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ORIGINAL ARTICLE

Familial Acute Myeloid Leukemia and Myelodysplasia in Hungary

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Abstract Although genetic predisposition to haematological malignancies has long been known, genetic testing is not yet the part of the routine diagnostics. In the last ten years, next generation sequencing based studies identified novel germline mutations in the background of familial aggregation of certain haematologic disorders including myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML). This is supported by the fact that the myeloid neoplasms with genetic predisposition represent a new category in the revised 2016 World Health Organization classification. According to the new classification, these disorders are subdivided based on the clinical and genetic features, including myeloid neoplasms with germline predisposition alone, or with pre-existing platelet disorder, cytopaenias or other organ failures. The predisposing genetic factors include mutations in the *RUNX1*, *CEBPA*, *GATA2*, *ANKRD26*, *ETV6*, *DDX41*, *TERC* or *TERT* and *SRP72* genes. The genes affected in these syndromes are important regulators of haemopoiesis and are frequently implicated in leukaemogenesis, providing deeper insight into the understanding of normal and malignant haemopoiesis. Despite the growing knowledge of germline predisposing events in the background of familial myeloid malignancies, the germline genetic component is still unknown in a subset of these pedigrees. Here, we present the first study of inherited myeloid malignancies in Hungary. We identified three families with apparent clustering of myeloid malignancies with nine affected individuals across these pedigrees. All tested individuals were negative for *CEBPA*, *GATA2*, *RUNX1*, *ANKRD26*, *ETV6*, *DDX41*, *TERC* or *TERT* and *SRP72* mutations, suggesting the presence of so far unidentified predisposing mutations.

Keywords Familial MDS/AML · Genetic predisposition · Germline mutation

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Introduction

Myelodysplastic syndromes (MDS) and acute myeloid leukaemias (AML) are generally considered sporadic diseases, however the importance of familial forms of these malignancies is being increasingly recognized [1] [2]. This has largely been facilitated by the recent next generation sequencing (NGS) based studies leading to identification of novel germline mutations predisposing to familial MDS/AML [3–6]. These familial cases are considered to be a rarity, but they are likely to be more common than currently appreciated. This is supported by the fact that familial MDS/AML represents a new category in the recently revised 2016 WHO classification of myeloid neoplasms and acute leukemia [7].

Familial MDS/AML cases often present in childhood and are commonly associated with peculiar clinical symptoms and syndromes. These syndromes are heterogeneous with regards

to their clinical presentation as well as the underlying causative genetic mutations, and need distinguished management with respect of the bone marrow donor selection as well as the monitoring of the affected individuals [8, 9]. The currently recognized familial leukaemia predisposition syndromes are classified according to the clinical symptoms and predisposing germline mutations [7]. AML or MDS caused by *CEBPA* or *DDX41* mutation occurs alone without other clinical symptoms or antecedent haematological condition [6, 10–13]. Familial MDS caused by *GATA2* mutations often occurs in the setting of cytopaenias, and rare immunological syndromes [3, 14, 15]. Mutations of *TERT* or *TERC* and *SRP72* manifested clinically in bone marrow failure syndromes associated with elevated risk of MDS/AML [4, 16]. Although the first symptom could be haematological malignancy in younger age, platelet dysfunction and variable bleeding disorders associated with elevated risk of MDS/AML are the most frequent clinical phenomena associated with the germline mutation of *RUNX1*, *ANKRD26* or *ETV6* in the background [5, 17, 18].

It is interesting to note that in some of the well-known clinical syndromes the germ-line predisposing event is not sufficient for the full blown malignant phenotype but secondary genetic events are necessary for manifestation of the overt malignancy [19–21]. Consequently, the recognition of the familial MDS/AML cases is hampered by the variable clinical presentation, incomplete penetrance and variable age of onset [22]. Although the spectrum of predisposing gem line mutations is wide and the number of novel susceptibility alleles demonstrated a rapid increase in the last few years (Fig. 1), the germline mutations identified to date explain only approximately 40–50% of the cases with inherited predisposition to myeloid malignancies, suggesting the existence of additional putative culprit genes [23]. Recognition of these families with genetic predisposition to MDS/AML is of critical importance, as they require distinct management and monitoring with respect to the bone marrow transplantation and the follow up strategy of the family members [9].

Here, we present our findings on the first collected Hungarian familial leukaemia cohort with three families with apparent clustering of myeloid malignancies with nine affected individuals across these pedigrees.

Materials & Methods

We identified three families with aggregation of myeloid malignancies with nine affected individuals within these pedigrees. Bone marrow or peripheral blood samples were collected from the affected members of these families. DNA isolation was performed with High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland) as recommended by the manufacturer. DNAs were tested for mutations in all currently known predisposition genes, including the full coding sequences of *CEBPA*, *GATA2*, *RUNX1*, *DDX41, TERT* and *TERC*, and for mutation hot-spots of *SRP62* (exons 6 and 10) and *ANKRD26* (5′-UTR region) genes. The sequence of the oligonucleotides used for polymerase chain reaction (PCR) amplification and direct Sanger sequencing is detailed in Supplementary Table 1. PCR amplification was performed using the AmpliTaq Gold® DNA Polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, USA), followed by digestion with ExoSAP-IT (Affymetrix, Santa Clara, California, USA). For bidirectional Sanger sequencing we used the BigDye® Terminator v3.1 Cycle Sequencing chemistry (Thermo Fisher Scientific, Waltham, Massachusetts, USA) followed by the analysis of the terminated fragments on a 3500 Genetic Analyser (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Results

Pedigree A

Three young individuals between age of 12 and 20 were diagnosed with MDS in family A (Fig. 2.). The proband, II-1, presented at age of 18 with three lineage cytopaenia and constant need of haemosupportation. The diagnostic laboratory evaluation referred severe pancytopaenia, her initial white blood cell (WBC) count was 2.4 G/L, haemoglobin (Hgb) level was 90 g/L and platelet count (Plt) was 27 G/L. The bone marrow biopsy demonstrated hypocellular MDS, megaloblastic erythropoiesis and dysplastic granulopoiesis. No cytogenetic alterations were detected. She underwent treatment with matched unrelated donor (MUD) transplantation. During the recovery, a grade IV intestine graft versus host disease (GVHD) occurred. After the recovery she achieved complete remission and is currently being monitored and followed. The older brother of the proband (II-2) was diagnosed with MDS at age of 20 during the bone marrow donor screening procedure of his sister (proband II-1). However, he didn't suffer from any clinical symptoms and his blood counts were normal (WBC: 5.11 G/L, Hgb: 132 g/L, Plt: 154 G/L) his screening bone marrow sample appeared to be dysplastic (normocellular haemopoiesis with mild to moderate dysplastic features of all cell lineages). In the following period his sister was transplanted with a matched unrelated donor (MUD) donor, and he is currently under watch and wait strategy. The younger brother of the proband (II-3) was diagnosed with MDS at age of 12. He presented with lymph node enlargement and considering the familial aggregation of haematologic disorders in his family he underwent a full clinical screening panel. His initial blood count was: WBC: 8.59 G/L, Hgb: 133 g/L, Plt: 157 G/L. His bone marrow biopsy showed mild to moderate dysplastic features of megakaryocyte cell line consistent with early dysplastic features. He is currently under watch and wait

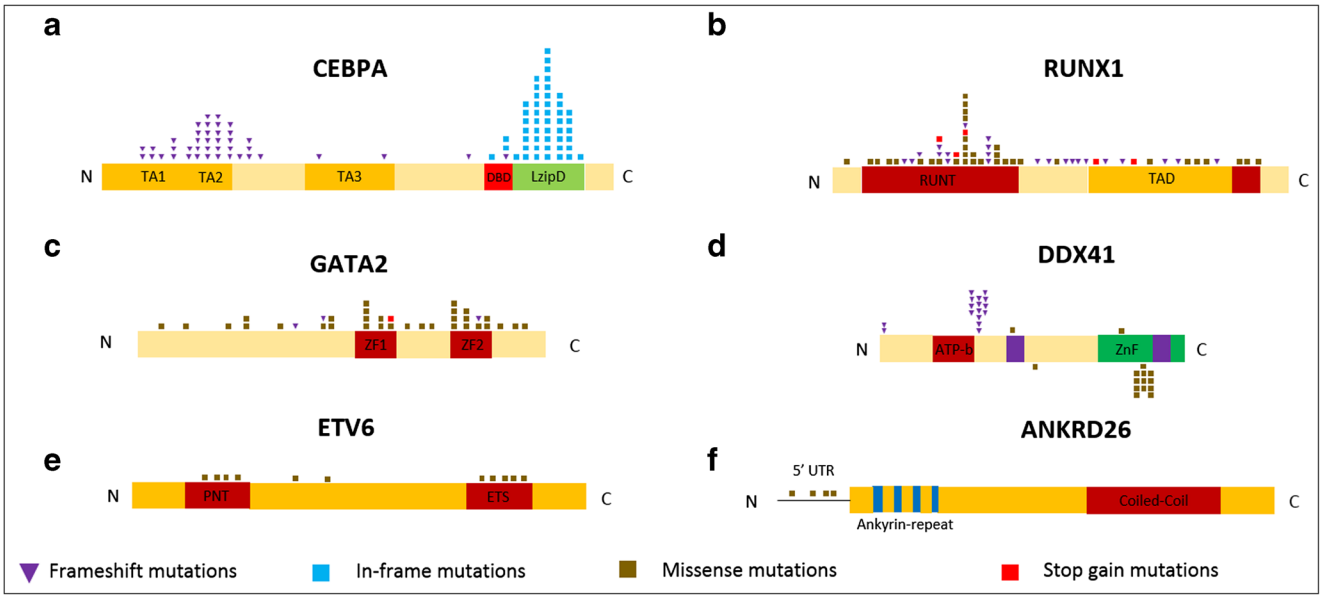

Fig. 1 Illustrated is the schematic protein structure and most frequently mutated regions of the currently known predisposition genes in familial myeloid malignancies: **a** CEBPA, **b** RUNX1, **c** GATA2, **d** DDX41, **e** ETV6 and **f** ANKRD26

strategy. All three individuals were tested for *CEBPA*, *GATA2*, *RUNX1*, *ANKRD26*, *ETV6*, *DDX41*, *TERC* or *TERT* and *SRP72* mutations and were found to be negative for these mutation targets.

Pedigree B

The diagnosis of MDS in the two young affected siblings of pedigree B (Fig. 3) was established simultaneously. The first proband (II-1) presented with moderate bleeding disorder and thrombocytopenia at age of 14. His initial blood counts showed marked thrombocytopenia, mild anaemia and low WBC count (WBC: 3.63 G/L, Hgb: 108 g/L, Plt: 93 G/L). Bone marrow aspirate and biopsy showed near total aplasia with erythropoietic remnant islets. Together with the latter biopsies, the microscopic picture was consistent with paediatric myelodysplastic syndrome. He is currently under strict clinical and laboratory monitoring. The second proband (II-2), the younger sister of II-1, also presented at the same time with bleeding symptoms and thrombocytopenia at age of 10. Her initial laboratory parameters were as follows: WBC

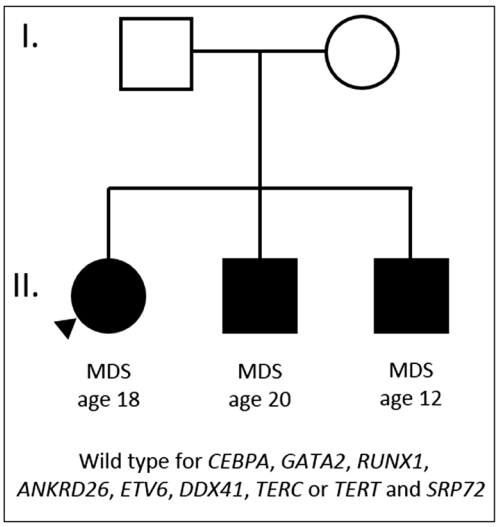

Fig. 2 Pedigree with three siblings diagnosed with MDS: Square denotes males and circle denotes females. Black boxes indicate affected individuals with MDS. The black arrow indicates the proband of the family. All three individuals were tested for *CEBPA*, *GATA2*, *RUNX1*, *ANKRD26*, *ETV6*, *DDX41*, *TERC* or *TERT* and *SRP72* mutations and were found to be negative for these mutation targets

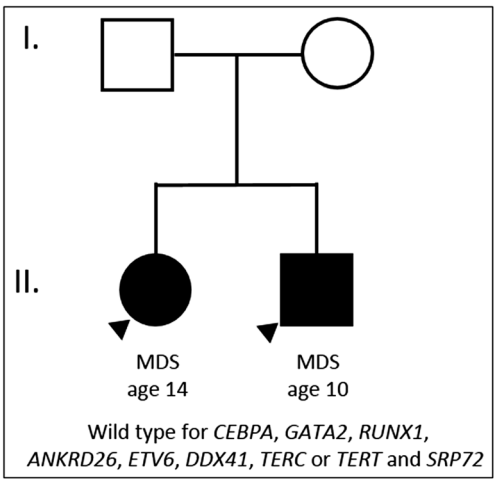

Fig. 3 Pedigree with two siblings affected with MDS. Square denotes males and circle denotes females. Black boxes indicates affected persons with MDS. Arrows indicates the probands of the family. We obtained DNA samples of all the individuals of the family and found no mutations in the analysed regions of the *CEBPA*, *GATA2*, *RUNX1*, *ANKRD26*, *ETV6*, *DDX41*, *TERC* or *TERT* and *SRP72* genes

2.76 G/L; Hgb 111 g/L; Plt 55 G/L. Bone marrow aspirate and biopsy were consistent with paediatric myelodysplastic syndrome of childhood. She is also being closely monitored. Both affected siblings were tested for *CEBPA*, *GATA2*, *RUNX1*, *ANKRD26*, *ETV6*, *DDX41*, *TERC* or *TERT* and *SRP72* mutations and displayed wild type genotypes.

Pedigree C

Four individuals between age of 53 and 82 in family C (Fig. 4) were diagnosed with AML. The proband II-1 was diagnosed with AML at age of 82. The disease represented M6 in the FAB category but harboured no cytogenetic or molecular abnormalities. The older brother of the proband was also diagnosed with AML at age of 53 and the younger sister of the proband also presented with AML at age of 57. The mother of the three affected individuals (I-2) was also diagnosed with AML at age of 64 in 1974. Unfortunately, no medical documentation was available for the latter mentioned member of this family. No germline variants were detected in the DNA obtained from the proband II-1 tested for *CEBPA*, *GATA2*, *RUNX1*, *ANKRD26*, *ETV6*, *DDX41*, *TERC* or *TERT* and *SRP72* mutations.

Discussion

Familial MDS/AML predisposition syndromes are rare inherited disorders characterized by significantly elevated risk of MDS and AML development [2, 7]. *RUNX1* and *CEBPA* represented the first two culprit genes identified as predisposing factors in background of familial platelet disorders with propensity to myeloid malignancy (FPD/AML) and 'pure' familial AML, respectively [13, 17]. In the last few years, we witnessed a steep increase in the number of predisposition genes discovered using NGS technologies, including *GATA2* [3, 14, 15], *ANKRD26* [5], *ETV6* [18, 24], *SRP72* [4], *DDX41* [6], *TERC* [25] and *TERT* [26].

Although several disorders with germline predisposition have been included in the revised 2016 WHO classification of myeloid neoplasms and acute leukemia as "myeloid neoplasms with germ line predisposition" [7], screening for the known germline mutations in the background of these syndromes is not part of the routine diagnostic algorithms. However, the most recent European LeukemiaNet(ELN) recommendation on diagnosis and management of AML recommends molecular testing using a gene panel that includes the currently known predisposing alleles if AML with genetic predisposition is suspected [27]. Recognition of the affected families with inherited AML/MDS is of critical importance as they not only provide rare and unique models to study the molecular pathogenesis of these diseases, but identification of an inherited mutation has indeed immediate clinical implications with regards to the management and monitoring of the affected family members [8, 9]. Nevertheless, it is believed that familial MDS/AML is still underdiagnosed and their frequency is considerably higher than currently appreciated.

Recognition of these families is indeed challenging and requires a heightened awareness and high index of suspicion from the clinicians. The diagnosis is complicated by the very variable clinical presentation, variable age of onset, incomplete penetrance and lack of family history. Given that many of these predisposition syndromes have only recently been described, clinical guidelines for screening and management of these families are in their infancy. Churpek and colleagues published the first proposed recommendations [28] recently updated by Churpek and Godley on behalf of the University of Chicago Hematopoietic Malignancies Cancer Risk Team with detailed guidelines on identification, genetic counselling and testing as well as clinical management of inherited myeloid malignancy syndromes [9]. To recognize these syndromes, complete patient and family history must be obtained

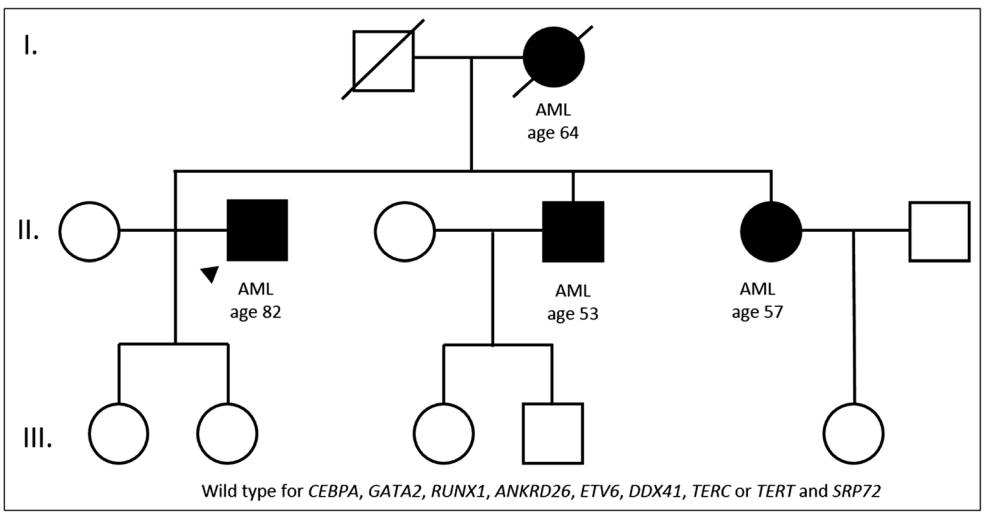

Fig. 4 Family with four affected individuals with AML. Square denotes males and circle denotes females. Black boxes indicates affected persons with AML. DNA was available from the proband of the family (II-1). We identified no mutation in the analysed regions of the *CEBPA*, *GATA2*, *RUNX1*, *ANKRD26*, *ETV6*, *DDX41*, *TERC* or *TERT* and *SRP72* genes

including data on previous malignancies and bleeding episodes (in cases with more than one first-degree relative affected with MDS/AML an underlying genetic predisposition should be suspected), as well as the known characteristics of the recognized familial MDS/AML syndromes. Identification of a germline mutation has clinical implications for stem cell transplantation including donor selection and conditioning regimen [8, 9]. It is recommended to screen all close relatives of an affected individuals to avoid transplantation of stem cells from a relative harbouring the same germline predisposition allele [22, 28]. All mutation carriers should also undergo a baseline bone marrow biopsy and a twice a year a complete blood cell count testing [22]. In families where a germline mutation can not be identified, but there is a high suspicion for familial involvement, a matched unrelated donor is preferable [8, 9].

Genes involved in the pathogenesis of familial MDS/AML affect a plethora of cellular processes, including master transcription factors governing haemopoiesis (*RUNX1, CEBPA, GATA2* and *ETV6),* telomerase regulation (*TERC/TERT*), ribosome assembly and translation (*DDX41)*, signal recognition (*SRP72*) with many of them also implicated in sporadic myeloid malignancies [29–32]. Novel candidate genes were identified in single pedigrees, including *DIDO1* and *KMT2C*, however these have not been confirmed yet in additional families [33, 34]. In spite of the rapid progress in the field of familial MDS/AML, mutations in these genes do not explain the genetic basis of all cases with the predisposing gene mutations remaining obscure in subset of these families, including the three pedigrees identified in our study. In pedigrees A and B, we identified five individuals affected with MDS with an early age of onset (10–20 years), while individuals in pedigree C demonstrated a considerably later age of onset (53–82). Standard sequencing assays to screen for the known predisposition alleles were established in Hungary. All individuals tested in these families harboured no mutations in the known predisposition genes, suggesting that the list of causative genes underlying familial myeloid disorders is not complete yet. Inclusion of families with unknown genetic background into genome wide screening studies utilizing whole genome or whole exome sequencing will likely facilitate identification of novel predisposition genes.

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