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The Clinical Picture of the ERCC6L2 Disease - from Bone Marrow Failure to Acute Leukemia

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Abstract:
Biallelic germline *ERCC6L2* variants strongly predispose to bone marrow failure (BMF) and myeloid malignancies characterized by somatic *TP53*-mutated clones and erythroid predominance. We present a series of 52 subjects (35 families) with *ERCC6L2* biallelic germline variants collected retrospectively in 11 centers globally, including follow-up of 1165 person-years. At initial investigations, 32 individuals were diagnosed with BMF and 15 with a hematological malignancy (HM). Subjects presented with 19 different variants across *ERCC6L2*, and we identified a founder mutation c.1424delT in the Finnish patients. The median age of subjects at baseline was 18 years (range 2-65). Changes in complete blood count (CBC) were mild despite severe bone marrow hypoplasia and somatic *TP53* mutations, with no significant difference between subjects with or without (HM). Signs of a progressive disease were increasing *TP53* variant allele frequency, dysplasia in megakaryocytes and/or erythroid lineage, and erythroid predominance in bone marrow morphology. The median age at onset of HM was 37.0 years (95% CI: 31.5–42.5; range 12–65). Overall survival (OS) at 3 years was 95% (95% CI: 85–100) and 19% (95% CI: 0–39) for patients with BMF and HM, respectively. Patients with myelodysplastic syndrome or acute myeloid leukemia with mutated *TP53* undergoing hematopoietic stem cell transplantation had a poor outcome: 3-year OS is 28% (95% CI: 0–61). Our results demonstrate the importance of early recognition and active surveillance of patients with biallelic germline *ERCC6L2* variants.

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Key points

- ERCC6L2 disease causes bone marrow failure requiring timely stem cell transplantation before progressing to a high-risk myeloid malignancy.
- Frequent somatic *TP53* mutation screening and bone marrow examinations are essential in the surveillance of ERCC6L2 patients.

Abstract

Biallelic germline *ERCC6L2* variants strongly predispose to bone marrow failure (BMF) and myeloid malignancies characterized by somatic *TP53*-mutated clones and erythroid predominance. We present a series of 52 subjects (35 families) with *ERCC6L2* biallelic germline variants collected retrospectively in 11 centers globally, including follow-up of 1165 person-years. At initial investigations, 32 individuals were diagnosed with BMF and 15 with a hematological malignancy (HM). Subjects presented with 19 different variants across *ERCC6L2,* and we identified a founder mutation c.1424delT in the Finnish patients. The median age of subjects at baseline was 18 years (range 2–65). Changes in complete blood count (CBC) were mild despite severe bone marrow hypoplasia and somatic *TP53* mutations, with no significant difference between subjects with or without HM. Signs of a progressive disease were increasing *TP53* variant allele frequency, dysplasia in megakaryocytes and/or erythroid lineage, and erythroid predominance in bone marrow morphology. The median age at onset of HM was 37.0 years (95% CI: 31.5–42.5; range 12–65). Overall survival (OS) at 3 years was 95% (95% CI: 85–100) and 19% (95% CI: 0–39) for patients with BMF and HM, respectively. Patients with myelodysplastic syndrome or acute myeloid leukemia with mutated *TP53* undergoing hematopoietic stem cell transplantation had a poor outcome: 3-

year OS is 28% (95% CI: 0-61). Our results demonstrate the importance of early recognition and active surveillance of patients with biallelic germline *ERCC6L2* variants.

Introduction

Excision repair cross-complementing 6 like 2 (*ERCC6L2*) is one of the most recently discovered genes linked to inherited bone marrow failure (BMF). The ERCC6L2 protein contributes to nucleotide excision repair and non-homologous end joining.[1-7] Additionally, ERCC6L2-depleted cells have been shown to exhibit increased reactive oxygen species levels, and ERCC6L2 is suggested to play a role in mitochondrial function.[1] Recessively inherited ERCC6L2 disease ranks highly among the drivers of BMF syndromes.[4] Like most of the BMF-causing germline gene defects, biallelic variants in *ERCC6L2* also predispose patients to development of myeloid malignancies.[8] We previously reported that all ERCC6L2-driven hematological malignancies (HM) harbored somatic *TP53* mutations, and the somatic mutagenesis seemed to occur already in the BMF phase.[8] Concerningly, the *TP53* mutations surreptitiously lead to a HM with extremely poor survival.[9] In distinction from other BMFs with leukemia predisposition, acute myeloid leukemias (AMLs) stemming from ERCC6L2 disease seem to be restricted to erythroid lineage.[8] The propensity for developing myeloid malignancy with *TP53* mutations in ERCC6L2 disease renders it a hematological disorder with extremely high-risk for morbidity and mortality.

Since the first depiction of patients with defective *ERCC6L2*, altogether 37 cases with biallelic germline *ERCC6L2* variants have been described in the literature (including 14 patients from Finland).[1-3,10-15] In prior studies among Finnish patients, all cases have been homozygous for the variant *ERCC6L2*(NM_020207.7): c.1424delT (p.Ile475ThrfsTer36,

rs768081343).[8,10] Moreover, a twenty-times-higher minor allele frequency of the variant in the Finnish population compared to the rest of the Europeans[16] suggests an accumulation of ERCC6L2 disease due to genetic drift as recognized in Finnish disease heritage (FDH).[17] Nevertheless, ERCC6L2 disease is not limited to Finland.

In this study, our multinational study group brings together detailed clinical and molecular features of both novel and previously identified ERCC6L2 patients (n=52). Our aim is to highlight the typical diagnostic clues and the course of ERCC6L2 disease providing clinicians means for recognition and planning of interventions in a timely manner.

Methods

Study Design

The study was approved by Helsinki University Hospital Ethics Committee (#206/13/03/03/2016 and #303/13/03/01/2011) and local Institutional Review Boards. All study subjects provided written informed consent. A retrospective chart review was performed in eleven centers globally. Individuals with a genetic diagnosis of biallelic germline variants in *ERCC6L2* were included with no additional restrictions (n=50). Two Finnish individuals had deceased prior to genetic confirmation but were additionally considered in the study. One presented with severe aplastic anemia (AA) and the other with AML M6. They had one and two siblings with verified biallelic *ERCC6L2* variants (Supplemental Table 1). The investigators from multiple centers reviewed the medical records and pseudonymized the patient data. Bone marrow (BM) examinations, including defining the BM cellularity, were carried out in each participating center according to local practices (detailed original hematopathology reports were available for most of the Finnish

patients, n=19/23). The disease courses and treatment responses are reported as defined by the local treating physicians. Family pedigrees were studied when possible (n=26/35 families). We performed a genealogical study to explore the possible founder effect of the variant *ERCC6L2* c.1424delT in Finland in accordance with previously described criteria.[18] We defined families as consanguineous if the parents were second cousins or closer.[19]

In this study, patients with a diagnosis of AA or a mention of hypocellular BM were defined as BMF. The MDS diagnoses were classified by the World Health Organization classification in 2016.[20] The diagnosis of AML was reported according to the standardized European LeukemiaNet 2022 criteria,[21] and if of erythroid root (AML M6) by morphological French-American-British classification. We considered a relative increase in the erythroid lineage of at least 50% of total BM cells as erythroid predominance. Individuals with biallelic *ERCC6L2* variants, but without a diagnosis of BMF, MDS, or AML, were included in the statistical analyses if the result of a BM examination was available.

Statistical analyses

We evaluated differences in continuous variables with an unpaired t-test and categorical variables with Chi-square test. All tests were two-tailed. P-values <0.05 were considered statistically significant. We studied the overall survival probability and the age of onset of HM by calculating Kaplan-Meier curves and compared with the log-rank test. We used Cox proportional hazard models for hazard ratios. Initial contact with a hematologist was set as the first time point in the follow-up. Death from any cause was defined as an event and surviving patients were censored on the last day known to be alive. Among the patients with

157 an initial diagnosis of BMF, we observed only two deaths during the follow-up, and
158 therefore report the mean survival time for them.
159
160 Data Sharing Statement
161 The data supporting the findings of this study is available upon request from the
162 corresponding authors (O.K., outi.kilpivaara@helsinki.fi and U.W.-K., ulla.wartiovaara-
163 kautto@hus.fi).
164
165 **Results**
166 Baseline characteristics of the subjects with biallelic *ERCC6L2* variants
167 The study included 52 individuals with *ERCC6L2* disease from nine countries and ten
168 different ethnic groups (including 33 previously reported cases). Table 1 and Figure 1
169 summarize the patient characteristics and the *ERCC6L2* mutation types. Out of the 35
170 families, 12 (34%) were consanguineous. The median age at referral to a hematologist was
171 18 years. There was no change in the median age even if individuals with biallelic *ERCC6L2*
172 variants but without diagnosis of BMF, MDS, or AML, were excluded. The most common
173 initial diagnosis was BMF (n=32; 62%). HM was diagnosed in every fourth individual at first
174 contact with a hematologist (MDS or AML n=14; T cell acute lymphoblastic leukemia [T-ALL]
175 n=1). The individual characteristics of study subjects are presented in Table 2 and in more
176 detail in Supplemental Table 1. The median age at the diagnosis of BMF was significantly
177 lower than for a HM (12.0 years and 29.0 years, respectively, p=0.0007; Table 3). Genetic
178 testing of the patients' families identified five siblings with biallelic *ERCC6L2* variants without
179 a previous diagnosis of a hematological disease (Table 2; Supplemental Table 1).
180

The major CBC values at baseline investigation are shown in Table 3 and Figure 2. Almost all study subjects presented initially with various degrees of thrombocytopenia (93%). Additionally, we observed anemia, macrocytosis, leukopenia, and neutropenia in 64%, 53%, 72%, and 68% of the subjects, respectively. Reticulocytes were within the normal range in 83% of the subjects. Patients in malignant and non-malignant stages of ERCC6L2 disease had similar CBC values at referral to a hematologist (Table 3, Figure 2).

We studied the original hematopathological reports of 19 Finnish patients from the first BM sample (trephine biopsy n=18, aspirate n=19; Supplemental Table 2). Six out of thirteen BMF patients (46%) and four out of six patients with HM (67%) had erythroid predominance in their BM. Dysplastic features were present in at least one cell lineage in nine patients with BMF (69%) and all the patients with HM. Dysplasia was restricted to erythropoiesis and megakaryocytes. Two patients initially experienced increased reticulin fibrosis and one patient developed severe fibrosis (grade 3/3) during the follow-up.

We detected a complex karyotype, defined as at least three independent chromosomal abnormalities, in 56% of patients with HM (n=9/16, including 14 initial diagnoses and two whose disease progressed during follow-up). *TP53* mutation analysis had been performed from BM/blood for 29 patients, 90% of whom had at least one mutated clone (n=26/29, not including copy number analyses in most of the patients). The number of observed mutations varied from 0 to 4, with a median of 1. The median of *TP53* VAF was 19.0 (range 1.3–94.0%). Patients without HM had significantly smaller *TP53* clones compared to those with malignant conditions: the median VAFs of *TP53* clones were 12.0 and 38.0%, respectively (p=0.002). Definite information on whether the *TP53* mutations were mono- or biallelic was

unavailable. Two patients had *TP53* mutation VAF of ≥ 50% and one patient had a chromosome 17 deletion, presumptive for biallelic *TP53* alteration.[22] Data on the few somatic mutations other than *TP53* was available for 22/52 patients (Table 2; Supplemental Table 1).

Genealogical study

Figure 1 illustrates the *ERCC6L2* variants present in the study subjects. The various types of mutations are dispersed across the gene. We identified a founder mutation (NM_020207.7) c.1424delT (p.Ile475ThrfsTer36) in the Finnish patients (homozygotes n=22, compound heterozygotes n=1). To further study the phenomenon, we performed a genealogical study in the Finnish families. We traced the ancestors from the Finnish Population Registries and microfilm copies accessible through the National Archives of Finland. The majority of the grandparents originated from an isolated region in Northeastern Finland (Figure 4A). Four out of the nine most geographically clustered families show a distant interfamilial relationship in a small rural village confirming the founder effect of the c.1424delT variant in *ERCC6L2* in the Finnish population (Figure 4B).

Extra-hematological features

Extra-hematological features are described for some ERCC6L2 patients: four had neurologic or neuropsychiatric conditions. Two of the four affected were from consanguineous families. Three had microcephaly. One had experienced recurrent bacterial and/or viral infections and two patients had been diagnosed with autoimmune diseases. Two adult patients had a history of solid tumor malignancy (melanoma and breast cancer, at the age of

21 and 35, respectively). The breast cancer patient suffered from a severe radiation injury following 50 Gy postoperative radiation (Supplemental Table 1).

Treatments and survival

We had treatment data available for 41 subjects. Thirteen individuals had surveillance with or without occasional transfusions as their only treatment strategy. Two BMF patients had androgen (oxymetholone and danazol) as the initial treatment. Treatment outcomes were not reported, but one of the patients underwent allogeneic hematopoietic stem cell transplantation (HSCT) subsequently. In addition, one BMF patient received immunosuppressive treatment of antithymocyte globulin, steroid and cyclosporine for eight days before the diagnosis of ERCC6L2 disease and proceeded to HSCT. A patient with MDS with fibrosis (MDS-F), treated initially with decitabine, reached a complete response prior to HSCT. One patient with MDS/AML with mutated *TP53* received investigational therapy within a clinical trial (anti-TIM3 antibody combined with a hypomethylating agent) reaching a complete morphologic remission with incomplete hematologic recovery before HSCT. All patients with AML M6 (n=6) went through induction chemotherapies (Supplemental Table 1). Three of the AML M6 patients reached remission but relapsed after HSCT (at four, seven, and 13 months, respectively) and three were resistant to chemotherapy and not transplanted (Supplemental Table 1). One patient presented with an initial diagnosis of T-ALL and achieved remission after induction chemotherapy. While continuing T-ALL therapy, the patient developed MDS/AML with mutated *TP53* and was carried to HSCT. As an initial treatment, 15 patients underwent HSCT (BMF n=9, MDS n=6).

We had follow-up data available for 40 individuals (77%) whose initial conditions were: BMF, n= 26; MDS or AML, n= 12; T-ALL, n=1; and one sibling with biallelic *ERCC6L2* mutation without a diagnosis of BMF or HM. The overall median follow-up time was 3.0 years (range 0.2–28 years). During the follow-up time, three patients with BMF developed an HM: one at four months and one at 25 years from diagnosis. For one case the progression time was not reported. In addition, three patients with an initial diagnosis of MDS progressed into AML at two months, six months, and eight months, respectively. Patients with an initial diagnosis of BMF had a mean survival of 26.7 years (95% CI 23.3–30.1; median not definable as only two patients succumbed during the follow-up) and three- and five-year overall survival (OS) of 95% (95% CI 85–100%). For MDS and AML patients, and one patient with an initial diagnosis of T--ALL who progressed to MDS/AML with mutated *TP53*, the three-year OS was 19% (95% CI 0–39) and median survival 1.6 years (95% CI 0.6–2.6) (Figure 3A). The initial diagnosis of HM (all with *TP53* status tested were *TP53*-mutated) was associated with a remarkable increase in the risk of mortality compared to the initial diagnosis of BMF (HR 34.5, 95% CI 4.3–273.8, p<0.001).

The median age at onset of a HM in all the patients was 37.0 years (95% CI 31.5–42.5; range 12–65; Figure 3B). Interestingly, patients with a homozygous *ERCC6L2* c.1424delT, Finnish founder mutation, were significantly older than other patients regarding the median age at onset of HM; 40.0 years (95% CI 36.1–43.9) and 22.0 years (95% CI 18.8–25.2), respectively (*p*=0.000026, Figure 3C). However, the OS was similar in both groups (*p*=0.267, Supplemental Figure 1).

Twenty-three subjects (44%) underwent HSCT (follow-up data available for 20/23). Two pediatric patients with BMF did not have *TP53* clones (aged 11 and 10 years at the time of testing) but all the others tested had *TP53* mutations. The median follow-up time after HSCT was 1.5 years (range 0.1–12.5). Figure 3D demonstrates the post-HSCT survival data: the mean survival time of BMF patients was 10.8 years (95% CI 7.6–13.9; n=9 with one death during the monitoring) and the median survival time was 1.9 years for those with HM at HSCT (95% CI 0.6–3.2; n=11 with six deaths during the follow-up). Patients with BMF had a one- and three-year OS of 88% (95% CI 64-100) compared to patients with HM at HSCT with one-year OS of 46% (95% CI 13–79) and two and -three-year OS of 28% (95% CI 0–61, p=0.07). We observed seven deaths after HSCT: four due to disease relapse (three AML and one MDS-F at the time of HCST) and three transplant-related mortality (two MDS and one BMF).

Discussion

Recessively inherited ERCC6L2 disease is a novel entity among life-threatening inherited BMFs. Since the first two patients were described in 2014,[1] a substantial amount of information has accumulated enabling us to draw the clinical picture of ERCC6L2 disease (Figure 5). In addition to the analysis of clinical data and foundational characteristics, we present follow-up data of 1165 person-years.

Classical inherited BMFs, such as Fanconi anemia (FA) and Shwachman-Diamond syndrome (SDS), are usually recognized in childhood. They characteristically present with extra-hematopoietic features, including visible skeletal abnormalities, suggestive of a congenital syndrome.[23] In comparison, our data propose that ERCC6L2 disease manifestations are

more disguised, leading to the identification of the underlying hematological condition later in life, usually in adolescence.

Suspicion of an underlying blood disease is generally raised based on changes in the patient's CBC. The vast majority (90%) of ERCC6L2 subjects presented with thrombocytopenia independent of the initial diagnosis. Notably in ERCC6L2 disease, we detected on average only modest alterations in CBC, which may be easily overlooked in most subjects. Furthermore, we did not observe reticulocytopenia in the patients with BMF. Despite the ambiguity of the CBC, our data shows that BM examination most often uncovered an underlying BM pathology in patients with biallelic *ERCC6L2* variants, especially evident in trephine biopsy. The inconclusiveness of the CBC in regard to the state of the BM is a phenomenon also described in SDS.[24]

A limitation of the study is the lack of central pathology review. Based on the subanalysis of hematopathological reports of 19 Finnish patients at initial investigations, we observed erythroid predominance in a considerable number of patients. The effect was more prominent in patients with HM. It is tempting to propose that the erythroid proliferation is a pathognomonic feature of ERCC6L2 disease indicating a susceptibility for HM of erythroid root (Figure 5).[8] Furthermore, we note a shift from hypocellular BM towards hypercellularity in patients with or progressing to a HM. Another potential ERCC6L2-specific feature is a tendency for developing secondary reticulin fibrosis in the BM, which was detected in six cases (Supplemental Table 1). The importance of the BM niche was advocated in a recent study using both patient samples and *ERCC6L2*-silenced cells which suggested that biallelic

321 *ERCC6L2* mutations also affect the mesenchymal stromal cells, which are paramount for
322 fibrogenesis.[14]
323
324 A prototype for somatic rescue is described in SAMD9/9L -mutated MDS with a nonrandom
325 loss of chromosome seven, which was identified in 61% of the patients in a recent study.[25]
326 Our data demonstrates that identifying somatically *TP53*-mutated clones at the initial
327 presentation is essential. The mutagenesis occurs in ERCC6L2 patients already at the stage
328 of BMF. Similar to SDS, we hypothesize that *TP53* mutations in ERCC6L2 disease act in
329 somatic restoration,[26] a phenomenon in which acquired somatic genetic alterations can
330 improve failing hematopoietic fitness[27]. We observed no other recurrent somatic mutations,
331 but further genetic studies are needed to validate our observations. Supporting the
332 hypothesis of somatic genetic compensation, at least three patients had experienced severe
333 pancytopenia in their childhood/teens but later regained a normal CBC (Supplemental Table
334 1). On the other hand, *TP53* mutagenesis most likely contributes to the strikingly younger
335 median age of onset of HM at 37 years in ERCC6L2 patients, in comparison to the median
336 age of onset at 60–70 in patients with MDS or AML with mutated *TP53*, but without
337 inherited predisposition.[9]
338
339 We found no specific genotype-phenotype association in ERCC6L2 disease. Notably, subjects
340 with homozygous c.1424delT mutation, the Finnish founder mutation, were significantly
341 older at the onset of HM than subjects with other biallelic *ERCC6L2* variants. Although the
342 age of onset for HM is different, the disease courses including *TP53* mutations and
343 aggressive HM with complex karyotype were alike across the ERCC6L2 genotypes. Selection
344 bias may, however, skew the results since ERCC6L2 disease is a novel entity among the

inherited BMF syndromes, and individuals with modest disease patterns may yet to be discovered. With increasing recognition of ERCC6L2 disease we aspire to clarify the genotype-phenotype correlation in more detail and in all the disease stages.

From our data, we were unable to conclude syndromic features in ERCC6L2 disease. First reports describe neurological involvement in subjects with biallelic *ERCC6L2* mutations in consanguineous families.[1,2] Thereafter, our studies among Finnish patients did not detect neurological symptoms.[8,10] Similarly, the great majority of subjects in this study (92%) presented no neurologic or neuropsychiatric symptoms. We suspect that neurological defects may be a result of reduced heterozygosity in consanguineous families, and we recommend awareness of these features. Regarding further extra-hematopoietic involvement, we marked only two ERCC6L2 patients with solid malignancies. Therefore, it is likely that cancer predisposition of ERCC6L2 disease is limited to HMs. However, considering the relatively young median age of disease onset and limited follow-up time, further surveillance and re-visiting the solid tumor predisposition is warranted.

We also want to highlight the young female patient with breast cancer who suffered from extreme radiation toxicities. ERCC6L2 involvement in DNA repair is well-established in the literature[1-7] and radiosensitivity in the loss of ERCC6L2 has also been reported in human haploid cell line HAP1.[6] Additionally, Zhang *et al*[2] observed that fibroblasts from a patient with biallelic *ERCC6L2* germline variant showed increased sensitivity to ionizing radiation. Although a single case in our series, the defective ERCC6L2 may translate to a tendency for excessive chemotherapy- or radiation-related toxicities and further monitoring of adverse events is needed. Susceptibility to infections or autoimmune conditions were not over-

represented in our study, noted only in three subjects. Nonetheless, Liu *et al*[5] showed that ERCC6L2 is required in immunoglobulin class-switching in murine B cell lines, suggesting that immunological impacts should be further investigated in ERCC6L2 disease.

The median OS time from birth was 49.6 years (95% CI 36.7-62.5, Supplemental Figure 2) for all ERCC6L2 subjects, placing ERCC6L2 disease in the middle among other inherited BMFs. The reported median OS times from birth are 67 years in Diamond-Blackfan anemia, 51 in dyskeratosis congenita, 41 in SDS, and 39 in FA.[28] The overall survival of ERCC6L2 individuals was significantly dependent on the disease stage at referral. The poor survival rate of patients with *TP53*-mutated myeloid malignancy is known and it is also reflected in our data. Similar OS is reported in SDS patients with MDS or AML.[29] Contributing to the same notion regarding disease rigor, most observed deaths were due to HM.

Myeloid malignancies with *TP53* mutations remain almost incurable.[9] Similarly to SDS,[30] this poses a major challenge in ERCC6L2 disease and performing allogeneic HSCT prior to HM ameliorates the prognosis. The survival data for patients who underwent HSCT is limited and needs to mature as the surveillance time is still relatively short (median 1.5 years) and the number of study subjects transplanted is small thus far. The timing of HSCT is essential and should be weighed together with excessive susceptibility for toxicities associated with DNA repair defects in general and transplant-related complexities, but also with individual psychosocial circumstances. Furthermore, clinically asymptomatic siblings considered as potential stem cell donors should be thoroughly examined.

While walking a tightrope with the timing of HSCT, ERCC6L2 patients require surveillance with repetitive blood and BM analyses, including the screening of *TP53* mutations. Uniformly with SDS, the CBC abnormalities may be minor or even absent, despite disease progression.[29] Thus, regular BM examination is needed to gauge the potential progression of the disease by detecting prominent changes in cellularity, increasing erythroid predominance or dysplasia, and somatic *TP53* mutations. Reticulin content is only shown in a trephine biopsy, which is also more accurate in the assessment of BM cellularity. Despite the potential discomfort for the patients, we suggest annual BM analysis (preferably with trephine biopsy) at least for those with *TP53* mutation(s). Regular BM monitoring is supported by Myers *et al*[29] who showed better OS for SDS patients with BM surveillance prior to development of malignancy compared to those without.

In this study, ERCC6L2 disease was markedly over-represented among individuals of Finnish ethnicity (44%), as compatible with the definition of FDH diseases. The allele frequency (AF) of c.1424delT in the Finnish population is 0.6%. In comparison, the European non-Finns AF is 0.0029%, indicating the allele enrichment in Finland (gnomAD v.3.1.2.).[16] Typical for FDH diseases, the AF is even higher in the Northeastern part of Finland, reaching up to 1.36% (Finngen DF5).[31] Additionally, we recognized 15 distinct Finnish core families in our study fulfilling the criteria for FDH diseases. Here, we conclude that the *ERCC6L2* c.1424delT is a Finnish founder variant and ERCC6L2 disease is the first cancer syndrome that can be added to the FDH.

In summary, our study draws the continuum of ERCC6L2 disease from an inconspicuous BMF to a dire HM (Figure 5). The findings indicate the importance of early recognition and active surveillance of patients with biallelic germline *ERCC6L2* variants. Until the

development of novel versatile therapies, HSCT is the only potentially curative treatment for ERCC6L2 disease, however, requiring a timely approach.

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Authorship Contributions

M.H. collected and analyzed the clinical data, drafted the manuscript and performed the statistical analyses; I.K. created the figures, performed the statistical analyses and contributed to the final content of the manuscript; S.P.M.D. shared collected data of Finnish patients and revised the manuscript; O.K. and U.W.-K. designed the study, coordinated data collection from multinational collaborators, and finalized the manuscript; T.Vu.; I.D.; J.S.; L.L.; R.P.L.; T.LB.; F.S.F.; T.S.; O.L.; E.H.L.; G.B.; B.T.; A.S.: F.B.; S.J.; A.A.K.; T.Z.; H.T.; C.M.; I.C.; K.J.; U.S.; R.N.; contributed to the recruitment of patients in the study and to their

management; T.Va. performed genealogical studies. All authors revised and approved the final version of the manuscript.

Conflict of Interest Disclosures

T.S. has provided consulting for Celgene, AbbVie, Janssen-Cilag, and Bristol-Myers-Squibb, and has had a congress fee provided by Novartis, Takeda; not related to this study. U.W.K. has received honoraria from Sanofi, Novartis, and Pfizer; and has provided consulting for Gilead, Pfizer, and Jazz; not related to this study. The remaining authors declare no relevant competing financial interests.

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548 **Table 1.** Summary of patient characteristics, n = 52.

Age at investigation	**Years (range)**
Median	18 (2 - 65)
Sex	**n (%)**
Female	32 (62)
Male	20 (38)
Ethnicity	**n (%)**
British Indian	1 (2)
British Pakistani	4 (8)
Druze	1 (2)
East African	3 (6)
Fijian Indian	1 (2)
Finnish	23 (44)
North African	1 (2)
Puerto Rican	1 (2)
Swedish	2 (4)
White (not specified)	15 (28)
Initial condition	**n (%)**
Sibling with biallelic *ERCC6L2* mutation without diagnosis of BMF, MDS or AML	5 (10)
BMF	32 (62)
MDS	9 (17)
MDS/AML*	2 (4)
AML	3 (6)
Other hematological malignancy (ALL)	1 (2)
***ERCC6L2* genotype**	**n (%)**
Homozygous	39 (75)
Compound heterozygous	13 (25)
Individuals by *ERCC6L2* mutation type	**n (%)**
Biallelic frameshift	29 (55)
Biallelic nonsense	5 (10)
Biallelic splicing	1 (2)
Biallelic exon deletion	4 (8)
Biallelic missense	4 (8)
Mixed	9 (17)

549 *According to the ELN2022 classification.[21]

550 Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BMF, bone
551 marrow failure; MDS, myelodysplastic syndrome.

552

553 **Table 2.** The individual characteristics of study subjects at initial investigations.

Patient ID	Family ID	Disease condition	Age (yrs)	ERCC6L2 genotype c.(NM_0202027.7)	ERCC6L2 genotype p.(NP_064592.3)	Somatic *TP53* clones in BM or PB (NM_000546.6; VAF)	Other somatic clones (VAF)
EP1*	EF1	BMF	2	c.[3409_3410del];[3763C>T]	p.[(Met1137GlufsTer7)];[(Arg1255Ter)]	N/A	N/A
EP2*	EF2	BMF	5	c.[1973G>A];[1973G>A]	p.[(Ser658Asn)];[(Ser658Asn)]	N/A	N/A
EP3*	EF3	BMF	6	c.[1930C>T];[1930C>T]	p.[(Arg644Ter)];[(Arg644Ter)]	Yes, variant N/A (4.8%)	No
EP4*	EF4	BMF	7	c.[2156del];[3675-2A>T]	p.[(Gly719AspfsTer50)];[(?)]	N/A	N/A
EP5	EF5	BMF	7	c.[3492+2T>G];[3492+2T>G]	p.[(?)];[(?)]	N/A	N/A
EP6*	EF6	BMF	8	c.[2734del];[2734del]	p.[(Glu912ArgfsTer8)];[(Glu912ArgfsTer8)]	N/A	N/A
EP7*	EF7	BMF	8	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	Not detected initially, at age 15 yrs c.638G>T p.(Arg213Leu) (3%)	N/A
EP8*	EF8	BMF	8	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	N/A	N/A
EP9	EF9	BMF	9	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	Not detected (latest screen at 10 yrs)	N/A
EP10*	EF7	BMF	9	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.742C>T, p.(Arg248Trp) (1.5%)	N/A
EP11*	EF10	BMF	10	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.659A>G, p.(Tyr220Cys) (36%); c.725G>T, p.(Cys242Phe) (3%)	No
EP12	EF11	BMF	11	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.723del, p.(Cys242AlafsTer5) (3%), c.376-2A>G (4.2%), c. 733G>A, p.(Gly245Ser) (41%)	*TET2* c.4102T>C, p.(Phe1368Leu) (2 %)
EP13	EF12	BMF	11	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	Not detected (latest screen at 11 yrs)	No
EP14	EF13	BMF	12	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.524G>A, p. (Arg175His)(7%)	N/A
EP15	EF14	BMF	12	c.[19C>T];[2156del]	p.[(Gln7Ter)];[(Gly719AspfsTer50)]	N/A	N/A
EP16*	EF2	BMF	12	c.[1973G>A];[1973G>A]	p.[(Ser658Asn)];[(Ser658Asn)]	N/A	N/A
EP17	EF15	BMF	12	c.[1930C>T];[1930C>T]	p.[(Arg644Ter)];[(Arg644Ter)]	c.742C>T, p.(Arg248Trp) (18.7%)	No
EP18*	EF4	BMF	13	c.[2156del];[3675-2A>T]	p.[(Gly719AspfsTer50)];[(?)]	N/A	N/A
EP19*	EF16	BMF	13	c.[1930C>T];[1930C>T]	p.[(Arg644Ter)];[(Arg644Ter)]	N/A	N/A
EP20*	EF17	BMF	14	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.646G>A, p.(Val216Met) (1.3%)	No
EP21*	EF18	BMF	17	c.[2156del];[3300_3303del]	p.[(Gly719AspfsTer50)];[(Asn1100LysfsTer12)]	N/A	N/A
EP22*	EF19	BMF	18	c.[1471C>T];[3796C>T]	p.[(Gln491Ter)];[(Arg1266Ter)]	N/A	N/A

EP23*	EF20	BMF	20	c.[1203_1206del];[1203_1206del]	p.[(Thr402CysfsTer2)];[(Thr402CysfsTer2)]	N/A	N/A
EP24	EF21	BMF	20	c.[1606_1751del];[1606_1751del]	p.[(Leu536GlyfsTer18)];[(Leu536GlyfsTer18)]	c.745A>T, p.(Arg249Trp) (15%); c.514G>T, p.(Val172Phe) (4%); c.737T>A, p.(Met246Lys) (2%)	No
EP25	EF22	BMF	21	c.[1606-141_1752-2788del];[1606-141_1752-2788del]	p.[(?)];[(?)]	c.569C>T, p.(Pro190Leu) (5%)	No
EP26	EF23	BMF	24	c.[1606-141_1752-2788del];[1606-141_1752-2788del]	p.[(?)];[(?)]	c.743G>A, p.(Arg248Gln) (35%)	No
EP27	EF24	BMF	30	c.[2474_2484delinsAAAG];[2474_2484delinsAAAG]	p.[(Thr825LysfsTer25)];[(Thr825LysfsTer25)]	Not detected	No
EP28	EF25	BMF	32	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.713G>A, p.(Cys238Tyr), (19%)	N/A
EP29	EF25	BMF	33	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.659A>G, p.(Tyr220Cys) (3%); later new clone c.716A>G p.(Asn239Ser) (5%)	No
EP30*†	EF26	BMF	34	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	N/A	N/A
EP31*	EF27	BMF	36	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.742C>T, p.(Arg248Trp) (15%); c.818G>T, p.(Arg273Leu) (2.5%); c.814G>A, p.(Val272Met) (2%); later new clone c.41T>C, p.(Leu14Pro) (1.5%)	No
EP32*	EF17	BMF	57	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.743G>A, p.(Arg248Gln) (5%); c.830G>T, p.(Cys277Phe) (23%); c.843C>A, p.(Asp281Glu) (11%); later new clone c.528C>G, p.(Cys176Trp) (9%)	No
EP33*	EF28	MDS	12	c.[2919_2923del];[2919_2923del]	p.[(Lys974HisfsTer3)];[(Lys974HisfsTer3)]	N/A	N/A
EP34	EF29	T-ALL	12	c.[1424del];[1930C>T]	p.[(Ile475ThrfsTer36)];[(Arg644Ter)]	c.730G>A, p.(Gly244Ser) (94%)	*TET2* c.5860G>A (p.Ala1954Thr) (52.4%)
EP35*	EF19	MDS	21	c.[1471C>T];[3796C>T]	p.[(Gln491Ter)];[(Arg1266Ter)]	N/A	N/A
EP36*	EF30	MDS	22	c.[814G>A];[814G>A]	p.[(Asp272Asn)];[(Asp272Asn)]	N/A	N/A
EP37*	EF23	MDS	22	c.[1606-141_1752-2788del];[1606-141_1752-2788del]	p.[(?)];[(?)]	Variant unknown (20%)	N/A
EP38*	EF31	MDS-F	25	c.[4421_4422del];[1424del]	p.[(Arg1474ThrfsTer10)];[(Ile475ThrfsTer36)]	c.742C>T, p.(Arg248Trp) (9%); c.818G>T, p.(Arg273Leu) (13%)	No
EP39	EF32	MDS	29	c.[1424del];[1847+1G>A]	p.[(Ile475ThrfsTer36)];[(?)]	c.524G>A, p.Arg175His (27%); c.818G>A, p.(Arg273His) (5%)	No

EP40*	EF33	MDS	36	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.517G>A, p.(Val173Met)(72%)	No
EP41	EF34	MDS	36	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.524G>A, p.(Arg175His) (6%)	*NRAS* c.38G>A p. (Gly13Asp) (2%); CBL c.1273_1281del p.(Gly425_Glu427del) (0.5%)
EP42*†	EF17	MDS	38	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	N/A	N/A
EP43*	EF33	MDS/AML	38	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.532C>G, p.(His178Asp) (35%)	No
EP44	EF35	MDS/AML	40	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.817C>T, p.(Arg273Cys) (38 %)	No
EP45	EF32	AML	20	c.[1424del];[1847+1G>A]	p.[(Ile475ThrfsTer36)];[(?)]	IHC TP53+++	NA
EP46*	EF17	AML	59	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.818G>T, p.(Arg273Leu) (VAF N/A)	N/A
EP47*	EF26	AML	65	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.577C>T, p.(His193Tyr); c.818G>A, p.(Arg273His) (VAF N/A)	N/A
EP48*	EF28	Sibling with biallelic *ERCC6L2* variants	3	c.[2919_2923del];[2919_2923del]	p.[(Lys974HisfsTer3)];[(Lys974HisfsTer3)]	N/A	N/A
EP49*	EF2	Sibling with biallelic *ERCC6L2* variants	4	c.[1973G>A];[1973G>A]	p.[(Ser658Asn)];[(Ser658Asn)]	N/A	N/A
EP50*	EF1	Sibling with biallelic *ERCC6L2* variants	11	c.[3409_3410del];[3763C>T]	p.[(Met1137GlufsTer7)];[(Arg1255Ter)]	N/A	N/A
EP51*	EF1	Sibling with biallelic *ERCC6L2* variants	18	c.[3409_3410del];[3763C>T]	p.[(Met1137GlufsTer7)];[(Arg1255Ter)]	N/A	N/A
EP52	EF35	Sibling with biallelic *ERCC6L2* variants	41	c.[1424del];[1424del]	p.[(Ile475ThrfsTer36)];[(Ile475ThrfsTer36)]	c.490A>G, p.(Lys164Glu) (9%)	No

554 * Previously reported; † Not tested

555 Note the updated transcript (NM_0202027.7), most previous studies reported variants in a previous
556 transcript version, hence the discrepancy in some cases.

557 Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; BMF, bone marrow failure; MDS,
558 myelodysplastic syndrome; N/A, not applicable; PB, peripheral blood; T-ALL, T-cell acute lymphoblastic
559 leukemia; VAF, variant allele frequency; yrs, years.

560 **Table 3.** Clinical features in ERCC6L2 subjects with and without hematological malignancy.

Variable	ERCC6L2 subjects without malignancy, n = 33	ERCC6L2 subjects with malignancy, n = 15	*P*	Data available, n
Age at diagnosis				
Median, years (range)	12 (2–57)	29 (12–65)	0.0007	33; 15
Complete Blood Count				
Median Leukocytes, 1x10⁹/L (range)	3.25 (1.3–7.7)	2.85 (1.2–5.8)	0.2891	28; 12
Median ANC, 1x10⁹/L (range)	1.16 (0.25–3.9)	0.65 (0.1–4.7)	0.6350	26; 12
Median Hemoglobin, g/dL (range)	10.80 (3.4–15)	10.10 (5.9–14)	0.1918	29; 13
Median MCV, fL (range)	101.5 (90–114)	98.0 (87–105)	0.3720	8; 5
Median Platelets, 1x10⁹/L (range)	63.50 (4–195)	80.0 (10–175)	0.9623	30; 13
Median reticulocytes, 1x10⁹/L (range)	58.0 (33.0–104.1)	29.1 (11.9–98.0)	0.0530	15; 6
TP53 status				
N of patients with TP53 clone (%)	15* (84.2)	11 (100)	0.1845	19; 11
N of mutations, range	0–4	1–2		18; 11
Median VAF % (range)	12.0 (1.3–36.0)	38.0 (6.0–94.0)	0.0020	16; 9
Bone marrow				
Hypocellular n (%)	25 (96)	4 (36)	<0.0001	26; 11
Normal n (%)	1 (4)	2 (18)		
Hypercellular n (%)	N/A	5 (45)		

561 *A clone was identified in one additional patient during follow-up

562 Abbreviations: ANC, absolute neutrophile count; MCV, mean corpuscular volume; VAF,
563 variant allele frequency.

564 **Figure legends**

565 **Figure 1. Germline *ERCC6L2* variants identified in the study subjects.** Various types of
566 mutations are detected across ERCC6L2 (NM_20207.7). A Finnish founder mutation
567 (p.Ile475ThrfsTer36) constitutes nearly half of the identified mutations. Figure created with
568 ProteinPaint proteinpaint.stjude.org.[32]

569 **Figure 2. Comparison of ERCC6L2 subjects with and without malignancy.** (A) Median age at
570 diagnosis with 95% CI, Mann Whitney test p=0.0007. (B-F) Complete blood count (CBC)
571 parameter median values in subjects with CBC data available with 95% CI, unpaired t-test
572 non-significant (ns). Blue-shaded area denotes Finnish reference values for CBC parameters.
573 Abbreviations: E6L2, ERCC6L2; ANC, absolute neutrophil count; Hb, hemoglobin; MCV, mean
574 corpuscular volume.

575 **Figure 3. Overall survival and cumulative incidence of hematological malignancy of**
576 **subjects with biallelic *ERCC6L2* variants.** (A) Kaplan-Meier curves displaying overall survival
577 of patients with biallelic *ERCC6L2* variants and initial diagnosis of bone marrow failure (BMF)
578 or myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML), including one patient
579 with T-ALL progressing to MDS/AML in this patient group. (B) Onset of a hematological
580 malignancy (HM) by age (n=48). (C) Onset of a HM by age in subjects with and without
581 homozygous mutation of *ERCC6L2* 1424delT (Finnish founder mutation). (D) Overall survival
582 of patients with biallelic *ERCC6L2* variants and allogeneic hematopoietic stem cell
583 transplantation (HSCT) according to the diagnosis at transplantation.

584 **Figure 4. Genealogical study of the Finnish families**. (A) Allele frequency (AF) distribution of
585 the *ERCC6L2* 1424delT variant in Finland and birthplaces of the grandparents of nine Finnish

586 families. Blue dots display the birthplaces of the grandparents. AFs according to the
587 gnomAD and Finngen databases.[16,31] (B) A pedigree of the four Finnish families with
588 *ERCC6L2* indices shows that the parents share a common ancestor in the 17th century.
589 Diamond symbol indicates the index.

590

591 **Figure 5. ERCC6L2 disease.** Characteristic features of ERCC6L2 disease are illustrated in a
592 three-phase continuum. In Phase 1 individuals present with hypocellular bone marrow with
593 or without small *TP53* mutated clonal hematopoiesis. Phase 2 depicts disease acceleration
594 including bone marrow failure (BMF) with increasing (possible biallelic) *TP53* mutations
595 progressing towards myeloid malignancy. Phase 3 represents overt hematological
596 malignancy with complex karyotype. Abbreviations: PB, peripheral blood; AML, acute
597 myeloid leukemia; M6, The French-American-British [25] subtype for acute erythroid
598 leukemia; MDS, myelodysplastic syndrome. Figure created with BioRender.com.

Figure 1.

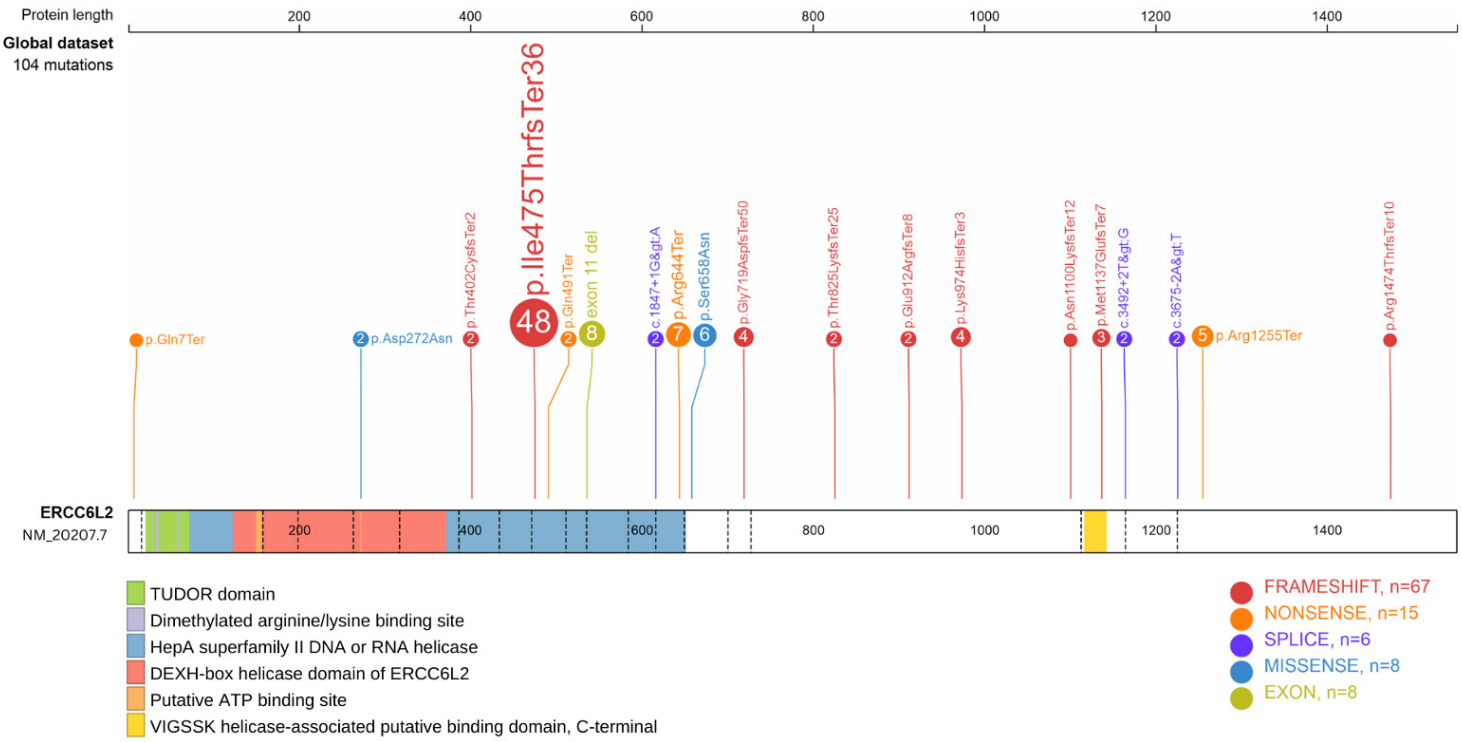


Figure 2.

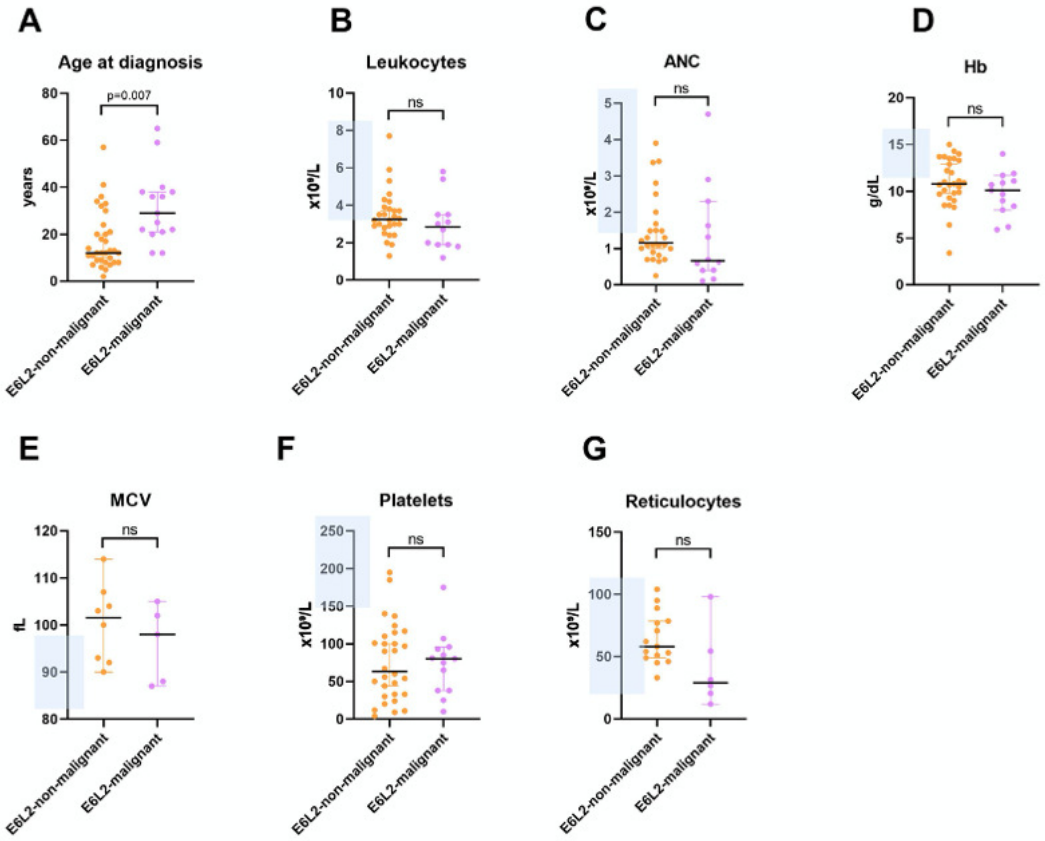
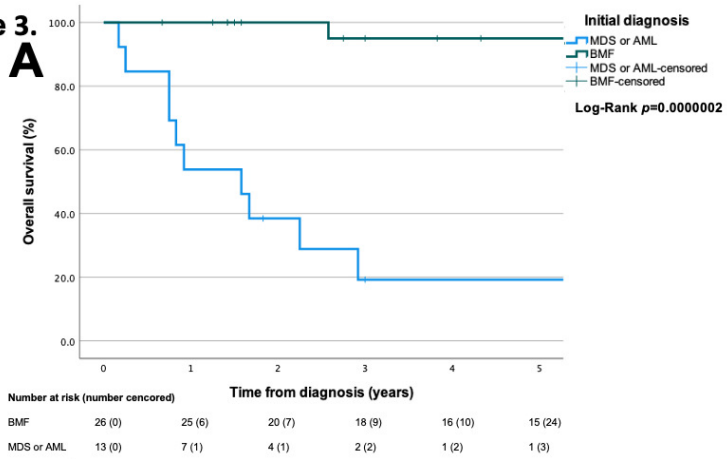
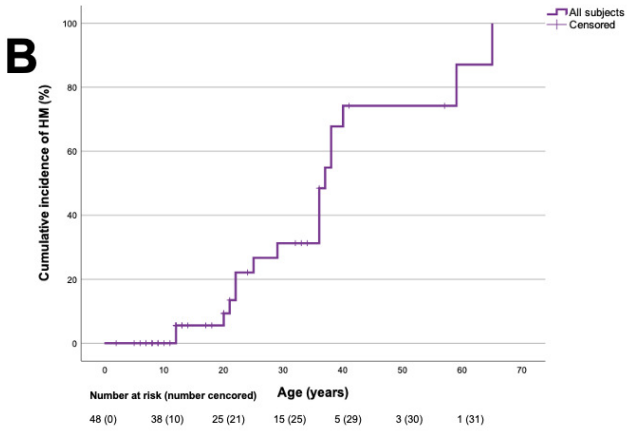
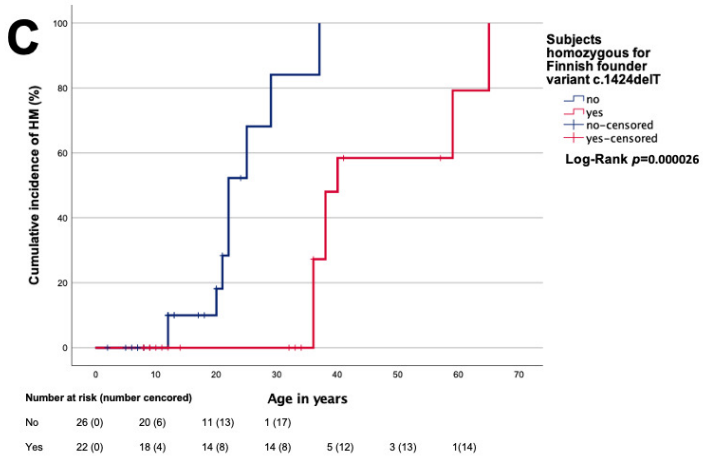


Figure 3.







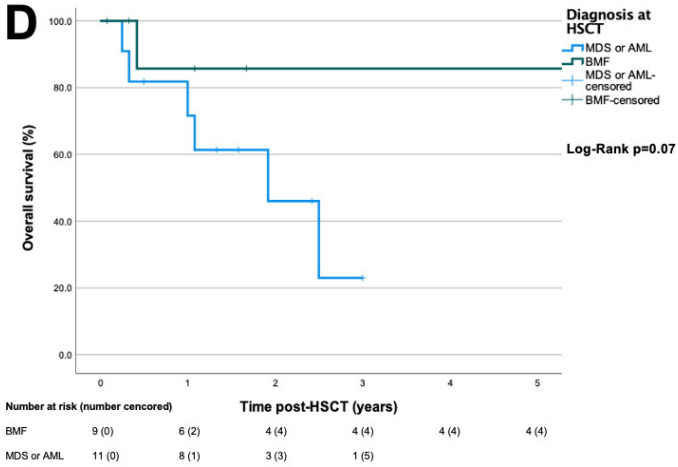
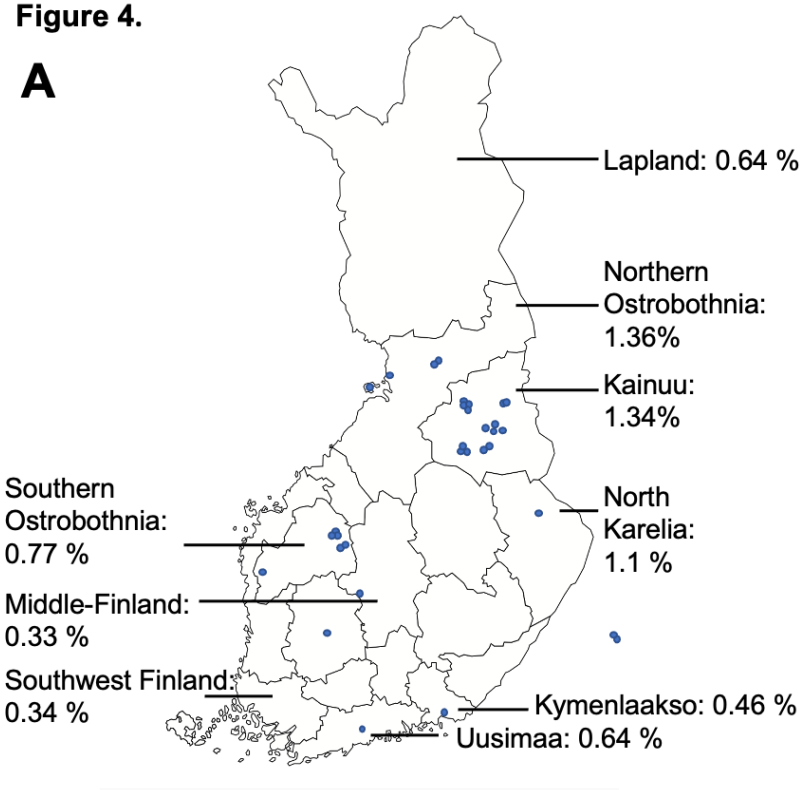


Figure 4.



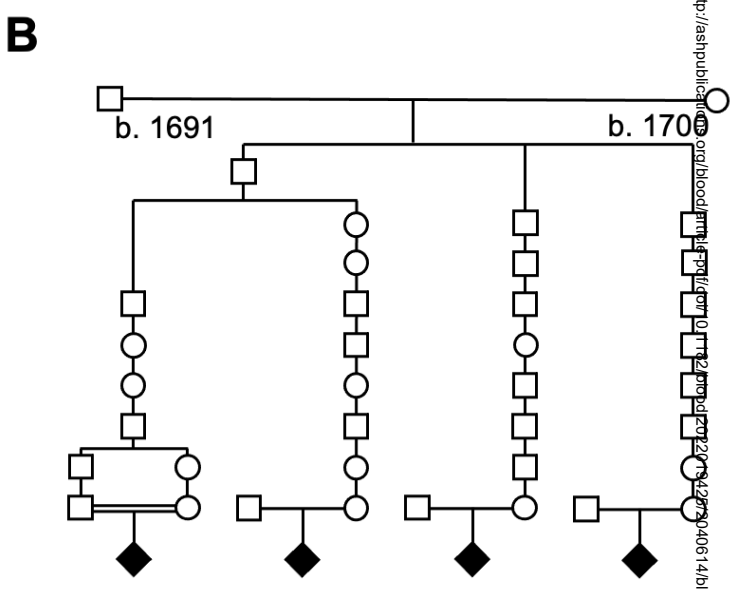


Figure 5.