Correct mRNA processing at a mutant TT splice donor in FANCC ameliorates the clinical phenotype in patients and is enhanced by delivery of suppressor U1 snRNAs.

The U1 small nuclear RNA (U1 snRNA) as a component of the major U2-dependent spliceosome recognizes 5′ splice sites (5′ss) containing GT as the canonical dinucleotide in the intronic positions +1 and +2. The c.165+1G>T germline mutation in the 5′ss of exon 2 of the Fanconi anemia C (FANCC) gene commonly predicted to prevent correct splicing was identified in nine FA patients from three pedigrees. RT-PCR analysis of the endogenous FANCC mRNA splicing pattern of patient-derived fibroblasts revealed aberrant mRNA processing, but surprisingly also correct splicing at the TT dinucleotide, albeit with lower efficiency. This consequently resulted in low levels of correctly spliced transcript and minute levels of normal posttranslationally processed FANCD2 protein, indicating that this naturally occurring TT splicing might contribute to the milder clinical manifestations of the disease in these patients. Functional analysis of this FANCC 5′ss within splicing reporters revealed that both the noncanonical TT dinucleotide and the genomic context of FANCC were required for the residual correct splicing at this mutant 5′ss. Finally, use of lentiviral vectors as a delivery system to introduce expression cassettes for TT-adapted U1 snRNAs into primary
FANCC patient fibroblasts allowed the correction of the DNA-damage-induced G2 cell-cycle arrest in these cells, thus representing an alternative transcript-targeting approach for genetic therapy of inherited splice-site mutations.

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