Germline Predisposition to Haematological Malignancies
Consensus guidelines meeting 28-29th April 2022

Background reading document

Contents
Introduction ............................................................................................................................. 1
Figure 1. Flowchart highlighting current challenges .............................................................. 3
Current pathway challenges ................................................................................................... 4
  From somatic detection to germline confirmation............................................................... 4
  Sample types ......................................................................................................................... 4
  Consent and sample acquisition in germline testing pathways .......................................... 5
  Donor selection ..................................................................................................................... 5
Genes conferring germline/constitutional (heritable) susceptibility to haematological malignancies ................................................................................................................................. 6
  DDX41 .................................................................................................................................. 6
  CEBPA ................................................................................................................................. 6
  RUNX1 ................................................................................................................................ 7
  ANKRD26 ............................................................................................................................ 7
  ETV6 ..................................................................................................................................... 7
  GATA2 ................................................................................................................................ 7
References ............................................................................................................................ 8

Introduction
Next generation sequencing (NGS) is now standard of care in the diagnostic work-up of patients with suspected haematological malignancies. In England, eligibility for testing is set by the National Genomic Test Directory (www.england.nhs.uk/publication/national-genomic-test-directories/). The identification of somatic gene variants can inform diagnosis, prognosis and therapeutic options. Somatic gene panels and Whole Genome Sequencing (WGS) can also identify individuals with potential or confirmed germline predisposition to haematological malignancy. Identifying these patients and setting up pathways for appropriate germline confirmation with the possibility of cascade predictive testing in family members is a relatively new area of expanding clinical and laboratory work. Current challenges within this include:

- Which genes and variants should be analysed and reported?
- What sample types are best used?
- What is the pathway for patients needing a clinical genetics referral?
The UK-CGG, CanGene-CanVar and the NHSE GLH Haematological Oncology Malignancies Working Group have convened these two half-day workshops to try to address these challenges. The aim of the meeting is to establish consensus guidelines on the clinical and laboratory pathways and the management of disease causing germline variation. Given this is a substantial topic, the meeting will focus on six genes: **DDX41, CEBPA, RUNX1, ANKRD26, ETV6, GATA2**. Hopefully any consensus reached will be applicable to an expanding number of other genes in this setting.
Figure 1. Flowchart highlighting current challenges

Patient presents to Haematology

Which genes to include?

NGS gene panel

Potential germline variant(s)?

GTAB
Review of somatic variants

Decisions on
- indication for germline testing
- sample type
- urgency

Clinical Genetics
Referral if germline variant(s)

Is blood relative a potential transplant donor?

Rapid
Priority germline test
Referral to clinical genetics
Rapid access predictive testing?

No transplant or unrelated transplant donor?

WGS

GTAB
Review of somatic + germline variants

Routine
Germline confirmation
Referral to clinical genetics
Predictive test option for relatives
Current pathway challenges

From somatic detection to germline confirmation

In practice, bioinformatic pipelines undertaken through somatic testing strategies usually highlight variants within three different classifications according to the American College of Medical Genetics (ACMG) variant interpretation guidelines: Variants of Uncertain Significance (VUS) (Class 3), Likely Pathogenic (Class 4) and Pathogenic (Class 5). There are no definitive guidelines about when to proceed to germline testing after somatic panel testing for haematological malignancy. Traditionally, in UK Clinical Genetics services, a threshold of 10% for finding a likely pathogenic/pathogenic germline variant has been used for considering germline testing (NICE, 2013). Germline VUS are generally not considered clinically actionable, with the exception of rare cases when further investigations may provide additional evidence to upgrade them to likely pathogenic.

In 2020, the European Society for Medical Oncology (ESMO) Precision Medicine Working Group sought to generate guidelines on germline-focussed analysis of tumour sequencing data, indications for germline testing and patient information/consent. Their published recommendations were based on the somatic to germline conversion rate in paired sequencing data from more than 16,000 solid cancers (Mandelka et al., 2019). In summary, these guidelines advise that germline focussed analysis should be offered where a variant is predicted to be likely pathogenic/pathogenic in the germline setting, highly penetrant and found at a Variant Allele Frequency (VAF) of >30% for single nucleotide variants or >20% for small insertions or deletions. Caution was advised regarding variants in genes that are not proven through research to be of high penetrance, or variants in genes associated with disorders unrelated to the malignancy for which testing was done. Confirmation of VUS was not recommended. Research evidence on patient preferences indicates that a majority of cancer patients would prefer to know of incidental germline findings irrespective of whether the associated condition is preventable (Yushak et al., 2016).

Sample types

In the solid cancer setting, DNA extracted from peripheral blood leukocytes is a commonly used sample for germline testing. Alternative sources of germline DNA are saliva (in which the predominant DNA source is leukocytes) or a buccal epithelial scrape. In the Haematology cancer setting, blood and saliva are unsuitable due to the predominance of tumour DNA. Sample options in this setting are a skin biopsy, T-cell purified DNA sample from blood, buccal scrape or remission bone marrow sample. A summary table of some advantages and disadvantages of these is below. Other samples such as urine, finger nails and skin swabs have been shown to yield insufficient DNA for testing (Padron et al., 2018). Advantages and disadvantages of different sample types which could potentially be obtained for germline analysis are outlined in Table One.
<table>
<thead>
<tr>
<th>Sample type</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin biopsy</td>
<td>High quantity DNA source</td>
<td>Invasive procedure</td>
</tr>
<tr>
<td></td>
<td>Cell culture option to increase yield</td>
<td>May not be possible in all centres</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Requirement for culture to avoid contamination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adds time delay to pathway</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If uncultured, chance of contamination by leukocyte-derived DNA</td>
</tr>
<tr>
<td>T-cell sorting</td>
<td>Easy accessible sample (blood)</td>
<td>Need specialist laboratory technique</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cannot be done in all centres</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Possibility of early somatic haematopoietic mutations misdiagnosed as germline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gold standard involves expensive FACS sorting otherwise risk of contamination</td>
</tr>
<tr>
<td>Buccal scrape</td>
<td>Easy access sample</td>
<td>Contamination by leukocyte-derived DNA*</td>
</tr>
<tr>
<td></td>
<td>Patients can self-sample at home</td>
<td></td>
</tr>
<tr>
<td>Remission bone marrow aspirate</td>
<td>Reduces the number of samples needing germline confirmation</td>
<td>May delay germline confirmation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not all patients have remission sampling</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not suitable in urgent cases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Possibility of residual clonal somatic haematopoietic mutations misdiagnosed as germline</td>
</tr>
</tbody>
</table>

*shown to be low if procedure optimised for epithelial cell recovery (Padron et al, 2018)

Table 1: Advantages/Limitations of sample types available for germline confirmation in patients with haematological malignancies

Consent and sample acquisition in germline testing pathways

The ESMO guidelines acknowledge that consent and sample storage for possible germline confirmation can be included ahead of somatic panel NGS, but is often a more staged approach, occurring after variant review and classification. Irrespective of timing, information provision and documented consent is conventional practice and a requirement prior to germline confirmatory testing. There are different approaches to confirmatory testing. There has been a general move towards mainstream germline testing in the solid cancer setting, with a referral for genetic counselling following confirmation of a germline pathogenic variant (Flaum et al, 2020). Survey-based qualitative research evidence in a separate study of nearly 1000 solid cancer patients showed high acceptability and satisfaction of a mainstream genetic testing model (Hamilton et al, 2021). Suggested models of care in the haematological setting also reflect the need for patient information and informed consent at various stages, with a requirement for formal genetic counselling around confirmation of a germline pathogenic variant (Godley & Shimamura, 2017; Tawana et al, 2018; Baliakas et al, 2019).

Donor selection

If treatment of haematological malignancy is to involve allogeneic hematopoietic stem cell transplantation, donor matching warrants consideration of any known germline predisposition (Godley & Shimamura, 2017). If an asymptomatic related donor is being considered, predictive testing for the known variant should be offered. Donor-derived leukaemias have been reported after transplant from a relative who was a carrier of a familial germline pathogenic variant in CEBPA (Xiao et al, 2011), DDX41 (Berger et al, 2017) and GATA2 (Galera et al, 2018). Consideration of the time-
sensitivity whilst upholding a relative’s right not to know would normally be addressed in genetic
counselling around predictive testing.

**Genes conferring germline/constitutional (heritable) susceptibility to haematological malignancies**

**DDX41**

The *DDX41* gene encodes a DEAD-box RNA helicase (Polprasert *et al*, 2015). Germline pathogenic variants in *DDX41* are associated with an increased risk of haematological malignancy, mainly Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukaemia (AML). The exact level of risk for *DDX41* carriers remains unclear. Cardoso *et al* (2016) compared the frequency of loss of function variants in MDS and secondary AML with the frequency seen in the Exome Aggregation Consortium (ExAC) database (Karczewski *et al*, 2017). Allowing for a background risk of developing MDS/AML as 1/100, they suggested a relative risk for *DDX41* carriers of 7.51. Studies in cohorts of MDS/AML patients with a germline *DDX41* pathogenic variant have found 27-43% have a family history of haematological malignancy or cytopenias (Sébert *et al*, 2019; Bannon *et al*, 2020), yet *de novo* germline variants are rare and have not been reported to date (Churpek *et al*, 2021). Diagnosis of malignancy is most commonly in the sixth or seventh decade, showing overlap with sporadic AML, and there is a higher penetrance in men compared to women (Quesada *et al*, 2019; Sébert *et al*, 2019). Heterozygous carriers of a germline pathogenic variant in *DDX41* often have normal blood counts until a malignancy develops (Lewinsohn *et al*, 2016). However, a personal history of cytopenia was reported in 22/33 *DDX41* carriers in the USA by Bannon *et al* (2020) and 15/33 *DDX41* carriers in France by Sébert *et al* (2020). No evidence-based guidelines for surveillance exist. Expert opinion has suggested Complete Blood Count (CBC) and clinical examination every 6-12 months, with recognition of a current lack of prospective data to inform this (Godley & Shimamura, 2017; Baliakas *et al*, 2019).

**CEBPA**

In 2004, the first family with a germline pathogenic variant in *CEBPA* was reported, including three relatives with AML (Smith *et al*, 2004). In 2015, analysis of 10 further families with pathogenic *CEBPA* variants was reported, including 24 individuals with AML (Tawana *et al*, 2015). Penetrance was 80% for development of AML in confirmed or obligate carriers. Median age of AML onset was 24.5 years (range, 1.75-46 years). Relapse risk was high (56% over 10 years) and characterised by an absence of *CEBPA* variants found in the diagnostic samples, with presence of different *CEBPA* variants in relapse samples, likely representing new leukaemic clones. From the limited number of families with a germline pathogenic variant, penetrance of AML appears to be high with onset at a younger age than sporadic AML. Most germline pathogenic variants have been identified in the N-terminal region of the gene. There is evidence to suggest that *CEBPA* pathogenic variants outside of the N-terminal region have lower penetrance of around 50% (Pathak *et al* 2016; Mendosa *et al* 2021). Heterozygous carriers of a germline pathogenic variant in *CEBPA* usually have normal blood counts until a malignancy develops (Tawana *et al*, 2017). Expert opinion has suggested CBC every 6-12 months, with bone marrow evaluation after CBC abnormalities (Tawana *et al*, 2010; Godley & Shimamura, 2017).
RUNX1
Germline pathogenic variants in RUNX1 have been established as a cause of familial platelet disorder and predisposition to haematological malignancy since the late 1990s (Song et al., 1999). Over 130 families with RUNX1-Familial Platelet Disorder and Associated Myeloid Malignancies (RUNX1-FPDMM) have been described, with a wide spectrum of variants and no obvious correlation of variant sub-type with penetrance or expressivity (Brown et al., 2020; DiFilippo et al., 2020). Median age of onset of haematological malignancy is 33 years, with a range from early infancy to later adulthood (Brown et al., 2020). Symptoms present as easy bleeding/bruising (~90%), MDS/AML (~25-50%) and eczema (up to 50%), with only a minority of carriers remaining symptom-free (Brown et al., 2020). Expert opinion guidelines recommend CBC every 3-4 month, clinical examination annually and bone marrow evaluation if either are abnormal, with dermatology review as needed (Deuitch et al., 2021).

ANKRD26
In 2011, six different pathogenic variants in ANKRD26 were described in nine families with autosomal dominant thrombocytopenia (Pippucci et al., 2011). Subsequent analysis of 21 families with ANKRD26 pathogenic variants showed that thrombocytopenia and bleeding tendency is usually mild (Noris et al., 2011). In 2013, a new series of 118 individuals who were confirmed or very likely ANKRD26 carriers showed that 10 of these individuals had developed myeloid malignancy (Noris et al., 2013). Combining this series with 108 ANKRD26 carriers already reported showed 4.9% had acute leukaemias, 2.2% had MDS and 1.3% had chronic myeloid leukaemia (CML). No evidence-based guidelines for surveillance exist. Expert opinion has suggested annual Complete Blood Count (CBC) with bone marrow evaluation if CBC is abnormal (Perez Botero et al., 2018).

ETV6
In 2015, three unrelated families were reported with different germline pathogenic variants in ETV6, demonstrating segregation with thrombocytopenia and haematological malignancy (Zhang et al., 2015). Subsequently, two unrelated families with ETV6 germline pathogenic variants segregating with thrombocytopenia and ALL were described after screening 588 paediatric leukaemia cases in a paediatric cancer database (Topka et al., 2015). No ETV6 germline pathogenic variants were identified in children with other types of cancer in this database. In a study of 4405 children with ALL, ETV6 germline analysis revealed 31 variants in 35 patients (0.79%), with 15/31 (48%) predicted to be deleterious variants (Moriyama et al., 2015). Children with an ETV6 germline variant and ALL were on average older than children with ALL and no ETV6 variants: 10.2 years (IQR 5.3-13.8) versus 4.7 years (IQR 3.0-8.7). Evidence so far has established the association of ETV6 with mild to moderate thrombocytopenia and a predisposition to acute leukaemias, whilst the degree of risk elevation remains unclear. No evidence-based guidelines for surveillance exist.

GATA2
A range of malignant and non-malignant phenotypes has been described in individuals and families with inherited heterozygous pathogenic variants in GATA2. Since 2011, there are more than 100 families and more than 122 carrier individuals reported with an associated phenotype (Brown et al., 2020). GATA2-deficiency syndrome is associated with disorders of haematopoiesis, lymphatics and immunity in children and adults, with overall penetrance estimated at over 90% in some families
(Collin et al, 2015). The risk of developing myeloid malignancy is estimated to be around 75%, although this is likely to include ascertainment bias, with median age of onset in the second decade (Wlodarski et al, 2017). GATA2 carrier families may be more easily identified clinically compared to other predisposition genes due to their syndromic features, although presentation is variable. No evidence-based surveillance exists, but suggested published guidelines advise serial CBC and annual bone marrow examination, with involvement of other medical specialties as needed (Collin et al, 2015).

References


