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GUEST EDITORIAL

Clinical and Molecular Significance of Splice Site Mutations in the Plakophilin 1 Gene in Patients with Ectodermal Dysplasia–Skin Fragility Syndrome

In 1997, McGrath et al. described the first case of a rare autosomal recessive inherited skin disease resulting from loss-of-function mutations in plakophilin 1 (PKP1), a component of desmosomes, cell–cell junctions found primarily in epithelial tissues (1). Plakophilin 1 is a member of the armadillo family of structural and signalling proteins and it contributes to the mechanical integrity and calcium stability of desmosomes (2). Clinically, loss of plakophilin 1 leads to trauma-induced skin fragility as well as developmental anomalies of hair and nails. These previously undocumented observations led to the introduction of a new term, ectodermal dysplasia-skin fragility syndrome (OMIM 604536), to describe such patients, and following the initial publication, six additional patients with *PKP1* gene mutations (from the UK, France, Japan, Israel and The Netherlands) were reported (3–7). In this issue of *Acta Dermato-Venereologica*, Zheng et al. present the clinical and molecular abnormalities of a further example of this genodermatosis, the first Chinese case to be described (p. 394, ref. 8).

This new case of ectodermal dysplasia-skin fragility syndrome is a compound heterozygote for two splice site mutations in *PKP1*, c.[1053T > A + 1054+1G > T] affecting the intron 5 donor splice site and an intron 10 acceptor splice site IVS10-2A > G. In general, splice site mutations account for about 15% of all disease-associated gene mutations, but in this syndrome they appear to be a much more common phenomenon. Specifically, thus far 13 of a possible 16 mutated alleles have involved splice site mutations of the *PKP1* gene, but just why the molecular pathology of ectodermal dysplasia-skin fragility syndrome should involve so many splice site mutations is difficult to fathom. Moreover, predicting the consequences of splice site mutations on RNA processing is also an uncertain matter and this has implications for establishing a paradigm for genotype-phenotype correlation and for providing accurate genetic counselling for patients and their families.

Of the reported patients with this desmosomal genodermatosis, keratinocyte cDNA has been examined in five cases (1, 3, 6–8). These analyses have demonstrated the protean effects of the splice site mutations with evidence of cryptic splice sites, exon skipping and retention of introns. Furthermore, some of these studies have implications for genotype-phenotype correlation. For example, RT-PCR across the *PKP1* splice site mutation IVS9+1G > A was shown to result in at least two different transcripts, one of which involved activation of a cryptic donor splice site within exon 9 leading to an in-frame deletion of 15 amino acids (7). This finding provided an explanation for the observation of some detectable plakophilin 1 protein in the skin by immunohistochemistry and for the somewhat milder clinical phenotype (e.g. relative lack of alopecia) compared with several of the other published cases. In the new report of ectodermal dysplasia-skin fragility syndrome in this issue, Zheng et al. also attempted to assess the effects of compound heterozygosity for their two new splice site mutations in *PKP1*. Unfortunately, they were unsuccessful in attempting to identify any *PKP1* transcripts using RNA recovered from formalin-fixed paraffin-embedded skin. This probably reflects very low levels of *PKP1* mRNA, as β-actin could still be amplified from their patient's skin.

Nevertheless, despite the lack of information from this mRNA analysis, it is now possible to make *in silico* predictions about the possible consequences of the splice site mutations c.[1053T > A + 1054+1G > T] and IVS10-2A > G. Specifically, over recent years, a number of computational tools have become available to assess mechanisms of normal and aberrant splicing. These include Automated Splice Site Analyses (https://splice.cmh.edu/index.html) or Splice Site Prediction by Neural Network (http://www.fruitfly.org/seq_tools/splice.html). Using the Delila™ software package (9), which scans genomic DNA sequences with weight matrices for sites with positive R_i (individual information content) values, as well as the Sequence Walker programme to display the results (10), it is possible to predict the consequences of the *PKP1* splice site mutations detected by Zheng et al. These analyses are illustrated in Fig. 1.

For the mutation c.[1053T > A + IVS5+1G > T], software analysis predicts that the T > A nucleotide substitution in the penultimate nucleotide of exon 5 actually strengthens the pre-existing donor splice site (Ri increased from 6.2 to 8.4 bits). In contrast, the IVS5+1G > T change reduces the information content of the natural donor splice site from 6.2 to −1.6 bits. The combination of both substitutions, therefore, abolishes the natural donor splice site (Ri decreased from 6.2 to

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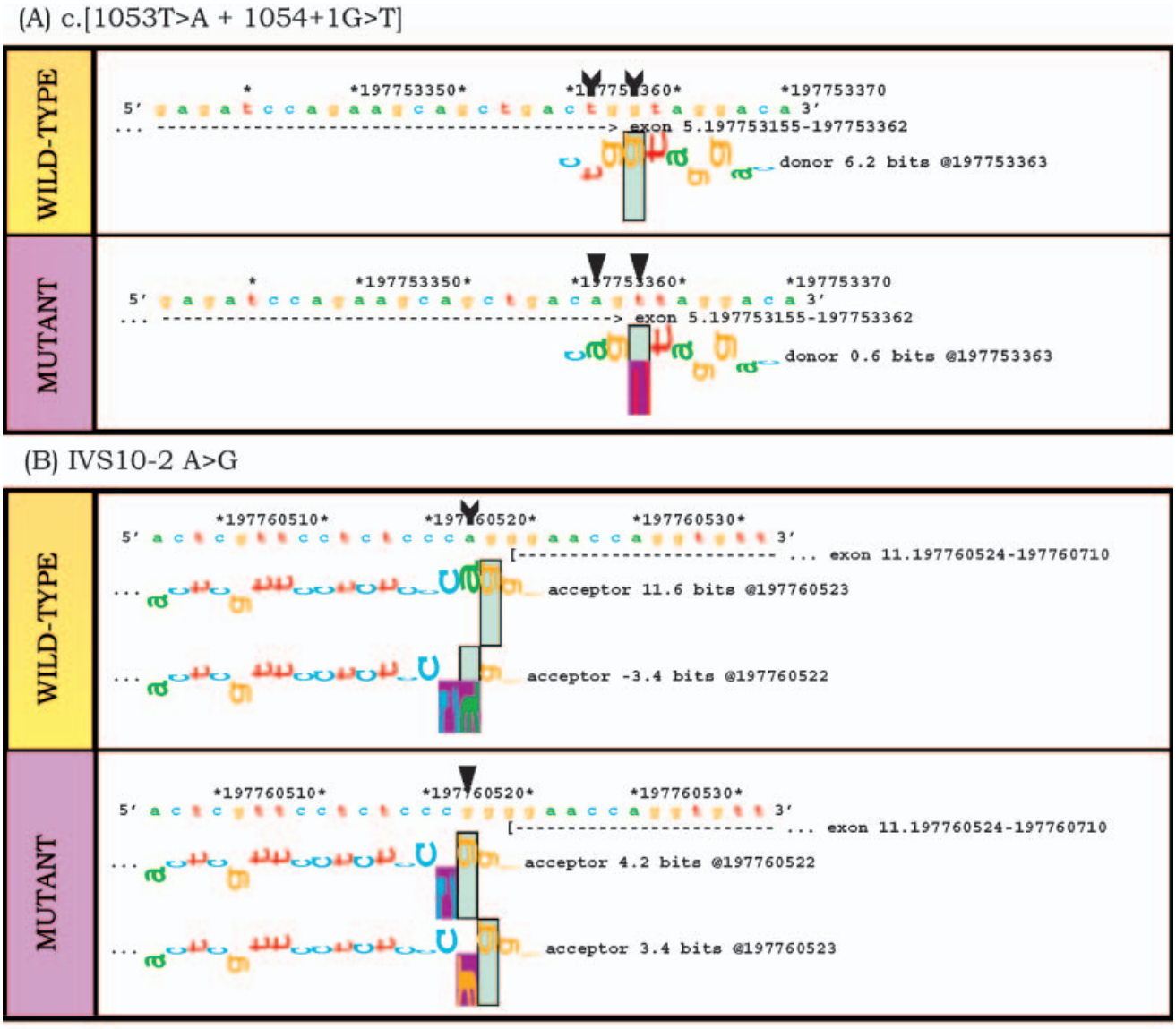

Fig. 1. In silico predictions of cryptic splicing in *PKP1*. (A) The c.[1053T > A + 1054+1G > T] mutation abolishes the natural intron 5 donor splice site, with Ri decreased from 6.2 to 0.6 bits This is predicted to result in intron 5 retention or exon 5 skipping. (B) In contrast, the IVS10-2A > G mutation results in a 'leaky' intron 10 acceptor splice site, with Ri reduced from 11.6 to 3.4 bits. However, this substitution also creates a new cryptic splice site at the last nucleotide of intron 10, with Ri increased from −3.4 to 4.2 bits (c and d). This predicts two outcomes for the mutation IVS10-2A > G: either reduced normal splicing of exon 11, or insertion of an additional single G into the beginning of exon 11 in the mRNA which would lead to a frameshift and downstream premature stop codon.

0.6 bits) and is predicted to result in intron retention and a premature termination codon 143 base pairs downstream within intron 5, or skipping of exon 5 (out-of-frame). For the other splice site mutation, IVS10-2A > G, the computational prediction is for a 'leaky' site with the R_i value reduced from 11.6 to 3.4 bits, but this substitution also creates a new acceptor splice site at the last nucleotide of intron 10 (Ri increased from −3.4 to 4.2 bits). This means that this acceptor splice site mutation should be able to produce some normal splicing of this intron-exon border, but there is also the possibility of a compromised normal splice site, with insertion of an additional single G nucleotide into the beginning of exon 11 (which would lead to a frameshift and a premature termination codon 333 base pairs downstream). Thus, although at the time of the skin biopsy in the patient being reported by Zheng et al. no *PKP1* mRNA or translated protein was detectable, the software tools predict that some normal splicing of the intron 10 acceptor splice site is in fact possible. In the future, therefore, it is conceivable that in this patient some full-length plakophilin 1 mRNA and protein may be generated and that this may result in a reduction in the severity of the clinical features in the patient and a somewhat milder phenotype compared with some of the other reported cases of ectodermal dysplasia-skin

fragility syndrome. Of course, the impact of the potentially normal *PKP1* gene processing on this patient's phenotype will depend on just how much plakophilin 1 protein is actually made. At present there are no satisfactory mathematical models capable of predicting this and the particular factors that govern the relative outcomes of leaky splice sites are not fully known.

Assessment of the other published *PKP1* gene splice site mutations using the Delila™ software reveals that one other acceptor splice site, IVS1-1G>A, also results in a leaky site. For this mutation, the R_i value of natural acceptor splice site of exon 2 is reduced from 16.4 to 8.8 bits, and it is also predicted to create a new cryptic splice site 1 base pair into exon 2, with Ri increased from 2.9 to 11.1 bits. As well as some normal splicing, published RT-PCR analyses have also confirmed this computer prediction by demonstrating deletion of the first base of exon 2 (leading to a frameshift and a premature termination codon 383 base pairs downstream in exon 2) as well as skipping of one or more downstream exons (3, 6). Interestingly, the mutation IVS1-1G>A has been reported in two different patients, a British child who is a compound heterozygote for this mutation and the nonsense mutation Y71X, and an Israeli child who is homozygous for IVS1-1G>A (3, 6). Both reports have shown similar clinical features and a complete absence of plakophilin 1 immunoreactivity on skin immunohistochemistry. However, it will be intriguing to assess follow-up in these individuals to see whether in future, given the leakiness of this particular splice site mutation, the skin phenotype improves in comparison with other cases in which no in-frame *PKP1* transcripts are predicted.

The computational models used to assess splicing integrity are also useful in determining whether sequence alterations outside consensus splice sites might have an effect on splicing. Sometimes this can lead to very unexpected and surprising results. For example, some apparently innocuous missense mutations can lead to formation of new donor or acceptor cryptic splice sites (11). However, for the three published *PKP1* gene mutations that occur outside consensus splice sites, Y71X, Q304X and 1132ins28 (1, 2), analysis with the Delila™ package does not predict any potential abnormalities in splicing.

The new splice site mutations in the *PKP1* gene reported by Zheng et al. (8) add to the growing database of the molecular pathology of ectodermal dysplasia-skin fragility syndrome (1, 3–7), although the unusual preponderance of splice site mutations cannot currently be explained. However, assessment of these mutations using modern computational tools predicts a diversity of consequences related to RNA processing. It will be fascinating to follow the progress of patients with this rare genodermatosis, both to observe the clinical consequences of the first inherited abnormality of desmosomes, and also to see whether the *in silico* predictions of leaky splice sites anomalies might in some way help strengthen genotype-phenotype correlation and improve genetic counselling.

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