and urosepsis. US revealed bilateral hydroureteronephrosis, urodynamics demonstrated high intravesical pressures, MCUG showed a large trabeculated bladder without VUR, and DMSA showed bilateral focal defects. His used intermittent catheterization *per* urethra. pCr was elevated (3.7mg/dl) at nine years. Sequencing *HPSE2* revealed a novel homozygous nonsense mutation, c.429T>A (p.Tyr143*) [NM_021828.4]; only then was his characteristic grimace recognised (Supplemental Figure 1G). His asymptomatic parents are heterozygous but his brother grimaces (Supplemental Figure 1H) and is homozygous; at 11 years, he has primary nocturnal enuresis. MCUG demonstrated bladder trabeculation but no VUR, and pCr was normal.

Family 6. The proband is a 17 year male with third cousin Saudi Arabian parents. Antenatal US was not undertaken and he grimaced as a young child. He had a NNNB and left-sided hydroureteronephrosis with renal parenchymal thinning. He underwent bladder augmentation and catheterized using a Mitrofanoff stoma. Examination by a Neurologist revealed facial weakness with abnormal movement of the mouth and eyes with expression. He had a shuffling gait with low muscle bulk in his legs. Brain magnetic resonance imaging was normal. Sequencing revealed a homozygous *HPSE2* variant, c.761_763del [NM_021828.4], an in-frame deletion of asparagine 254. Family samples were unavailable.

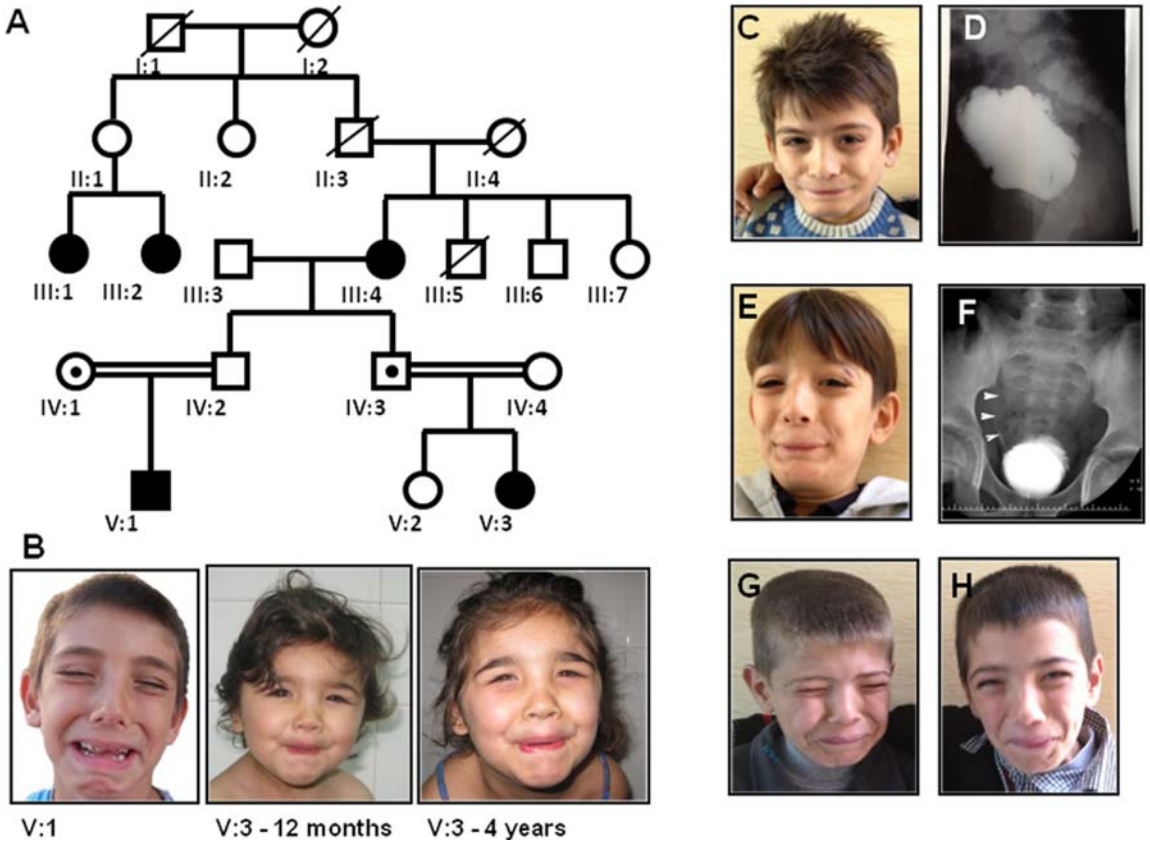
Family 7. The male proband from Kosovo presented with primary diurnal and nocturnal enuresis and urosepsis aged seven years. Investigations revealed bilateral hydroureteronephrosis, the left kidney contributing 39% of total function. Cystoscopy showed

a trabeculated bladder with outlet hypertrophy, and urodynamics showed high intravesical pressures and residual urine. Aged nine years, the left ureter was reimplanted when pCr was 0.7mg/dl. Bladder dysfunction and enuresis persisted. pCr was 1.39mg/dl at 13 years when self-catheterization *via* an umbilical Mitrofanoff stoma was initiated. Nevertheless, kidney function deteriorated with pCr 1.94mg/dl at 16 years. He grimaces when smiling. Sequencing *HPSE2* revealed the homozygous nonsense mutation, c.457C>T [NM_021828.4] (p.Arg153*).

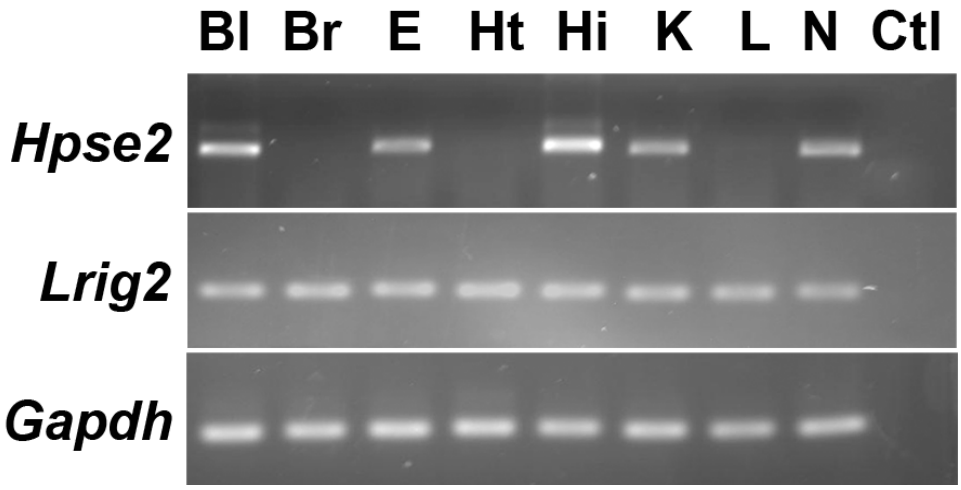
Supplemental Table 1. Rare (<1%) heterozygous missense variants in *HPSE2* in familial VUR cohorts.

Variant	Segregation of variant with VUR phenotype	Frequency in Variome Databases	Predicted Consequence
c.980C>A, p.Thr327Lys	Seen in three affected siblings and an apparently unaffected mother.	None	Align GVGD - Class C0 – less likely SIFT – Deleterious score 0.04
c.1021C>T, p.Arg341Trp	Seen in two affected female siblings, an apparently unaffected sister and their apparently unaffected father whose brother had VUR and a nephrectomy.	Not in databases. Adjacent to variant c.1022G>A, p.Arg341Gln causing change in same amino acid on ESP (0.03%).	Align GVGD – Class C65 – most likely SIFT – Deleterious score 0.00
c.1258C>T, p.Arg420Trp	No	Reported once in European American chromosomes on ESP (0.008%).	Align GVGD – Class C65 – most likely SIFT – Deleterious score 0.00
c.1618G>C, p.Val540Leu	No	dbSNP - rs140066668, Present in ESP (0.3%).	Align GVGD – Class C0 – less likely SIFT – Deleterious score 0.00
c.1769G>A, p.Arg590His	No	dbSNP - rs138098027. Present in ESP (0.01%)	Align GVGD – Class C0 – less likely SIFT – Deleterious score 0.00

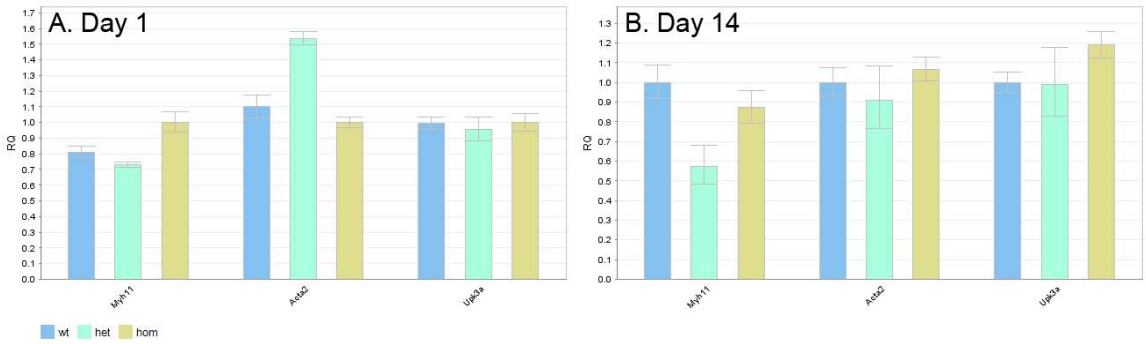
Legend: Each variant has only been seen once. Databases checked are dbSNP build 137 and NHLBI Exome Sequencing Project (ESP) Exome Variant Server. Prediction software used Align GVGD - http://agvgd.iarc.fr/, SIFT - http://sift.jcvi.org/, Human Splicing Finder - http://www.umd.be/HSF/



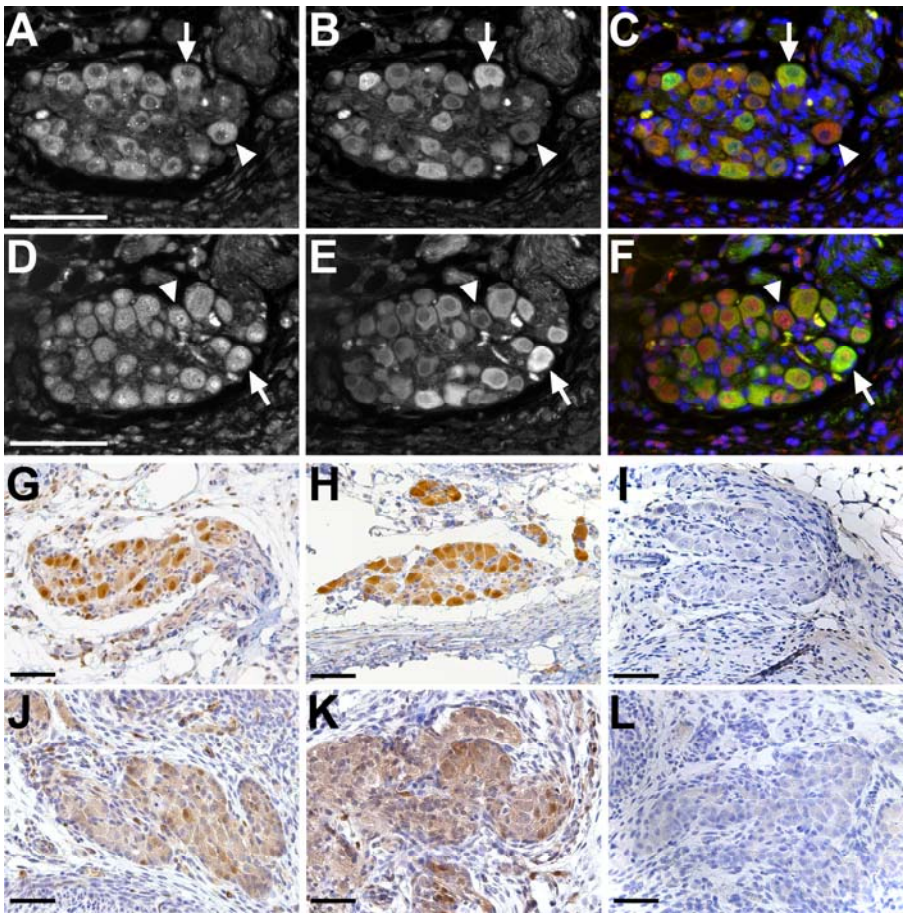
Supplemental Figure 1. Clinical details of selected families. A. Pedigree of Family 2. **B.** Faces of V:1 and V:3 from Family 2 showing grimace upon smiling. Note the simultaneous contraction of the corners of the mouth and eyes. **C.** Face of proband from Family 4. **D.** MCUG of proband from Family 4; note bladder trabeculation and pseudodiverticulae. **E.** Face of younger brother from Family 4. **F.** MCUG showing unilateral VUR (white arrowheads). **G.** Face of proband from Family 5. **H.** Face of affected sibling from Family 5



Supplemental Figure 2. RT-PCR analyses of wild-type E17 mouse organs. Note *Hpse2* expression in urinary bladder, eye, hindgut, kidney and neural tube. *Lrig2* was detected in all organs examined. *Gapdh* is included as a housekeeping control. Key: Bl, urinary bladder; Br, brain; E, eye; Ht, heart; Hi, hindgut; K, metanephric kidney; L, liver; N, neural tube; and Ctl, negative control with no cDNA.



Supplemental Figure 3. Quantitative PCR of bladder transcripts. RNA was extracted from whole bladders and smooth muscle *(Myh11* and *Acta2)* and epithelial *(Upk3a*) transcripts were quantified. Their levels are depicted after factoring for *Hprt1*, used as a reference housekeeping transcript. Note that levels of the muscle transcripts were similar in homozygous mutant (hom) compared with wild-type (wt) littermates at postnatal Days 1 and 14, thus providing no support for a myogenic pathogenesis of the bladder phenotype. Results are shown as average values, and the bars are SDs, relating to three replicate measurements of each transcript.



Supplemental Figure 4. Phospho-ERK in pelvic ganglia. Longitudinal histology sections through pelvic ganglia in two week old (**A-I**) and two day old (**J-L**) mice. **A-F.** A ganglion was imaged by immunofluorescence. **A**. Immunostaining for heparanase 2 (white) shows neural cell bodies containing the protein; **B.** same section immunoprobed for pERK (white) detects this protein in a subset of neural cell bodies; and **C.** merged image with all nuclei stained blue using DAPI, heparanase 2 in red and pERK in green. Note that some cells, such as the one indicated by the arrowhead, were positive for heparanase 2 but not pERK, whereas others, such as the one indicted by the arrow, contained both proteins. **D**. Immunostaining for LRIG2 (white) shows neural cell bodies containing the protein; **E.** same section immunoprobed for pERK detects this protein (white) in a subset of neural cell bodies; and **F.**

is a merged image with all nuclei stained blue using DAPI, the LRIG2 red and pERK in green. Note that certain cells (arrowhead) were positive for LRIG2 but not pERK, whereas others (arrow) contained both proteins. **G-I.** Brightfield images of pelvic ganglia with nuclei stained blue with hematoxylin. Two week old wild-type ganglion (**G**) immunostained for pERK (brown) shows a similar pattern to that of a homozygous *Hpse2* mutant ganglion (**H**). **I.** Two week old wild-type ganglion without primary antibody. **J-L.** Images of pelvic ganglia from two day old mice; wild-type immunostained for pERK (**J**); homozygous mutant immunostained for pERK (**K**); wild-type ganglion without primary antibody (**L**). Note pERK immunoreactivity is more diffuse than at two weeks of age, with similar appearances of wild-type and mutant tissues. Scale bars are 50 μm.