Urofacial syndrome (UFS) is an autosomal-recessive disease characterized by congenital urinary bladder dysfunction, associated with a significant risk of kidney failure, and an abnormal facial expression upon smiling, laughing, and crying. We report that a subset of UFS-affected individuals have biallelic mutations in LRIG2, encoding leucine-rich-rich repeats and immunoglobulin-like domains 2, a protein implicated in neural cell signaling and tumorigenesis. Importantly, we have demonstrated that rare variants in LRIG2 might be relevant to nonsyndromic bladder disease. We have previously shown that UFS is also caused by mutations in HPSE2, encoding heparanase-2. LRIG2 and heparanase-2 were immunodetected in nerve fascicles growing between muscle bundles within the human fetal bladder, directly implicating both molecules in neural development in the lower urinary tract.

Lower-urinary-tract (LUT) and/or kidney malformations occur in 1–2 out of 1,000 pregnancies and are common causes of childhood renal failure. Advances have been made regarding genetic causes of kidney malformations, but less is known about LUT malformations despite the fact that some, including nonsyndromic vesicoureteric reflux (VUR), are common and familial. Autonomic nerve activity controls the bladder’s ability to act as a low-pressure reservoir, which intermittently and completely expels its contents per urethra. Several congenital disorders feature dysfunctional bladders. Bladder muscle is weak in prune belly syndrome (PBS [MIM 100100]), a condition represented by at least 10-fold coverage. Single-nucleotide substitutions and small insertion and/or deletion variants were identified with our in-house variant-calling pipeline (Table S1, available online), and exome variant profiles were analyzed with a model of a rare autosomal-recessive disorder. In the same gene in both individuals, we prioritized rare putative loss-of-function variants that were absent from variome databases, including dbSNP build.

LRIG2 Mutations Cause Urofacial Syndrome


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Figure 1. Identification of Mutations in LRIG2 in Three Families Affected by UFS
Pedigrees and LRIG2 mutation analysis in families 1 (A), 2 (B), and 3 (C).
(A) In family 1, a homozygous frameshift (c.1230delA [p.Glu410Aspfs*6]) in exon 10 was identified.
(B) In family 2, affected child II-1 is shown at the age of 6 months. Her voiding cystourethrogram shows a trabeculated bladder and severe left-sided VUR (Bi). A compound-heterozygous frameshift (c.2088delC [p.Ser697Hisfs*11]) (Bii) and a compound-heterozygous

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A second consanguineous Turkish family (family 3; Figure 1C) has two siblings affected by UFS. II:1 is a 9-year-old girl with facial features of UFS (Figure S1) and constipation. At 4 years old, she presented with urosepsis and enuresis when a low-capacity, overactive bladder and bilateral VUR were detected. At the age of 6 years, she underwent a left nephrectomy for Wilms tumor.13 At the age of 2 years, her younger sister, II:2, presented with enuresis and a low-capacity, overactive bladder and VUR and subsequently underwent bladder augmentation. Autozygosity mapping identified in chromosomal region 1p13.2 a 8.5 Mb segment overlapping that in family 1, and Sanger sequencing of LRIG2 identified homozygous nonsense mutation c.2125C>T (p.Arg709*) in exon 15. This mutation segregated with the disease in the family and was absent from variome databases, 116 local exomes, and 94 healthy Turkish controls. Previous studies have implicated the loss of a 1 Mb locus in chromosomal region 1p13 in Wilms tumor,14 but LRIG2 lies outside the critical region.

The identified nonsense and frameshift LRIG2 mutations were predicted to result in loss of function via NMD of transcripts. cDNA analysis showed that the large insertion within exon 14 of family 2 results in an in-frame skipping of this exon from the transcript; this is likely due to disruption of exon splice enhancers, consistent with the effects of previously reported exonic insertions of AluYa5 elements.15 The transcript was not subject to NMD but was predicted to result in loss of most of the second immunoglobulin (Ig)-like domain and most conserved part of the gene within the LRIG family (Figure S2).16 Despite somewhat variable LUT phenotypes, there were no consistent clinical differences between these UFS individuals with LRIG2 mutations and those previously reported to have HPSE2 mutations.8 At present, it is difficult to draw a conclusion about the relative contribution of each gene to UFS. To date, of the 14 families affected by classical UFS, we identified nine (64.3%) with mutations in HPSE2,
three (21.4%) with mutations in LRIG2, and two (14.3%) with mutations in neither gene. Other groups have only reported individuals with mutations in HPSE2.9 Bladder dysfunction in UFS is similar to that found in Hinman syndrome, or “non-neurogenic neurogenic bladder.”17,18 Accordingly, we screened LRIG2 in 23 individuals (13 multiethnic British and 10 previously reported Turkish cases) with Hinman syndrome.19 Of these, variants in LRIG2 were found in a single individual, a white British male who, at the age of 10 years, presented with a 2 year history of secondary enuresis. He was hypertensive and had mild renal impairment. Investigations revealed a trabeculated bladder with a thickened wall and bilateral severe hydronephrosis. A micturating cystourethrogram showed no obstruction, and urodynamic analysis revealed a poorly compliant bladder with detrusor overactivity and detrusor sphincter dyssynergia. Renal failure progressed despite bladder augmentation. He lacks the facial features of UFS. He has compound heterozygous missense variants c.1648C>T [p.Arg550Cys] (exon 13) and c.2554A>T [p.Ile852Phe] (exon 16), in affected individual II:1.

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LRIG2 modulates cell turnover and adhesion in neural support cells via altered growth-factor signaling,21 and altered LRIG2 localization occurs in various types of neural tumors.22–24 Detection of LUT abnormalities antenatally and abnormal grimacing in infancy implies that pathogenic mechanisms underlying UFS initiate during development. Indeed, in embryonic mice, VII nerve ganglia express Lrig2.16 The human bladder initiates at 7 weeks of gestation.5,25 At this stage, we detected by immunohistochemistry neither LRIG2 nor heparanase-2 (data not shown). Later in the first trimester, detrusor muscle differentiates25 and is invaded by autonomic nerves.26 At this stage, both LRIG2 and heparanase-2 were immunodetected within nerve fascicles located between muscle bundles (Figures 3A–3D). LRIG2 was also weakly immunolocalized in smooth-muscle bundles themselves (Figure 3A). Coimmunostaining with β3-tubulin, a neuronal axonal cytoskeletal protein, demonstrated that LRIG2 appears to be localized in cells adjacent to neurons (Figures 3E–3G). These might be Schwann-like cells within preganglionic parasympathetic nerves, the neurons of which synapse with second-order neurons within intramural ganglia.4 Furthermore, we detected LRIG2 transcripts in 12-week-postconception human fetal bladders and ureters, but not in kidney tissue (Figure S4), whereas the three transcripts of HPSE227 were detected in the bladder, but not in ureter or kidney tissue. Heparanase-2 immunoreactivity partially overlapped with β3-tubulin (Figures 3H–3J), consistent with its being present in neurons themselves. Evidence already implicates heparanase-1 in neural biology,28–30 and its enzymatic activity is inhibited by heparanase-2.10 Collectively, the evidence indicates that both LRIG2 and heparanase-2 are required for normal LUT innervation.

**Figure 2. Identification of Mutations in LRIG2 in an Individual with Nonsyndromic Dysfunctional Voiding**

and/or neural function. It is curious why loss-of-function mutations in these two genes, expressed more widely in the peripheral nervous system, should result in a specific phenotype confined to elimination and facial expression. Mild degrees of dysfunctional bladder voiding, including overactivity, are common in otherwise healthy children after the age when conscious bladder control has normally been acquired. Nonsyndromic VUR, which affects 1% of children, is commonly familial. We speculate that the biological pathways mediated by LRIG2 and the heparanases might be implicated in these common disorders of elimination.

Supplemental Data

Supplemental Data include Supplemental Material and Methods, three figures, and two tables and can be found with this article online at http://www.cell.com/AJHG/.

Acknowledgments

H.M.S. is funded through a Wellcome Trust Clinical Training Fellowship. N.A.H. is a Wellcome Trust Senior Fellow in Clinical Science. The study was also funded by project grants from Kidney Research UK and Kidneys for Life and was also supported from the Manchester Biomedical Research Centre.

Received: September 26, 2012
Revised: October 23, 2012
Accepted: December 5, 2012
Published: January 3, 2013

Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.omim.org
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
SIFT, http://sift.jcvi.org/

Accession Numbers

The GenBank accession number for the LRIG2 Alu insertion reported in this paper is JX891452.