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Blood 142 (2023) LBA-6–LBA-8

The 65th ASH Annual Meeting Abstracts

LATE BREAKING ABSTRACTS

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ERG Is a New Predisposition Gene for Bone Marrow Failure and Hematological Malignancy

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There remain gaps in our knowledge of hereditary and sporadic causes of hematological malignancy (HM) and bone marrow failure (BMF) that prevent optimal diagnosis, disease surveillance and treatment. Here we report the discovery of *ERG* as a novel predisposition gene for BMF and HM. *ERG* is a known oncogene, typically via gene-fusions, leading to dysregulated ERG overexpression in blood and solid cancers. We identified a germline ERG ETS domain variant p.Y373C segregating with thrombocytopenia in a mother, who progressed to AML (27 yr) and then therapy-related MDS (35 yr), and in her 2 sons. All three showed copy neutral loss of heterozygosity of all or part of chromosome 21q, including the ERG locus, with the oldest son showing at least 2 somatic genetic rescue (SGR) events. The possibility of causal *RUNX1* variants were ruled out, with the smallest somatic cnLOH event beginning within the *RUNX1* gene, but not encompassing the RUNT domain where the majority of pathogenic missense variants are located. *ERG*, a highly constrained gene (LOEUF <0.33), is critical for definitive hematopoiesis, adult hematopoietic stem cell (HSC) function and platelet maintenance. An identical corresponding heterozygous germline variant (p.Y343C) in *ERG's* closest gene by homology, *FLI1*, causes platelet-type bleeding disorder-21 (BDPLT21, OMIM #617443).

Through global collaborations, we have identified 15 heterozygous variants in the *ERG* gene, 13 of which are missense and 2 truncating variants, in 17 individuals with cytopenia and/or HM (mainly myeloid) or lymphedema (Table). Onset of hematological symptoms ranged from birth to 38 years for truncating and constrained ETS domain variants. Of these 15 variants, 12 have been confirmed germline including 2 *de novo*. Only 4 meiotic transmissions are observed. None of the missense variants in the highly conserved ETS domain of ERG which mediates DNA binding, protein-protein interactions and nuclear localization, are present in gnomAD. We have functionally characterized 19 ERG variants, 12 potentially pathogenic, 1 known mouse pathogenic variant and 3 population controls demonstrating that most ETS domain missense variants display loss-of-function (LOF) characteristics disrupting transcriptional transactivation (Figure), DNA-binding and/or nuclear localization *in vitro*. Robust preliminary data from *ex vivo* models of ERG overexpression in mouse fetal liver cells in tissue culture (cytokine-independence), a mouse transplant assay and previous germline mutant *Erg* mouse models are concordant with ETS domain missense variants being LOF compared to wildtype ERG and benign controls. Together, these data provide clinical, *in vitro* and *ex vivo* functional studies implicating LOF variants in hematological disease predisposition. LOF *ERG* mutations also occur in sporadic cases of HM.

Recently, as part of a Genomics England Research Consortium population study, 4 truncating *ERG* variants were described in 7 individuals across 4 families with 3 meiotic transmissions and a *de novo* case with primary lymphedema (1) and we add 2 novel missense variants here. One patient showed SGR across the ERG locus in blood. Blood phenotypes were not described. Our results demonstrate that germline *ERG* variants predispose to diverse cytopenia, BMF and HM in both children and adults. In our family mentioned above, the mother received an unrelated alloHSCT due to t-MDS while her 2 sons with cytopenias continue to be monitored. The natural history of this new syndrome will require careful identification of germline lesions with additional longitudinal studies in more patients and families needed. This ERG syndrome parallels GATA2 deficiency syndrome (HM and lymphedema) and *RUNX1* Familial Platelet disorder-myeloid malignancy (thrombocytopenia and HM). Like the well-known disease genes *GATA2* and *RUNX1*, ERG is also a member of the transcription factor heptad involved in HSC maintenance and differentiation. *ERG* adds to a growing list of genes whose unregulated expression contributes to HM and other cancers.

Identification of causal germline *ERG* variants like those outlined in this study, has direct clinical implications for patient and family management including diagnosis, counselling, surveillance and treatment strategies such as selection of bone marrow transplant donors and potential for targeted therapies including gene and cell therapy.

Reference

1. Greene D *et al*. Nat.Med. 29:679-688 2023

Disclosures Scott: *Roche:* Consultancy, Membership on an entity's Board of Directors or advisory committees. **Sicre De Fontbrune:** *Alexion, AstraZeneca Rare Disease:* Honoraria, Research Funding; *Novartis:* Honoraria, Research Funding; *Sobi:* Honoraria, Research Funding; *Samsung:* Honoraria, Research Funding; *Pfizer:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees. **Mutsaers:** *AstraZeneca:* Research Funding; *GlaxoSmithKline:* Consultancy. **DeMille:** *ARUP Laboratories:* Current Employment. **Crawford:** *Illumina:* Current Employment. **Soulier:** *STRM.Bio:* Membership on an entity's Board of Directors or advisory committees; *Rocket Pharmaceuticals:* Consultancy, Honoraria.

Table. Information on patients carrying ERG variants.

Patient ID	Gender	ERG variant (NP_891548.1) (479 aa)	VAF (%)	gnomAD v2.1.1	COSMIC	REVEL	CADD	Hematological-related Phenotype	Non-hematological Phenotype	Age onset of first phenotype (yr)	Germline ERG (inherited/ de novo)	Somatic mutations
1	M	p.E20Vfs*13	60	0	0	-	-	MDS		38	Yes	
2	F	p.P116R	56	2	0 (1x P116G)	0.36	31	Thrombocytopenia, Thrombocytopathy, platelet aggregation disorder	Hypertension, diabetes, cataract	73	Not available	-20q no cnLOH
3		p.I126T	59	0	0	0.512	27.5	Chronic thrombocytopenia since childhood. AML (40 yr), relapse post HSCT (51 yr), died (52 yr). Familial history of dominant thrombocytopenia.	None in clinical records	<40	Yes (also *RUNX1* deletion)	FLT3-ITD IDH1
4	M	p.R302C	40	2	2 (5x R302H)	0.365	34	CLL	None in clinical records	Unknown	Yes	
5		p.P306L		0	1	0.394	34	None	Lymphedema		Yes	
6	F	p.M341V	44	0	0 (2x M341I)	0.45	25.7	Severe congenital aplasia and abnormal B cells. Child received allo-HSCT.	Prematurity for acute fetal distress (33 weeks)	Birth	Yes (inherited)	del(7)(q21.3q12.3) 42.8 Mb
7		p.D345N	24	0	4 (1x D345E)	0.3149	33	MDS			Not available	
8		p.D363A	34	0	3 (1x D363G)	0.6209	28	MDS			Not available	
9	M	p.R370H	46	0	0 (1x R370G, 2x R370L)	0.881	27.7	Neutropenia (at birth), pancytopenia (18 yr). Family history of dominant cytopenia, aplastic anemia, AML.	Not reported in clinical records	Birth	Yes	None (Blood at 18 yr)
10	M	p.R370P	44	0	1 (2x R370C)	0.888	28.2	MDS (asymptomatic thrombocytopenia and leukopenia)	Bilateral inguinal hernias, avascular necrosis of femoral head, severe aortic valve insufficiency with secondary heart failure	29	Yes	No
11		p.Y372*	48	0	0	n/a	38	Congenital pancytopenia, bone marrow failure	Telomeropathy		Yes (*de novo*)	
12	M	p.Y373C	17	0	0	0.852	31	Thrombocytopenia, neutropenia	None in clinical records	21	Yes (inherited)	RUNX1 Two 21q cnLOH
13	M	p.Y373C	44	0	0	0.852	31	Thrombocytopenia, neutropenia	None in clinical records	19	Yes (inherited)	21q cnLOH
14	F	p.Y373C	40	0	0	0.852	31	AML, Thrombocytopenia, t-MDS	None in clinical records	27	Yes (inherited)	IDH1 TP53 GATA2 21q cnLOH
15		p.K380N	46	0	0 (1x K380E)	0.642	22.9	Anemia, Thrombocytopenia, Pancytopenia, Macrocytic anemia, Abnormality of spleen, Eosinophilic infiltration of esophagus.	Frontal bossing, Hypoalbuminemia, Abnormal vitamin B12 level, Hemangioma, Hepatosplenomegaly, Vomiting, Diarrhea, Exocrine pancreatic insufficiency, Gastrointestinal inflammation, Erythema, Protein-losing enteropathy, Posteriorly rotated ears, Capillary malformation, Generalized-onset seizure, Autistic behavior, Delayed speech and language development, Weight loss.		Yes (*de novo*)	
16		p.Y388C		0	1	0.90	28.6	None	Lymphedema		Yes	
17		p.G394W	15	0	1 (8x G394R)	0.661	35	t-AML after treatment for DLBCL and prostate cancer	DLBCL and prostate cancer		Yes	
Controls												
gnomAD	37M,69F	p.M219I		106	1	0.05799	22.5	n/a		n/a	Yes	n/a
gnomAD	81M,17F	p.P275S		98	1	0.223	22.4	n/a		n/a	Yes	n/a
PMID: 18500845	n/a	p.S322P		0	0 (2x S322L)	0.574	31	ALL, thrombocytopenia		n/a	Yes	n/a
PMID: 21880795	n/a	p.R370S		0	0 (6x R370G/ P/C/L)	0.803	33	ETV6 variant (ALL)		n/a	No	n/a
COSMIC	unknown	p.R385H		0 (1xR385C)	4 (5x R385C)	0.674	27.6	n/a	2x BrCa, 1x Biliary, 1x Upper aerodigestive tract	n/a	No	n/a
gnomAD	16M,10F	p.P404A		26	0 (1x P404S)	0.086	19.6	n/a		n/a	Yes	n/a

Figure. Transactivation assays of ERG variants. K562 cells were transfected with pcDNA3 empty vector (EV), pcDNA3-ERG (WT) and pcDNA3-ERG mutants. All constructs were co-transfected with a luciferase reporter plasmid driven by a *ITGA2B* promoter with quadruplicate replicates, repeated 3 times. Fold change (mean ± S.E.M.) compared to the WT is plotted. A 1-way analysis of variance (ANOVA) with multiple comparison was performed to compare each variation to WT (P <0.0005, *).

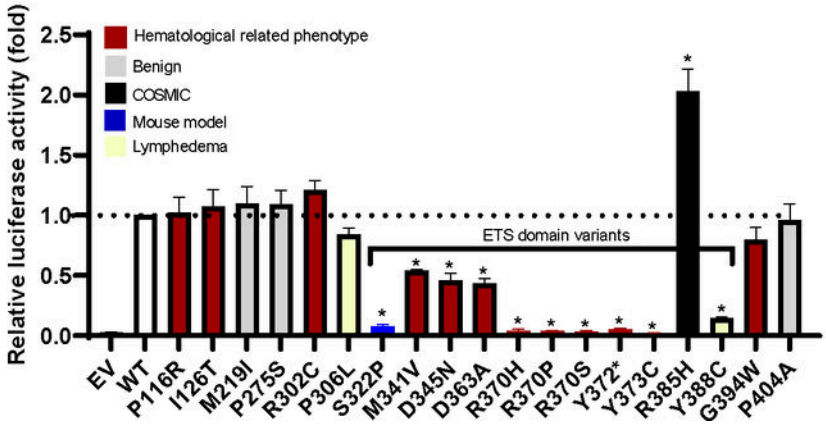

Figure 1

https://doi.org/10.1182/blood-2023-191986