5. Kim J, Lahl K, Hori S et al. Cutting edge: depletion of Foxp3+ cells leads to induction of autoimmunity by specific ablation of regulatory T cells in genetically targeted mice. *J Immunol.* 2009;183(12):7631-7634.
6. Liston A, Farr AG, Chen Z et al. Lack of Foxp3 function and expression in the thymic epithelium. *J Exp Med.* 2007;204(3):475-480.
7. Manrique SZ, Correa MA, Hoelzinger DB et al. Foxp3-positive macrophages display immunosuppressive properties and promote tumor growth. *J Exp Med.* 2011;208(12):2561.
8. De Klerck B, Carpentier I, Lories RJ et al. Enhanced osteoclast development in collagen-induced arthritis in interferon-gamma receptor knock-out mice as related to increased splenic CD11b+ myelopoiesis. *Arthritis Res Ther.* 2004;6(3):R220-R231.
9. Ho VW, Sly LM. Derivation and characterization of murine alternatively activated (M2) macrophages. *Methods Mol Biol.* 2009;531:173-85.
10. Baumgarth N, Roederer M. A practical approach to multicolor flow cytometry for immunophenotyping. *J Immunol Methods.* 2000;243(1-2):77-97.

To the editor:

Genetic defects in PRC2 components other than *EZH2* are not common in myeloid malignancies

Several genetic alterations affecting loci encoding epigenetic regulators have been discovered in myeloid malignancies in the last few years. Previously, we and others identified novel mutations in the histone methyltransferase gene *EZH2*.[1-3] *EZH2* encodes the catalytic subunit of the polycomb repressive complex 2 (PRC2), which mediates the methylation of lysine 27 of histone H3 (H3K27). Trimethylation of H3K27 is an epigenetic modification associated with gene silencing. Loss-of-function mutations in *EZH2* were found in 6% of patients with myelodysplastic syndromes (MDS), 6% of patients with myeloproliferative neoplasms (MPN) and 12% of patients belonging to the MDS/MPN intermediate group.[1,2]

The PRC2 complex consists of 3 core components, EZH2, SUZ12 and EED, which are required for the complex to execute its function (supplemental Figure 1A, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). A knock-out of any one of these components leads to early embryonic lethality in mice, demonstrating their importance during early development.[4] Shen et al showed that EZH1, a homolog of EZH2, might substitute for EZH2, because it interacts with the same PRC2 components and likewise needs EED and SUZ12 for its histone methyltransferase activity. Moreover, EZH1 and EZH2 bind to a largely overlapping set of target genes.[5] Considering these data, we hypothesized that mutations in *EED*, *SUZ12* and *EZH1* might be present in patients with myeloid malignancies, leading to a similar phenotype as loss-of-function mutations in *EZH2*. Furthermore, our recent single nucleotide polymorphism (SNP) array analysis of 102 MDS patients identified one patient with an acquired 5-Mb deletion at 6p22.3 encompassing 15 genes, including *JARID2* (supplemental Figure 1B), as well as one patient who displayed uniparental disomy of this region (supplemental Table

Table 1. Genetic variations in PRC2 components

UPN	Disease	DNA variation	Protein variation	Hetero-/homozygous	Acquired/inherited
SUZ12					
MDS-3	MDS-RAEBt	c.211G > A	p.V71M	Heterozygous	N/A
MDS-14	MDS-RA	c.211G > A	p.V71M	Heterozygous	Inherited
MDS-121	MDS-RAEB	c.196G > A	p.A66T	Heterozygous	Inherited
PV-20	PV	c.211G > A	p.V71M	Heterozygous	Inherited
PV-31	PV	c.211G > A	p.V71M	Heterozygous	N/A
PMF-3	PMF	c.66A > C	p.G22G	Heterozygous	N/A
CMML-13	CMML-1	c.1220C > T	p.T407I	Heterozygous	N/A
CMML-36	CMML-1	c.211G > A	p.V71M	Heterozygous	N/A
CMML-40	CMML-2	c.1569A > T	p.T523T	Heterozygous	N/A
MDS/MPN-U-19	MDS/MPN-U	c.98C > T	p.A33V	Heterozygous	N/A
EED					
MDS-41	MDS-RA	c.363T > C	p.V121V	Heterozygous	N/A
MDS-45	MDS-RAEB	c.1030C > T	p.R344C*	Heterozygous	Inherited
PV-11	PV	c.12G > A	p.R4R	Heterozygous	N/A
CMML-60	CMML-2	c.374A > T	p.E125V	Heterozygous	N/A
EZH1					
–	–	–	–	–	–
JARID2					
MDS-27	MDS-RARS	c.3504C > T	p.H1168H	Heterozygous	N/A
MDS-28	MDS-RAEB	c.1474C > T	p.R492C	Heterozygous	N/A
MDS-50	MDS-RA	c.1474C > T	p.R492C	Heterozygous	Inherited
MDS-50	MDS-RA	c.3504C > T	p.H1168H	Heterozygous	N/A
MDS-55	MDS-RA	c.3504C > T	p.H1168H	Heterozygous	N/A
MDS-86	MDS-RA	c.1674C > T	p.P558P	Heterozygous	N/A

None of the MDS patients with a genetic variation in *SUZ12*, *EED* or *JARID2* carried a mutation in *EZH2*. For the PV, PMF, CMML and MDS/MPN-U patients the *EZH2* status was not determined.

UPN indicates unique patient number; RAEBt, refractory anemia with excess blasts in transformation; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RARS, refractory anemia with ringed sideroblasts; and N/A, not analyzed due to a lack of available T cells or because of a silent variation.

* Variation detected in an exon which is only present in EED isoform ENST00000351625.

1).[6] As JARID2 can interact with the core components of PRC2 and is involved in the recruitment of PRC2 to specific target genes in embryonic stem cells,[7] mutations in this gene might perturb PRC2 function as well.

To determine whether mutations in PRC2 components other than *EZH2* occur in patients with various myeloid malignancies, we sequenced the entire coding region and splice donor and acceptor sites of *SUZ12* (n = 256), *EED* (n = 326), *EZH1* (n = 197) and *JARID2* (n = 99; supplemental Table 2). We collected bone marrow and blood from patients with various myeloid malignancies after obtaining informed consent according to the Declaration of Helsinki. This was approved by the medical-ethical committee of the Radboud University Nijmegen Medical Centre. None of the described genes harbored frameshift or nonsense mutations, but we detected 13 novel variants (7 missense, 6 silent) in 20 patients (Table 1). Sequencing of available constitutional (T cell) material showed that 4 of these variants (all missense) were not somatically acquired. For the remaining 9 variants (3 missense, 6 silent), T cells were not available. Among these was a p.E125V variation in EED, which may still be interesting because it is located in a WD40 domain involved in binding to EZH2 and thus could potentially have consequences for PRC2 function. It therefore remains possible that PRC2 components other than *EZH2* are mutated, albeit at a very low frequency ($< 1\%$).

In conclusion, our results indicate that, although the PRC2 complex can be impaired in myeloid malignancies because of mutations in EZH2, other important PRC2 proteins are not frequently affected. This suggests that EZH2 is the most vulnerable component of this complex, whose proper function is important for normal hematopoiesis.

Leonie I. Kroeze
Laboratory of Hematology, Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre and Centre for Molecular Life Sciences, Nijmegen, The Netherlands

Gorica Nikoloski
Laboratory of Hematology, Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre and Centre for Molecular Life Sciences, Nijmegen, The Netherlands

Pedro da Silva-Coelho
Laboratory of Hematology, Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre and Centre for Molecular Life Sciences, Nijmegen, The Netherlands

Patricia van Hoogen
Laboratory of Hematology, Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre and Centre for Molecular Life Sciences, Nijmegen, The Netherlands

Ellen Stevens-Linders
Laboratory of Hematology, Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre and Centre for Molecular Life Sciences, Nijmegen, The Netherlands

Roland P. Kuiper
Department of Human Genetics, Radboud University Nijmegen Medical Centre and Centre for Molecular Life Sciences, Nijmegen, The Netherlands

Susanne Schnittger
MLL Munich Leukemia Laboratory, Munich, Germany

Torsten Haferlach
MLL Munich Leukemia Laboratory, Munich, Germany

Heike L. Pahl
Department of Experimental Anaesthesiology, University Hospital Freiburg, Centre for Clinical Research, Freiburg, Germany

Bert A. van der Reijden
Laboratory of Hematology, Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre and Centre for Molecular Life Sciences, Nijmegen, The Netherlands

Joop H. Jansen
Laboratory of Hematology, Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre and Centre for Molecular Life Sciences, Nijmegen, The Netherlands

*L.I.K., G.N. and P.d.S.-C. contributed equally to this article.

The online version of this article contains a data supplement.

Acknowledgments: Financial support was obtained from Stichting Life Science Health (NIRM) and the Portuguese FCT (SFRH/BD/60391/2009).

Contribution: L.I.K., G.N., P.d.S.-C., B.A.v.d.R., and J.H.J. designed experiments; R.P.K. performed and analyzed the SNP arrays; L.I.K., G.N., P.d.S.-C., P.v.H., and E.S.-L. performed sequence analysis and T-cell cultures; H.L.P., S.S., and T.H. provided subject material; and J.H.J. and L.I.K. wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: J. H. Jansen, Laboratory of Hematology, Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre, Nijmegen Centre for Molecular Life Sciences, PO-box 9101, 6500 HB Nijmegen, The Netherlands; e-mail: j.jansen@labgk.umcn.nl.

References

1. Ernst T, Chase AJ, Score J, et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet.* 2010;42(8):722-726.
2. Nikoloski G, Langemeijer SM, Kuiper RP, et al. Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. *Nat Genet.* 2010;42(8):665-667.
3. Makishima H, Jankowska AM, Tiu RV, et al. Novel homo- and hemizygous mutations in EZH2 in myeloid malignancies. *Leukemia.* 2010;24(10):1799-1804.
4. Pasini D, Bracken AP, Jensen MR, Lazzerini Denchi E, Helin K. Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. *EMBO J.* 2004;23(20):4061-4071.
5. Shen X, Liu Y, Hsu YJ, et al. EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. *Mol Cell.* 2008;32(4):491-502.
6. Langemeijer SM, Kuiper RP, Berends M, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. *Nat Genet.* 2009;41(7):838-842.
7. Pasini D, Cloos PA, Walfridsson J, et al. JARID2 regulates binding of the Polycomb repressive complex 2 to target genes in ES cells. *Nature.* 2010; 464(7286):306-310.