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Supplemental Data

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

Table S1. Filtering strategy for the called variants

Filtering step	Count
Unfiltered	672,588
High quality (Phred-like consensus quality score >15)	517,303
Not in dbSNP	80,475
Not covered by 1000 Genomes Project data	59,020
Not in in-house database of recurrent variants	48,811
Frequency of variant allele > 75% (i.e. “homozygous”)	5,110
Protein changes + SpliceSite (SS) (+/- 25nts intronic)	38
Predicted loss-of-function mutation in linkage interval	1

Using MAQ and SAMtools, in total 672,588 variations were detected. After filtering for a minimal phred-like consensus quality score >15, 517303 variations remained. A stringent filter step discarding known variations using dbSNP, 1000 genomes data and the CCG in-house database with about 100 exomes reduced the list to 48811. Further filtering for homozygosity (allele frequency > 75%) together with changes in the protein sequence or location +/- 25 nts inside 3’/5’ splice sites reduced the list to 38 candidate variations. Finally, only 2 variations were found to overlap the previously identified regions of significant linkage – a frameshift mutation in *CHRM3* and a missense change in *HEATR1*.

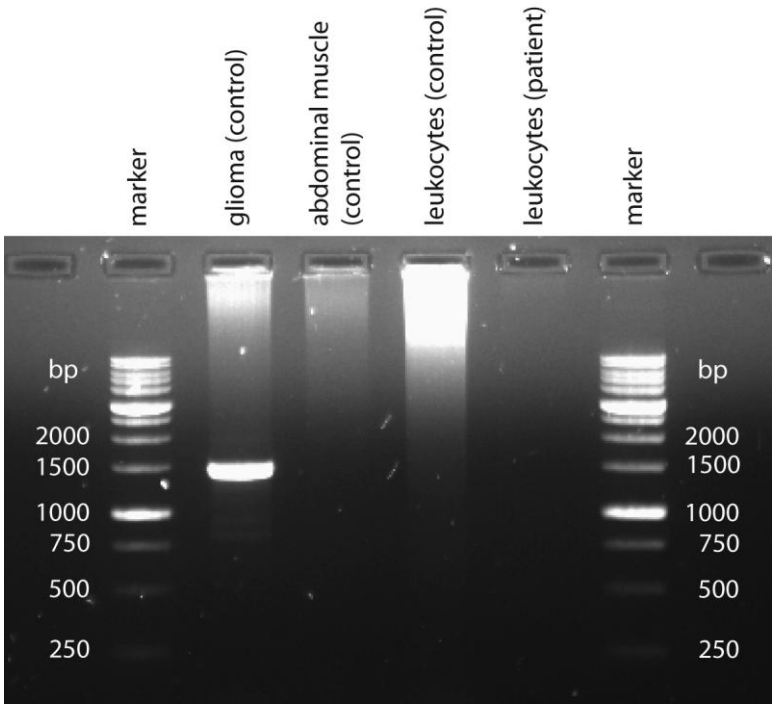

Figure S1. *CHRM3* - cDNA from different tissues (Agarose Gel 1%)

Line **1** shows the expected band of 1.450 bp from an amplified **glioma** cDNA control probe. cNDA amplification of control **abdominal muscle** (line **2**), control **leukocytes** (line **3**), and patient **leukocytes** (line **4**) did not yield the desired product

PCR conditions:

Primers were chosen to be located in exons 4 and 5 to securely avoid amplification of genomic DNA. The quality of leucocyte cDNA has been tested by successfully amplifying house keeping genes. Annealing temperature 58 °C, 5 min elongation time, 35 cycles, Expand Long Template PCR Sytem (Roche), DNA Ladder 1kb (AppliChem).

Primer sequences:

CHRM3-cDNA-Forward	GGAGCTGGTCTCTTGGGCAG
CHRM3-cDNA-Reverse	CCACGGCTGACTCTAGCTG