

**Disclosures**

No Conflicts of interest to disclose.

**Contributions**

None.

**References**

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## The arrival of personalized genomics in bone marrow failure

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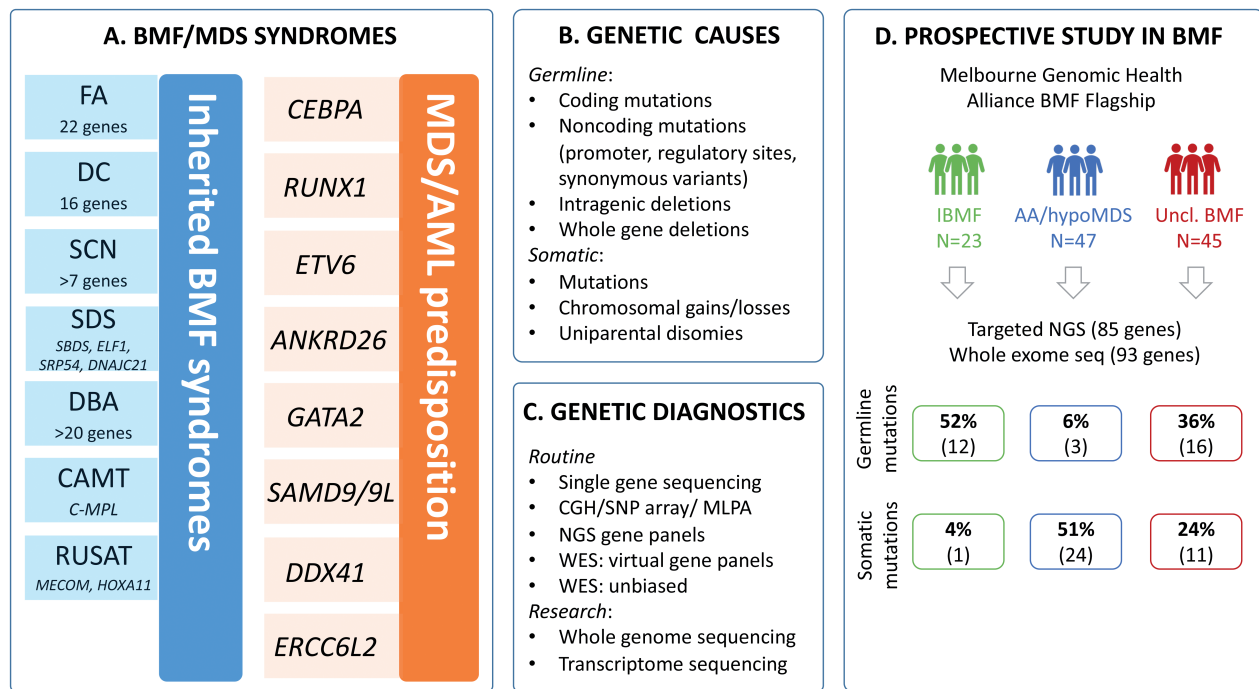
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From the description of a first genetic bone marrow failure (BMF) syndrome (dyskeratosis congenita) in the literature<sup>1</sup> it took over 80 years to discover *FANCA* as the first gene causing BMF in humans.<sup>2</sup> Using a laborious process of positional cloning, which starts by finding patients with similar phenotype patterns, performing linkage analysis using polymorphic markers to finally localize the affected cDNA, a number of additional BMF genes have been identified in the following years. These include *DKC1* discovered in 1998,<sup>3</sup> *RPS19* in 1999<sup>4</sup> and *ELANE* in 1999,<sup>5</sup> to name just a few. Fueled by mapping the human genome and rapid advances in genome-wide technologies, a large proportion of genes predisposing to BMF and myelodysplastic syndromes (MDS) were identified using next-generation sequencing (NGS) technologies in the 21<sup>st</sup> century. Overall, roughly 100 genes have, thus, so far been associated with BMF/MDS syndromes. Classical inherited BMF syndromes can be categorized into Diamond-Blackfan anemia (DBA), Fanconi anemia (FA), severe congenital neutropenia (SCN), dyskeratosis congenita (DC), Shwachman-Diamond syndrome, and congenital thrombocytopenias (Figure 1A). From a biological perspective, all these entities are caused by loss of function of fundamental cellular pathways such as DNA repair, ribosome or telomere maintenance. In recent years we have witnessed a series of new discoveries on hereditary conditions predisposing to MDS.<sup>6-8</sup> The majority of these MDS predisposing genes are essential hematopoietic transcription factors and result in heterogeneous phenotypes that can bridge over to

the classical BMF spectrum.

The landscape of germline genetic changes in BMF/MDS syndromes includes mutations in coding regions or non-coding alterations in promoters, regulatory elements, synonymous mutations, small deletions spanning single exons, and whole gene deletions (Figure 1B). Somatic alterations are mostly point mutations in leukemia driver genes and chromosomal gains or losses, but revertant uniparental disomies are also frequently found. Most BMF experts perform a phenotype-driven approach by first obtaining a family and medical history, followed by detailed physical examination and analysis of blood counts and bone marrow cellularity, morphology and cytogenetics. This approach most often guides the selection of an appropriate genetic testing platform to identify the causative gene (Figure 1C). For example, deletions (whole gene or intragenic) are a common cause of DBA and FA and for their detection, a well-established clinical copy number method is required (such as high resolution comparative genomic hybridization/single nucleotide polymorphism [CGH/SNP] array or multiplex ligation-dependent probe amplification [MLPA]). On the other hand, intronic non-coding mutations in *GATA2* might escape standard whole exome sequencing (WES) diagnostics because of coverage gaps, or simply due to missing expert knowledge about such lesions. The diagnostic genetic process for BMF and hereditary MDS is often challenging due to the continuous addition of novel genotypes and the evolving phenotype spectrum, but also the clinical need for a rapid turnaround



**Figure 1. Genomics of bone marrow failure and hereditary myelodysplastic syndromes.** (A) Syndromes and associated genes. (B) Genetic changes encountered in bone marrow failure/ hereditary myelodysplastic syndromes (BMF/MDS). (C) Genetic methods required to identify causative genetics. (D) Outline of study by Blombery *et al.* 2020. FA: Fanconi anemia; DC: dyskeratosis congenita; SCN: severe congenital neutropenia; SDS: Shwachman-Diamond syndrome; DBA: Diamond Blackfan anemia; CAMT: congenital amegakaryocytic thrombocytopenia; RUSAT: radioulnar synostosis with amegakaryocytic thrombocytopenia; IBMF: inherited BMF; AA/hypoMDS: aplastic anemia/hypocellular MDS; Uncl. BMF: unclassified BMF; CGH: comparative genomic hybridization; SNP: single nucleotide polymorphism; MLPA: multiplex ligation-dependent probe amplification; NGS: next-generation sequencing; WGS: whole exome sequencing.

time to guide therapeutic decisions.

Blombery and co-workers<sup>6</sup> are now presenting a very innovative multi-institutional prospective study from the Melbourne Genomic Health Alliance BMF Flagship (Figure 1D). They analyzed a series of 115 pediatric and adult patients diagnosed consecutively with BMF at four institutions in Australia between May 2017 and August 2018. The main purpose was to assess the impact of comprehensive genomic evaluation in this heterogeneous cohort. The authors performed a tour de force analysis using a genomic pipeline consisting of WES, targeted NGS, and in selected cases additional RNA sequencing (RNAseq) to confirm variant effect on RNA expression and droplet digital PCR (ddPCR) technique to validate small somatic clones. Every patient received personalized genetic counseling and the pathogenic variants identified through this study were used for clinical decision making. Before starting genetic analysis, patients were grouped into three diagnostic categories: inherited BMF (23 patients), acquired aplastic anemia (AA)/hypocellular MDS (47 patients) and unclassified BMF (45). The analysis resulted in a change of diagnostic category in 26% of the cohort. Most notably, the authors discovered germline causes in 3 of 47 and 16 of 45 of patients with pretest diagnoses of AA/MDS and clinically unclassifiable BMF, respectively. Additionally, somatic mutations were discovered in 51% of AA/MDS group, 24% of unclassified BMF, and 4% of inherited BMF (Figure 1D).

Recent studies have demonstrated the utility of comprehensive genomic analysis in diagnosing BMF and hereditary MDS. Zhang and colleagues employed an NGS panel

of 85 genes to find that 8 of 71 patients with idiopathic BMF/MDS carry damaging germline mutations in *GATA2*, *RUNX1*, *DKC1* or *LIGIV*.<sup>9</sup> They also demonstrated the utility of NGS panels to detect genomic copy variants in BMF. Another study focused on finding copy number variants in a large cohort consisting of BMF patients and found that 16% of them carry pathogenic copy number variants.<sup>10</sup> A landmark study by a French group in 2018 investigated 179 patients with suspected but genetically unresolved BMF. Using WES sequencing of fibroblast DNA, they found pathogenic mutations affecting 28 genes in 48% of this cohort.<sup>11</sup> Finally, in a most recent study using WES performed in 86 families with MDS/AML, known genes were found mutated in 57% of families, and 65 new candidate loci were discovered in 43% of cases.<sup>12</sup> An important finding of this work was that there is no single gene that accounts for a large number of unresolved cases with familial disease. However, aforementioned studies were of retrospective nature in cohorts collected over many years to decades. This leads to several questions: What is the immediate benefit to patient care? Should investigators approach families about pathogenic variants found in research only studies and if yes, how do we do this?

In the current study, Blombery *et al.*<sup>13</sup> used an accredited genomic pipeline where results can be returned immediately after testing to clinicians and patients. This approach had important implications for clinical management, including the choice to perform allogeneic stem cell transplantation and optimal sibling donor selection, treatment (such as corticosteroids in DBA), identification of at risk family members and implementation of disease-specific

surveillance. Several examples showcased the importance of such a real-time prospective study for patients with BMF. In one family, the identification of a pathogenic *TINF2* mutation permitted family screening and implementing DC-specific surveillance. In another family, diagnosis of homozygous *RAD51C* Fanconi-like syndrome had implications for breast and ovarian cancer screening in family members. Interestingly, in one case with a newly identified homozygous *FANCA* mutation (that resulted in exon skipping based on RNAseq), the chromosomal fragility test was ambiguous. This emphasizes the clinical impacts of personalized genomics in BMF patients, especially when the clinical features and functional testing do not completely align with a BMF syndrome, to obtain an accurate diagnosis and avoid missed or delayed diagnosis.

NGS technologies have already fulfilled their promise as a diagnostic tool in BMF/MDS. Now, we should ride on this impressive momentum and take advantage of international consortia to implement a rapid diagnostic pipeline for these rare disorders in a prospective setting, as has already been demonstrated for pediatric cancers and seriously ill infants.<sup>14,15</sup> The virtue will be to use rapid “all-in-one” genomic platforms such as WGS complemented by RNAseq allowing simultaneous detection of all types of disease-relevant genomic lesions. Personalized genomics for patients with BMF has finally arrived.

#### Disclosures

No Conflicts of interest to disclose.

#### Contributions

None.

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