Germline RUNX1 mutations/deletions and genetic predisposition to haematological malignancies

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Mutations/délétions germinales de RUNX1 *et prédisposition génétique aux hémopathies malignes*

Acute leukaemia, genetic predisposition, *RUNX1*, thrombocytopaenia, FPD/AML

Abstract

The *RUNX1* gene encodes for the alpha subunit of the core binding factor, a heterodimeric transcription factor complex involved in haematopoietic differentiation. *RUNX1* germline mutations and deletions are implicated in familial platelet disorder with predisposition to acute myeloid leukaemia (FPD/AML). They have been described in more than 200 families and it is estimated that more than 6,000 families worldwide are affected by this genetic predisposition. The classic phenotype of individuals carrying this constitutional abnormality includes mild to moderate thrombocytopaenia with normal volume, abnormal platelet functions (associated with or without a tendency to bleed),[1] dysmegakaryopoiesis, and a predisposition to develop haematological malignancies of various phenotypes (acute myeloid leukaemia, myelodysplastic syndromes, acute T-cell lymphoblastic leukaemia) at any age. When the phenotype is suggestive, but sequencing remains negative, the diagnostic algorithm should include a copy number analysis to look for large deletions. Haematopoietic stem cell transplantation is the only curative therapy but involves taking precautions when selecting intrafamilial donors. Monitoring individuals who have not yet developed a haematological malignancy has not yet been codified but regular follow-up with complete blood count is recommended, coupled with screening for additional molecular abnormalities predicting a malignant transformation.

Résumé

Le gène *RUNX1* code la sous-unité α du core binding factor, complexe transcriptionnel hétérodimérique impliqué dans la différenciation hématopoïétique. Les mutations et délétions germinales de *RUNX1* sont responsables des thrombopénies familiales avec prédisposition aux leucémies aiguës myéloïdes (FPD/AML). Elles ont été décrites dans plus de 200 familles et l'on estime que plus de 6 000 seraient concernées par cette prédisposition génétique dans le monde. Le phénotype classique des individus porteurs de cette anomalie constitutionnelle inclut une thrombopénie d'intensité légère a modérée à plaquettes de taille normale, des anomalies des fonctions plaquettaires (associées ou non à une tendance hémorragique), une dysmégacaryopoïèse et une prédisposition au développement d'hémopathies malignes de phénotypes divers (leucémie aiguë myéloïde, syndromes myélodysplasiques, leucémie aiguë lymphoblastique T) pouvant survenir à tout âge. En cas de phénotype évocateur et de séquençage négatif, l'algorithme diagnostique devra inclure une recherche d'anomalie du nombre de copies du gène, afin de mettre en évidence une éventuelle délétion. La greffe de cellules souches hématopoïétiques est la seule thérapie curative mais implique des précautions pour la sélection des donneurs intrafamiliaux. Si la surveillance des individus indemnes d'hémopathie n'est pas encore complètement codifiée, il est conseillé de proposer un suivi hématologique régulier (hémogramme) complété d'une recherche d'anomalies moléculaires additionnelles annonciatrices d'une transformation maligne.

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Preamble

A genetic predisposition is defined as a condition that makes an organism more susceptible to a particular disease. It involves the intervention of internal or external aggravating factors capable of triggering the disease in question.
Although the first descriptions of "familial leukaemias" date back to the 1950s, [1] their genetic component was largely identified with the development of molecular biological techniques from the 2000s onwards. This is, therefore, a relatively recent field of study in the treatment of patients with haematology. In 2016, the World Health Organization recognised haemopathies occurring in the context of an underlying genetic syndrome as a separate category [2]. For physicians and patients, there is a two-fold interest in identifying a genetic predisposition to haematological malignancies. On the one hand, it is possible to explain the occurrence of a pathology that is often considered to result from chance due to a lack of an identified environmental factor. On the other hand, special precautions are implied regarding patient care, particularly when a haematopoietic stem cell (HSC) transplant from a family donor is envisaged. For researchers, the understanding of the physiopathology and the phenomena that contribute to the developement of leukaemias in these syndromes can often be extrapolated to sporadic diseases, which may involve the same genes.
Germline mutations and deletions of *RUNX1* were the first genetic abnormalities reported to predispose individuals to the development of haematological malignancies. Consequently, these abnormalities are the most widely described in the literature. They can arise in many circumstances (acute leukaemia, myelodysplasia, chronic thrombocytopaenia, family context), and at all ages. It is important to note at this stage that investigation of a predisposition syndrome should be carried out within the framework of genetic counselling and with informed consent. The identification and management of affected patients and their families therefore requires knowledge of the clinical and biological characteristics and the risks of progression associated with these changes. Close collaboration between haematologists, paediatricians, haemobiologists, biologists and geneticists is also required [3]. However, monitoring methods and treatment recommendations remain to be determined.

The discovery of constitutional defects in *RUNX1*

The first publication reporting the association between chronic thrombocytopaenia and high occurrence of haematological malignancies in the same family dates from the late 1970s [4]. The authors reported on a sibling group of 10 children in which three siblings died of leukaemia, while at least three others, as well as their mother, had chronic thrombocytopaenia and various biological signs of platelet dysfunction. In the mid-1980s, physicians and researchers in Boston defined the outline of familial platelet disorder with a predisposition to acute myeloid leukaemia (FPD/AML, OMIM #601399) based on the observation of a large family (192 individuals over seven generations). In this family, 29 members had thrombocytopaenia of varying severity, with autosomal dominant inheritance, sometimes with a tendency towards bleeding, while six had developed a haematological malignancy between the ages of 10 and 62 [5]. It took more than 10 years to identify a minimal region in this family responsible for the observed phenotype, located on the long arm of chromosome 21 and containing five candidate genes [6]. Suspicions quickly focused on *RUNX1* (formerly AML1), which had been known since the early 1990s for its involvement in 8;21 translocation (t [8;21]), observed in sporadic acute myeloid leukaemia (AML) [7]. Finally, it was in 1999 that the same team formally identified the *RUNX1* gene as the site of constitutional mutations–or, more rarely, large deletions–associated with FPD/AML syndrome in six distinct families (including the family described in Boston in

1985, characterised by a constitutional deletion of the gene) [8]. Since then, more than 200 *RUNX1* mutated families have been reported in the literature [9] and it is estimated that more than 6,000 families worldwide are affected by this genetic predisposition [10].
It should be noted that although the terms "FPD/AML" or "FPD/MM" (for myeloid malignancies) are widely used to describe patients with a constitutional defect in *RUNX1*, the spectrum of associated haemopathies is not restricted to myeloid haemopathies, and constitutional abnormalities affecting other, more recently, discovered genes (*ANKRD26, ETV6*) are also associated with familial thrombocytopaenia with a predisposition to haematological malignancies [11, 12].

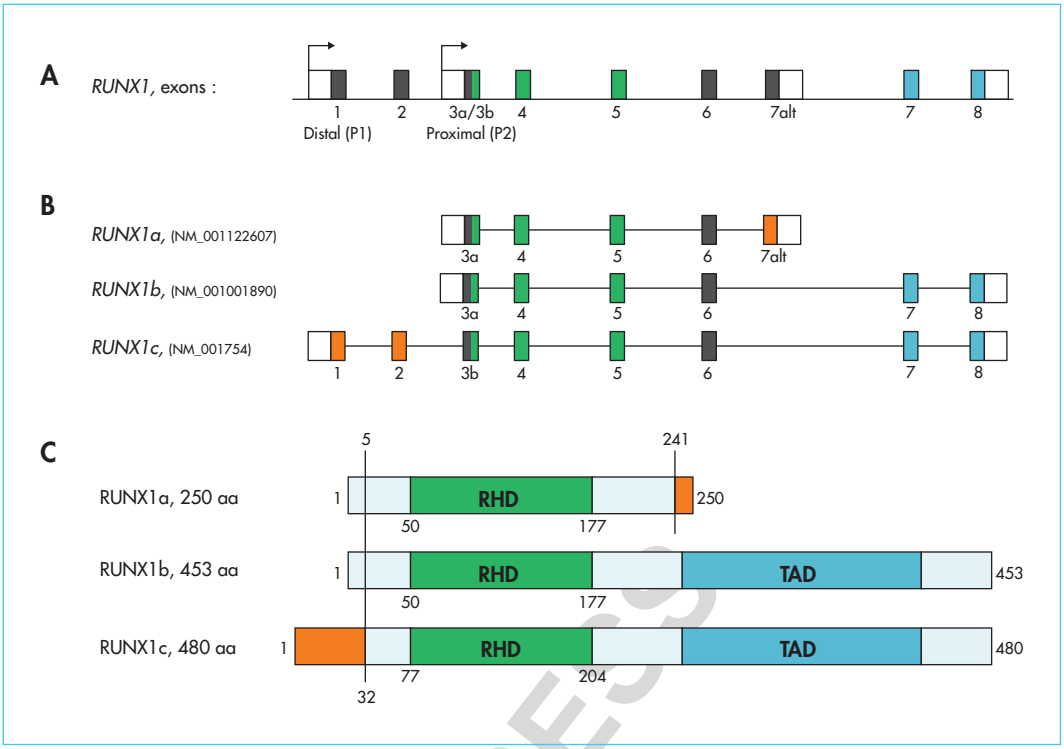
Genomic organisation and function of *RUNX1*

The *RUNX1* gene is located on the long arm of chromosome 21 (at 21q22) and spans 260 kb. It encodes the α subunit of the *core binding factor* (CBF), a heterodimeric transcriptional complex (*RUNX1*/CBFβ) involved in haematopoietic differentiation and the emergence of HSCs [13]. *RUNX1* knockout (KO) mice die after 12.5 days of embryonic development, due to a complete absence of definitive haematopoiesis [14]. The conditional deletion of *RUNX1* reveals that it is not essential for the maintenance of haematopoiesis in adulthood, but plays an essential role in the differentiation of B and T lymphocytes and megakaryocytes [15]. Mice develop myelodysplastic [16] or myeloproliferative syndromes over time [17].
Transcription of the *RUNX1* gene is dependent on two promoters located upstream of exon 1 (the distal P1 promoter) and exon 3 (the proximal P2 promoter) leading to three main isoforms, the expression of which is finely regulated spatiotemporally during embryogenesis [18]. The two longest isoforms, *RUNX1*b (453 amino acids) and *RUNX1*c (480 amino acids), are distinguished by their 5' end, according to whether the transcription is initiated by P1 or P2 (*figure* 1). A third, shorter (250 amino acids) *RUNX1*a isoform is produced from P2 and truncated in its 3' part, conferring antagonism towards the other isoforms. The N-terminal part of the three isoforms is characterised by the presence of a highly evolutionarily-conserved 128-amino acid domain, the runt homology domain (RHD). The RHD is essential for binding *RUNX1* to CBFβ and to target DNA, this binding being critical to increase the affinity of *RUNX1* to DNA, and to protect *RUNX1* from degradation by the proteasome [19]. *RUNX1* usually acts in cooperation with other transcription factors (including PU.1 and CEBPα) via this RHD [13]. The C-terminal part of *RUNX1*b and *RUNX1*c isoforms also contains the transactivation domain (TAD) involved in recruiting co-activators or repressors, which modulate its transcriptional activity. The CBF complex thus controls the expression of many genes that are key in:

– granulo-monocytopoiesis: genes for interleukin-3 (IL-3), *granulocyte-macrophage colony-stimulating factor* (GM-CSF), *colony stimulating factor 1 receptor* (CSF1R), and myeloperoxidase [20-23]
– T lymphopoiesis: α and δ T-cell receptor (TCR) chains [24, 25],
– megakaryocytopoiesis: cyclin inhibitor CDKN2D/p19INK4D and non-muscle myosin 10 heavy chain (MYH10), involved in megakaryocyte polyploidisation, and transcription factor NFE2/p45 [26-28].

In addition to its primary role as a transcription factor, *RUNX1* is also considered to be a mediator of epigenetic regulation [29]. Although it lacks enzymatic activity, it is able to interact with many regulatory proteins, including those involved in post-translational modifications of histones and conformational changes of chromatin [30]. It therefore has a two-fold function in terms of gene expression, by directly

Figure 1



A) Genomic organisation of *RUNX1* and localisation of the P1 and P2 promoters. **B**) Main transcriptional isoforms of *RUNX1*. **C**) Corresponding protein isoforms: a, b and c. Sequences that differ between isoforms are marked in orange.

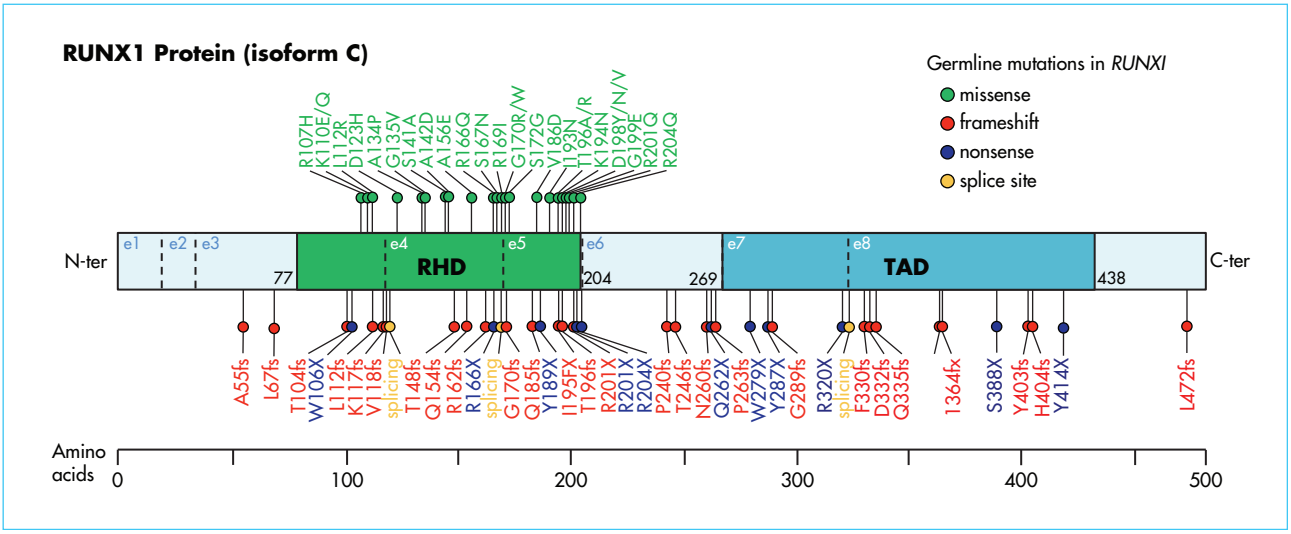
recruiting the transcriptional machinery at the promoters of target regions and by altering chromatin accessibility at local and distal regulatory regions [31].

Constitutional abnormalities of the *RUNX1* gene and general basis for interpretation

There are two main categories of mutations in *RUNX1* (*figure* 2). The first are missense variants resulting in the substitution of an amino acid. These mutations are almost always located in the RHD domain and most often affect the amino acids that interact with the target DNA. Outside the RHD domain, care should be taken when considering a missense variant of *RUNX1* as disease-causing [32]. The second category includes nonsense or frameshift mutations. These mutations are identified throughout the gene and involve the production of a truncated protein and/or rapid degradation. Outside the coding regions, splice variants have also been described in more than a dozen families (most often at the canonical splice sites of exons 4 and 5) [33].

A number of families or individuals present partial or complete deletions of the gene, or even more complex rearrangements (tandem duplication, exon inversion, translocation) [34, 35]. The frequency of these situations is probably under-estimated due to the lower efficiency of current sequencing techniques used to detect them and the lack of systematic analysis of the number of gene copies in diagnostic algorithms. The use of alternative techniques–chromosomal analysis by DNA chip (comparative genomic hybridisation [CGH-array], polymorphism arrays [SNP-array]), multiplex ligation-dependent probe amplification (MLPA), genome

FIGURE 2



Distribution of constitutional mutations in *RUNX1* reported in the literature. The main functional domains and corresponding exons are depicted on the protein. The mutations are described for the *RUNX1*c isoform (NM_001754).

sequencing–in suggestive cases with initial negative sequencing is therefore recommended [36-38].

Depending on the type of genetic defect identified, two mechanisms may be involved. In the case of a deletion or truncated protein unable to bind to CBFβ and/or DNA, the residual activity of wild-type *RUNX1* is retained, resulting in haploinsufficiency. In cases of mutations (missense or nonsense) that still allow the protein to bind to CBFβ and/or DNA, the residual activity of wild-type *RUNX1* is decreased because mutated *RUNX1* sequesters CBFβ, leading to a dominant-negative effect [39].

In 2019, recommendations for the annotation and interpretation of *RUNX1* variants were established [10]. It is recommended that variants are annotated using the longest *RUNX1*c isoform (NM_001754; transcribed from Pl), which represents the isoform most expressed in definitive haematopoiesis. Interestingly, several FPD/AML families are characterised by the constitutional deletion of the first exons of *RUNX1*c involving Pl (thus leaving *RUNX1*a and *RUNX1*b intact), suggesting that it is also the most important isoform in pathophysiology [9]. Interpretation of the variants should take into account the frequency of polymorphisms based on public databases, the segregation of the variant in the family, whether the variant is de novo or not (usually a rare occurrence) [40, 41], and the location and type of the variant (missense or premature stop codon). In exceptional cases, functional tests may be necessary to reach a conclusion [39], to evaluate the in vitro impact of the variant on DNA binding, heterodimerisation with CBFβ, stability of the complex, intracellular localisation, and transactivational capacity of target genes. It should be noted that some constitutional mutations have been simultaneously identified in several unrelated individuals (codons Gl35, R166, R201, R204) [9, 42]. The germline nature of the variant must be confirmed based on a sample of constitutional origin, ideally a fibroblast culture. Blood and marrow are considered to be insufficient, since *RUNX1* is also the target of somatic (acquired) mutations which are recognised as constitutional variants. These somatic mutations are globally observed in 6–20% of myeloid haemopathies: AML [43] chronic myelomonocytic leukaemia (CMML) [44] and myelodysplastic syndromes (MDS) [45]. In practice, extra-haematopoietic samples may not be

required when hereditary transmission is confirmed (*e.g*. by studying the parents' blood).

Description of familial thrombocytopaenia syndrome with a predisposition to acute myeloid leukaemia

Clinical presentation

The presentation leading to the diagnosis is highly variable, even between individuals in the same family. The classic FPD/AML phenotype is defined by the triad:

– mild to moderate thrombocytopaenia,
– functional platelet abnormalities,
– a predisposition to the development of haematological malignancies.

Patients often present with a personal or family history of thrombocytopaenia, typically from childhood. The haemorrhagic syndrome, secondary to thrombocytopaenia and thrombopathy, is moderate (epistaxis, ecchymosis or menorrhagia) or absent. Affected individuals generally do not require specific treatment, except in high-risk situations (surgery, trauma) [46]. A certain proportion of these individuals are therefore only diagnosed with FPD/AML when a haemopathy is discovered in the individual or in their family circle. Extra-haematopoietic abnormalities such as eczema, psoriasis and arthritis are consistently reported at a higher frequency than in the general population (more often in families with a nonsense variant of *RUNX1*, although this remains to be confirmed) [9, 47]. Genome-wide association studies (GWAS) have also shown that *RUNX1* could be a susceptibility gene for these diseases [48]. These disorders would thus constitute one of the clinical characteristics of the FPD/AML spectrum. Also, FPD/AML may be part of a more complex clinical syndrome (with dysmorphia, intellectual disability, cardiac disorders, *etc*.) with large deletions of the 21q22 locus involving *RUNX1* and contiguous genes [49, 50].
Finally, a case of spontaneous reversion, demonstrated by progressive decrease in the allelic ratio of the constitutional mutation of the *RUNX1* mutation in childhood (resulting in about 10% of total haematopoiesis at 12 years of age), has recently been reported [51]. The patient also had a normal platelet count while other family members presented an FPD/AML phenotype. While it is impossible to know how common this is and whether it reduces the risk of developing a haemopathy, this description may have important implications for molecular diagnosis.

Thrombocytopaenia and platelet dysfunction

Chronic mild-to-moderate thrombocytopaenia with normal platelet volume is the most obvious feature of FPD/AML. Typical platelet count values range from 70 to 145 G/L. However, some individuals may have low values within the normal range on a single blood count, but thrombocytopaenia will usually be evident on repeat sampling. Its absence on multiple samples should call into question the diagnosis (*e.g*. in a familial segregation study of a variant of undetermined significance). The platelets show a decrease in the number and content of dense granules and a partial deficit of α granules. Platelet aggregation tests are disrupted, particularly when collagen and epinephrine are used as agonists [27, 46, 52]. The intraplatelet persistence of MYH10–physiologically repressed by *RUNX1* during megakaryocyte polyploidisation and almost undetectable in normal platelets–also provides a simple functional test [53] for diagnostic purposes or to study the deleterious nature of a *RUNX1* variant when its significance is uncertain.

Dysmegakaryopoiesis

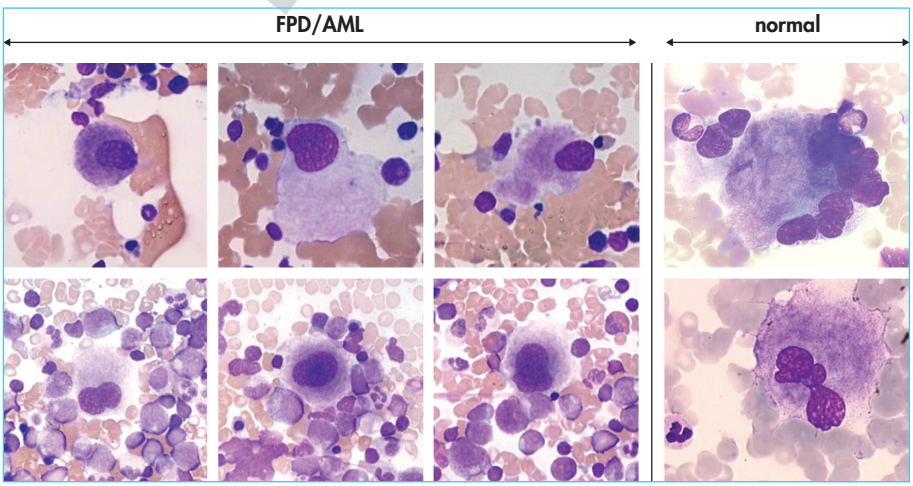
If a bone marrow investigation is performed, it reveals normocellular or hypocellular bone marrow with isolated megakaryocytic involvement (in the absence of progression to haematological malignancy). Megakaryocytes mostly appear small with a hypolobulated nucleus *(figure 3)* [40, 54]. Eosinophilia may be noted but there is no dysplasia of other lineages or abnormal cells (the presence of which should raise suspicion of malignant progression).

In vitro studies using primary patient cells have shown a decrease in the generation of megakaryocytes from progenitors [8]. Megakaryocytes show a maturation defect, decreased ploidy and abnormal proplatelet formation. Platelets isolated from patients' blood show structural abnormalities with a variety of sizes and the presence of giant granules and vacuoles [55]. These alterations can be partly explained by a defect in the regulation of *RUNX1* target genes such as p19INK4D [26] or the myosins MYL9, MYH9 and MYH10 [55]. Studies using primary CD34+ cells and induced pluripotent stem cells (iPSCs) derived from FPD/AML patients have demonstrated that the observable defects in megakaryopoiesis are directly caused by the loss of *RUNX1* activity, secondary to the constitutional defect [53, 55].

Malignant haemopathies

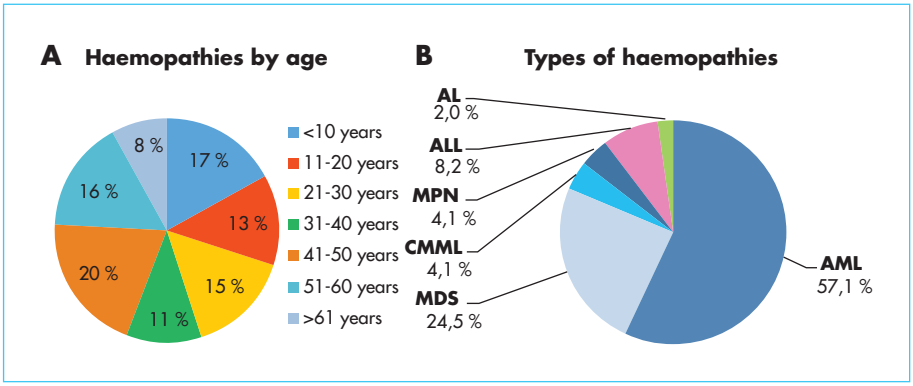
The discovery of one or more cases of haematological malignancies in a family frequently leads to the diagnosis of FPD/AML, probably contributing to an overestimation of this risk in these patients. Age at diagnosis and type of haemopathy vary widely, even within the same family, making genetic counselling difficult. Anticipation (the phenomenon that members of younger generations present with the disease at a younger age than those of previous generations) has been rarely reported, but not conclusively as a biological reality [56]. These observations are probably due, at least in part, to disparities in diagnostic treatment between young and elderly subjects (the constitutional genetic component is more easily evoked when the haemopathy occurs in a young patient than when it occurs at an advanced age). The median age at progression to haematological malignancy is about 30 years but with very high variability (from

FIGURE 3



Dysmegakaryopoiesis, a characteristic of FPD/AML patients. At least 10% (sometimes much more) megakaryocytes are dystrophic, exhibiting a small size, scanty cytoplasm, and hypolobulated or unilobed nucleus.

FIGURE 4



A) Age at onset. **B**) Types of haematological malignancies observed in patients with a constitutional defect in *RUNX1* (from [58]). AL: acute leukaemia with no specification; ALL: acute lymphoblastic leukaemia; AML: acute myeloid leukaemia; CMML: chronic myelomonocytic leukaemia; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasia.

less than five to more than 85 years) *(figure 4A);* this distinguishes FPD/AML from other predisposing syndromes [57, 58]. Approximately 40% of individuals develop a haemopathy before the age of 50, and almost half of families feature at least one paediatric case (<18 years). The haemopathies that develop are mainly of the myeloid type (AML and MDS), but there are also cases of T-phenotype acute lymphoblastic leukaemia (T-ALL) in almost a quarter of families (*figure 4B)* [42, 58, 59]. Furthermore, there is no correlation between the type of haemopathy and type of constitutional *RUNX1* abnormality, and members of the same family can develop both myeloid and lymphoid haemopathies.

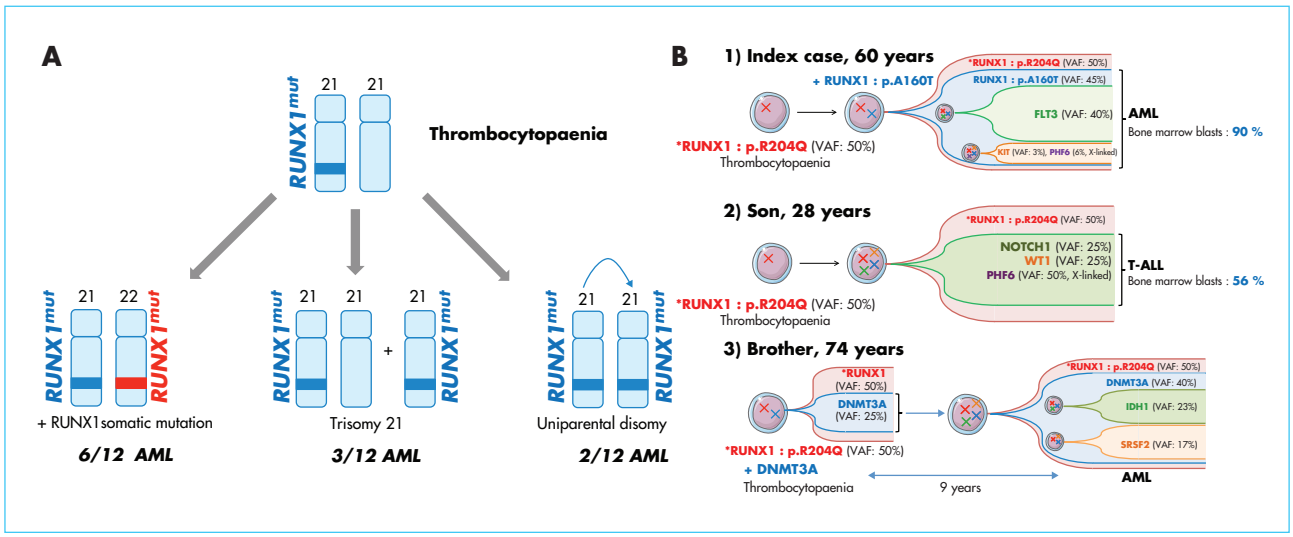
Some studies, however, suggest that the type of variant may modulate the risk of haemopathy, although this may not always be the case. Individuals with a dominant-negative mutation would thus have a higher risk of developing a haemopathy than patients with *RUNX1* haploinsufficiency [39, 46, 53, 60]. A study of several iPSC lines derived from *FPD/AML* patient cells and embryonic stem (ES) cell lines, in which *RUNX1* is inhibited by RNA interference, showed that the residual *RUNX1* dosage plays an important role in the disease phenotype. Haploinsufficiency would lead to the development of megakaryocytic and platelet abnormalities, while an even greater decrease through the dominant-negative effect would be associated with an amplification of the granulomonocytic compartment and genomic instability linked to a decrease in the expression of p53 target genes, compatible with a pre-leukaemic state [53].

However, haemopathies also occur in families with *RUNX1* deletions, indicating more complex mechanisms of disease progression.

Leukaemic transformation: a model of leukaemogenesis

Incomplete penetrance, the latency period and the variety of haematological malignancies observed in FPD/AML patients rapidly prompted the suggestion of the existence of additional (acquired) abnormalities capable of inducing transformation and possibly directing towards a distinct phenotype. Secondary defects in *RUNX1* are by far the most common during transformation to AML [42, 61]. These involve either a mutation on the second allele or a duplication of the germline mutation (via trisomy 21 or uniparental disomy). In a series of French patients (updated data [36, 37, 40, 42, 61]), 11 of the 12 patients who developed AML had acquired a secondary defect in *RUNX1 (figure 5*A, B, *case 1);* six with

FIGURE 5



Additional abnormalities acquired during leukaemic transformation in FPD/AML patients [36, 37, 40, 42, 61]. **A)** Secondary *RUNX1* abnormalities in AML secondary to FPD/AML. **B)** Representative examples of leukaemogenesis observed in FPD/AML patients within the same family: **1)** index case, **2)** son, **3)** brother). The age at diagnosis of acute leukaemia is indicated.

mutation in the second allele and five with duplication (three with trisomy 21 and two with uniparental disomies). These abnormalities appear to be events which initiate leukaemia and again attest to the importance of *RUNX1* assays in leukaemogenesis [53], but are never observed at the stage of isolated thrombocytopaenia [9, 42]. From a mechanistic point of view, it is assumed that the acquisition of a second *RUNX1* defect under selection pressure leads to significant genetic instability, the impairment of DNA repair pathways and the very rapid acquisition/accumulation of other molecular events [62]. These frequently include mutations in *SRSF2, PHF6, WT1, TET2* and *BCOR/BCORL1*, as well as the subclonal acquisition of cell proliferation-activating mutations (*RAS* pathway and receptor tyrosine kinases) [9, 42]. The mutations in *ASXL1* (frequently associated with sporadic AML with *RUNX1* mutations) are rare in AML secondary to FPD/AML [9]. A Japanese team has shown that *CDC25C* acquired mutations frequently contribute to leukaemogenesis through the deregulation of cell cycle checkpoints and the acquisition of additional abnormalities [63]. However, these mutations have not been confirmed in French, Australian and American series [9, 42, 64]. Cytologically, it is interesting to note that while somatic biallelic mutations in *RUNX1* are very strongly associated with non-maturing AML (AML0 based on FAB classification) [65, 65], AMLs occurring in an FPD/AML setting are more likely to show signs of differentiation (AML1, AML2, AML4 and AML5) [61].

In T-ALL, acquired defects in *RUNX1* are rare, while those in *NOTCH1* or *JAK3* are more frequent, demonstrating the role of secondary abnormalities in determining the leukaemia phenotype [42, 66, 67] *(figure 5*B*, Case 2).*

There are usually no associated abnormalities prior to malignant transformation thus sequencing panels are useful for monitoring patients. Mutations in DNMT3A, TET2 or SRSF2, which are characteristic of age-related clonal haematopoiesis (ARCH) or clonal haematopoiesis of indeterminate potential (CHIP) [68], are nevertheless noted in FPD/AML patients without haematological malignancies and occur earlier and more frequently than in the general population, which could be due to increased genetic instability [42, 64, 66, 69]. These mutations may precede

the diagnosis of haemopathy by several years, even when the allelic ratio is high (*figure 5*B, *Case 3*: AML occurring after a long period [more than nine years] of *DNMT3A*-mutated clonal haematopoiesis). Recent studies suggest that the selection and emergence of these clones may be prompted by the (previously underestimated) pro-inflammatory environment in FPD/AML patients, thus contributing to the increased risk of haematological malignancies [62].

Treatment and monitoring

HSC transplantation is the only curative therapy for FPD/AML. It should not be proposed in the absence of a proven haematological malignancy, although the use of preventive treatment is the subject of much debate [70]. The only recommendation here is to warn against using family members as donors, who should be tested for the constitutional variant as part of genetic counselling. Although the management and monitoring of `healthy' (haemopathy-free) individuals with a genetic predisposition to haematological malignancies and their relatives has not yet been fully codified, there is some consensus among experts [71]. A haemobiological consultation is necessary in cases of scheduled surgical or invasive procedures in order to minimise the risk of bleeding. To monitor the risk of transformation, a blood count once or twice a year seems reasonable. Monitoring may be proposed using high-throughput sequencing panels for additional mutations, as their occurrence should be considered as a sign of transformation. The presence of mutations associated with CHIP (*DNMT3A, TET2*) is more difficult to interpret as it is not yet clear whether their presence is synonymous with transformation, however, these are observed in FPD/AML transformation. In any case, for a clone with acquired abnormalities, more regular monitoring may be indicated in order to follow progression. A myelogram should be performed if new cytopaenias and/or abnormal cells appear. It is advisable to perform a myelogram, together with cytogenetic and molecular biological tests, when predisposition is revealed, to serve as a reference for subsequent follow-up. In particular, this is useful for estimating cytological evolution when the question of MDS transformation arises. Diagnostic criteria for MDS specific to FPD/AML patients have been proposed [40, 54]. These criteria emphasise that thrombocytopaenia and isolated (characteristic) dysmegakaryopoiesis are not suggestive of MDS in these patients (*table 1*). In all cases, these recommendations should be adapted according to the wishes of the patients and according to their personal or intra-family experiences.

Table 1

Diagnostic criteria of MDS in FPD/AML patients.

Major diagnostic criteria
1. Anaemia and/or neutropaenia
2. Exclusion of reactional causes of cytopaenias
3. Blood and bone marrow blasts <20%
Minor diagnostic criteria
a. Dyserythropoiesis and/or dysgranulopoiesis
b. Appearance of at least one additional cytogenetic or molecular anomaly
All the major and at least one minor criteria are necessary to establish a diagnosis of MDS

Conflicts of interest: the authors have no conflicts of interest to report in relation to this article.]

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