Mutation of Murine Adenylate Kinase 7 Underlies a Primary Ciliary Dyskinesia Phenotype

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Online Data Supplement

Supplementary Materials and Methods

AK7-deficient Mice

The mutant mouse strain described here arose serendipitously in the process of generating transgenic mice harboring a tetracycline-regulatable heme oxygenase 1 (HO-1) construct. Microinjections were performed in FVB/N strain pronuclei by the Brigham and Women's Hospital Core Transgenic Mouse Facility (Boston) and founder animals were crossed to the FVB/N strain. Transgenic progeny was identified by polymerase chain reaction (PCR) analysis on genomic DNA isolated from tail biopsies. Progeny of one founder (transgenic line TH67) exhibited phenotypic characteristics consistent with the acquisition of a recessive mutation due to the serendipitous disruption of a locus by the transgene insertion event. This line was further characterized genetically, and the insertion locus was identified by standard molecular methods (Supplementary Fig. E1). Southern analysis determined that a singular transgene insertion locus was present in the mutant and a restriction map of the locus, generated by endonuclease *Apa I*, revealed a 2.76 kB junction fragment. *Apa I*-digested genomic DNA was subjected to preparative gel electrophoresis and DNA fragments in the 2kB - 4kB range were isolated and ligated under conditions favoring intramolecular circle formation. The ligated DNA products served as templates for nested PCR, using primers on the human HO-1 sequences present in the junction fragment. Primary PCR employed primers LJ (5'-TCGGGTTGCGGACGCTCCAT) & HO-JF-4 (5'-GCCAGGTGACCCGAGACGGC) and secondary PCR employed primers LJ-2 (5'-CGCTGAGGACGCTCGACCAA) & HO-JF-2 (5'-

CTATGTGGCCCTGGAGGAGGAGAT). PCR products corresponding to the expected size of 2.38 kb for the amplicon representing the junction fragment were cloned and sequenced. The sequence of the genomic region was used to query the Mouse Genome database, identifying intron 4 of the Ak7 gene as the insertion locus.

Northern blot analysis, RT-PCR & Genomic RCR

Total tissue RNA was isolated using Qiagen RNeasy Mini kits (Qiagen, CA, USA) and 1 to 2 µg was reverse transcribed using the Superscript first strand synthesis system for RT-PCR (Invitrogen Life Technologies, CA, USA). The primers used to amplify exons 1 to 4, exons 4 to 12 and exons 12 to 18 were the following: Ex1F, 5'-ATGGCTGAAGAGGAAGTTCC-3';
Ex4R, 5'-GATCTCGCCCATGTCATCAC; Ex4F, 5'-GTGATGACATGGGCGAGATC; Ex12R, 5'-TGTACTCTTTGAGGATGCTG; Ex12F, 5'-CAGCATCCTCAAAGAGTACA; Ex18R, 5'-ACTCCTCCCAGCGGGCTATC.
Expression of the Ak7 gene transcripts was analyzed with the forward Ex1F primer and the following reverse primers:
In10R, 5'- GCTAGCATAGGAAAGCTAGTA; In18R, 5'-CCTCCTTTTAATGGTGCGC and Ex20R, 5'- GGCTCTACTGCATTTCAGG.
Genomic DNA was purified from brain using QIAam DNA kit (Qiagen, CA, USA) and amplified using Epicenter Biotechnologies Fail Safe System (Epicenter Biotechnologies, WI, USA), with the following primers:
4467Rjd2, 5'-TTCCCCATGGCTTTGAGATCAGGT and 4467Lju2, 5'-

CTCATAGCAATAACCAGACAGGCTA located in Ak7 intron 5 and flanking the transgene insertion point. Amplified products from wild-type and Ak7 mutants are 478 and 419 bp long respectively. Exons 5, 6, 11 and 18 were amplified from genomic DNA using the following primers: Ex5F, 5'- TCAGTGGAGAATCACCCTAGCATTC;

In5R, 5'- CTTGCTGCTTTGCTTGCTTCCATG;

Ex6F, 5'- CACAGAAGAGGATTATCGAAGAAGG;

In6R, 5'-TGGATCATAATCTGGACCAGGAAC;

Ex11F, 5'- CCCTGGAAAGATCCAGAAAGTG;

In11R, 5'-TAGCTATGTGTCCACCAAGCCT;

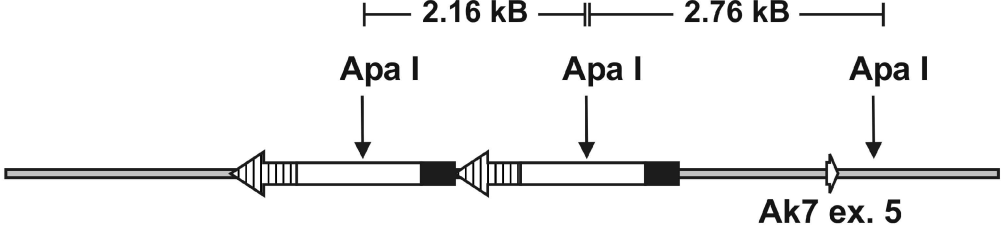
Ex18F, 5'- GATAGCCCGCTGGGAGGAGT

and In18R, 5'- ATTAGATGCCCTGGACCTAGAGCC.

Supplementary Figure Legends

Figure E1. Schema of the methods used to identify the Ak7 locus as the transgene insertion point in transgenic line TH67. The position of Ak7 exon 5 (white arrowhead) is shown for orientation purposes. The two tandem copies of the transgene construct are present in a singular locus and are depicted as large arrows. *Black box*: the prokaryotic *tet* operator; *white box*: human HO-1 cDNA; *striped arrowhead*: SV40 large T-antigen polyadenylation region.

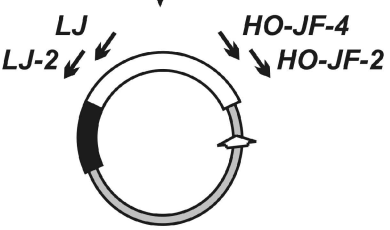
Figure E2. The murine Ak7 locus and associated transcripts. (*A*) Exon-intron organization of the Ak7 gene. Boxes represent exons and introns are represented by solid lines. Arrows indicate the position of putative translation initiation (up arrow) and termination (down arrow) codons. (*B*) Ak7 gene transcripts. Ak7a, b, & c were detected in these studies. Ak7a and Ak7b correspond to GenBank entries with accession numbers XM_994344 and XM_911194, respectively. Ak7c is not represented in GenBank databases. (*C*) Schematic representation of splicing events occurring in Ak7 transcripts. Differential splicing/polyadenylation events in exons 10 and 18 generate truncated transcripts Ak7c and Ak7b, respectively. Black rectangles represent coding regions and gray rectangles represent non coding regions of the respective exons.



Apa I digestion of line TH67 genomic DNA

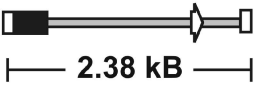
Gel isolation of genomic DNA fragments in the 2-4 kB size range

Ligation at low DNA concentration to favor circle formation



Nested PCR using primers on the human HO-1 transgene

Isolation and cloning of the amplicon corresponding to the junction fragment



Sequencing

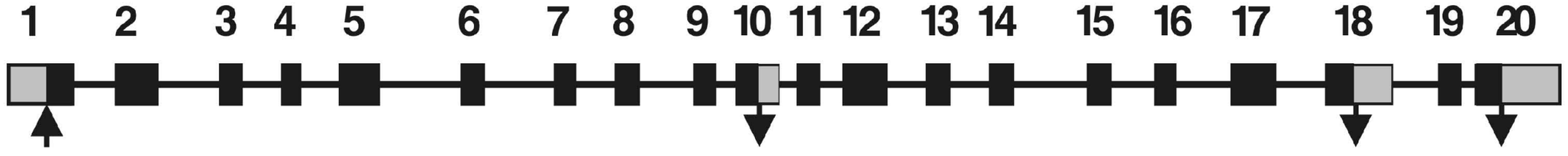
Mouse Genome dB query

Ak7 locus homology

Fernandez-Gonzalez et al., Fig. E1

A

AK7 locus



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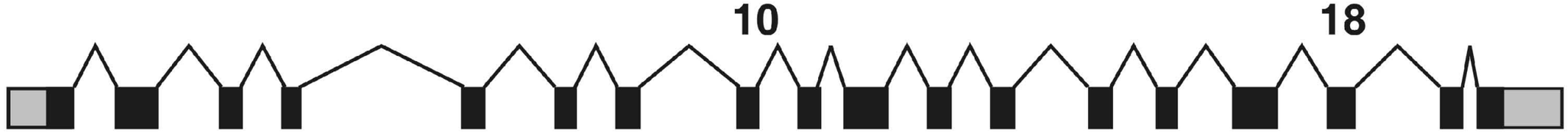
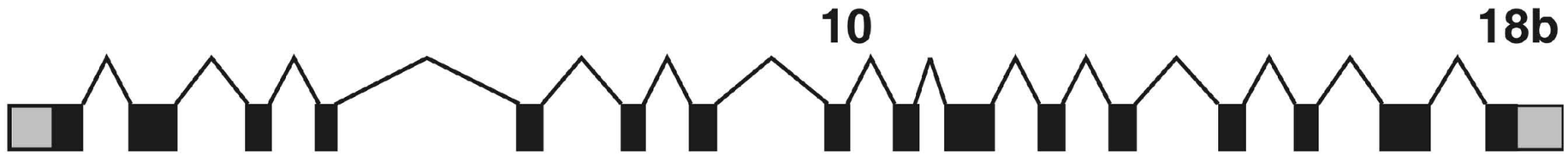
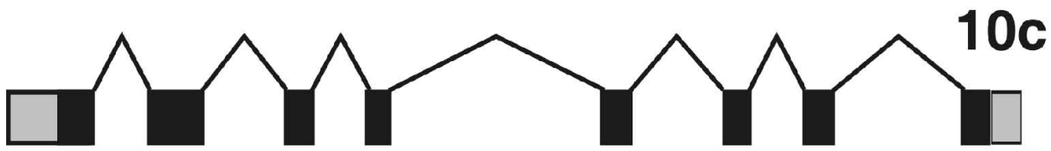
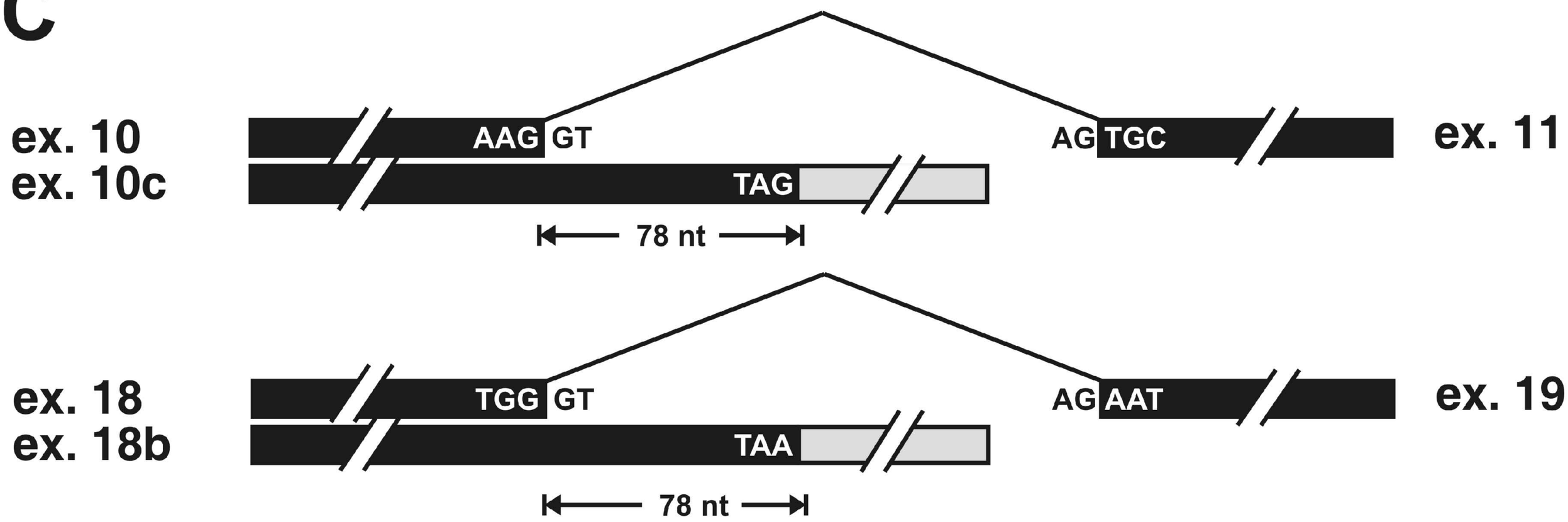






C



Fernandez-Gonzalez et al., Fig. E2