Challenges of reporting potentially germline variants by cancer laboratories

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Introduction

- Approximately 8% of adult patients with cancer have a pathogenic germline variant. Many patients with these germline mutations lack a family history consistent with cancer predisposition syndrome

- 13.6% of AML patients were shown to be carriers of 1 or more P/LP germline variants in genes associated with either cancer susceptibility or haematological malignancies in a recent study of a cohort of adult AML patients (Yang et al. Blood 2022)

- Clinical suspicion for a familial predisposition syndrome, along with genetic screening, should ***not*** be restricted to children or adolescents:
    - Germline mutations in ***DDX41*** define families with an increased risk of MDS and AML that can present in adulthood

Germline variants and MDS

- Aplastic Anaemia, bone marrow failure (BMF) and MDS or AML diagnosed in **infancy**:
  - 18% of individuals had deleterious variants in BMF syndromes
  - 19–29% had deleterious variants in hereditary MDS/AML genes

- Screened Pediatric MDS patients diagnosed **< 18 yrs old**:
  - 17% deleterious variants in *SAMD9* or *SAMD9L*
  - 7% deleterious variants in *GATA2*

- Germline Pathogenic/Likely Pathogenic variants in MDS/AA patients diagnosed between **18-40 yrs old**
  - The frequency of P/LP germline variants in adults aged 18–40 diagnosed with MDS/AA is high, and the associated germline syndrome is often not apparent from the patient's medical or family history

1. Churpek JE, Pyrtel K, Kanchi K-L, Shao J, Koboldt D, Miller CA,et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. Blood. 2015;126:2484–90.
2. Guidugli L, Johnson AK, Alkorta-Aranburu G, Nelakuditi V,Arndt K, Churpek JE, et al. Clinical utility of gene panel-based testing for hereditary myelodysplastic syndrome/acute leukemia predisposition syndromes. Leukemia. 2017;31:1226–9.
3. Schwartz JR, Ma J, Lamprecht T, Walsh M, Wang S, Bryant V,et al. The genomic landscape of pediatric myelodysplastic syndromes. Nat Commun. 2017;8:1557.
Leukemia volume 35, pages2439–2444 (2021)

National Genomic Test Directory

Document first published: 3 August 2018
Page updated: 21 April 2022
Topic: Commissioning, Genomics, Specialised commissioning
Publication type: Guidance

The National Genomic Test Directory specifies which genomic tests are commissioned by the NHS in England, the technology by which they are available, and the patients who will be eligible to access to a test. The National Genomic Test Directory for rare and inherited disorders and cancer can be accessed below.

If you have any questions about the genomic testing available in your area, please contact your local genomic laboratory hub.

Document

National Genomic Test Directory for rare and inherited disease

Microsoft Excel 175 KB

Summary

The National Genomic Test Directory for rare and inherited diseases specifies the genomic tests commissioned by the NHS in England for rare and inherited disorders, the technology by which they are available, and the patients who will be eligible to access to a test.

Updated 21 April 2022.

Document

Rare and inherited disease eligibility criteria

PDF 3 MB 391 pages

Summary

This eligibility criteria document supplements the National Genomic Test Directory by setting out which patients should be considered for testing under that indication, and the requesting specialties is a list of the clinical specialties who would be expected to request the test.

Updated 21 April 2022.

Document

National Genomic Test Directory for cancer

Microsoft Excel 490 KB

Summary

The National Genomic Test Directory for cancer specifies the genomic tests commissioned by the NHS in England for cancer, the technology by which they are available, and the patients who will be eligible to access to a test.

Updated 21 April 2022.

Genes included in Next Generation Sequencing (HaemOnc Panel)

- *ABCA1, ABL1, ACD, AKT1, ANKRD26, ARAF, ARID1A, ASXL1, ATM, ATR, ATRX, BCL11B, BCL2, BCL6, BCOR, BCORL1, BCR, BIRC3, BOD1L1, BRAF, BRCA1, BRCA2, BRCC3, BTK, CALR, CARD11, CBL, CBLB, CCND1, CCND2, CCND3, CD274, CD79B, CDKN2A, CDKN2B, CEBPA, CHEK2, CREBBP, CSF3R, CSNK1A1, CTC1, CTCF, CUX1, CXCR4, DCLRE1C, DDX41, DIS3, DKC1, DNAJC21, DNM2, DNMT3A, DNMT3B, DPYD, DUSP22, EED, ELANE, EP300, ERBB3, ETNK1, ETV6, EZH2, FAM46C, FANCL, FBXW7, FLT3, FOXO1, G6PC3, GATA1, GATA2, GFI1, GNAS, GNB1, GPRC5A, HAX1, HRAS, IDH1, IDH2, IKZF1, IL7R, IRF4, JAGN1, JAK1, JAK2, JAK3, KDM5A, KDM6A, KIT, KLF2, KMT2A, KMT2C, KMT2D, KMT2E, KRAS, LAMB4, LMO1, LMO2, MAP2K1, MAP3K1, MECOM, MEF2B, MET, MPL, MYB, MYC, MYCN, MYD88, NCOR1, NCOR2, NF1, NF2, NFE2, NOTCH1, NOTCH2, NPM1, NRAS, NRD1, NSD1, NT5C2, NTRK1, NTRK2, NTRK3, NUDT15, NUP98, PALB2, PAX5, PDGFRA, PDS5B, PHF6, PIGA, PIK3CA, PIK3CD, PIK3R1, PLCG2, PMS1, PMS2, PPM1D, PRPF8, PTEN, PTPN11, PTPRT, RAD21, RAD50, RAD51C, RAD51D, RHOA, RIT1, RPL5, RPL10, RUNX1, SAMD9, SAMD9L, SAMHD1, SBDS, SETBP1, SF1, SF3B1, SH2B3, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5B, SUZ12, TAL1, TERC, TERT, TET1, TET2, TINF2, TP53, TPMT, TRAF2, U2AF1, U2AF2, USP7, VPS45, WAS, WT1, XPO1, XRCC2, ZRSR2*

Panel	Genes
ALL	*ETV6,NOTCH1,FBXW7,TP53,PTEN,KRAS,NRAS,HRAS,ABL1*
AML and ALL	*NPM1,CEBPA,RUNX1,FLT3,IDH1,IDH2,KIT,WT1,ASXL1,SRSF2,STAG2,RAD21,TP53,KRAS,NRAS,KMT2A,PPM1D,ETV6,NOTCH1,FBXW7,PTEN,HRAS,ABL1, GATA1, DDX41, BCL2*
AML and MDS	*ASXL1, BCOR, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, NRAS, NPM1, KMT2A, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, STAG2, SRSF2, TET2, TP53,U2AF1, WT1, PPM1D, GATA1, DDX41, BCL2*
MPN limited	*JAK2, CALR, MPL*
MPN and MDS	*KRAS, NRAS, TP53, JAK2, CALR, MPL, ASXL1, CBL, CHEK2, CSF3R, CUX1, DNMT3A, EZH2, IDH1, IDH2, IKZF1, KIT, NFE2, SF3B1, SH2B3, SRSF2, TET2, U2AF1, HRAS, RUNX1, SETBP1, ZRSR2, BCOR, PTPN11, ABL1, GNB1,KMT2C*
Myeloid All Genes	*ASXL1, ABL1, BCL2, BCOR, CBL, CHEK2, CSF3R, CUX1, CEBPA, DDX41, DNMT3A, EZH2, FLT3, GATA1, GNB1, HRAS, IDH1, IDH2, IKZF1, JAK2, CALR, KIT, KMT2A, KMT2C, KRAS, MPL, NFE2, NF1, NRAS, NPM1, PPM1D, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, STAG2, SH2B3, SRSF2, TET2, TP53, U2AF1, WT1, ZRSR2*
Histiocytosis	*BRAF, MAP2K1, NRAS, KRAS, HRAS, ERBB3, ARAF, MAP3K1, PIK3CA, PIK3CD*
Myeloma	*KRAS, NRAS, BRAF, TP53, DIS3, FAM46C, IRF4*
CLL	*TP53, BTK, PLCG2, BCL2, NOTCH1, ATM, SF3B1, BIRC3*
Chronic B lymphoid	*ARID1A, BRAF, BTK, CARD11,CREBBP, CXCR4, EP300, EZH2, FOXO1, MAP2K1, MEF2B, MYD88, PLCG2, TP53, CCND3, TCF3, ID3, ATM*
Chronic T lymphoid	*RHOA, DNMT3A, IDH2, TET2, STAT3, STAT5B, TP53*

Virtual Panels

Mutated genes with well-known predisposition to hereditary haematological malignancies (HHM) detected and referred to clinical genetics service upon detection

- *ANKRD26*
- *DDX41*
  - The reported germline alterations are commonly frameshift mutations
- *CEBPA*
  - Nearly 10% of AML patients with biallelic *CEBPA* mutation also harbour a germline *CEBPA* mutation
  - These germline mutations are commonly frameshift or nonsense mutations
- *ETV6*
  - Predisposition to ALL and myeloid malignancies
- *GATA2*
- *RUNX1*
- Genes associated with Noonan Syndrome

Please note that this assay can not exclude the germline origin for the ***Gene*** c.xxx p.(xxx) variant.
Referral to clinical genetics service for family history and further assessment is recommended.

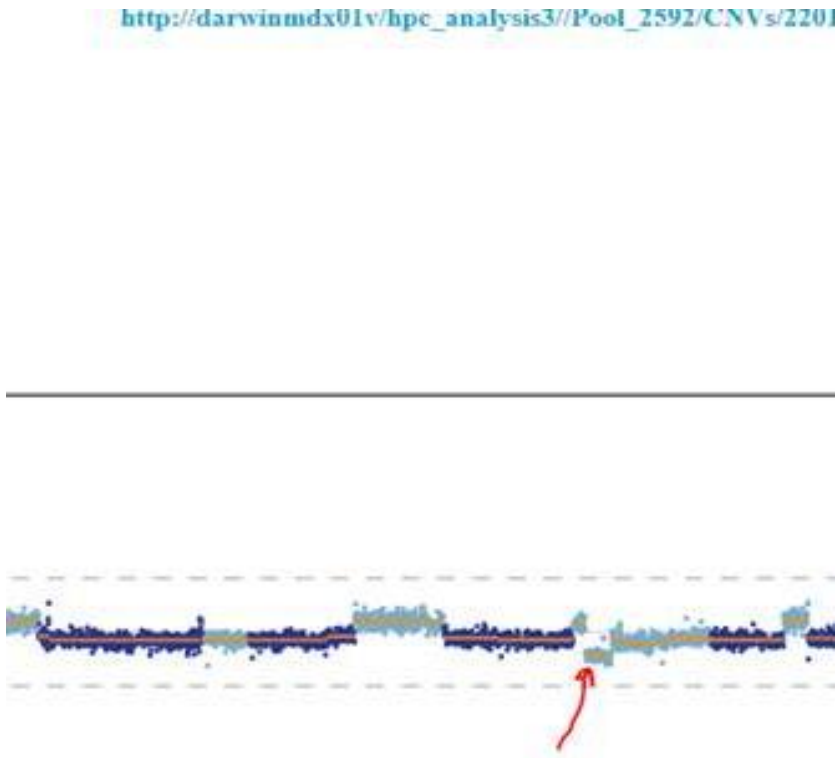
Does a low VAF excludes germline origin?

When to suspect a germline origin in a somatic variant screen?

- Germline variant maybe suspected when MAF is 40-60%, however
  - The VAF should not be used as a filter during the investigation of a potentially incidentally detected HHM as multiple sequencing variables may lead to inaccurate VAFs, such as:
    - loss of heterozygosity,
    - variable probe binding
    - other bioinformatic pipeline issues
- For this reason, Roloff et al propose to perform germline testing in anyone with a P/LP variant in an HHM-associated gene on somatic NGS profiling (JCO 2021)

Case 1

- 5 year old male with B-ALL – BM shows B-lymphoid blasts at 90%.
- Cytogenetics and our CNV data shows a partial loss of chr12 (*ETV6*).
- On NGS we have an *ETV6* missense variant c.1046T>C p.(Leu349Pro) at 5.8% VAF.
- This variant has been reported in Kindred analysis to confer susceptibility to ALL.
- *ETV6* variant, germline or Somatic?

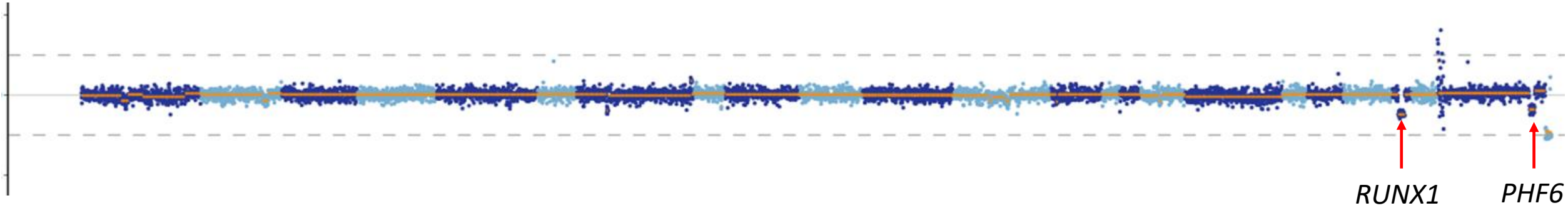


PMID: 26102509 - Topka S, Vijai J, Walsh MF, et al. Germline ETV6 Mutations Confer Susceptibility to Acute Lymphoblastic Leukemia and Thrombocytopenia. PLoS Genet. 2015;11(6):e1005262. Published 2015 Jun 23. doi:10.1371/journal.pgen.1005262

Copy number alteration and germline origin

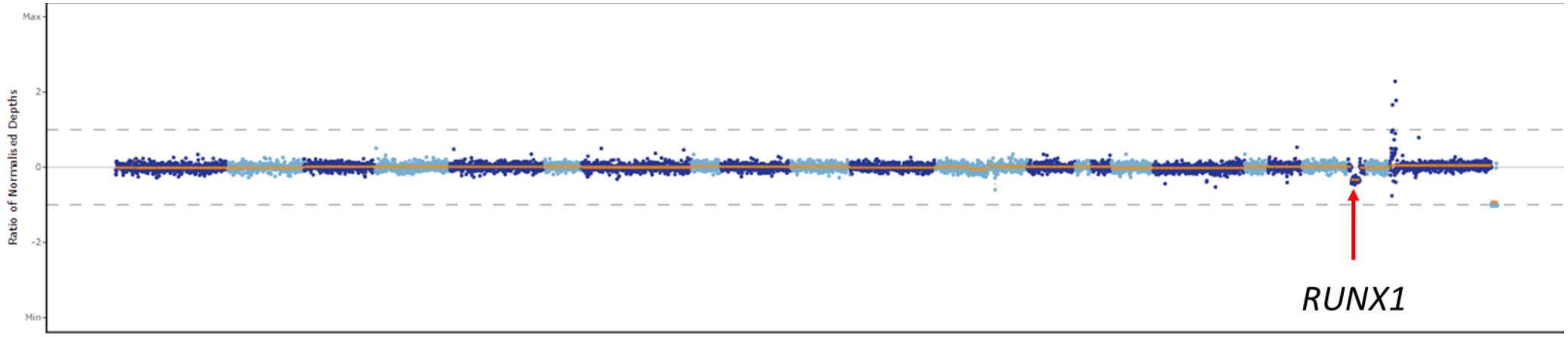
Case 1

- 61 y, investigated for MDS,
- Daughter died of AML
- Morphology of the BM: Myeloid precursors are seen at all stages of maturation with no overt dysplasia. There is dyserythropoiesis with n/c asynchrony and nuclear budding seen in >10% of precursors. Blasts account for 1% of nucleated cells.
- *BCOR*, c.1005dup p.(Ser336LeufsTer45): 33.8%
- *EZH2*, c.448del p.(Ile150Ter): 34.3%
- Is the *RUNX1* copy number alteration of germline origin?



Case 2

- 68 y, investigated for myelofibrosis,
- Morphology of the BM: not available
- 46,XX apparently normal (female) karyotype.
- *ASXL1, G646WfsTer12): 44.9%*
- *KRAS, G12S: 29.6%*
- *NRAS, G12D: 6.7%*
- *TET2, Q770Ter: 46.0%*
- *EZH2, I146T and A103_S106del): 46.6% and 41.0% respectively.*
- Is the *RUNX1* copy number alteration of germline origin?



article

Haploinsufficiency of *CBFA2** causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia

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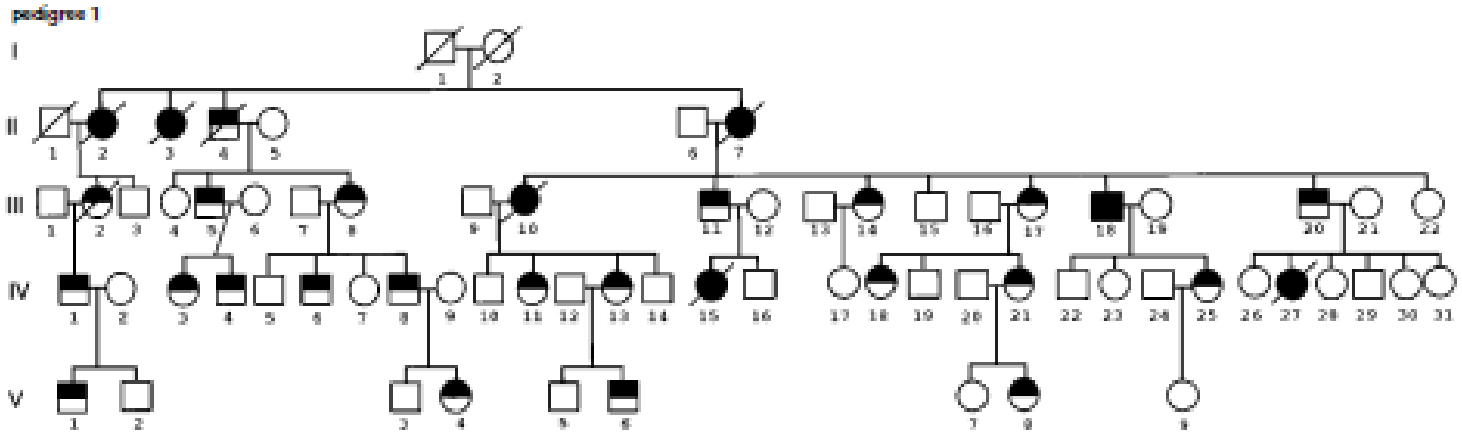
Linkage to markers on chromosome 21q identified an 880-kb interval containing the disease gene. Mutational analysis of regional candidate genes showed nonsense mutations or intragenic deletion of one allele of the haematopoietic transcription factor *CBFA2* (formerly *AML1*) that co-segregated with the disease in four FPD/AML pedigrees. We identified heterozygous *CBFA2* missense mutations that co-segregated with the disease in the



*RUNX1

Suspicious germline variants detected among the samples analysed by HaemOnc panel

CANCER GENETICS

Inherited Susceptibility to Hematopoietic Malignancies in the Era of Precision Oncology

Gregory W. Roloff, MD[1]; Michael W. Drazer, MD[2]; and Lucy A. Godley, MD, PhD[3]

review articles

abstract

As germline predisposition to hematopoietic malignancies has gained increased recognition and attention in the field of oncology, it is important for clinicians to use a systematic framework for the identification, management, and surveillance of patients with hereditary hematopoietic malignancies (HHMs). In this article, we discuss strategies for identifying individuals who warrant diagnostic evaluation and describe considerations pertaining to molecular testing. Although a paucity of prospective data is available to guide clinical monitoring of individuals harboring pathogenic variants, we provide recommendations for clinical surveillance based on consensus opinion and highlight current advances regarding the risk of progression to overt malignancy in HHM variant carriers. We also discuss the prognosis of HHMs and considerations surrounding the utility of allogeneic stem-cell transplantation in these individuals. We close with an overview of contemporary issues at the intersection of HHMs and precision oncology.

JCO Precis Oncol 5:107-122. © 2021 by American Society of Clinical Oncology

INTRODUCTION

For most individuals, the development of cancer occurs at an advanced age and is propagated by the acquisition of somatic driver mutations. However, beginning in the late 1960s, clinicians noted families with cancer clusters. The first kindred, described by Li and Fraumeni,[1] was afflicted with sarcomas and breast cancers, and subsequent laboratory studies described pathogenic germline variants in *TP53* as the molecular etiology. Pathogenic germline variants predisposing to the development of solid malignancies are now estimated to account for as many as 10%-15% of new cancer diagnoses.[2] Although families with numerous cases of hematopoietic neoplasms have been recognized for several decades,[3] elucidating the molecular etiologies of hereditary hematopoietic malignancies (HHMs) has accelerated in recent years with advances in next-generation sequencing (NGS). Clinically, a HHM diagnosis provides opportunities for testing and education of family members who are unaffected variant carriers and may benefit from cancer screening efforts. Similarly, providers may avoid using hematopoietic stem and progenitor cells (HSPCs) from a related, unaffected donor who also carries the pathogenic variant of interest, thereby avoiding the risk of donor-derived leukemia.[4] On a research basis, identifying patients at risk for HHMs informs efforts to understand HHM biology and leukemogenesis while enhancing our understanding of normal hematopoiesis. Table 1 lists recognized HHMs, includes references to seminal HHM publications, and cites contemporary review articles. In this review, we overview mechanisms of leukemogenesis in HHMs, discuss the current utility of hematopoietic stem-cell transplantation (HSCT), and highlight real-world considerations regarding patient identification, evaluation, and testing.

How Does Leukemogenesis Occur in HHMs?

The incidence of leukemia increases with age and is generally driven by the accumulation of pathogenic somatic mutations in HSPCs.[45] Clonal hematopoiesis (CH) is the process by which HSPCs acquire leukemogenic somatic mutations, but with normal peripheral blood cell counts and no morphologic evidence of malignancy.[46] CH is observed in approximately 10% of older adults and is a leukemogenic risk factor (hazard ratio of 12.9).[47]

Carriers of likely pathogenic or pathogenic (P/LP) germline variants in HHM-associated genes experience variable risk for progression to bone marrow failure, myelodysplastic syndrome (MDS), and/or acute leukemia. The implications of CH in HHMs are not fully elucidated. For example, it is unclear if CH is a required leukemic precursor in HHMs or if HHM variant carriers with CH have a significantly increased risk for the development of malignancies. To date, no interventions have been demonstrated to suppress CH clones and/or impede leukemogenic progression from CH to hematopoietic malignancies.

ASSOCIATED CONTENT
Appendix
Author affiliations and support information (if applicable) appear at the end of this article.
Accepted on November 2, 2020 and published at ascopubs.org/journal/po on January 11, 2021: DOI https://doi.org/10.1200/PO.20.00387

What should the decision be on reporting the pathogenic/likely pathogenic variants in the genes for which there are some evidence for germline susceptibility to cancer but they are not among the clinically recommended genes for further investigation?

TABLE 1. Overview of Pertinent HHMs

Molecular Function	Gene	Inheritance Pattern	Hematopoietic Manifestations
Transcriptional machinery	*ARID1A*	AD	MM
	CEBPA	AD	AML
	ETV6	AD	B-ALL and MM
	GATA2	AD	MDS and AML
	IKZF1	AD	B-ALL
	MECOM/EVI1	AD	Amegakaryocytic thrombocytopenia and aplastic anemia
	MRTFA	AR	Megakaryoblastic leukemia and thrombocytopenia
	PAX5	AD	B-ALL
	RUNX1	AD	B-ALL, MM, and T-ALL
	TET2	AR and AD	MM and lymphomas
	TP53	AD	MDS, AML, B-ALL, lymphoma, and T-ALL
DNA repair pathways	*ATM*	AD	CLL, T-ALL, and lymphoma
	BLM	AR	Lymphomas, ALL, MDS, and AML
	CHEK2	AD	CLL and MM
	ERCC6L2	AR	Bone marrow failure, MDS, and AML
	*Fanconi genes**	AR, except *FANCB* (X-linked), and *FANCR* (AD)	ALL, MDS, and AML
	KDM1A	AD	Multiple myeloma and AML
	MBD4	AD	MDS and AML
	NBN	AR	Non-Hodgkin Lymphoma, and T-ALL
	RECQL4	AR	Lymphomas and T-ALL
	USP45	AR	Multiple myeloma
Telomere biology	*DKC1*	X-Linked	Bone marrow failure, MDS, and AML
	NAF1	AD	
	TERC	AD	
	TERT	AD and AR	
	RTEL1	AD and AR	

TABLE 1. Overview of Pertinent HHMs (Continued)

Molecular Function	Gene	Inheritance Pattern	Hematopoietic Manifestations
Ribosomopathy	*DNAJC21*	AR	MDS and AML
	EFL1	AR	Bone marrow failure
	NPM1	AD	Bone marrow failure
	SBDS	AR	MDS and AML
Cellular proliferation	*CBL*	AD	JMML
	CSF3R	AD	Chronic neutrophilic leukemia, and myeloid and lymphoid malignancies
	JAK2	AD	Myeloproliferative neoplasms
	MPL	AD and AR	Thrombocytosis
	PTPN11	AD	JMML and ALL
	RBBP6	AD	Myeloproliferative neoplasms
	SH2B3	AR	B-ALL and myeloproliferative neoplasms
	SAMD9	AD	MDS and AML
	SAMD9L	AD	MDS and AML
Emerging biology	*ANKRD26*	AD	MDS and AML
	ARID1A	AD	Multiple myeloma
	DDX41	AD	MM and B-cell malignancies
	DIS3	AD	Multiple myeloma
Other	*CST3, FGA, GSN, LYZ, and TTR*	AD	Hereditary amyloidosis
	NF1	AD	JMML and MDS
	RBM8A	AR	Thrombocytopenia
	SRP72	AD	MDS and bone marrow failure
	WAS	X-linked	MDS and AML

The difficulty of reporting variants which might be of germline origin by somatic labs and some recommendations

- The germline origin can not be excluded for some variants in particular at VAF between 40 to 60% using only tumour samples, however suspicion should not be limited to this range and should be raised for genes with known germline susceptibility to cancer
- Familial history and detailed clinical features for most of the cases sent to somatic lab are not available
- Should always be a recommendation on referral to clinical genetics service for any pathogenic/likely pathogenic variants associated with cancer in the genes in the panels?
- Should the recommendation for referral in the report be discussed first with the local clinical genetics service before being added to the report by a somatic lab? How does it affect clinical genetic services?
- Should the analysis of constitutional material for germline variants (such as CD3) be done in parallel with tumour samples for AML and MDS cases as a part of routine diagnostic process?

acknowledgement

Molecular pathology

Andrew George

April Hallett

Hood Mugalaasi

Martijn van der Linde

Michael Hubank

Mikel Valganon Petrizan

Oluwatosin Taiwo

Sanna Hulkki Wilson

Sara Ribeiro

Suzanne MacMahon

Victoria Smith

Yunis Khan

Yvonne Morgan

Clinal Genetics

Terri McVeigh

Bioinformatics

Lina Yuan

Ridwan Shaikh

Sabri Jamal

Haematology

David Taussig

Dima El-Sharkawi

Sunil Iyengar