Hereditary acute myeloid leukemia associated with C-terminal CEBPA germline variants

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Short Report

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Abstract

Acute myeloid leukemia with germline *CEBPA* mutation is a subtype of acute myeloid leukemia that is associated with a favorable prognosis. Most of the reported cases of acute myeloid leukemia with *CEBPA* germline variants involve a germline variant in the N-terminus and a somatic variant in the C-terminus. There are only a few reported cases where the *CEBPA* germline variant has been identified in the C-terminus and the somatic variant in the N-terminus. This case report and review of the literature illustrates that, although acute myeloid leukemia with *CEBPA* N- or C-terminal germline variants have certain similarities such as atypically young age at diagnosis, frequent relapse, and favourable overall prognosis, there are also significant differences such as lower life-time penetrance of acute myeloid leukemia and shorter time to relapse for germline C-terminal cases. These findings add important information on the natural history and clinical outcomes of acute myeloid leukemia with germline *CEBPA* C-terminal variants and these findings should be considered in the management of patients and their family members.

Introduction

The CCAAT enhancer binding protein alpha (*CEBPA*) is a single exon gene located on chromosome 19 and consists of two N-terminal transactivating domains, a basic DNA binding domain and a C-terminus bZIP leucine-zipper dimerization domain [1]. The protein product, C/EBP-α, is an important transcription factor that plays a role in the differentiation of myeloid cells and regulates the expression of granulocyte specific genes[2]. Likely pathogenic and pathogenic germline *CEBPA* variants predisposing to acute myeloid leukemia (AML) are recognized as a distinct subtype of AML [2–4]. Germline *CEBPA* variants predisposing to AML account for approximately 0.65% of all new AML cases and 7–11% of AML with bi-allelic *CEBPA* mutations, and are associated with a favorable prognosis, despite frequent relapses, compared to AML with bi-allelic *CEBPA* mutations without a germline predisposing variant[2, 5, 6]. In the majority of described AML cases with germline *CEBPA* variants, a germline variant was found on the N-terminus of *CEBPA* and a second, somatic, variant found on the C-terminus [6]. The penetrance for N-terminal *CEBPA* germline variants is nearly 100% for the development of AML[7]. There are only a few reported cases of hereditary AML associated with *CEBPA* C-terminal germline variants and therefore less is known about the prognosis for these patients. In this case report and review of the literature, we aim to add to the literature of germline *CEBPA* C-terminal variants predisposing to AML by describing a family from Atlantic Canada with a *CEBPA* C-terminus germline variant (c.932A > C, p.Gln311Pro) and summarize the clinical characteristics of all published *CEBPA* C-terminus germline variants to help further advance our understanding of this AML subtype and its implications for the management of patients and their families.

Methods

Germline *CEBPA* variant testing

Germline genetic testing for the 40-year-old male with AML was conducted using a commercial 41-gene Hereditary Leukemia Next Generation Sequencing (NGS) Panel from BluePrint Genetics. DNA was extracted from cultured skin fibroblasts and a heterozygous germline *CEBPA* c.932A>C, p.Gln311Pro was identified at a variant allele frequency (VAF) of 48%. Targeted familial variant testing for the germline *CEBPA* c.932A>C, p.Gln311Pro variant was also done through BluePrint Genetics. DNA for the familial variant testing was obtained from a peripheral blood sample and from saliva for the sister and parents, respectively.

CEBPA variant search and review of the literature

A search for published cases of AML with *CEBPA* germline variants in the C-terminal was performed using PubMed and Google Scholar. The human genomic variant search engines VarSome®, ClinVar, and Mastermind® were also used to search for reported variants in the CEBPA (NM_ 004364.5) C-terminus, where the C-terminus was defined as amino acids 278 to 358. Each reported *CEBPA* C-terminus variant was inputted into the Mastermind® search engine to identify any published articles citing the particular variant. Each article was reviewed to determine if the *CEBPA* C-terminus variant was 1) germline origin and 2) associated with AML.

Informed consent

All individuals in this case report provided written consent to be a part of an Inherited Predisposition to Hematologic Malignancies research study at our institution. This study was reviewed and approved by the Nova Scotia Health research and ethics board. Consent for publication of clinical information in medical journals for research purposes was obtained at the time of enrolment in the study.

Results

Case description

A 40-year-old Caucasian male of Scottish ancestry from Prince Edward Island, Canada, whose past medical history was significant for vitamin B12 deficiency, presented to medical attention with progressive fatigue and complete blood count (CBC) revealed normocytic anemia (hemoglobin (HGB) 83 g/L), thrombocytopenia (platelets (PLT) 93 x 10^9 g/L) and leukopenia (white blood cell count (WBC) 2.3 x 10^9 g/L) with 45% circulating blasts suggestive of acute leukemia. Bone marrow aspirate and biopsy confirmed the diagnosis of AML and ancillary testing identified normal cytogenetics (46 XY), four *CEBPA* variants, and a *TET2* variant (Table 1). Germline testing using DNA extracted from cultured skin fibroblasts was done given the patient's young age at diagnosis and identified that one of the four *CEBPA* variants [CEBPA (NM_ 004364.5) c.932A>C, p.Gln311Pro] was germline in origin. The *CEBPA* (NM_ 004364.5) c.932A>C, p.Gln311Pro variant, located on the C-terminus of CEBPA, was present in a heterozygous state at variant allele frequency (VAF) of 48%. The patient received

"3+7" (Daunorubicin-Cytarabine) induction chemotherapy and achieved a complete remission (CR), which was then consolidated with three cycles of high dose cytarabine (HiDAC) chemotherapy.

Table 1. Summary of patient's bone marrow aspirate molecular profile at time of diagnosis and at first relapse. VAF = variant allele frequency

	Molecular profile at diagnosis	Molecular profile at relapse
Germline variant	c.932A>C, p. Gln311Pro VAF 48%	*CEBPA* c.932A>C, p. Gln311Pro VAF 50%
	CEBPA c. 233_234dup, p.Ala79fs VAF 44%	*CEBPA* c. 233_234dup, p.Ala79fs VAF 25%
Somatic *CEBPA* variants	*CEBPA* c.555_593del, p. Pro186_Pro198del VAF 12% *CEBPA* c. 541delT, p.Tyr 181fs VAF 11%	
Other somatic variants	*TET2* c. 2902_290insT, p.Gln986fs VAF 8%	*TET2* c. 4160G>C, p.Arg1387Pro VAF 22%

The patient's family history was significant for a maternal first cousin once removed (II.9) who was diagnosed with breast cancer in her late 50s followed by acute leukemia in her early 60s as well as a paternal grandfather with prostate cancer (I.1) (Figure 1). The patient's younger sister (III.2), mother (II.4) and father (II.3) all consented to undergoing variant specific testing. At ages 70 and 40 years, respectively, the mother (II.4) and sister (III.2) were found to be healthy, asymptomatic, heterozygous carriers of the *CEBPA* c.932A>C, p.Gln311Pro variant whereas the father (II.3) was wild-type.

Seven months after achieving first CR, the patient's AML relapsed. Next generation sequencing at the time of relapse revealed persistence of the germline *CEBPA* c.932A>C, p.Gln311Pro variant as well as one of the previous three somatic variants, *CEBPA* c.233_234dup, p.A79fs; disappearance of the other two somatic *CEBPA* variants; and a new *TET2* variant (Table 1). Re-induction chemotherapy with fludarabine, cytarabine and filgrastim (FLAG) was given and a second CR was achieved. This was followed by a myeloablative matched-unrelated allogeneic hematopoietic stem cell transplant (HSCT) using peripheral blood stem cells. Although the patient's sister was a 10/10 human leukocyte antigen match, an unrelated donor was selected to reduce the risk of a donor derived leukemia, as the sister was known to carry the same deleterious germline *CEBPA* variant as the patient. At seventeen months post HSCT, the patient remains in a CR.

Discussion

Most of what is known about the clinical features and outcomes of AML with germline *CEBPA* variants is based on cases of N-terminal germline variants. Our case report and comprehensive review of the literature highlights that, although there are some similarities between AML with germline N- and C-terminal *CEBPA* variants, there are also important differences. To date, a total of nine unique germline C-terminal *CEBPA* variants associated with AML have been reported in eleven unrelated families (Figure 2, Table 2)[1, 5, 8–12]. Of these, the *CEBPA* c.932A>C, p.Gln311Pro variant that was identified in our case is one of only two variants to have been reported in separate, unrelated families. It is classified as a likely pathogenic variant according to the American College of Medical Genetics /Association for Molecular Pathology (ACMG/AMP) criteria[13]. Of the other eight variants, three are classified as either pathogenic or likely pathogenic and five as a variant of uncertain significance (Table 2, Figure 2) [1, 5, 8–13].

Please see separate submitted file for table 2

Table 2. Summary of the published cases of acute myeloid leukemia (AML) with germline C-terminus *CEBPA* variants [NM_004364.5]. CR= complete remission, mo = months, NA= not available, NR = not reported, NT = not tested, VUS = variant of uncertain significance, yo= year old. [1, 5, 8–13]

*Further variant details not available

†Familial AML defined as more than 1 individual in a family diagnosed with AML

In terms of similarities, deleterious germline variants in both N- and C-terminal regions of *CEBPA* predispose to *de novo* AML, without any preceding dysplastic or cytopenic phase, and compared to sporadic AML, which has a median age at diagnosis of 68 years, the affected individuals typically develop AML at a relatively young age, often less than age 50 years [2, 6, 7]. The median age at AML diagnosis for individuals with a *CEBPA* C-terminal germline variant, based on our case and those reported in the literature to date, is 30 years, with a range of 6 to 60 years old (Table 2). This median age and age range are comparable to the median age of diagnosis of 25 years and range of 2 to 50 years reported for individuals with a *CEBPA* N-terminal germline variant [2, 6, 7]. While it is challenging to determine the prognosis of AML associated with a *CEBPA* C-terminal germline variant based on the limited information in the small number of published cases, it appears that, just as in AML with *CEBPA* N-terminal germline variant, the prognosis is favorable. The ten-year overall survival (OS) for AML with *CEBPA* N- terminal germline variants is 67% [7]. In the cases of AML with *CEBPA* C-terminal germline variants, the follow up data was variable however, of the eight patients with follow up data, five (62.5%) were still alive at time of last follow up (Table 2).

Family	Individual	Diagnosis	Germline c-terminal *CEBPA* variant	ACMG/AMP Criteria	Somatic variants	Familial AML†	Penetrance	Treatment	T
Family 1	27 yo male	AML	c.890G>T, p.Arg297Leu Missense	Likely pathogenic PS4; PM1; PM2	*CEBPA* c. 936_937insCAG, p.Gln312fs	No	2/2 (100%)	NR	N
	59 yo female	AML	c.890G>T, p.Arg297Leu Missense	Likely pathogenic PS4; PM1; PM2	*GATA2* c.961C>T, p. Leu321Phe *CEBPA* c.936_937insCAG, p.Gln312fs	Yes	2/2 (100%)	NR	N
Family 2	59 yo	AML	c.890G>T, p.Arg297Leu Missense	Likely pathogenic PS4; PM1; PM2	*CEBPA* c.940_941insAAG, p.Lys313_val314insGlu	No	NA	NR	N
Family 3	58 yo male	AML	c.932A>C, p.Gln311Pro Missense	Likely Pathogenic PM1; PM2;PP1_mod	NT	Yes	6/13 (46%)	Yes	N
	20 yo female	AML	c.932A>C, p.Gln311Pro Missense	Likely Pathogenic PM1; PM2;PP1_mod	NT	Yes	6/13 (46%)	CR1 after induction Relapse #1: 9 months post-CR1 Relapse #2: 3 months post-CR2	N
	11 yo female	AML	c.932A>C, p.Gln311Pro Missense	Likely Pathogenic PM1; PM2;PP1_mod	NT	Yes	6/13 (46%)	CR1 after induction Relapse #1: 8 months post-CR1 Relapse #2: 7 months post-CR2	N
	22 yo female	AML	c.932A>C, p.Gln311Pro Missense	Likely Pathogenic PM1; PM2;PP1_mod	NT	Yes	6/13 (46%)	CR1 after induction Relapse #1: approx. 12 months after CR1	Y R #
Family 4	40 yo male	AML	c.932A>C, p. Gln311Pro Missense	Likely Pathogenic PM1; PM2;PP1_mod	*CEBPA* c.233_234dup.; p.Ala79fs *CEBPA* c.555_593del, p. Pro186_Pro198del *CEBPA* c.541delT, p.Tyr 181fs *TET2* c.2902_290insT, p.Gln986fs	Yes	1/3 (33%)	CR1 after induction Relapse #1: 7 months post-CR1	Y R #
Family 5	9 yo male	AML	c.937A>G, p.Lys313Glu Missense	VUS PM1; PM2	*CEBPA* c.908_925dup, p.Ala303_Val308dup *EZH2*, GATA2*, KIT**	No	1/2 (50%)	NR	N
Family 6	51 yo	AML	c.950T>C, p.Leu317Pro Missense	VUS PM1; PM2; PP3	*CEBPA* c.332_339del, p.ala111glyfsTer56	No	NA	NR	N
Family	11 yo	AML without	c.971T>G,	VUS	*CEBPA* c.97_112del; p.	No	NA	NR	Y

7	female	maturation	p.Leu324Arg Missense	PM1;PM2: PP3	Phe33AlafsTer122 *WT1* c.1221_1222insC, p.Phe408LeufsTer2 *GATA2* c.953C > T, p.Ala318Val *KIT* c.1914G > C, p.Met638Ile FLT3-ITD*				
Family 8	41 yo female	AML	c.985_988dup, p.Gln330ArgfsTer74 Frameshift	Pathogenic PVS1; PM1; PM2	*CEBPA* c.330-339del, p.Gly114AlafsTER43	Yes	1/4 (25%)	NR	N
Family 9	17 yo female	AML with myelodysplastic changes	c.994_998dup, p.Glu334AlafsTer90 Frameshift	Pathogenic PVS1; PM1; PM2	*NPM1* c.860_863dup, p.Trp288CysfsTer12 *TET2* c.4075C > T, p.Arg1359Cys *FLT3-ITD**	Yes	NA	NR	Y
Family 10	33 yo	AML	c.1018G>A, p.Gly340Ser Missense	VUS PM1; BP4	*FLT3^{TKD}** *NPM1**	No	NA	NR	N
Family 11	60 yo female	AML	c.1073del, p.Ala358GlyfsTer64 Frameshift	VUS PVS1_mod;PM2	*CEBPA* c.937_939dup, p.Lys313dup	No	1/2 (50%)	CR achieved after 2nd induction	N

While the prognosis for AML with germline *CEBPA* mutation is favorable, there are high rates of AML relapse, including both relapse of original disease or occurrence of a second *de novo* AML. There is an estimated 56% incidence of AML relapse at 10 years in individuals with a *CEBPA* N-terminal variant [7]. Although the number of reported cases is low, from our literature review, the rate of AML relapse in individuals with a *CEBPA* C-terminal variant was lower, at 27%, but with a shorter median duration of follow up of 2 years (range 7 months to 11 years) (Table 2). Another notable difference was the time to first relapse; with C-terminal germline variant patients having a shorter time to first relapse, with a median of nine months (range 7-12 months), compared to those with an N-terminal variant, with a median of 27 months [7].

The most distinctive difference, however, between AML associated with *CEBPA* C- versus N-terminal germline variants is the degree of penetrance of the variants. *CEBPA* N-terminal germline variants are highly penetrant, with a reported life-time incidence of AML between 90 and 100% [7]. Conversely, *CEBPA* C-terminal germline variants appear to have incomplete penetrance [6]. A penetrance of 46% (i.e. 12 of 26 confirmed germline carriers of a *CEBPA* germline C-terminal variant developed AML), was found based on data from our case and all other currently published cases (Table 2). Another difference is the types of variants themselves. Germline *CEBPA* C-terminal variants are mostly missense variants (67%) compared to N-terminal variants, which are most frequently frameshift variants (Figure 2; Table 2) [6].

Identification of germline predisposition variants in AML has important clinical implications for the prognosis and management of patients. There should be a high degree of suspicion with pursuit of clinical germline genetic testing for individuals with AML in which molecular testing reveals two or more *CEBPA* variants in the leukemic clone and/or have persistence of a *CEBPA* variant at a VAF of ~50% at the end of induction therapy despite achieving a CR. When the presence of a germline *CEBPA* variant is suspected, the patient should ideally be referred to a centre with expertise in germline predisposition syndromes for appropriate genetic counselling and germline testing [14].

Our case and literature review highlight the importance of early consideration of HSCT in first CR for patients with AML with *CEBPA* C-terminal germline variants. While this subtype of AML appears to be sensitive to chemotherapy and has a favorable prognosis, there remains a high risk of early relapse, as occurred in our patient's case [1]. An HSCT in CR is the only potential therapy that can rid the bone marrow of the predisposing germline variant, thereby decreasing the risk of future relapse and the need for further intensive induction chemotherapy [14]. Given the short interval between CR and first relapse observed in individuals with AML and *CEBPA* C-terminal germline variants, early identification of the germline variant and testing any potential related stem cells for presence of the same variant is of critical importance to avoid delays in donor selection and ultimately delay in HSCT. Donor-derived leukemias have been reported with the use of stem cells from donors carrying a deleterious germline *CEBPA* variant [15]. It is therefore recommended that such donors be excluded and, if no suitable related donor is available, that a matched unrelated donor is selected [14].

There are also important considerations for family members of individuals with AML and a *CEBPA* C-terminal germline variant. These individuals should be referred to a centre with expertise in germline predisposition syndromes to receive genetic counselling and consideration for genetic testing [14]. Follow up and surveillance of *CEBPA* C-terminal variant carriers is based on available data and expert opinion and consists of routine monitoring of CBCs for early detection of a possible myeloid malignancy as well as early bone marrow aspirate and biopsy if any signs of new unexplained cytopenia(s) arise [6, 14].

In summary, our case and review of the literature sheds new light on the natural history and clinical outcomes of AML with *CEBPA* C-terminal germline variants, which has both similarities but also important differences compared to the more common AML with *CEBPA* N-terminal germline variants. Both entities have certain overlapping features such as atypically young age at diagnosis, early and frequent relapse, and favourable overall prognosis. However,

there appear to be significant differences with a lower life-time penetrance of AML and shorter time to relapse for C-terminal cases that must be considered when managing patients with AML with a *CEBPA* C-terminal germline variant and counselling their family members.

Declarations

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Informed consent: As per our institution's Research and Ethics Board policy, written informed consent was obtained from all individuals whose personal medical information was included in the case report prior to submission of the manuscript

Data availability: The authors declare that data supporting the findings of this study are available within the article.

Author contributions: AT identified the cases and obtained written informed consent for the case report as well as supervised and revised the manuscript. AH performed the literature review, wrote the manuscript, and participated in the revisions of the manuscript. All authors edited, read, and approved the final manuscript.

References

1. Pathak A, Seipel K, Pemov A, et al (2016) Whole exome sequencing reveals a C-terminal germline variant in CEBPA-associated acute myeloid leukemia: 45-year follow up of a large family. Haematologica 101:846. https://doi.org/10.3324/HAEMATOL.2015.130799
2. Brown AL, Hahn CN, Scott HS (2020) Secondary leukemia in patients with germline transcription factor mutations (RUNX1, GATA2, CEBPA). Blood 136:24–35. https://doi.org/10.1182/BLOOD.2019000937
3. Arber DA, Orazi A, Hasserjian RP, et al (2022) International Consensus Classification of Myeloid Neoplasms and Acute Leukemia: Integrating Morphological, Clinical, and Genomic Data. Blood. https://doi.org/10.1182/BLOOD.2022015850/485730/INTERNATIONAL-CONSENSUS-CLASSIFICATION-OF-MYELOID
4. Döhner H, Wei AH, Appelbaum FR, et al (2022) Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood 140:1345–1377. https://doi.org/10.1182/BLOOD.2022016867
5. Taskesen E, Bullinger L, Corbacioglu A, et al (2011) Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for CEBPA double mutant AML as a distinctive disease entity. Blood 117:2469–2475. https://doi.org/10.1182/BLOOD-2010-09-307280
6. Tawana K, Rio-Machin A, Preudhomme C, Fitzgibbon J (2017) Familial CEBPA-mutated acute myeloid leukemia. Semin Hematol 54:87–93. https://doi.org/10.1053/J.SEMINHEMATOL.2017.04.001
7. Tawana K, Wang J, Renneville A, et al (2015) Disease evolution and outcomes in familial AML with germline CEBPA mutations. Blood 126:1214–1223. https://doi.org/10.1182/BLOOD-2015-05-647172
8. Simon L, Spinella JF, Yao CY, et al (2020) High frequency of germline RUNX1 mutations in patients with RUNX1-mutated AML. Blood 135:1882–1886. https://doi.org/10.1182/BLOOD.2019003357
9. Zhang Y, Wang F, Chen X, et al (2020) Companion gene mutations and their clinical significance in AML with double mutant CEBPA. Cancer Gene Ther 27:599–606. https://doi.org/10.1038/S41417-019-0133-7
10. Kim HS, Han E, Jang W, et al (2019) Germline CEBPA mutations in Korean patients with acute myeloid leukemia. Leuk Res 76:84–86. https://doi.org/10.1016/J.LEUKRES.2018.12.003
11. Rio-Machin A, Vulliamy T, Hug N, et al (2020) The complex genetic landscape of familial MDS and AML reveals pathogenic germline variants. Nature Communications 2020 11:1 11:1–12. https://doi.org/10.1038/s41467-020-14829-5
12. Gutman JA, Hoffner B (2012) A novel CCAAT/enhancer binding protein α germline variant in a case of acute myeloid leukemia. Leuk Lymphoma 53:1006–1007. https://doi.org/10.3109/10428194.2011.638718/SUPPL_FILE/DISCLOSURE.ZIP
13. Richards S, Aziz N, Bale S, et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405–424. https://doi.org/10.1038/GIM.2015.30
14. Godley LA, Shimamura A (2017) Genetic predisposition to hematologic malignancies: management and surveillance. Blood 130:424–432. https://doi.org/10.1182/BLOOD-2017-02-735290
15. Xiao H, Shi J, Luo Y, et al (2011) First report of multiple CEBPA mutations contributing to donor origin of leukemia relapse after allogeneic hematopoietic stem cell transplantation. Blood 117:5257–5260. https://doi.org/10.1182/BLOOD-2010-12-326322

Figures

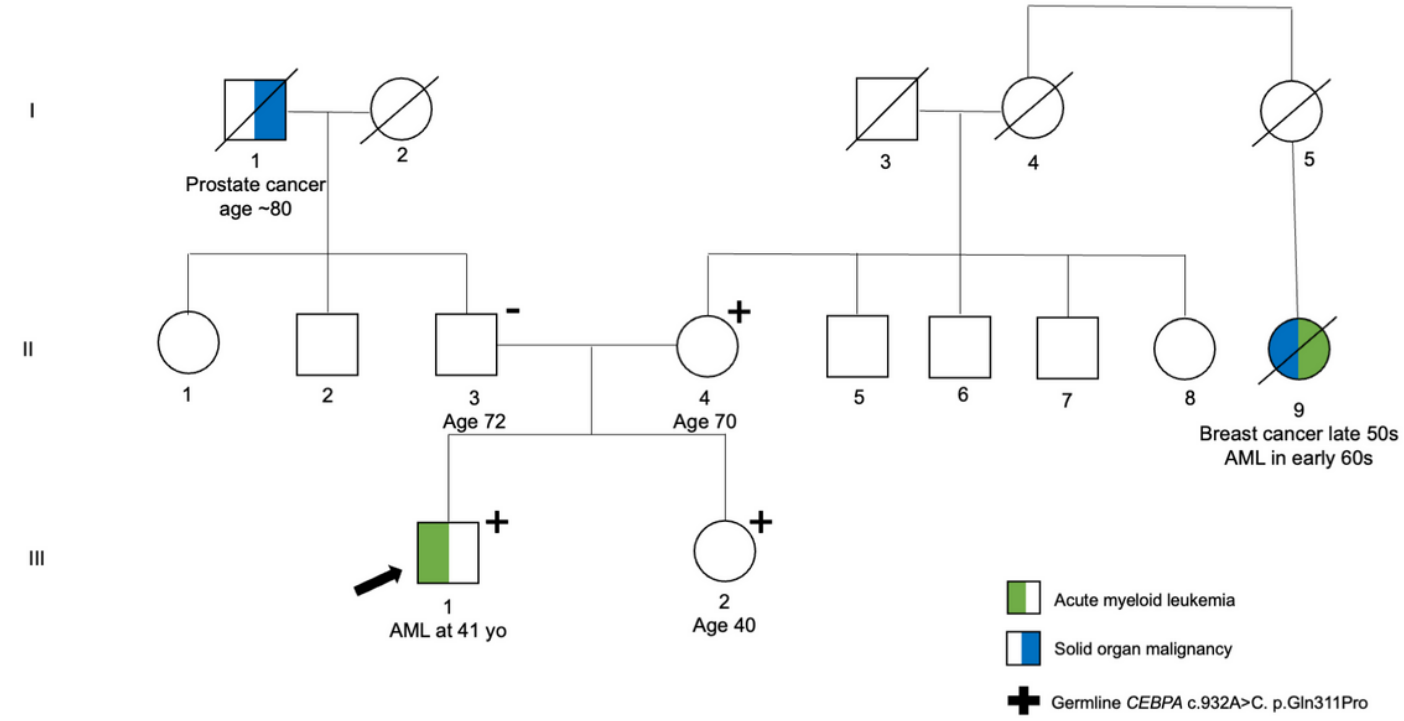

Figure 1

Pedigree of described germline *CEPBA* c.932A>C, p.Gln311Pro variant family originating from Atlantic Canada. Roman numerals indicate generations and Arabic numbers indicate individuals. The proband is indicated by an arrow. Individuals with the germline *CEBPA* c.932A>C, p.Gln311Pro are marked with a +. Individuals tested for the *CEPBA* c.932A>C, p.Gln311Pro and found to be wild type are indicated by -.

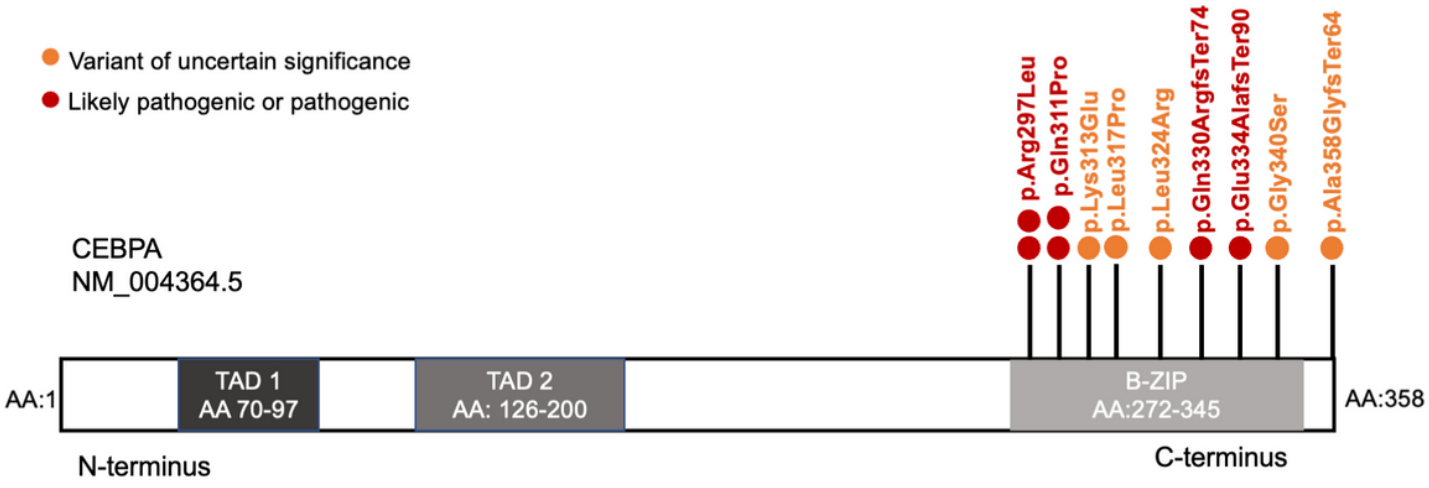

Figure 2

Protein schematic representation of all published c-terminal germline *C*EBPA variants to date including the p.Gln311Pro variant in our case (as of April 2022). [Transcript ID: NM_004364.5]. C-terminus encompasses AA 278 to 358. AA= amino acid, B-ZIP = basic leucine zipper domain, TAD = transactivating binding domain. Number of circles corresponds to the number of reported families with the variant.