Potential role of STAG1 mutations in genetic predisposition to childhood hematological malignancies

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Dear Editor,

Cohesin ring is a multi-protein complex that plays an essential role in a wide range of cellular processes: besides its canonical role in sister chromatids cohesion and segregation [1], the complex gives a fundamental contribution to DNA repair and maintenance of genome integrity [2], and in transcriptional regulation [3]. Cohesin genes are classified as encoding core subunits (SMC1A, SMC3, RAD21, and the paralogs STAG1/STAG2), and cohesin regulatory factors (e.g., NIPBL, HDAC8, and others) [1, 2].

Among these, STAG1 encodes for a key subunit of the complex, essential for chromatids cohesion [1, 4]. Germline mutations of cohesins lead to Cohesinopathies [5], while recurrent somatic mutations in multiple components of the complex are known in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) [6], as well as solid tumors [7].

A correlation between Cohesinopathies and cancer predisposition has not been established yet. However, the reports of three Cornelia de Lange patients (CdLS) affected respectively by Down syndrome-like acute megakaryoblastic leukemia (AMKL) [8], acute lymphoblastic leukemia (ALL) [9] and myelodysplastic syndrome (MDS) [10], suggest that germline mutations in Cohesins could constitute a predisposing factor to hematological disorders.

The present study aims to characterize germline Cohesins variants in pediatric patients affected by hematological diseases.

We screened 120 childhood ALL consecutive diagnoses: 107 B-ALL (89.1%), 11 T-ALL (9.2%), and 2 mixed phenotype acute leukemia cases (MPAL) (1.7%). Additionally, we sequenced 19 sporadic pediatric patients referred by our clinicians for having a familial recurrence of cancer (n = 8), syndromic features (n = 9) associated with either ALL (n = 15) or AML (n = 2); two additional cases were rare pediatric MDS.

A custom Next-Generation Sequencing panel was used, including 39 genes associated with predisposition and leukemogenesis. (Supplementary Table 1). We sequenced DNA extracted from bone marrow mononuclear cells during the disease and remission phase, the latter defined by a minimal residual disease (MRD) value below 10^{-4}. NGS data that support the findings of this study are available in the ArrayExpress database (www.ebi.ac.uk/arrayexpress), reference numbers E-MTAB-11757 and E-MTAB-11760.

We focused on Cohesins variants and two previously uncharacterized heterozygous variants have been identified in the STAG1 gene. The first STAG1 variant (Arg1167Gln) was found in a 2 years old male patient affected by B-ALL (negative for common translocations; central nervous system negative; medium risk for MRD). He was enrolled in the AIEOP-BFM ALL2009 protocol, he experienced a late combined relapse (BM and CNS) and underwent HSCT. The patient had no comorbidities nor syndromic stigmata.

The second variant (STAG1 Arg1187Gln) was identified in a 14 years old male patient affected by MDS with an excess of blasts (MDS-EB1), with complex karyotype (47, XY, +8, del(16)(q22)[19]/46, XY[1]), who received HSCT. The patient did not show any syndromic features.

Both STAG1 variants are located in a highly conserved region of the gene, frequently affected by mutations known to be implicated in oncogenesis (Pecan database, https://pecan.stjude.cloud/STAG1) (Fig. S1A).

The Arg1167Gln (c.3560G > A; rs777032446) is a germline missense alteration (VAF 44.6% at diagnosis and 41.3% in remission), classified as VUS in InterVar and Varsome. It is the only variant identified, among genes included in the NGS panel.

The Arg1187Gln (c.3560G > A; rs777032446) is a germline missense variant (VAF 51.9% at diagnosis) predicted as likely pathogenic in Varsome and VUS in InterVar. We validated the germline origin by PCR and Sanger sequencing of DNA isolated from liver biopsy, collected after HSCT transplantation (Fig. S1G).

The MDS patient carried also a somatic Arg953* variant (c.2857C > T) in the paralog STAG2 gene, annotated in InterVar, Varsome, and COSMIC as pathogenic in cancer (Fig. S2).

In order to set up an in vitro model to investigate the role in predisposition of STAG1 germline mutations, two Lymphoblastoid Cell Lines (LCLs) were generated through the immortalization of PB B-lymphocytes from the Arg1167Gln mutated patient (L-STAG1) and from the Arg1187Gln mutated patient (M-STAG1). As a control, four different LCLs were generated from healthy donors.

First, we confirmed by PCR and Sanger sequencing that both L-STAG1 and M-STAG1 have maintained the genetic profile of interest (Figs. S1E–H; S2C), while the CTRs’ LCLs were wild type.

Second, we established the absence of other abnormalities in L-STAG1 and M-STAG1 LCLs, by karyotype and NGS custom panel analysis.

To evaluate the correlation between the different STAG variants and cancer, we analyzed the allele frequency (VAF) of the mutated positions across non-tumor and tumor cohorts. Known variants are combined and analyzed for the gene STAG1, transcript ID ENST00000383202. Minor allele frequencies of all coding germline variants present in STAG1 in a global, healthy population, taken from the gnomAD database, are summed up codon-wise (Fig. S1B) and the VAF of STAG1 p.1167 and p.1187 indicates that these mutations are rare in the general population (details in Supplementary Data).
An analogous model was applied to STAG2 R953* somatic alteration, rarely germline mutated in the non-cancer population (gnomAD database, AF < 10^{-5}) (Fig. S2D).

To investigate the functionality of STAG1 variants on DNA stability, we evaluated the status of chromatin exchanges during the mitotic division.

All LCLs were treated with phytohemagglutinin, to stimulate T lymphocyte growth (T0), incubated with BrdU (T24), which is incorporated only during the first mitotic division, and blocked in metaphase with Colchicine after the second generation (T72). Fluorescence microscopy after Hoechst staining showed that both M-STAG1 and L-STAG1 LCLs are characterized by a significantly higher number of abnormal chromatin exchanges. The average number of exchanges per nucleus is equal to 4.31 for L-STAG1 and 4.8 for M-STAG1, while the mean value for the four control LCLs is 3.05 (range 2.66 to 3.50; p < 0.0001) (Fig. 1A).

Furthermore, the population of cells that had one or more chromosomes with double exchanges is higher in both patients-derived LCLs: in L-STAG1 the percentage was 20.79%, compared to a mean of 12.66% for the control LCLs (n = 4; range 4.57 to 19.09%, p > 0.05), while in M-STAG1 the percentage was 22.17% (p = 0.0069) (Fig. 1B).

We also aimed to estimate the capability of LCLs to repair DNA after double-strand breaks (DSBs) induced by ionizing radiations. We evaluated the phosphorylation level of histone γH2AX, a common marker of DNA double-strand breaks damage [2, 11]. LCLs were seeded at different concentrations, to guarantee the exponential growth phase, they underwent X-ray irradiation at 3Gy and 6Gy and then were marked with Phospho-Histone H2AX antibody at different timepoints of incubation (T0, T24, T48 after irradiation).

γH2AX phosphorylation status in M-STAG1 is significantly higher in basal conditions (Fig. 2A). The differential phosphorylation further increases after radiation at 3Gy or 6Gy. Fig. 2B shows a representative experiment at 3Gy, demonstrating a significantly lower capability of M-STAG1 to repair DNA after damage, compared to control LCLs.

Overall, control LCLs have a reduction of γH2AX phosphorylation at 48 h after irradiation (indicating a successful DNA repair), while M-STAG1 has an increased phosphorylation level, thus corresponding to a defective DNA repair capability.

In addition, a highly positive pH2AX subpopulation (namely pH2AX++) can be discriminated only in M-STAG1 even at basal conditions, and its level progressively increases at the timepoints after irradiation, thus confirming the previous result (Fig. 2C).

Similar findings have been obtained also after 6Gy irradiation (Fig. S7).

Therefore we identified two germline variants of the STAG1 gene in two pediatric patients, affected by B-ALL and MDS, respectively. Those variants are located in a highly conserved
region where multiple variants associated with solid tumors were mapped.

For the first time, we specifically explored the functional role of germline STAG1 variants in oncogenesis, by evaluating how they can corrupt a pre-leukemic clone, making it genetically unstable and more prone to further somatic mutations.

We demonstrated that the STAG1-mutated LCLs have a higher number of both single and double chromatids exchanges compared to control LCLs. This is a common indicator of poor chromosomal stability and spontaneous chromosome instability, which is associated with failure of DNA repair and accumulation of DNA damage events. Similarly, higher SCE have been already found in other familial cancers, such as BRCA1/2 mutated breast cancer [12].

Moreover, M-STAG1 cells displayed an increased DNA damage sensitivity, with a significantly lower DNA repair capability after X-ray irradiation. These results are consistent with studies by Bauerschmidt et al., who demonstrated that repair of radiation-induced DNA DSBs was reduced in SMC1- or RAD21-depleted cells [13].

The germline status of the identified variants in non-syndromic patients is compatible with their effects on DNA stability and DNA damage repair mechanisms, compatible with life but predisposing to oncogenesis.

Although the preliminary evidence on STAG1 therapeutic potential [14], further biological studies are needed before considering the clinical relevance of STAG1 germline variants and any therapeutic translation as a preemptive intervention. Taken together, our study provides strong evidence in support of the involvement of STAG1 germline variants in predisposition to onco-hematological diseases in childhood.

If confirmed, cases carrying a STAG1 germline variant would merit genetic counseling for the patient and its family, in order to make appropriate decisions for any therapeutic program (i.e., radiotherapy, selection of Hematopoietic Stem cell donor), as well as for any surveillance. It would be crucial to assess whether those variants have a de novo origin or silent carriers are present in the family.

In the future, we cannot exclude a similar scenario also involving other cohesin genes.

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AUTHOR CONTRIBUTIONS
CS, GF, and GC contributed to study conception and design; CS and SR performed experiments and collected data; GG contributed to SCE experiments; EE and NP contributed to cell cycle analysis; SR, FA, and UF performed the bioinformatic analysis; LRB collected clinical data; CS, SR, LRB, and GF contributed to data analysis and interpretation; VM and JH reviewed data analysis and interpretation; CS, SR, GF, and GC wrote the manuscript. AB and GC supervised the study; all authors revised the paper and approved the submitted version.

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COMPETING INTEREST
The authors declare no competing interests.

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