Identification of homozygous deletion in ACAN and other candidate variants in familial classical Hodgkin lymphoma by exome sequencing

perinöllinen syöpä, klassinen Hodgkinin lymfooma, eksomisekvensointi

Avainsanat – Nyckelord – Keywords

Säilytyspaikka – Förvaringställe – Where deposited

Muita tietoja – Övriga uppgifter – Additional information

Hodgkin lymphoma (HL) is a lymphoid neoplasm that normally originates from germinal centre B-cells. It is the most common cancer in adolescents aged 15–19 years, and the sixth most common in children aged 0–14 years (http://www.cancer.org/Research/CancerFactsStatistics/CancerFactsFigures2014/cancer-facts-and-figures-2014.pdf) The global annual incidence of HL in all age groups is 3 in 100,000, and it represents about 30% of lymphomas (Stein, 2008). HL is divided into classical Hodgkin lymphoma (CHL), which accounts for 95% of cases, and nodular lymphocyte predominant Hodgkin lymphoma accounting for the remaining 5%. A particular characteristic of CHL is that the clonal tumour cells, the so-called Hodgkin-Reed-Sternberg (HRS) cells, constitute only about 1% of the tumour mass, the majority of the tissue being composed of benign immune and inflammatory cells. Because of this, identification of somatic mutations that might play a role in CHL tumourigenesis is challenging.

The aetiology of CHL is not completely understood. Epstein–Barr virus (EBV) infection is associated with CHL, with about 40% of CHL cases expressing EBV-encoded RNAs, and the role of EBV in HL pathogenesis is further supported by molecular biology studies (Kuppers, 2009). However, given that EBV is one of the most common viruses, the role of genetic factors in HL susceptibility should also be acknowledged. The familial clustering of CHL has been reported (Goldin et al, 2004), however, a germline translocation affecting the KLHDC8B gene is currently the only known candidate for a penetrant CHL predisposing mutation (Salipante et al, 2009).

In this work, we studied a family of Middle Eastern origin whose clinical description has been reported earlier (Kamper et al, 2005). Three out of five children were diagnosed with EBV-positive CHL within a 6-year period (Fig 1). The index case, Patient I, is a male diagnosed with nodular sclerosis CHL
(NSCHL) at the age of 5 years, Patient II is a female diagnosed with NSCHL at 12 years of age and Patient III is a male diagnosed with mixed cellularity CHL when aged 12 years. The affected siblings share human leucocyte antigen class I haplotypes A26B38;A9B5 and their tumour cells were EBV-positive. Based on serology in February 2012, the unaffected siblings also had a prior EBV infection.

**Figure 1.** The family pedigree and ACAN c.2836_2892del status.

To identify the putative CHL predisposing genetic defects, we sequenced the exomes from the affected siblings’ blood samples using 101 bp paired-end reads. Using a control set of 3891 exomes and genomes (in-house Finnish patients with other phenotypes and multiethnic public datasets), and accepting only variants shared by affected individuals, resulted in 35 variants. Then 35 genes containing these variants were then prioritized (i.e. their relatedness to lymphoma was assessed) and the deleteriousness of each variant was predicted computationally. Applying the selection criteria (Data S1) resulted in ten missense and three other type CHL candidate variants (Table 1). The full list of shared variants can be found in Table SI and the list of HL-associated genes used in prioritization in Table SII.

**Table 1.** Candidate classical Hodgkin lymphoma predisposing variants.

The only homozygous variant was a cluster of closely located single nucleotide variants in the ACAN gene. Interestingly, Sanger sequencing of the region revealed that the aberrant signal arose from a homozygous 57-base pair in-frame deletion c.2836_2892del, leading to a deletion of 19 amino acids (Figure S1). Both parents and one unaffected child were found to be heterozygous for the deletion, and the other unaffected sibling displayed wild type ACAN (Fig 1). ACAN encodes a proteoglycan called aggrecan, which is an essential element of the extracellular matrix in cartilaginous tissue. It contains a long tandem repeat sequence consisting of 19 repeats of 19-amino acid motif. The deletion identified in ACAN removes part of the repeat sequence as well as nine amino acids from the unique sequence. ACAN has not been associated with
tumourigenesis or tumour predisposition but recent research has suggested that noncellular tumour microenvironment components may promote the oncogenic effects of EBV (Cader et al, 2013).

Because the pedigree structure is compatible with a recessive mode of inheritance, we determined the ratio of homozygous and heterozygous variants of the affected siblings (Table SIII), to establish whether it could provide support for the trait to be recessive. For controls, the same ratio was also determined in 12 randomly selected Finnish in-house samples. Indeed, homozygosity was shown to be higher in the affected individuals than in Finnish controls, which is noteworthy because of the inbreeding in the Finnish population.

Besides the ACAN deletion, the candidate variant set contains a nonsense variant in KIAA0141, and a removal of stop codon in LY75-CD302. LY75-CD302 is a fusion protein resulting from read-through transcription between the lymphocyte antigen 75 (LY75) and CD302 molecule (CD302) genes, and interestingly, was originally identified in HRS cell lines (Kato et al, 2003). The stop codon removal was found to be present in all affected individuals and their unaffected father. The change results in addition of 22 amino acids to LY75-CD302 and CD302 proteins. KIAA0141 is a death-associated protein 3 binding apoptosis signal enhancer (Harada et al, 2010). The observed change results in a premature stop in the last exon 12, which truncates the protein 13 amino acids before the reference stop codon.

Of the 10 genes containing missense-type candidate variants (TNS1, USP10, AFTPH, IPO9, CCDC64, MIEF1, OR52N2, VPS36, FAT2 and KLHL35; listed in descending prioritization order), USP10 seems most interesting, based on its function. It is a deubiquitinating protease, acting as a regulator of TP53 in DNA damage response and tumour development (Jochemsen & Shiloh, 2010). Gene descriptions for the remaining candidates can be found in Table SI.

The candidate genes above provide a platform for validation studies in extended sets of familial CHL samples. Robust identification of additional mutations in families with this intriguing disease-susceptibility phenotype would
be a breakthrough in CHL risk prediction and provide valuable clues to the molecular basis of HL.

Acknowledgements

We thank Inga-Lill Svedberg for excellent technical assistance. This work was supported by grants from the Academy of Finland (Finnish Centre of Excellence Program 2012–2017, and personal grant no. 137680 for OK), the Finnish Cancer Society (SL and LA), the Sigrid Juselius Foundation (SL and LA), Helsinki University Central Hospital and the Karen Elise Jensens Foundation (FA). This study makes use of data generated by the UK10K Consortium, derived from samples from ALSPAC and TwinsUK. A full list of the investigators who contributed to the generation of the data is available from www.UK10K.org. Funding for UK10K was provided by the Wellcome Trust under award WT091310.

Author contributions

PK and FdA recruited the patients and are responsible for the clinical data; SL and LAA designed the study and co-ordinated the research; HR, OK, MT, and SS carried out the laboratory experiments and performed the genetic analyses; HR drafted the manuscript with assistance from others; All authors approved the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

Heikki Ristolainen¹,²
Outi Kilpivaara¹,²,⁶
Peter Kamper³,⁶
Minna Taskinen²,⁴
Silva Saarinen¹,²,⁵
Sirpa Leppä⁵,⁶
Francesco d'Amore³
Lauri A. Aaltonen*¹,²

¹Department of Medical Genetics, University of Helsinki, Helsinki, Finland, ²Genome-Scale Biology Research Program, Research Programs Unit, University of Helsinki, Helsinki, Finland, ³Department of Hematology, Aarhus University Hospital, Aarhus, Denmark, ⁴Helsinki University Central Hospital Cancer Center, Department of Oncology Helsinki, Finland, ⁵Department of Clinical Genetics, University of Helsinki and Helsinki University Central Hospital, ⁶These authors contributed equally to this work

*E-mail: lauri.aaltonen@helsinki.fi

Biomedicum Helsinki
PO Box 63 (Haartmaninkatu 8)
FI-00014 University of Helsinki, Finland
Tel: +358-2941-25595
Fax: +358 2941 25610

References


Figure 1. The family pedigree and ACAN c.2836_2892del status.
<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Ensembl Gene ID</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Rank¹</th>
<th>PolyPhen2 Prediction</th>
<th>SIFT prediction</th>
<th>Coverage²</th>
<th>Variant % of Total coverage</th>
<th>Zygosity</th>
<th>Quality Score²</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNS1</td>
<td>ENSG00000079308</td>
<td>c.389G&gt;A</td>
<td>p.(Tyr130Cys)</td>
<td>2</td>
<td>probably_damaging(0.999)</td>
<td>deleterious(0)</td>
<td>58</td>
<td>0.49het</td>
<td></td>
<td>67</td>
</tr>
<tr>
<td>ACAN</td>
<td>ENSG00000157766</td>
<td>c.2836_2892del</td>
<td>p.(Gly46_Glu64del)</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td></td>
<td>**</td>
<td>**hom</td>
<td>52</td>
</tr>
<tr>
<td>USP10</td>
<td>ENSG00000103194</td>
<td>c.1769G&gt;A</td>
<td>p.(Arg586Gln)</td>
<td>6</td>
<td>possibly_damaging(0.892)</td>
<td>deleterious(0.01)</td>
<td>41</td>
<td>0.53het</td>
<td></td>
<td>66</td>
</tr>
<tr>
<td>AFTPH</td>
<td>ENSG00000119844</td>
<td>c.167A&gt;T</td>
<td>p.(Lys56Met)</td>
<td>9</td>
<td>probably_damaging(0.999)</td>
<td>deleterious(0)</td>
<td>60</td>
<td>0.50het</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>IPO9</td>
<td>ENSG00000198700</td>
<td>c.523C&gt;T</td>
<td>p.(Arg175Cys)</td>
<td>11</td>
<td>probably_damaging(0.949)</td>
<td>deleterious(0.03)</td>
<td>91</td>
<td>0.45het</td>
<td></td>
<td>97</td>
</tr>
<tr>
<td>CCDC64</td>
<td>ENSG00000135127</td>
<td>c.1573A&gt;G</td>
<td>p.(Arg525Gly)</td>
<td>14</td>
<td>possibly_damaging(0.739)</td>
<td>deleterious(0.02)</td>
<td>20</td>
<td>0.53het</td>
<td></td>
<td>77</td>
</tr>
<tr>
<td>KIAA0141</td>
<td>ENSG00000081791</td>
<td>c.1509C&gt;G</td>
<td>p.(Tyr503Stop)</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td></td>
<td>18.49het</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>MIJF1</td>
<td>ENSG000000100335</td>
<td>c.794G&gt;A</td>
<td>p.(Thr265Arg)</td>
<td>16</td>
<td>possibly_damaging(0.816)</td>
<td>deleterious(0.03)</td>
<td>32</td>
<td>0.46het</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>OR52N2</td>
<td>ENSG00000180988</td>
<td>c.512G&gt;A</td>
<td>p.(Cys171Tyr)</td>
<td>24</td>
<td>possibly_damaging(0.488)</td>
<td>deleterious(0.02)</td>
<td>202</td>
<td>0.53het</td>
<td></td>
<td>76</td>
</tr>
<tr>
<td>VPS36</td>
<td>ENSG00000136100</td>
<td>c.665T&gt;A</td>
<td>p.(Leu222Gln)</td>
<td>27</td>
<td>probably_damaging(0.999)</td>
<td>deleterious(0)</td>
<td>148</td>
<td>0.50het</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>FAT2</td>
<td>ENSG000000086570</td>
<td>c.8278A&gt;G</td>
<td>p.(Thr2760Aia)</td>
<td>29</td>
<td>probably_damaging(0.973)</td>
<td>deleterious(0.04)</td>
<td>118</td>
<td>0.47het</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>KLHL35</td>
<td>ENSG00000149243</td>
<td>c.1486G&gt;T</td>
<td>p.(Gly496Cys)</td>
<td>30</td>
<td>probably_damaging(1)</td>
<td>deleterious(0)</td>
<td>18</td>
<td>0.58het</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>LY75-CD302</td>
<td>ENSG00000248672</td>
<td>c.5620T&gt;G</td>
<td>p.(Stop1874Glu)</td>
<td>*</td>
<td>-</td>
<td>-</td>
<td>89</td>
<td>0.49het</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

¹ Rank according to sum of ToppGene and Endeavour ranks (see Table S1)
² These are averages of three samples; variant quality scores are by GATK UnifiedGenotyper
* Prediction scores could only be calculated for SNPs
* Endeavour did not recognize Ensembl gene ID of LY75-CD302, so prioritization could only be done by ToppGene
** this information for ACANs not available, since the homozygous deletion was discovered using Sanger sequencing

Table 1. Candidate classical Hodgkin lymphoma predisposing variants.