Whole genome sequencing reveals *DICER1* as a candidate predisposing gene in familial Hodgkin lymphoma

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Dear Editor,

Hodgkin lymphoma (HL) originates from germinal center B-cells and accounts for about 10% of newly diagnosed lymphomas and 1% of all *de novo* neoplasms worldwide with an incidence of about 3 cases per 100,000 people in western countries.[1] HL is the most common cancer in adolescents aged 20–30 years with a lower incidence in adults older than 55 years. HL is classified into classical HL (cHL), which accounts for 95% of all cases and the rare nodular lymphocyte-predominant HL (NLPHL). Though familial risk for HL is among the highest of all cancers,[2] not many genetic risk factors have been identified besides associations with the human leukocyte antigen (HLA) complex and few germline variants in familial cHL and NLPHL.[3–6] Here we report our findings from whole-genome sequencing of a family with HL revealing a novel missense mutation in *DICER1* gene resulting in impaired expression of tumor-suppressing miRNAs suggesting a possible mechanism underlying HL biogenesis in this family.

The 7-year-old proband (III-1; Fig. 1*a*) was diagnosed with Stage II A cHL (nodular sclerosis HL) at the children's hospital, University of Heidelberg, Germany. Her mother (II-2) was also diagnosed with Stage II A cHL (lymphocyte rich HL) at the age of 34, while her 42-year-old father (II-1) and 66-year-old grandmother (I-1) were healthy. In order to identify possible HL predisposing variants, we collected blood samples and clinical information from the subjects with a written informed consent and ethical review board approval in accordance with the tenets of the Declaration of Helsinki. Whole-genome sequencing of germline genomes of the two patients (II-2 and III-1) and one healthy family member (II-1), mapping, variant calling, annotation and filtering were done using standard methods. Our in-house developed pedigree-based DNA sequencing pipeline was applied in ranking, segregation, conservational screening, and evaluation of deleteriousness of the coding variants (Supporting Information Methods). After excluding the variants that are common, present in the healthy father, synonymous and with the combined annotation dependent depletion score <10, 1331 variants remained (Supporting Information Table). We focused on the 70 non-synonymous exonic variants and after applying our pipeline criteria and literature review, ended up with 5 potential candidates (*DICER1, HLTF, NOTCH3, PLK3* and *RELB*; Supporting Information Fig. 1). To confirm the variant segregation and assess the mutational status of the healthy grandmother whose DNA was available by then, we performed Sanger sequencing of the four family members and found that all the above variants were also present in the grandmother except *DICER1*, indicating that *DICER1* variant may contribute to the neoplastic phenotype in this family (Fig. 1*b*). Analysis of whole exome sequencing data of the largest cohort of HL families reported to date (65 families)[6] and whole genome sequencing data of two independent HL families (unpublished data) did not reveal any *DICER1* mutations. Lee *et al*. analyzed somatic mutations of *DICER1* gene in 930 hematologic tumors and found only a mutation in one non-Hodgkin lymphoma patient.[7] To check the frequency of *DICER1* variants in HL, we sequenced exon 24 of *DICER1* spanning amino acids 1699–1788 (ENST00000526495) from 760 germlines of unselected HL patients recruited within the German Hodgkin Study Group during 1998–2007 and did not find any mutations confirming that this is a rare variant, consistent with data in the ExAC database (allele frequency 0 among 277,264 tested alleles; http://exac.broadinstitute.org/gene/ENSG00000100697).

Dicer is an endoribonuclease required for microRNA (miRNA) biogenesis and several other RNA interference phenomena. The Dicer protein contains highly conserved tandem endonuclease domains, RNase IIIA and RNase IIIB (Fig. 1*c*). Mutations are heavily concentrated in the base pairs encoding metal-ion-binding residues D1709 and E1813, other hotspots being D1705, D1713 and G1809, all of which bind to the metal ions or are adjacent to the binding sites. The *DICER1* variant that we identified (ENST00000526495: exon24: c.T5133G: p.I1711M) is a novel heterozygous missense mutation located in the highly conserved region adjacent to the metal-binding residues E1705, D1709 and the mutation hotspot D1713 within the

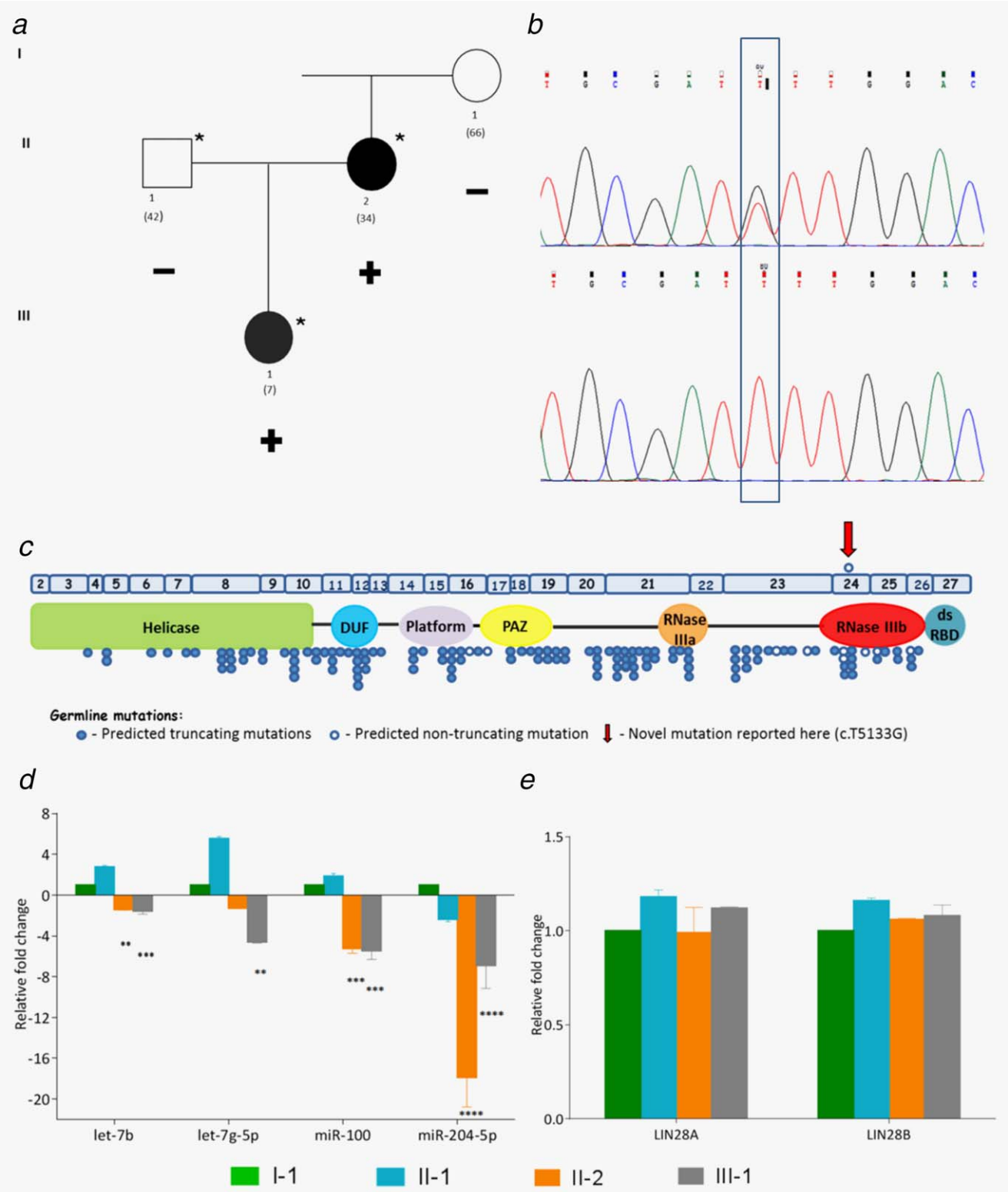

Figure 1. (*a*) Pedigree of the analyzed family. Filled in symbols represents the family members with Hodgkin lymphoma. *Indicates individuals that were analyzed by whole-genome sequencing, ± signs indicate the presence or absence of the *DICER1* variant, respectively. For affected individuals age at diagnosis is given in the brackets whereas for the unaffected individuals age at time of this study is reported. (*b*) Sanger sequencing of *DICER1* exon 24. Representative electropherogram of affected (upper panel) and unaffected (lower panel) family members. The position of the novel variant we identified is highlighted in the box. (*c*) Graphic representation of *DICER1* structure highlighting the published germ-line variants. The red arrow indicates the position of the variant. (*d*) qRT-PCR expression of selected miRNAs in family members with and without *DICER1* mutation. (**$p < 0.005$; ***$p < 0.0005$; ****$p < 0.00005$). (*e*) qRT-PCR expression of *LIN28A* & *LIN28B* in family members with and without *DICER1* mutation.

RIIIB domain (Fig. 1*c*). Heterozygous germline *DICER1* mutations have been associated with an autosomal dominant syndrome, named "*DICER1* syndrome" (Online Mendelian Inheritance in Man (OMIM) reference: 601200), characterized by an increased risk of developing different early onset benign and malignant tumors. Familial *DICER1* germline mutations have also been linked to several isolated neoplasms. Interestingly, occurrence of HL of the T cell phenotype was recently reported in a family with *DICER1* syndrome bearing two distinct *DICER1* variants: a single frameshift deletion in exon 24 (c.5299delC) and a likely benign variant (c.4616C>T; p.Thr1539Met) in exon 23 of the gene.[8] Unfortunately, we have no information available of possible other cancers in our family; however the early onset of HL in III-1 may suggest a *DICER1* syndrome background.

Recurrent somatic mutations in the metal-binding residues of the *DICER1* RIIIB domain required for cleavage of dsRNA have been shown to strongly impair maturation of 5p miRNAs. Several reports have shown a selectively decreasing expression of mature 5p miRNAs in tumors bearing *DICER1* RIIIB domain mutations compared to both *DICER1* wild type tumors and adjacent normal tissue.[9] Among them, many miRNAs have been reported to act as tumor suppressors through inhibition of oncogenic signaling pathways (RAS/MAPK, HMGA2, Cyclin D 1/2/3, Cyclin A, CDK 4/6, c-myc, Lin28 and STAT5). In order to evaluate the functional effect of our novel variant, we checked for the expression of known tumor suppressor miRNAs (let-7b, let-7g 5p, miR-100 and miR-204 5p) by qPCR in the blood samples of the family members. The results showed that these miRNAs were significantly down-regulated in *DICER1* mutated compared to wild-type individuals (Fig. 1*d*). As it has been reported that *LIN28* negatively regulates let-7 family members,[10] we evaluated the expression levels of *LIN28A* and *LIN28B* mRNA by qPCR in the family members without evidence of any significant difference (Fig. 1*e*) suggesting that the observed miRNA changes are not due to *LIN28* regulation but due to reduced function of *DICER1*.

In conclusion, we identified a novel heterozygous missense germline mutation in *DICER1* cancer predisposition gene segregating with familial HL. We showed significant down-regulation of tumor suppressor miRNAs in *DICER1*-mutated family members, suggesting that this mechanism might trigger the development of HL in this family.

Acknowledgements

Authors thank the Genomics and Proteomics Core Facility (GPCF) of German Cancer Research Center (DKFZ), for providing excellent library preparation and sequencing services. S.G. acknowledges the financial support from the University of Milan & Italian Ministry of Instruction, University and Research (M.I.U.R).

Authors Contribution

O.R.B., A.F. and K.H. conceived and designed the study. W.B. and M.W.-H. provided the HL family samples. A.E. provided the samples for unselected HL cases. N.P., M.S., O.R.B., S.G. and A.K. analyzed the data. O.R.B. and S.G. performed the experiments and wrote the first draft of the manuscript. All authors read, commented on and approved the manuscript.

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Grant sponsors: University of Milan & Italian Ministry of Instruction, University and Research (M.I.U.R), Harald Huppert Stiftung, TRANSCAN-ERA-NET, BMBF, Deutsche Krebshilfe

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