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COMMENT

The cause of Jones syndrome put to REST: a mutation in the *REST* gene causes gingival fibromatosis and hearing loss

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In 1977, Jones and coworkers reported a dominantly inherited syndrome characterized by gingival fibromatosis and progressive hearing loss [1]. The Jones study was based on the identification of affected individuals in a single family, and it was followed by descriptions of additional familial cases that were likewise characterized by co-inheritance of gingival fibromatosis and progressive hearing loss [2, 3]. Thus, Jones syndrome became recognized as a bona-fide clinical entity. However, the underlying genetic defect was not identified. In this issue of *EJHG*, Elisa Rahikkala and coworkers provide evidence that Jones syndrome co-segregates with heterozygosity for a frameshift mutation in the last exon of the repressor element 1 (RE1)-silencing transcription factor (*REST*) gene [4].

The REST protein is a transcriptional repressor; it binds to RE1 sequence motifs in hundreds of genes and recruits co-repressors to the binding sites. The resulting protein complexes act on REST target genes, altering the acetylation and methylation status of histones near the RE1 motifs [5]. In most cell types, the REST complex is active and target genes of REST are repressed. Notable exceptions are neurons, pancreatic β-cells, and mechanosensory hair cells (HCs) of the inner ear [6–8]. In these three cell types, target genes of REST are expressed because activity of the REST complex is minimal. The mechanisms underlying inactivation of this complex differ between HCs and the other two cell types. In HCs the inactivation is due to splicing of an alternative exon of *REST* (i.e., exon 4, previously called exon N) into the mature mRNA (Fig. 1A), whereas in β-cells and fully differentiated neurons it is caused by transcriptional silencing of *REST* [6–8]. In neurons undergoing differentiation, both mechanisms play a role, but the effect of transcriptional silencing predominates [9].

REST is a vital transcription factor. In mice, ubiquitous deletion of both *Rest* alleles (*Rest*$^{-/-}$) causes upregulation of neuronal transcripts in non-neuronal cells and embryonic lethality at ~E11 [10]. Humans homozygous for loss-of-function *REST* mutations have not been identified; however, heterozygosity for *REST* mutations is now associated with the following four disorders: Wilms tumor (a childhood kidney cancer), Jones syndrome, non-syndromic gingival fibromatosis, and non-syndromic hearing loss [4, 8, 11, 12]. Based on this clinical diversity, specific groups of *REST* variants have been proposed to affect the molecular function of REST differently. Some of the identified pathogenic *REST* variants may not encode any protein product (Fig. 1B, “no protein”), others are likely to encode truncated proteins that preserve some interactions of the wild-type protein (Fig. 1B, bottom 7 variants in left column), and one of the identified variants interferes with the alternative splicing of *REST* (Fig. 1B, rounded bracket).

The Wilms tumor-associated germline mutations in *REST* appear not to cause either gingival fibromatosis or hearing loss [12]. Most are located in regions of the gene that encode the RE1-binding zinc finger domains (Fig. 1A, gray arrows) [12]. Some are nonsense or frameshift mutations that cause insertion of premature stop codons more than 50 nucleotides (nts) upstream of the last exon-exon junction in the mRNA [12]. These stop codons are likely to prevent production of the REST protein by targeting the transcript to the nonsense-mediated mRNA decay pathway (Fig. 1B, marked as “no protein”). Other Wilms tumor-associated *REST* mutations cause amino acid substitutions within or near the zinc finger domains (Fig. 1A, gray arrows). These substitutions have been suggested to reduce the affinity of the encoded protein for RE1 motifs [12], and none of the encoded proteins have been found to be redirected to novel DNA binding sites. Each of these mutations is inherited from parents without Wilms tumors, indicating that the penetrance is incomplete, and neither the patients nor their parents have been diagnosed with gingival fibromatosis or hearing loss [12]. Thus, the key consequence of loss-of-function mutations in the zinc finger-coding regions of *REST* seems to be predisposition to Wilms tumors. Notably, in mice heterozygosity for deletion of the first coding exon of *Rest* (*Rest*$^{+/-}$) is not associated with tumors in the kidney or other organs. Thus, in mice inactivation of one of the two alleles of *Rest* is insufficient to drive tumorigenesis.

In contrast to Wilms tumor cases, in Jones syndrome both gingival fibromatosis and progressive hearing loss are defining clinical characteristics [4]. However, the hearing loss is easily missed because it is limited to an inability to perceive high-frequency sounds. The newly described Jones syndrome-associated mutation shifts the translational reading frame in exon 5 of *REST* (Fig. 1A, red arrow) such that the encoded protein lacks one of the 9 zinc finger domains of wild-type REST, as well as one of the two repressor domains that mediate the recruitment of co-repressors (Fig. 1B, mutation in red) [4]. Although the functionality of this truncated REST form was not evaluated by Rahikkala and coworkers, the effects of similar mutations on REST activity had been tested previously by Chen and colleagues [13]. This group had identified REST-truncating mutations in patients with hereditary non-syndromic gingival fibromatosis (Fig. 1A, green

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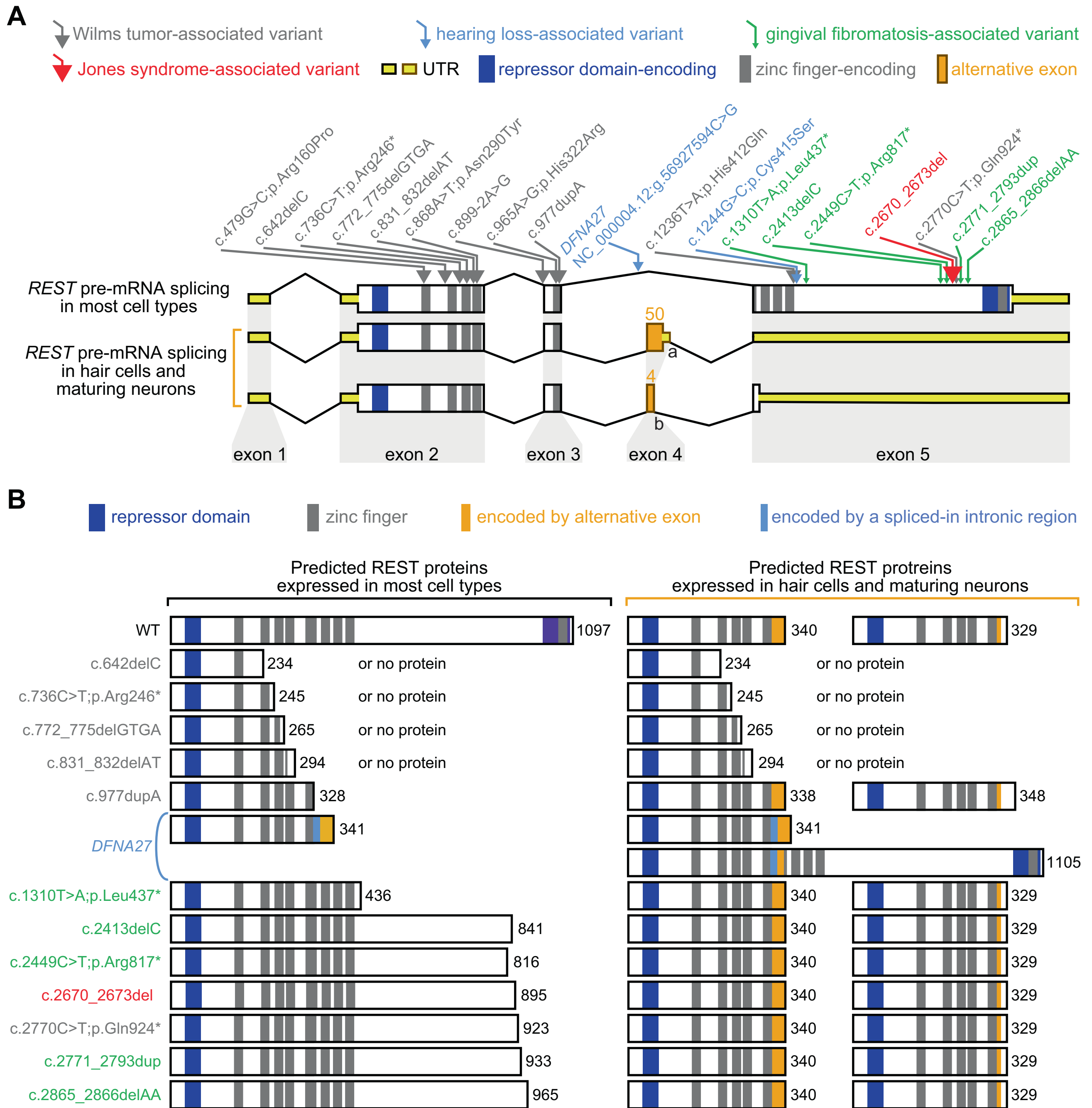

Fig. 1 Hereditary diseases associated with pathogenic forms of *REST*. **A** Schematic of exon-intron structure of *REST* isoforms, illustrating the locations of disease-causing mutations (arrows) and various domain-encoding regions (filled rectangles). The Jones syndrome-associated mutation is shown in red. Also indicated are the constitutively spliced exons (shapes framed in black), the alternative exon (exon 4), the splice donor sites of exon 4 ("a" and "b"), the number of nucleotides in exons 4a and 4b (orange numbers), and UTRs (yellow). Tented lines represent joining of exons in the maturing pre-mRNA. Orange bracket indicates that either exon 4a or (in a smaller percentage of *REST* mRNAs) exon 4b is spliced into the transcript in hair cells and maturing neurons but not in most other cell types. **B** Schematic of protein products of the indicated *REST* variants. Black numbers indicate the number of amino acids in the predicted proteins. Filled rectangles represent repressor domains (dark blue), zinc finger domains (gray), exon 4-encoded regions (orange), and an intronic sequence-encoded region that occurs in the DFNA27 versions of REST (light blue). The name of Jones syndrome-associated mutation is shown in red. Square brackets indicate the cell types in which protein products are typically found. "WT": wild type REST. "no protein": the variant-encoded mRNA is predicted to be targeted to the non-sense mediated mRNA decay pathway.

arrows). They had also shown that these truncated proteins impair the repressor activity of co-expressed wild-type REST and suggested that the truncated proteins compete with wild-type REST for DNA binding [13]. Therefore, the effects of these mutations are likely dominant negative. Given the genetic and clinical similarities between these non-syndromic gingival fibromatosis cases and Jones syndrome, Rahikkala and colleagues recommend that all patients who are heterozygous for REST-truncating mutations receive detailed oral and hearing examinations [4]. This would ensure that the diagnosis of high-frequency hearing loss is not missed in patients with a form of gingival fibromatosis that appears to be non-syndromic.

One cause of non-syndromic progressive hearing loss is deafness autosomal dominant 27 (*DFNA27*), a mutation that lies in an intronic region of *REST* [8]. *DFNA27* differs from other published *REST* mutations in that it interferes with the alternative splicing of exon 4 of *REST*. Specifically, *DFNA27* creates a novel splice acceptor site upstream of exon 4. The novel acceptor site is constitutively spliced to the end of exon 3, and it increases the length of exon 4 by 20 nts [8]. Two splice donor sites exist at the 3′ end of exon 4 of *REST*: "a" and "b" (Fig. 1A). Whereas splicing of wild-type exon 4 at the "a" donor site produces a 50-nt exon with a stop codon (Fig. 1A), splicing of the DFNA27 version of exon 4 at this site generates a 70-nt exon that also contains a stop codon [8]. Also, whereas splicing of wild-type exon 4 at the "b" donor site produces a 4-nt frameshifting microexon (Fig. 1A), splicing of the DFNA27 version of exon 4 at this site generates a 24-nt exon that does not truncate the protein-coding sequence of *REST* [8]. Splicing of the DFNA27 version of exon 4 at the "b" donor site requires splicing factors that are expressed specifically in HCs and neurons [8]. Given that the DFNA27 version of exon 4b does not inactivate REST, this splicing event likely results in abnormally high REST activity in HCs, a condition that is detrimental to HC survival and hearing in a mouse model of the disease [8]. Splicing of the DFNA27 version of exon 4 at the "a" donor site does not require cell type-specific factors and results in inactivation of the encoded protein [8]. Thus, *DFNA27* is a gain-of-function mutation in HCs, and perhaps also in maturing neurons, but a loss-of-function mutation in most other cell types.

Another cause of non-syndromic progressive hearing loss in a family is a missense mutation in exon 5 of *REST* (c.1244 G > C;p.C415S, Fig. 1A) [14]. As in the case of Jones syndrome, and in contrast to that of DFNA27 patients, the extent of this hearing loss is not uniform across audible frequencies. Thus, the *DFNA27*-associated hearing loss is clinically distinct from the hearing losses caused by missense and truncating mutations in exon 5 of *REST*.

Why are the inner ear and the gingiva so sensitive to the effects of mutations in exon 5 of *REST*? Do these mutations cause abnormally high expression of REST target genes? If yes, which are the pathogenic target genes of REST in these tissues? Answering these questions will likely require analysis of animals genetically modified to mimic human mutations in *REST* exon 5; however, no such mutant animals have been generated. Although *Rest*$^{+/-}$ mice are available they do not mimic the effects of the REST-truncating mutations in exon 5 because the *Rest*$^{-}$ allele does not encode a C-terminally truncated protein. Moreover, gingival fibromatosis has not been observed in these mice and evidence for hearing loss is conflicting [8, 15]. This supports the notion that new animal models of REST deficiency will be required to better understand the pathogenesis of diseases caused by mutations in exon 5 in *REST*. Such studies promise to enable the design of novel therapies for treating these diseases. In conclusion, the current report by Rahikkala et al. puts the cause of Jones syndrome to REST and, together with other studies, reveals a need to define the molecular effects of pathogenic *REST* variants in tissues (inner ear and gingiva) that have not been at the center of REST-related research.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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