ERCC6L2 defines a novel entity within inherited acute myeloid leukemia

Suvi P. M. Douglas,[1,2] Pihla Siipola,[1,2] Panu Kovanen,[3] Marja Pyörälä,[4] Sakari Kakko,[5] Eeva-Riitta Savolainen,[6] Urpu Salmenniemi,[7] Katri Orte,[8] Soili Kytölä,[9] Esa Pitkänen,[10] Kimmo Porkka,[11] Outi Kilpivaara[1,2*] and Ulla Wartiovaara-Kautto[11,1*]

[1]Applied Tumor Genomics / Research Programs Unit, University of Helsinki, Helsinki, Finland
[2]Department of Medical and Clinical Genetics / Medicum, University of Helsinki, Helsinki, Finland
[3]Department of Pathology, University of Helsinki and HUSLAB, Helsinki University Hospital, Helsinki, Finland
[4]Department of Hematology, Kuopio University Hospital, Kuopio, Finland
[5]Hematology-Oncology Unit, Oulu University Hospital, Oulu, Finland
[6]Hematology Laboratory, Nordlab Oulu, Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland
[7]Department of Clinical Hematology and Stem Cell Transplantation Unit, Turku University Hospital and Turku University, Turku Finland
[8]Laboratory of Molecular Hematology and Pathology, TYKS Laboratory Division, Turku, Finland
[9]Laboratory of Genetics, Helsinki University Hospital, Helsinki, Finland
[10]Genome Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany
[11]Department of Hematology, Helsinki University Hospital Comprehensive Cancer Center and University of Helsinki, Helsinki, Finland

*O.K. and U.W-K. contributed equally to this study.

Correspondence: Outi Kilpivaara, Applied Tumor Genomics, Research Programs Unit, University of Helsinki, Biomedicum Helsinki 1, Rm B322b, PO Box 63, 00014 University of Helsinki, Helsinki, Finland; e-mail: outi.kilpivaara@helsinki.fi; and Ulla Wartiovaara-Kautto, Helsinki University Hospital, Comprehensive Cancer Center, Department of Hematology, PO Box 372, 00029 HUS, Helsinki, Finland; e-mail: ulla.wartiovaara-kautto@hus.fi

Text 1182 words, 1 figure, 1 table, 20 references

TO THE EDITOR:

ERCC excision repair 6 like 2 (*ERCC6L2*) is a newly identified gene with an impact on hematological disease development. Lack of ERCC6L2 results in defects in the transcription-coupled nucleotide excision repair (TC-NER) pathway, leading to genome instability.[1] It also affects mitochondrial function, increasing reactive oxygen species levels and altering cellular homeostasis.[2]

Biallelic germline mutations in *ERCC6L2* were recently reported to cause bone marrow failure (BMF).[2–6] The first article described two consanguineous families where affected children suffered from developmental delay and microcephaly in addition to BMF.[2] Subsequent studies have, however, excluded these extra-hematopoietic manifestations from the disease phenotype.[1,3,4] Järviaho *et al.* reported the *ERCC6L2* c.1457delT, p.Ile486ThrfsTer36 mutation (NM_001010895.2, GRCh37; rs768081343) in two Finnish BMF cases.[3]

Most BMF syndromes predispose to myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Four cases of MDS and/or AML have been reported among 24 patients with biallelic *ERCC6L2* mutations.[1,4] These patients were diagnosed with MDS or AML in childhood or as young adults (aged 2-22), with only one alive at the time of original reports. [1,4] AML/MDS subtypes were not reported, however, all patients were described to carry monosomy 7. [1,4] This is a common abnormality in therapy-related, secondary, erythroid, and germline predisposed leukemias.[7,8]

The definition of acute myeloid leukemia with erythroid characteristics (AML M6 by French-American-British (FAB) classification) has been under debate.[9,10] In practice, AML M6a and AML M6b are considered as MDS or “AML, NOS, non-erythroid subtype” or “AML, NOS, erythroid leukemia (pure erythroid type)”, in the current World Health Organization (WHO) classification of myeloid malignancies.[11] For clarity we use FAB nomenclature here.

We report causality of a germline homozygous *ERCC6L2* c.1457delT, p.Ile486ThrfsTer36 mutation (Supplemental data, Figure S1) resulting in early somatic *TP53* mutations and AML with erythroid characteristics resembling AML M6.

Initially we discovered three families with a homozygous germline *ERCC6L2* c.1457delT mutation, and validated the result in a series of AML M6 patients (n=7, excluding AML M6 arising after chemotherapy or radiation treatment of another malignancy), identified in the Finnish Hematology Registry (FHR) (Figure 1B). A series of AML of other subtypes with whole exome sequencing data available (n=165) was used as a control set (Figure 1C). In the discovery families we identified six individuals with the homozygous mutation (Figure 1A), all of whom had AML M6 (n=3) or BMF (n=3) (Table 1). Only patients with BMF were alive. Additionally, one individual (#1459, Family 2), whose bone marrow morphology data but not tissue or DNA samples were available had succumbed to AML M6.

In the series of seven other ML M6 patients, we found one patient with the same homozygous *ERCC6L2* mutation. Clinical characteristics of all *ERCC6L2*-mutated patients are detailed in Table 1 (and Supplemental data). *ERCC6L2* c.1457delT was identified heterozygous in three patients (consistent with gnomAD MAF of .005) in the control set of 165 AMLs of other subtypes. No other *ERCC6L2* mutations were present in the AML germline exomes, nor did we detect any biallelic *ERCC6L2* mutations. In summary, four of the ten tested AML M6 cases carried the homozygous *ERCC6L2* mutation in comparison to 0/165 in the control group of other subtypes of AML ($P = 9.734\times10^{-5}$, only statistically independent cases (n=3) included). We also investigated germline *ERCC6L2* variants in 10,389 cancer patients (including 142 AML cases) available in the TCGA PanCanAtlas dataset.[12] No homozygous protein-truncating rare (<5% MAF) variants were found.

Somatic tumor protein p53 (*TP53*) mutations are prevalent in AML M6 at 36% compared to 11% in other AML subtypes.[9,13] Two out of three BMF cases and all AML patients with biallelic *ERCC6L2* mutation had acquired somatic *TP53* mutations in their bone marrow each, already before AML diagnosis (Table 1). No other somatic mutations in myeloid genes with recurrent mutations in AML or MDS were found.

Median age at diagnosis of AML M6 in our *ERCC6L2*-mutated patients was 49 years (39 years if including #1459). In the other AML M6 patients the median age was 67 years, consistent with previously reported median of 68 years.[13] Despite the lower age of leukemia onset in *ERCC6L2*-mutated patients, no one survived, which reflects the dismal prognosis of AML M6 diagnosis.[7] None of the AML M6 patients were known to suffer from

BMF preceding leukemia and no blood count data is available from the time before AML diagnosis, however, relatives of #1439 and #1459 reported both individuals having suffered from anemia in their youth. This may be explained by the (symptomless) mild cytopenias sometimes observed in BMF.

Although a notably high penetrance was observed here, a larger sample series is needed for a more refined assessment of penetrance. The identified *ERCC6L2* mutation seems to be a founder mutation in Finland (gnomAD Finns MAF=.005 vs global MAF=.0005) and may be enriched in certain areas explaining the inheritance pattern in Family 2 with ancestors from the same region (Figure 1).[14]

We also identified individuals in earlier phases of the disease continuum from BMF to leukemia. Interestingly, two out of the three BMF patients had somatic *TP53* mutations representing clonal evolution. This was also reflected in the *ERCC6L2*-mutated AML M6 cases as they all were carriers of one or two somatic *TP53* mutations at the time of leukemia diagnosis, suggesting a strong positive selection. *TP53* alterations in AML M6 are not rare but we suggest that, at least in the setting of ERCC6L2-driven leukemogenesis, they represent the early steps towards malignancy and lead to poor leukemia therapy results. This may be similar to Shwachman-Diamond syndrome, which is another well-known BMF syndrome with strong leukemia predisposition.[15,16] The order of molecular changes is in contrast to e.g. 5q- MDS's where *TP53* alterations are thought to occur after chromosomal rearrangements.[8] How ERCC6L2-deficiency predisposes to *TP53* mutations warrants further studies. Notably, recent reports demonstrate the high impact of somatically mutated *TP53* clones in the dynamics of leukemogenesis.[17,18]

We report a direct association of a homozygous truncating germline mutation in *ERCC6L2* with a specific high-risk leukemia subtype characterized by *TP53* mutation(s) and erythroid predominance resembling AML M6 by FAB classification. Certain genes have been previously associated with AML M6 predisposition but the study families have also presented with other types of leukemias and/or hematological malignancies indicating a less lineage-restricted predisposition.[19,20] To our knowledge, this is the first time a germline alteration is suggested to cause a strictly specific subset of acute leukemia. In the era of precision medicine, our findings suggest that AML with somatic *TP53* mutations and

erythroid predominance stemming from biallelic *ERCC6L2* mutations forms a new entity of AML within myeloid neoplasms with germline predisposition.

Our families have acknowledged for years that acute myeloid leukemia with a dismal prognosis runs among them. This study has finally discovered the culprit and has also given a relieving “verdict” for some family members. Based on our findings and previous reports on *ERCC6L2*-driven BMF, we suggest hematologists to consider careful follow-up and prompt planning of HSCT being thus far the only potentially life-saving possibility for *ERCC6L2*-deficient patients with BMF at risk of leukemia.

The study was approved by the Ethics Committee of Helsinki University Hospital (#206/13/03/03/2016 and #303/13/03/01/2011). All living subjects have given an informed written consent to participate in the study.

Acknowledgements

This study was supported by grants from Academy of Finland (#274474, #312492, and #284538), Helsinki University Hospital Comprehensive Cancer Center Research Funding, Cancer Society of Finland, Signe and Ane Gyllenberg Foundation, and Väre Foundation for Pediatric Cancer Research. This study couldn’t have been carried out without extremely skillful technical help by Lotta Katainen. We would like to thank L. A. Aaltonen and P. Vahteristo for valuable comments on the manuscript. We are extremely grateful to the patients and their families for participating in this study.

S.P.M.D. is a PhD candidate at University of Helsinki. This work is submitted in partial fulfillment of the requirement for the PhD.

The sponsors of this study are public or nonprofit organizations that support science in general. They had no role in gathering, analyzing, or interpreting the data.

Authorship

Contribution: S.P.M.D. analyzed the whole exome sequencing data, P.S. and S.P.M.D. did the capillary sequencing and analyzed the results, P.K. re-examined the BM aspirate slides and biopsy specimen. U.W-K. collected the patient samples and clinical data together with M.P., S.Kakko, E-

R.S., U.S. and K.O.. S.Kytölä analyzed and interpreted the NGS panel data. E.P. did the statistical analysis and analyzed the online datasets for truncating *ERCC6L2* mutations. K.P. provided the control exome data. S.P.M.D. drafted the manuscript. O.K. and U.W-K. designed the study, supervised the experiments and finalized the manuscript. All authors revised and approved the final version of the manuscript.

Conflict-of-interest disclosure:
M.P.: Travel, Accommodations, Expenses: Amgen, Pfizer
U.S.: Consultancy: Mylan;
K.O.: Travel, Accommodations, Expenses: Novartis, Novartis Oncology, Astra Zeneca:
U.W.-K.: Consultancy: Pfizer, Sanofi-Genzyme;
The other authors declare no competing financial interests.

Correspondence: Outi Kilpivaara, Applied Tumor Genomics, Research Programs Unit, University of Helsinki, Biomedicum Helsinki 1, Rm B322b, PO Box 63, 00014 University of Helsinki, Helsinki, Finland; e-mail: outi.kilpivaara@helsinki.fi; and Ulla Wartiovaara-Kautto, Helsinki University Hospital, Comprehensive Cancer Center, Department of Hematology, PO Box 372, 00029 HUS, Helsinki, Finland; e-mail: ulla.wartiovaara-kautto@hus.fi

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predisposition to erythroid MDS/erythroleukemia. *Leukemia*. 2016;30:2242.

Table 1. Patients with a homozygous *ERCC6L2* mutation.

Family	Patient	Disease course	Age at hematologic diagnosis	Previous conditions	Family history of malignancies	Bone marrow karyotype	Somatic mutations in bone marrow			Somatic mutation analysis
							FLT3/ NPM1	*TP53,* VAF	Other	
1	**1445**	MDS -> AML M6 -> relapse soon after allogeneic HSCT, deceased	MDS at 38, AML M6 at 39	No	Father died of CRC at 50's; paternal cousin died of pancreatic cancer at 40's; #1458	Hypodiploid 41-43, -5, -7 -17, -18, -19, -20	-/-	c.532C>G p.(His178Asp) 35%	None	Whole exome sequencing
1	**1458**	MDS -> AML M6 -> relapsed on chemotherapy, deceased	MDS at 36, and AML M6 at 37	Melanoma in situ x 2 (operated)	Father died of CRC at 50's; paternal cousin died of pancreatic cancer at 40's; #1445	Hypodiploid 43; -7, -12, 5q-	-/-	c.517G>A p.(Val173Met)	N/A	Capillary sequencing of TP53
2	**1450**	BMF, alive	14	No	Two paternal aunts died of AML M6	CN	-/-	None	None	NGS panel
2	**1439**	AML M6 -> relapsed 13 months after allogeneic HSCT, deceased	59	No	Sister (#1459) died of AML M6; other sister (#1463) has BMF	t(3;12;?),t(12;?), -7,-5, t(5;?)	-/-	c.577C>T p.(His193Tyr), c.818G>A p.(Arg273His)	N/A	Capillary sequencing of TP53
2	**1459***	AML M6-> refractory disease, deceased	38	No	Sister (#1439) died of AML M6; other sister (#1463) has BMF	Complex (specific data N/A)	N/A	N/A	N/A	N/A
2	**1463**	Mild neutropenia and thrombocytopenia -> BMF, alive	cytopenias at 47, BMF at 59	No	Two sisters (#1459 and # 1439) died of AML M6	CN	-/-	c.743G>A p.(Arg248Gln) 5% , c.830G>T p.(Cys277Phe) 23%, c.843C>A p.(Asp281Glu) 11%	None	NGS panel
3	**1438**	AA, spontaneous recovery -> marginal neutropenia and thrombocytopenia and severe BMF 20 years later	AA at 11, mild cytopenias at 20, BMF at 31	Cerebral vein thrombosis, Rathke's cyst	Two cousins (mother's side) with some hematological symptoms, grandmother died of leukemia NOS, 6/7 of grandmother's siblings died of solid tumors, 2/13 father's siblings died of cancer (CRC and liver).	CN	-/-	c.659A>G p.(Tyr220Cys) 31%	None	NGS panel
4	**1443**	AML M6 -> relapsed soon after allogeneic HSCT, deceased	AML M6 at 65	Tubular adenoma with dysplasia in rectum at 59	Sister died of AA at 34	42-46; Del(5)(q31), Dup(5)(q31)/t(5;5), -7, 11q23/MLL amplification or translocation; -4	-/-	c.818G>A p.(Arg273His), c.856G>A p.(Glu286Lys)	N/A	Capillary sequencing of TP53

All *TP53* mutations reported in NM_000546.5. Variant allele frequency (VAF) not available for capillary sequencing data. Only hot spot exons 5-9 were checked with capillary sequencing (see supplemental methods). AA indicates aplastic anemia; AML, acute myeloid leukemia; BM, bone marrow; BMF, bone marrow failure; CN, normal chromosomes; CRC, colorectal cancer; HSCT, hematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; M6, acute erythroid leukemia (FAB); NOS, not otherwise specified; N/A, not available; -, mutation negative; +, mutation positive; *not tested.

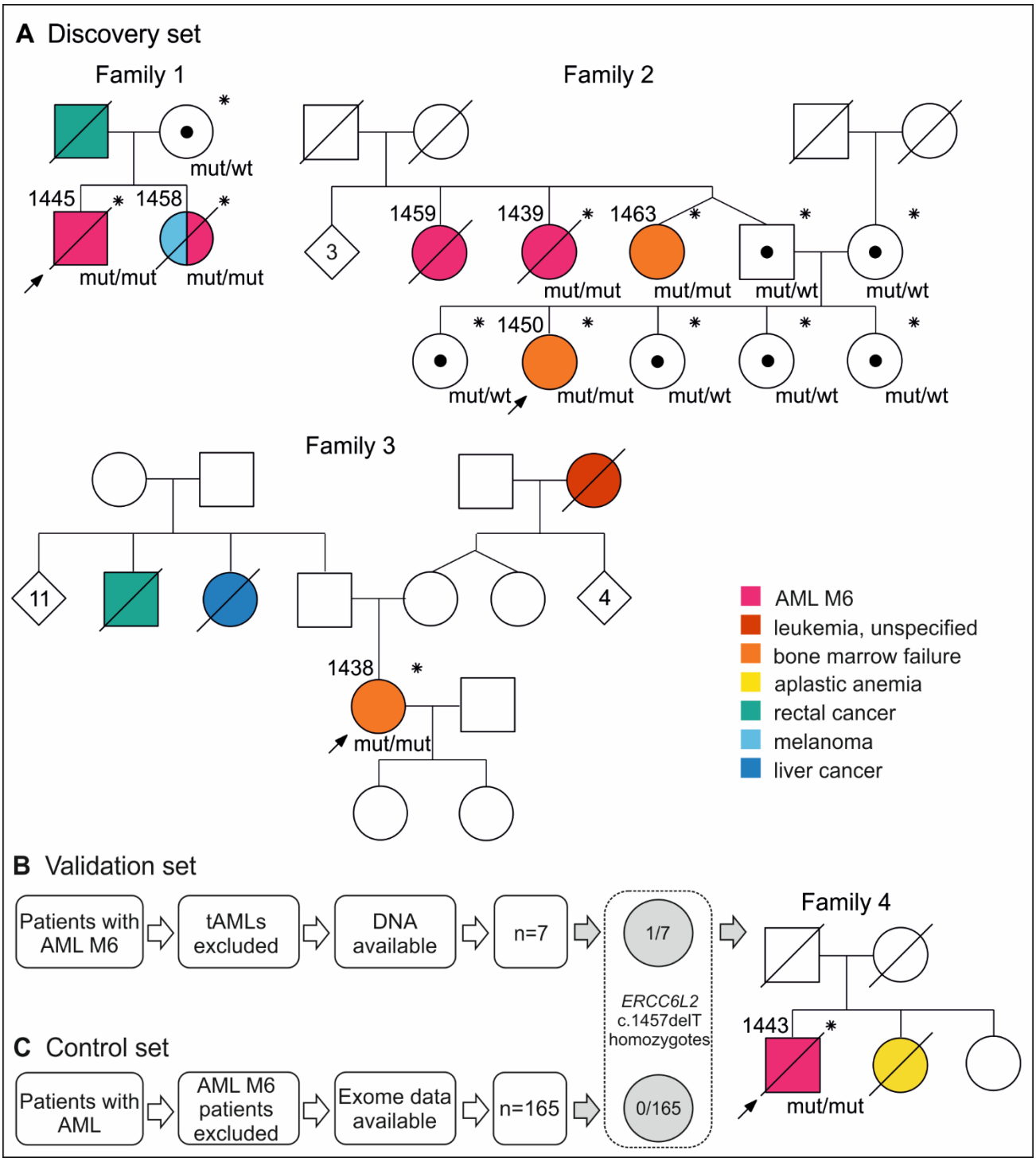

Figure 1. Families in this study and the selection process of patient data. Individuals that have been genotyped for the *ERCC6L2* c.1457delT mutation are marked with an asterisk (*). Black dots (•) represent heterozygous carriers of the mutation. Probands in each family are marked with arrows. AML indicates acute myeloid leukemia; mut, mutated; tAML, therapy-related AML and wt, wild type. (A) Families (1-3) in the discovery set. *Family 1:* #1445 was 38 years old when referred to hematologist due to pancytopenia. His bone marrow (BM) was dysplastic with strong erythroid predominance and an excess of myeloid blasts. Aiming at allogeneic hematological stem cell transplantation (HSCT) his 36-year-old sister (#1458) was examined as a donor candidate. Tests revealed peripheral blood cytopenias. The following BM examination revealed MDS which quickly progressed to AML M6. She died of refractory leukemia. #1445 underwent HSCT from a registry donor but relapsed quickly with a therapy-resistant AML M6 and succumbed to the disease. *Family 2:* The index patient (#1450) aged 18 was diagnosed with bone marrow failure (BMF) of unknown origin and referred to the hematology department in 2018. Her two paternal aunts (#1459 and #1439) had deceased of AML M6. The twin sister (#1463) of the index's father had mild thrombocytopenia and was diagnosed with BMF and three acquired *TP53* mutations along with this study. *Family 3:* #1438 had spontaneously recovered from aplastic anemia in her childhood. At 31 years, while pregnant, she was identified to suffer from persistent thrombocytopenia. An NGS myeloid gene panel on her peripheral blood sample detected a somatically mutated *TP53* clone. BM samples showed severe BMF. (B) Analysis of the validation set. One (#1443) out of seven AML M6 patients was found homozygous for *ERCC6L2* c.1457delT. *Family 4:* #1443 was 65 when diagnosed with AML M6. His sister had succumbed to severe aplastic anemia (or bone marrow failure) at a young age. (C) No *ERCC6L2* c.1457delT homozygotes were found in the control set of 165 AML patients with other subtype.