Inherited bone marrow failure syndromes: retrospective review of NGS panel testing in affected individuals

Päivi Kokkonen,[2] Joe Jacher,[1] Alicia Scocchia,[1] Elina Hirvonen,[2] Emma Mårtensson,[2] Hatice Duzkale,[2] Kim Gall,[1] Zöe Powis,[1] Inka Saarinen,[2] Johanna Sistonen,[2] Juha Koskenvuo,[2] Lotta Koskinen,[2] Tero-Pekka Alastalo[1]

[1] Blueprint Genetics Inc, Seattle, USA; [2] Blueprint Genetics, Espoo, Finland

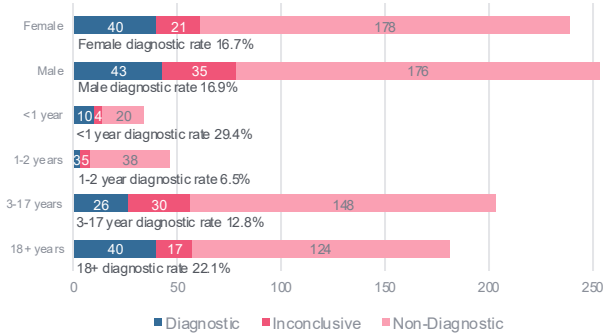
Introduction

- Inherited bone marrow failure syndromes (IBMFS), recently classified as inborn errors of immunity, represent a genetically heterogenous group of conditions characterized by aplastic anemia, congenital malformations, and increased risk to develop malignancies.
- Identifying the molecular etiology of IBMFS can allow for personalized management, surveillance, and risk estimation for the patient and their family members. Limited information exists regarding patients undergoing testing for IBMFS.
- Comprehensive next-generation sequencing (NGS) panel testing can be a useful molecular diagnostic tool, where broad inclusion of genes associated with IBMFS along with robust analysis of both multigenic and intragenic copy number variation (CNV) are expected to significantly contribute to diagnostic yield.
- To determine the diagnostic efficacy of a broad panel test including robust CNV analysis, we conducted a retrospective review of test results from patients with suspected IBMFS.

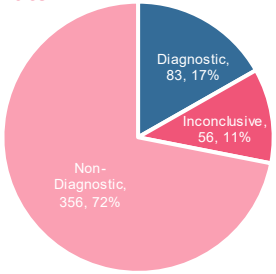
Methods

- We reviewed clinical reports from 495 consecutive patients with an indication of suspected IBMFS who underwent clinical panel testing at Blueprint Genetics (a CLIA-certified diagnostic laboratory).
- The Bone Marrow Failure Syndrome panel was utilized for testing, which contains 135 genes and includes sequence variant, CNV, and targeted noncoding variant analysis.
- CNV analysis was performed bioinformatically from NGS data using two variant calling algorithms, including a proprietary method specific for small, intragenic, exon-level CNVs.
- Variant interpretation was performed according to ACMG guidelines.
- Statistical analysis was performed using Fisher's exact test.

Demographic information by result



Diagnostic rate



Diagnostic- Pathogenic and/or likely pathogenic variants consistent with patient's reported phenotype and with associated disease inheritance (one for dominant, two for recessive conditions)

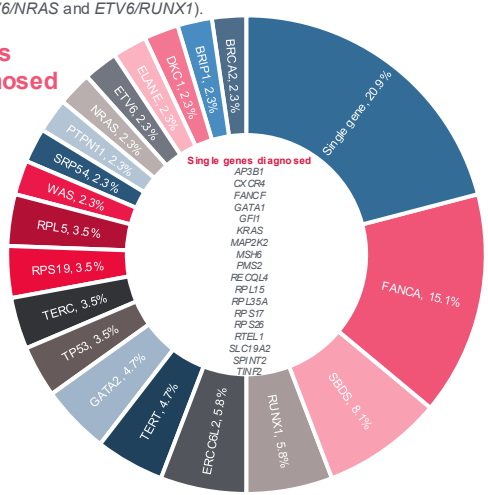
Inconclusive- Variants of uncertain significance

Non-Diagnostic- Likely benign, benign or no variants of interested reported

Results

- No statistical differences found in diagnostic rate, although patients tested before age one trend towards a higher diagnostic rate (p=0.0546) and patients tested between ages 1-2 trend towards a lower diagnostic rate (p=0.0604).
- Variants in *FANCA* were the most frequently reported diagnostic finding (13/86, 15.1%). Variants in *SBDS* were the second most frequently reported diagnostic finding (8/85, 9.4%). 12/14 (85.7%) were variants in exon 2 and a indel (c.183_184delinsCT) was also identified.
- The most common diagnoses were Fanconi anemia (18, 20.9%), Shwachman-Diamond syndrome (9,10.5%), Diamond-Blackfan anemia and Rasopathies (both 6, 7%). Two patients were reported with dual diagnoses (*ETV6/NRAS* and *ETV6/RUNX1*).

Genes diagnosed



Genes Diagnosed	Associated conditions
AP3B1	Hermansky-Pudlak syndrome
BRCA2	Fanconi anemia, Medulloblastoma, Glioma susceptibility, Pancreatic cancer, Wilms tumor, Breast-ovarian cancer, familial
BRIP1	Fanconi anemia, Breast cancer
CXCR4	Warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome
DKC1	Hoyeraal-Hreidarsson syndrome, Dyskeratosis congenita
ELANE	Neutropenia
ERCC6L2	Bone marrow failure syndrome 2
ETV6	Thrombocytopenia 5
FANCA	Fanconi anemia
FANCF	Fanconi anemia
GATA1	Anemia, without thrombocytopenia, Thrombocytopenia with beta-thalassemia,, Dyserythropoietic anemia with thrombocytopenia
GATA2	Myelodysplastic syndrome, Chronic neutropenia associated with monocytopenia, evolving to myelodysplasia and acute myeloid leukemia, Acute myeloid leukemia, Emberger syndrome, Immunodeficiency
GFI1	Neutropenia, severe congenital, 2 autosomal dominant, Neutropenia, nonimmune chronic idiopathic, of adults
KRAS	Noonan syndrome, Cardiofaciocutaneous syndrome
MAP2K2	Cardiofaciocutaneous syndrome
MSH6	Endometrial cancer, Mismatch repair cancer syndrome, Colorectal cancer, hereditary nonpolyposis
NRAS	Noonan syndrome
PMS2	Mismatch repair cancer syndrome, Colorectal cancer, hereditary nonpolyposis
PTPN11	Noonan syndrome, Metachondromatosis
RECQL4	Baller-Gerold syndrome, RAPADILINO syndrome, Rothmund-Thomson syndrome
RPL15	Diamond-Blackfan anemia
RPL35A	Diamond-Blackfan anemia
RPL5	Diamond-Blackfan anemia
RPS17	Diamond-Blackfan anemia
RPS19	Diamond-Blackfan anemia
RPS26	Diamond-Blackfan anemia
RTEL1	Pulmonary fibrosis and/or bone marrow failure, Dyskeratosis congenita
RUNX1	Platelet disorder, familial, with associated myeloid malignancy
SBDS	Aplastic anemia, Shwachman-Diamond syndrome, Severe spondylometaphyseal dysplasia
SLC19A2	Thiamine-responsive megaloblastic anemia syndrome
SPINT2	Diarrhea 3, secretory sodium, congenital, syndromic
SRP54	Shwachman-Diamond syndrome
TERC	Aplastic anemia, Pulmonary fibrosis and/or bone marrow failure, telomere-related, Dyskeratosis congenita
TERT	Aplastic anemia, Pulmonary fibrosis and/or bone marrow failure, telomere-related, Dyskeratosis congenita
TINF2	Revesz syndrome, Dyskeratosis congenita
TP53	Colorectal cancer, Li-Fraumeni syndrome, Ependymoma, intracranial, Choroid plexus papilloma, Breast cancer, familial, Adrenocortical carcinoma, Osteogenic sarcoma, Hepatoblastoma, Non-Hodgkin lymphoma
WAS	Neutropenia, severe congenital, Thrombocytopenia, Wiskott-Aldrich syndrome

CNV analysis

- CNVs ranged from 241 bp to 2.2 Mb.
- CNVs contributed to the diagnosis of 16.9% (14/83) of diagnostic findings.
- 64.3% (9/14) of these CNVs were intragenic.

Conclusions

- Genetic testing using multi-gene panels can lead to molecular diagnoses and potential changes in treatment for patients with IBMFS.
- While not statistically significant, patients tested before age one trend towards a higher diagnostic rate, suggesting that testing patients with an early onset, possibly severe phenotype can be especially effective.
- Next-generation sequencing panel testing that includes high-quality CNV and indel analysis contribute to a 20.3% increase in the diagnostic yield among patients with IBMFS.

Conflict of interest statement: All authors are employed by Blueprint Genetics.

Blueprint Genetics