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LETTER TO THE EDITOR

RNA-binding protein (*RBFOX1*) inherited polymorphism rs8051518 is not associated with splice factor mutations in myelodysplastic syndromes and myeloproliferative neoplasms

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Dear Editor,

In recent years, many DNA polymorphisms were linked to the risk of tumor development or poor outcome in cancer patients [1–3]. Large publicly available datasets of whole exome and whole genome sequencing projects like The Cancer Genome Atlas (TCGA) or the International Cancer Genome Consortium (ICGC) enable the analysis of polymorphisms in thousands of cancer patients in silico. In a very detailed work, Carter et al. [4] analyzed nearly 6000 cancer patients from the TCGA and ICGC databases and found over 400 genetic interactions between germline polymorphisms and major somatic events, like mutations in driver genes. One noteworthy interaction is an 8-fold increase of *SF3B1* mutations when a germline variant in intron 4 (rs8051518) of the RNA-binding protein (*RBFOX1*) was present. Furthermore, the authors showed an increase of *RBFOX1* mRNA expression and differentially spliced exon-exon junctions in the presence of the minor allele (rs8051518). This interaction is proposed to have functional relevance for the development of the disease [4].

Because the TCGA and ICGC cohorts consist mainly of solid tumors, we were interested if the described interaction between *RBFOX1* and *SF3B1* could also be found in myelodysplastic syndromes (MDS) and myeloproliferative neoplasia (MPN). These disease subtypes of myeloid neoplasms are well known to harbor splice factor mutations, including *SF3B1* mutations [5], but comprehensive datasets of these diseases are not present in TCGA or ICGC. Furthermore, we wanted to examine if this genetic interaction could also be found in *SRSF2* and *U2AF1* mutated patients.

We selected from the archive of the Institute of Pathology altogether 690 cases, including MDS ($n = 245$), MPN ($n = 153$), MDS/MPN-overlap ($n = 264$), and 2nd AML ($n = 28$) with known splice factor mutations (*SRSF2* mutated, $n = 325$; *SF3B1* mutated, $n = 93$; *U2AF1* mutated, $n = 59$) or wild-type for the three investigated splice factor genes ($n = 213$) (Table 1). All samples were formalin-fixed paraffin-embedded bone marrow trephines. Mutation profiles of these cases were available from the routine workup in our institute which includes the sequencing of 25 genes important in myeloid neoplasms [6]. *RBFOX1* allele status was determined by pyrosequencing as described [7]. Examples for *RBFOX1* pyrosequencing results are shown in Fig. 1a.

As shown in Table 1 and Fig. 1b, we were not able to validate the observed genetic interaction of *RBFOX1* rs8051518 and the presence of a *SF3B1* mutation. In our *SF3B1* mutated cohort, the homozygous minor allele status (aa, 6.5%) is actually lower compared with the wild-type cohort (11.7%). The same is true for *SRSF2* and *U2AF1* with 5.8% aa and 5.1% aa, respectively. Figure 1c depicts the relative frequencies of splice factor mutations for a given RBFOX1 genotype of the patient samples. It is shown that 48.1% of minor allele genotype (aa) has no detectable mutation in *SRSF2*, *SF3B1*, and *U2AF1*. A statistical analysis with a one-way ANOVA and Kruskal-Wallis test (GraphPad Prism, Version 5.0) revealed no difference between the three splice factor gene mutated cohorts compared with the wild-type cohort (overall p value = 0.3467). Comparison of the RBFOX1 genotype with different disease subtypes (Table 1, lower rows) shows a decreased minor allele frequency in MPN disease (2.8%). However, in a one-way ANOVA, the differences between the disease subtypes are not significant (overall p value = 0.1072).

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Table 1 Patient sample cohorts with different splice factor mutations related to *RBFOX1* allele status and disease subtype

		RBFOX1 genotype rs8051518			Disease subtype			
	Total cases	AA	Aa	aa	MPN	MDS	MDS/MPN	2nd AML
SRSF2^{mut}	$n = 325$	183	123	19	88	50	175	12
		56.3%	37.8%	5.8%	27.1%	15.4%	53.8%	3.7%
SF3B1^{mut}	$n = 93$	51	36	6	6	54	32	1
		54.8%	38.7	6.5%	6.5%	58.1%	34.4%	1.1%
U2AF1^{mut}	$n = 59$	31	25	3	12	24	19	4
		52.5%	42.4%	5.1%	20.3%	40.7%	32.2%	6.8%
Wild-type	$n = 213$	102	86	25	47	117	38	11
		47.9%	40.4%	11.7%	22.1%	54.9%	17.8%	5.2%
MPN	$n = 153$	86	63	4				
		56.2%	41.2%	2.6%				
MDS	$n = 245$	121	100	24				
		49.4%	40.8%	9.8%				
MDS/MPN	$n = 264$	145	99	20				
		54.9%	37.5%	7.6%				
2nd AML	$n = 28$	16	8	4				
		57.1%	28.6%	14.3%				

mut gene mutation in the specific splice factor gene

Furthermore, we tried to validate the increased mRNA expression level in patients with *RBFOX1* minor allele genotype. However, in replicate analysis of 18 FFPE RNA samples, 9 cases from the *SF3B1* mutated and 9 from the wild-type cohort (3 times *RBFOX1* AA, Aa, aa, respectively), we were not able to detect *RBFOX1* transcript in qPCR with three exon-exon spanning primer pairs (exon 5-6, 6-7 [8], 10-11). Because it is known that *RBFOX1* expression is high in brain tissue [9], we tested 4 FFPE RNA samples from glioblastoma patients in replicate analysis and found robust *RBFOX1* expression (mean CT values exon 5-6: 31.9 SD 3.8; exon 6-7: 30.6 SD 3.4; exon 10-11: 32.5 SD 3.3), whereas the three tested housekeeping genes *TRIM27* (mean CT-value 28.9, SD 2.8 in the bone marrow samples vs. 27.9, SD 2.3 in the

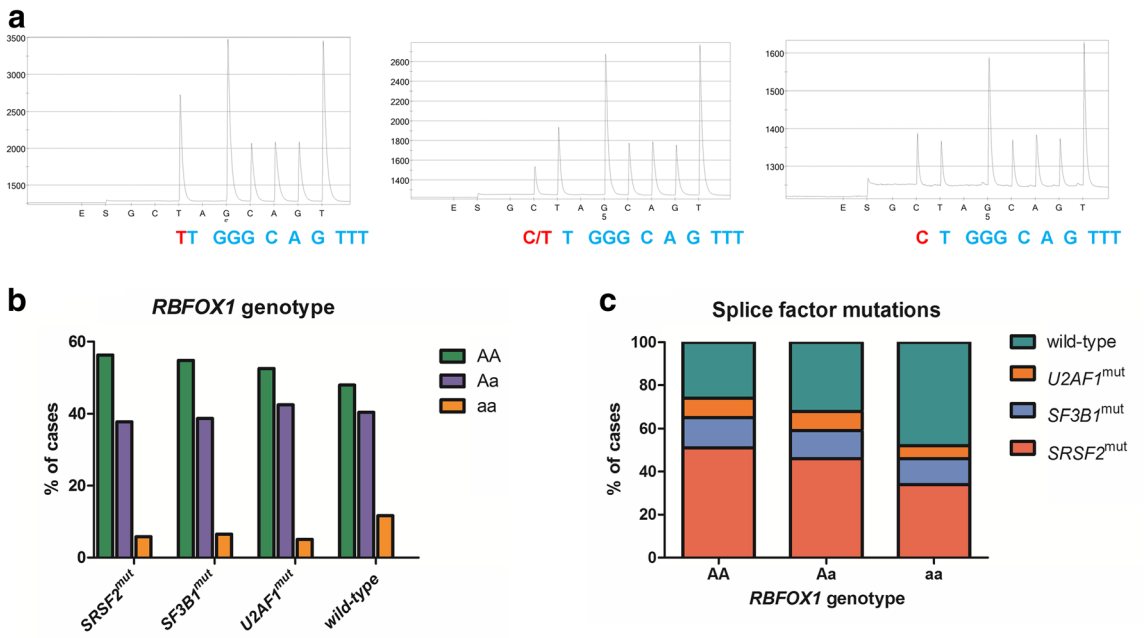

Fig. 1 **a** Pyrosequencing results for AA (homozygous T), Aa (heterozygous, C and T allele), and aa (homozygous C, rs8051518) allele status of *RBFOX1* intron 4 (HGVS NM_018723.3:c.27+218416). **b** Relative frequencies of *RBFOX1* allele status in the three cohorts with splice factor mutations and the wild-type cohort. **c** Relative frequencies of splice factor mutated patient samples for given *RBFOX1* genotypes

glioblastoma tissue), *FBXW2* (mean CT-value 31.3, SD 2.9 vs 28.8, SD 2.8), and *PSMB2* (mean CT value 28.4, SD 3.2 vs. 25.5 SD 1.8) show comparable expression between bone marrow and glioblastoma tissue, demonstrating the validity of our qPCR approach.

Because of the absent or very low expression of *RBFOX1* in the bone marrow, we conclude that it is unlikely that *RBFOX1* plays a role in developing splice factor mutations.

In conclusion, we were not able to confirm the described genetic interaction [4] of *RBFOX1* minor allele with the presence of a *SF3B1* mutation. Also, *SRSF2* and *U2AF1* mutations are not associated with the minor *RBFOX1* allele. Our results highlight the importance of the replication of reported genetic interactions in large independent cohorts and the cell-type as well as the disease-type specificity of genetic associations. The field of (intronic) genetic polymorphisms and their influence in development and outcome of cancer is very interesting, but a lot of research is required to enlighten these associations.

Compliance with ethical standards

The study was approved by the Ethics Committee of Hannover Medical School

Conflict of interest The authors declare that they have no conflict of interest.

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