Muscarinic Acetylcholine Receptor M3 Mutation Causes Urinary Bladder Disease and a Prune-Belly-like Syndrome

Stefanie Weber,1,14,* Holger Thiele,2 Sevgi Mir,3 Mohammad Reza Toliat,2 Betül Sozeri,3 Heiko Reutter,4,5 Markus Draaken,4,6 Michael Ludwig,7 Janine Altmüller,2 Peter Frommolt,2,8 Helen M. Stuart,9 Parisa Ranjzad,9 Neil A. Hanley,9 Rachel Jennings,9 William G. Newman,10 Duncan T. Wilcox,11 Uwe Thiel,16 Karl Peter Schlingmann,12 Rolf Beetz,13 Peter F. Hoyer,1 Martin Konrad,12 Franz Schaefer,14 Peter Nürnberg,2,8,15 and Adrian S. Woolf9

Urinary bladder malformations associated with bladder outlet obstruction are a frequent cause of progressive renal failure in children. We here describe a muscarinic acetylcholine receptor M3 (CHRM3) (1q41-q44) homozygous frameshift mutation in familial congenital bladder malformation associated with a prune-belly-like syndrome, defining an isolated gene defect underlying this sometimes devastating disease. CHRM3 encodes the M3 muscarinic acetylcholine receptor, which we show is present in developing renal epithelia and bladder muscle. These observations may imply that M3 has a role beyond its known contribution to detrusor contractions. This Mendelian disease caused by a muscarinic acetylcholine receptor mutation strikingly phenocopies Chrm3 null mutant mice.

Lower urinary tract and/or kidney malformations account for 40% of childhood end-stage renal failure.1 Advances have been made in unraveling the genetic pathogenesis of kidney anomalies,2 but less is known about the genetic origin of malformations of the lower tract. Congenital bladder outlet obstruction (BOO) has several causes, the commonest being posterior urethral valves (PUVs), with a high risk of developing chronic renal insufficiency.1,3 Recently, mutations of HPSE2 (MIM 613469; see Web Resources), encoding a heparanase inhibitor expressed in developing urinary tracts, were described in urofacial syndrome (UPS [MIM 236730]), which presents with a dysmorphic, poorly emptying bladder.4 Also associated with congenital bladder dysfunction is prune belly syndrome (PBS [MIM 100100]). In its rare complete form, it comprises megacystis with disorganized detrusor muscle, cryptorchidism and thin abdominal musculature with overlying lax skin. PUV and PBS generally occur sporadically, although families have been reported with more than one affected member.3,5,6

We here describe a homozygous loss-of-function mutation of muscarinic acetylcholine receptor M3 (CHRM3) (1q41-q44) in five brothers with a PBS-like syndrome (Figures 1 and 2). CHRM3 (MIM 118494) encodes the M3 subtype of muscarinic acetylcholine (ACh) receptors, the major receptor mediating urinary bladder contraction upon micturition.7 We restudied a family reported in 20055 when there were four surviving boys with congenital BOO born to consanguineous Turkish parents. A fifth affected male sibling had died soon after birth as a result of renal failure and urosepsis (II-1). He and one affected surviving brother (II-4) displayed marked abdominal wall distension and were assigned as having PBS, whereas their three brothers were considered to have “PUV” because cystoscopy noted urethral valve-like structures. Recently, a sixth brother (II-7) was born, also with a malformed bladder. Evaluation of available cystograms showed massive and irregular-walled bladders with diverticulae (Figure 1). Vesicoureteric reflex was a variable feature. Urethral patency appeared normal in available cystograms. Cystometry performed in four affected boys in the first years of life revealed detrusor hyporeflexia with high residual volumes after micturition. Thus, the collective features in this kindred are most consistent with the PBS spectrum. Affected individuals also had bilaterally impaired pupillary constriction to light (Figure 1) and dry mouths. These examinations were performed after informed consent was obtained from the patients and their parents. Ethical approval for this study was obtained from the ethics review board at the Ruprecht-Karls-University Heidelberg and the collaborating institutions, according to the Declaration of Helsinki.

*Correspondence: stefanie.weber@uk-essen.de
DOI 10.1016/j.ajhg.2011.10.007. ©2011 by The American Society of Human Genetics. All rights reserved.
Using a SNP chip-based genome-wide linkage approach, we previously identified two regions of homozygosity on chromosomes 1 (35 cM) and 11 (9 cM) in four affected surviving males of this family (Figure 2). Exon capture and massively parallel sequencing now lead to the identification of the causative genetic defect in **CHRM3**, located in the chromosome 1 critical interval (Figure 2). DNA of index patient II-4 was enriched for protein-coding genes with the Agilent SureSelect Human All Exon 38 Mb kit and ran on two lanes of the Illumina GAIIx Sequencer with the paired-end protocol and a read length of 95 bp at each end, generating ~202,3106 raw reads of which ~193,3106 could be mapped to the human reference sequence. Alignment and variant calling was performed with MAQ for SNP detection (version 0.7.1) and BWA-short (version 0.5.7) in combination with SAMTOOLS (version 0.1.7) for indel detection. On average, 90% of the exome was covered at least 30-fold and 97% ten-fold. Scripts developed in-house were applied to detect protein changes, affected splice sites, and overlaps with known variants (Table S1 available online). In detail, using MAQ and SAMtools, we identified a total of 672,588 variations. After filtering for a minimal phred-like consensus quality score > 15, 517303 variations remained. A stringent filter step discarding known variations with dbSNP, 1,000 genomes data, and the CCG in-house database with ~100 exomes reduced the list to 48811. Further filtering for homozygosity (allele frequency > 75%) together with changes in protein sequence or location ±25 nts inside 3'/5' splice sites reduced the list to 38 candidate variations. Finally, only two variations were found to overlap with the previously identified regions of significant linkage—a missense change in **HEATR1**: c.6037A > G;p.Lys2013Glu (RefSeq accession number NM_018072.5) and an insertion-deletion frameshift mutation in **CHRM3**: c.1173_1184 delinsT; p.Pro392Ala*43 (RefSeq accession number NM_000740.2). Both variations were not present at the Exome Variant Server, NHLBI Exome Sequencing Project (ESP, Seattle, WA; see Web Resources). **HEATR1** encodes BAP28, a protein involved in rRNA transcription and processing, and knockdown of **Heatr1** in zebrafish causes embryonic death due to CNS degeneration, a phenotype considerably different from the one observed in our index patient. **CHRM3**, however, encodes the human muscarinic acetylcholine receptor M3 (hereafter simply called M3), a G protein-coupled receptor with seven transmembrane domains that mediates the actions of acetylcholine. The American Journal of Human Genetics 89, 668–674, November 11, 2011
Transmembrane domains and the major receptor involved in mediating urinary bladder contraction upon micturition. Both the obvious loss-of-function nature of the CHRM3 mutation and the striking similarity of the human phenotype to Chrm3 null mutant mice (discussed below) underscored the likelihood of this mutation being the causal genetic lesion in our family. The p.Pro392Ala fs*43 mutation induces a premature termination codon (PTC) at position 435. If mutant RNA transcripts were stable and translated, a truncated M3 protein would be generated lacking part of the third cytosolic loop, transmembrane domains VI and VII and the C terminus of the receptor. Three-dimensional structure predictions of mutant M3 suggest perturbed folding of the remaining third intracellular loop (M3R) in addition to loss of transmembrane helices VI and VII in mutant M3. Three-dimensional protein structure modeling was obtained as a homology (comparative) protein structure with (PS)2-v2 using Protein Data Bank (PDB) entries 2rh1, 2rh1A and 1r5sA as template. Structures were processed with Jmol. Model reliability is ≥75% for wild-type and mutant (excluding the mutant C terminus) and 55%–75% for the mutant C terminus.

Transmembrane domains and the major receptor involved in mediating urinary bladder contraction upon micturition. Both the obvious loss-of-function nature of the CHRM3 mutation and the striking similarity of the human phenotype to Chrm3 null mutant mice (discussed below) underscored the likelihood of this mutation being the causal genetic lesion in our family. The p.Pro392Ala fs*43 mutation induces a premature termination codon (PTC) at position 435. If mutant RNA transcripts were stable and translated, a truncated M3 protein would be generated lacking part of the third cytosolic loop, transmembrane domains VI and VII and the C terminus of the receptor. Three-dimensional structure predictions of mutant M3 suggest perturbed folding of the remaining third intracellular loop (M3R) in addition to loss of transmembrane helices VI and VII in mutant M3. Three-dimensional protein structure modeling was obtained as a homology (comparative) protein structure with (PS)2-v2 using Protein Data Bank (PDB) entries 2rh1, 2rh1A and 1r5sA as template. Structures were processed with Jmol. Model reliability is ≥75% for wild-type and mutant (excluding the mutant C terminus) and 55%–75% for the mutant C terminus.
M2 and M3), 16,17 only M3 is the critical receptor for mRNA of two muscarinic acetylcholine receptor subtypes. Although male and female adult human bladders express contractions stimulated by parasympathetic nerves, and to expel urine per urethra. The latter is driven by detrusor contractility to carbachol ex vivo is impaired and (only) male mutant adult bladders are grossly distended in vivo. Although it is unknown whether megacystis is present in younger Chrm3 mutant mice, the hypotonic bladder phenotype is very similar to humans with CHRM3 mutations, described above. Of note, only male Chrm3 mice will develop clinical disease. This is, at least in mice most probably, related to differences between male and female urethral anatomy with male bladders being more contractile to force the urine through the longer urethra. Alternatively, female mice (but not males) might have other compensatory physiological mechanisms allowing them to empty their bladders independent of M3 action. Expression and function of M3 in rat and guinea pig urinary bladders do not seem to differ considerably between male and female animals and with regard to overall muscarinic responsiveness this situation appears to be similar in humans. In humans, we can neither rule out nor confirm a male-limited phenotype: the pedigree of the presented index family is not helpful to delineate the phenotype of female homozygous CHRM3 mutation carriers given that no female individual is affected by such a mutation. Mother and sister of the index person are both heterozygous mutation carriers with apparently normal bladder anatomy and function. With respect to PBS, the full clinical picture, including BOO, cryptorchidism, and abdominal wall distension, is limited to males; however, incomplete forms of PBS have also been described in females.

The etiology of abdominal muscle deficiency in individuals with PBS has been a matter of controversy. Either a common underlying defect of mesodermal development is proposed or, as other authors suggest, abdominal muscle deficiency seems to be a nonspecific anatomic defect that is secondary to fetal abdominal wall distention (caused by megacystis, hydronephrosis or constipation). In the present study, RT-PCR experiments were performed on RNA extracted from human mature abdominal wall musculature, but no M3 expression was apparent.
These data are in favor of abdominal wall distension being a secondary event after megacystis and/or urinary stasis due to impaired urinary bladder contraction early in fetal life.

Failure of bladder contractility before birth in humans with homozygous (or compound heterozygous) CHRM3 mutation might contribute to the observed bladder dysmorphology postpartum. Bladder dysfunction is likely to persist in individuals with CHRM3 mutation throughout life, constituting an important risk factor for the progression of renal insufficiency. Chrm3 mutant mice also display impaired salivation and dilated pupils, consistent with a role for muscarinic ACh actions in serous salivary secretion and pupillary constriction, and a similar phenotype is present when human CHRM3 is mutated.

Deletions within 17q12 encompassing hepatocyte nuclear factor 1B (HNF1B [MIM 189907]) have been reported in sporadic PBS, but whether HNF1B itself or a nearby disrupted gene is causative remains unclear, and HNF1B mutations have not yet been implicated in familial PBS. Furthermore, although HNF1B mutations are a not uncommon cause of dysplastic kidneys, such patients rarely have overt bladder lesions. Importantly, in our CHRM3 mutation family, HNF1B was normal by sequencing and seeking copy number variants (data not shown). Mice mutant for b2 and b4 or a3 subunits of the nicotinic ACh receptor display enlarged, hypocontractile bladders, as a result of compromised neural relaying via autonomic ganglia, and may serve as models for humans with congenital BOO who do not have CHRM3, HPS2, or HNF1B mutations. We suggest that the next decade

(Figure S1).
will witness the unraveling of genetic causes of bladder malformations, thus providing insights into the mechanisms of both normal development and explaining how bladder dysmorphology arises in the context of PBS, PUV, UFS and other related syndromes.\(^3\)

**Supplemental Data**

Supplemental Data include one table and one figure and can be found with this article online at [http://cell.com/AJHG/](http://cell.com/AJHG/).

**Acknowledgments**

We thank the patients and their family for participating in this study, Bettina Cirkel and Christian Becker for excellent technical support and Anne Deix for critical advice. Financial grant support was received from the Kidney Research UK (W.G.N. and A.S.W.), Kids Kidney Research and Kidneys for Life (A.S.W.), Manchester NIHR Biomedical Research Centre (A.S.W., R.J., N.A.H., H.S., and W.G.N.), and The Wellcome Trust (A.S.W. and N.A.H.). S.W. and E.S. received financial support from the Fritz Thyssen grant. H.R. and M.D. are members of the German network for congenital uro-rectal malformations (CURE-Net), which is supported by research grant (01GM08107) from the German Federal Ministry of Education and Research. The 4C-Kidney Disease Study is supported by research grants of the KfH Foundation for Preventive Medicine, ERA-EDTA and IFB Transplantation.

Received: August 2, 2011
Revised: October 17, 2011
Accepted: October 19, 2011
Published online: November 10, 2011

**Web Resources**

The URLs for data presented herein are as follows:

- The Cardiovascular Comorbidity in Children with Chronic Kidney Disease Study, [http://www.4c-study.org](http://www.4c-study.org)
- Exome Variant Server, [http://snp.gs.washington.edu/EVS](http://snp.gs.washington.edu/EVS)
- GUDMAP, [http://www.gudmap.org](http://www.gudmap.org)
- Mouse Genome Informatics, [http://www.informatics.jax.org](http://www.informatics.jax.org)
- Online Mendelian Inheritance in Man (OMIM), [http://www.omim.org](http://www.omim.org)

**References**