Poster L-7

SNPs in NAGNAG Acceptors Are Highly Predictive for Variations of Alternative Splicing

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Short Abstract: We identified 64 SNPs that probably influence alternative splicing at NAGNAG acceptors and demonstrated their splice-relevance by searching EST/mRNA databases and own experiments. We showed that the NAGNAG motif is necessary and sufficient for alternative splicing. Thus, the effect of these SNPs on alternative splicing is highly predictive.

Long Abstract:
Splicing mutations have been suspected to be the most frequent cause of hereditary diseases and an increasing number of SNPs has been described that cause diseases by a change or disruption of the normal splicing pattern. Since the impact of SNPs on splicing is hard to predict in silico and is difficult to analyze experimentally, silent or intronic SNPs that may cause a phenotype or a disease by changing splicing patterns are often not investigated. Therefore, the identification of genetic variations that cause changes in the splicing pattern of a gene is important. Recently, we described the widespread occurrence of alternative splicing at NAGNAG (or tandem) acceptors. Here, we performed a genome-wide screen and identified 121 SNPs that affect NAGNAG acceptors. To extract those SNPs that probably influence alternative NAGNAG splicing, we proposed a simple classification scheme that aims at dividing all tandem acceptors into those that are alternatively spliced and those that are not. We propose to subdivide all tandem acceptors into 'plausible' (HAGHAG, H stands for A, C, or T) and 'implausible' (GAGHAG, HAGGAG, or GAGGAG) acceptors. This classification is based on several reasons: (i) 31% of the plausible but only 1.7% of the implausible NAGNAGs have EST evidence for alternative splicing, (ii) GAG acceptors are very rare, (iii) only plausible but not implausible NAGNAGs have the same bias towards intron phase 1 as experimentally confirmed NAGNAGs, (iv) 77% of the SNPs in a HAGGAG acceptor affect the non-functional GAG and not the HAG, and (v) of the HAGGAGs that are not conserved in the chimpanzee genome the GAG is affected in 83%. Using this classification, we extracted 64 from the total of 121 SNPs and demonstrated their splice-relevance by searching EST/mRNA databases and own experiments. Using SNPs that comprise NAGNAG-acceptor and non-NAGNAG-acceptor alleles as ‘knockout experiments made by nature’, we showed that the NAGNAG motif is necessary for this type of alternative splicing. Furthermore, we found that this motif is also sufficient, i.e. a single mutation that changes a non-NAGNAG to a NAGNAG acceptor is sufficient to enable alternative NAGNAG splicing. Thus, the effect of these SNPs on alternative splicing is highly predictive. Interestingly, 23% (15 of 64) of these SNPs are translationally non-silent and, thus, introduce a novel dimension of variability on the protein level by changing the NAGNAG acceptor and the protein sequence. Whereas homozygotes express either one or two isoforms, heterozygosity results in three different proteins. For example, rs17173698 in PAPSS2 results in a change from Glu to the oppositely charged Lys and potentially in the deletion of the charged residue by NAGNAG splicing. The disease relevance of a NAGNAG SNP is

demonstrated for the ABCA4 gene (Maugeri et al. 1999) where the acceptor site is changed from TAGGAG to TAGCAG. This mutation has a much higher frequency in patients with Stargardt disease 1 (STGD1) and that is assumed to be a mild mutation that causes STGD1 in combination with a severe ABCA4 mutation. Maugeri et al. found that only the TAGCAG alleles produce two splice forms. Our study exactly predicts this mutation outcome. We found that 18 of these 64 SNPs occur in known disease genes, including genes that are associated with Alzheimer disease, Down syndrome, Breast Cancer and Asthma. In another interesting example, a SNP changes an acceptor of GOLGA1 from AAATAG to AAGTAG. Alternative splicing at this NAGNAG acceptor would result in an inframe TAG insertion and, thus, a truncated protein. GOLGA1 codes for an autoantigen associated with Sjogren syndrome and it remains to be elucidated whether this SNPs plays a role in the pathogenesis of this disease. Thus, the SNPs identified in this study are preferable candidates for more-detailed functional analysis and association studies to link alternative splicing with diseases. (Maugeri et al. 1999): Maugeri A et al. “The 2588G->C mutation in the ABCR gene is a mild frequent founder mutation in the Western European population and allows the classification of ABCR mutations in patients with Stargardt disease”. Am J Hum Genet 64:1024–1035, 1999