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CYLD mutations differentially affect splicing and mRNA decay in Brooke-Spiegler syndrome

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ABBREVIATIONS

BSS       Brooke-Spiegler syndrome
NHEK      Normal human epidermal keratinocytes
PBMC      Peripheral blood mononuclear cells
PTC       Premature termination codon
qRT-PCR   Quantitative reverse transcriptase polymerase chain reaction
RT-PCR    Reverse transcriptase polymerase chain reaction
EDITOR,

Brooke-Spiegler syndrome (BSS; OMIM 605041), also known as familial cylindromatosis (OMIM 132700), is an autosomal dominant tumour predisposition disorder characterised by the occurrence of cylindromas, trichoepitheliomas, and spiradenomas. BSS is caused by heterogenous mutations in the CYLD gene. To date, different CYLD mutations have been reported, most of them resulting in a premature termination codon (PTC). Among these, thirteen splice site mutations have been described. However, it remains largely elusive how such mutations affect splicing.

Here, we studied three unrelated Caucasian patients with BSS who were initially diagnosed both clinically and histopathologically (Table 1). To unequivocally confirm the diagnosis, we performed mutation analysis of the CYLD gene which revealed three splice site mutations. In order to select appropriate tissues for RNA studies, we assessed the expression of CYLD in normal human epidermal keratinocytes (NHEK), leukocytes and peripheral blood mononuclear cells (PBMC) by quantitative reverse transcriptase (qRT)-PCR. In all patients, residual mRNA levels were measured in RNA specimens derived from whole blood.

We detected three CYLD splice site mutations, designated c.2108+1G>A (Fig. 1a), c.2242-2A>G (Fig. 1b), and c.2109-2A>C (Fig. 1c). These mutations were associated with distinct phenotypes, respectively (Table 1). Interestingly, the first patient carried a novel mutation
that was not detectable in either parent. Paternity was confirmed by haplotype analysis (data not shown). To the best of our knowledge, c.2108+1G>A represents the first de novo mutation reported in BSS. All mutations were shown by restriction enzyme digestion to be absent from 100 unrelated, unaffected Caucasian control individuals (data not shown).

Comparison of mRNA expression by RT-PCR revealed that CYLD is expressed at almost equal levels in NHEK, leukocytes and PBMC (data not shown). In the patients carrying mutations c.2108+1G>A and c.2242-2A>G, RT-PCR on RNA derived from PBMCs revealed the wild-type product containing 430 base pairs (bp) and a smaller additional band of 363 and 321 bp, respectively. Automated DNA sequencing showed that the smaller bands represented fragments excluding exons 15 and 17, respectively (data not shown). Both exon skipping events lead to putative out-of-frame translation and generation of a PTC. For mutation c.2108+1G>A the PTC is located 30 codons downstream of the mutation site (p.Pro682Gln31fs*) and for c.2242-2A>G, 47 codons downstream of the mutation (p.Ala748Leufs48*). Of note, we did not detect an aberrant splicing pattern for c.2109-2A>C. This finding suggested that the transcript of the mutated allele is subject to nonsense-mediated mRNA decay.

To study the role of nonsense-mediated mRNA decay in the three splice site mutations detected here, we measured CYLD mRNA expression in peripheral blood leukocytes by qRT-PCR. Whereas mutations c.2108+1G>A and c.2242-2A>G do not result in a significant decrease of mRNA levels, mutation c.2109-2A>C is associated with approximately 60% mRNA decay when compared to a control (Fig. 1d).

The patient harbouring mutation c.2109-2A>C presented with cylindromas exclusively. This mutation has been previously reported in a family with trichoepitheliomas only, which suggests that the residual amounts of CYLD mRNA seem to be unrelated to the distinct phenotypes of individuals with this CYLD splice site defect. To date, fifteen splice site...
mutations have been reported to underlie different phenotypes, comprising Brooke-Spiegler syndrome (30%), familial cylindromatosis (35%), and multiple familial trichoepithelioma (35%). These previous reports and our own data confirm the previous notion that there is no apparent genotype-phenotype correlation in diseases caused by CYLD mutations. Possibly, as of yet unknown environmental factors, modifier genes or epigenetic events could contribute to the different phenotypes observed.

REFERENCES


LEGENDS TO TABLE AND FIGURES

Table 1  CYLD splice site mutations in this study, associated phenotypes, and the structural and functional consequences of these mutations.

Figure 1  a-c) Splice site mutations detected in this study, designated c.2108+1G>A; c.2242-2A>G; and c.2109-2A>C. d) Quantitative real-time PCR indicates that mutations C.2108+1G>A and C.2242-2A>G are not associated with relevant mRNA decay. By contrast, mutation C.2109-2A>C leads to substantial mRNA decay.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>c.2108+1G&gt;A</th>
<th>c.2242-2A&gt;G</th>
<th>c.2109-2A&gt;C</th>
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<tbody>
<tr>
<td>Phenotype</td>
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<td>Cylindromas and trichoepitheliomas</td>
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<td>Exon skipping</td>
<td>Nonsense-mediated mRNA decay</td>
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<td>Levels of mRNA</td>
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<td>Normal</td>
<td>40% of normal</td>
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