Undertaking germline confirmation of somatic findings

West Midlands Regional Genetics Laboratory experience (C&S GLH):
Paula Page

Viapath Molecular Pathology at King's College Hospital experience (SEGLH):
Nick Parkin

Haematological Malignancies Consensus Workshop 28th April 2022

Introductions: WMRGL experience

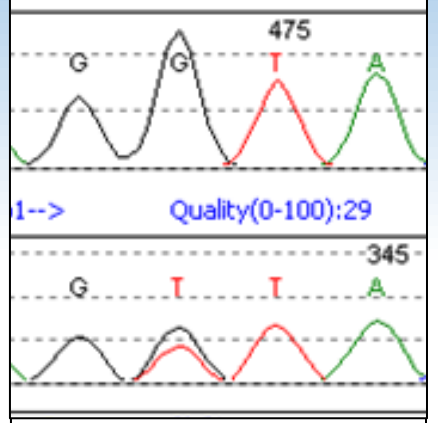
2007: WMRGL first offered testing for germline variants in *RUNX1* (plus *CEBPA* for >10 years).

2015: Collaborated with Familial MDS/AML research group at Barts to create small Familial AML NGS panel. Batching an issue.

2017: Commenced panel testing for myeloid neoplasia to detect somatic variants – with awareness that some variants would be germline (!)

2019: Incorporated 13 relevant cancer susceptibility genes (CSG) into the somatic myeloid panel – batching no longer an issue

- Within Rare Disease TD, Haematology was awarded to C&S GLH - most testing delivered by Oxford. Retention of R347 (Inherited predisposition to AML) and R366 (Inherited susceptibility to ALL) panels in Birmingham due to our prior experience
- Haem-Onc team work closely with staff in Familial Cancer section to ensure cases handled appropriately: accurate ACGS classification and interpretation of variants
- Good local infrastructure with Haematology consultants and Clinical Genetics



RUNX1 pathogenic splice site variant c.270+1G>T identified in large pedigree with FPD. Reported in 2010

F-AML referrals over last 5 years	No. samples
Diagnosis	130
Confirmation	41
Family studies	16
Pre-symptomatic	13
Banking	39
Total	**239**

WMRGL: Identification of potential germline variants using somatic panels – who to follow-up?

- Variant allele frequency (VAF): Apply threshold to limit follow-up of potential germline variants in pre-identified 'familial' genes.
  - >40% VAF (being aware of CN status)
- If variant in CSG identified, classify according to somatic variant guidelines (AMP) and ACGS BPG for germline variant classification - gene specific if available

Only variants classified as pathogenic or likely pathogenic in the germline setting are highlighted on the report for potential follow-up. **May report a VUS in patients potentially eligible for stem cell transplant (SCT)**

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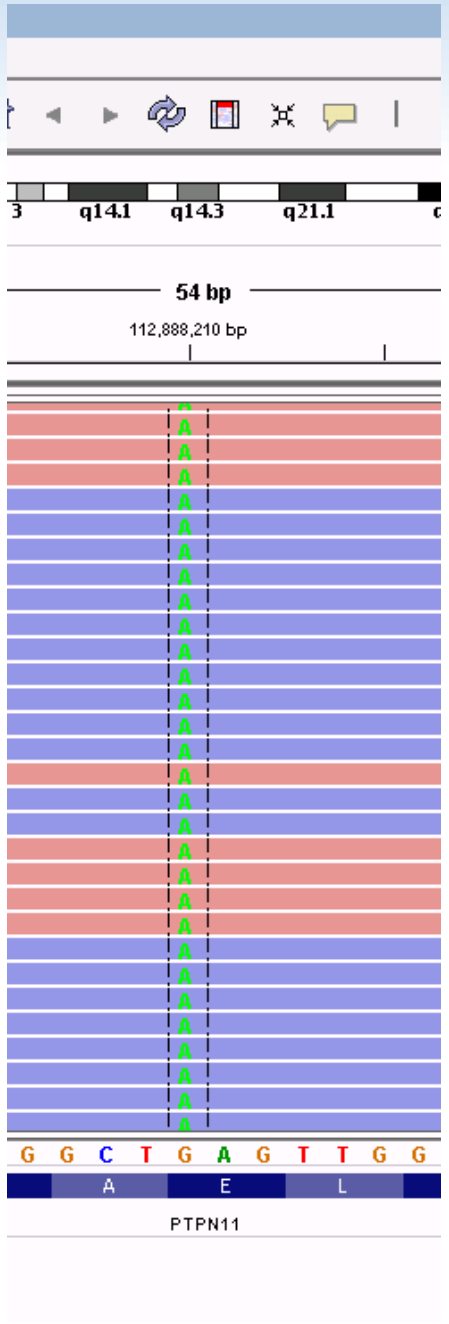
ClinGen Myeloid Malignancy Variant Curation Expert Panel recommendations for germline *RUNX1* variants

- Report states that variant could be somatic or germline in origin and make recommendations for investigation:
  - request tumour-free tissue (cultured fibroblasts or blood/marrow in remission)
  - recommend referral to clinical genetics if germline status established
- Predictive testing can be offered to at risk relatives once germline status confirmed in proband, ideally following appropriate input from Clinical Genetics

Case 1

- Marrow from 3 year old female in Jan 2021
  - Presented with high WCC (~70), also had chicken pox at the time, large liver and spleen. No monocytosis.
  - Flow consistent with CP-CML: *BCR::ABL1* and *JAK2* V617F negative
  - Suspected diagnosis revised to JMML
- ANK [20]
- No evidence of monosomy 7 by FISH
- NGS using paediatric targeted NGS panel (38 genes)
  - c.226G>A p.(Glu76Lys) in *PTPN11*
    - VAF of 44%.
  - Seen previously in-house and classified as clinically significant
  - VAF at a level that could be germline in origin – ACMG classification performed
  - Pathogenic



Case 1 NGS report

- It is not known whether this *PTPN11* variant is somatic or germline in origin.
- *PTPN11* variants are recurrent in myeloid neoplasia, including JMML, MDS and AML.
- In a germline setting they are also associated with Noonan Syndrome (Kratz et al. Blood 2005 106:2183-2185; Tartaglia et al. BJH 2005 129:333-339).
- In order to investigate further, recommend patient referred to Clinical Genetics, to undertake an assessment and organise the appropriate follow-up studies.

Case 1 Follow-up investigations

March 2021 –?Transformation. Another marrow taken.

- Clinical genetics now involved.
  - Patient to have phenotype assessed
  - SCT planned for end of May, brother potential donor.
- ANK[20]
- Same *PTPN11* variant observed at 36%
  - ?neoplastic origin
  - ?somatic mosaicism (reported in RASopathies)
- Management of de novo JMML versus RASopathy –related JMML are very different
  - De novo JMML very aggressive - SCT
  - RASopathy-related JMML often spontaneously regress – W&W
- Many conversations between clinical teams and lab: extremely urgent that we rule out/confirm germline origin of variant
- Lab advised skin biopsy is preferable but bleeding issues. Lab were asked to investigate other tissues, agreed with some reluctance.

Case 1 Follow-up investigations *PTPN11* c.226G>A p.(Glu76Lys)

If positive – confirms germline somatic mosaicism

If negative – germline origin has not been excluded due to site sampling differences

Tissue	VAF	Significance	Outcome
CD3+ T-cell fraction (PB)	8% (48/589 reads)	?germline mosaic ?early neoplastic event in haematopoietic stem cells ?contamination due to T-cell impurity	Did not establish potential origin of the *PTPN11* variant therefore not reported
Nail clippings	3% (11/292 reads)	Not present in any other patients in this run – increases likelihood it is genuine ?contamination with leukocytes	Result reported as equivocal Recommended we needed to test DNA from cultured fibroblasts to comment further on origin

Nail clipping results

Hereditary Predisposition to Hematopoietic Neoplasms: When Bloodline Matters for Blood Cancers

Abhishek A. Mangaonkar, MBBS, and Mrinal M. Patnaik, MD

https://doi.org/10.1016/j.mayocp.2019.12.013

Supplementary table 2: Table highlighting advantages and disadvantages of sources of tissue for germline confirmation of hereditary predisposition syndromes.

Source of germline tissue	*Advantages*	*Disadvantages*
Skin fibroblast	Increased accuracy, higher DNA yield	Invasive procedure, prolonged time (3-6 weeks), cumbersome, possibility of false positives due to blood contamination (especially if patients are thrombocytopenic), culturing process can introduce genetic changes.
CD3+ T cells	Easily available, may not require a repeat blood draw	Cannot be used for malignancies with a lymphoid predisposition or cell of origin at the hematopoietic stem cell level.
Buccal Swab	Convenient	False positives due to contamination with hematopoietic cells.
Hair follicle	Convenient	Lower DNA yield.
Nail Clippings	Convenient	Lower DNA yield, poor DNA quality, contamination with leukocytes.

Identifying potential germline variants from sequencing hematopoietic malignancies

Ira L. Kraft[1] and Lucy A. Godley[1,2]

2498 blood® 26 NOVEMBER 2020 | VOLUME 136, NUMBER 22

Cultured skin fibroblasts or hair bulbs are considered to the best source for germline DNA.[5,6] Skin biopsies may be performed simultaneously with a BM biopsy, when the skin is sterile and anesthetized. Unfortunately, culturing fibroblasts requires several weeks and may be technically challenging, and hair bulbs may be scarce in oncology patients and do not yield large quantities of DNA, limiting downstream testing. In addition to

dictions as a result of clonal hematopoiesis (CH) and other rearrangements. Buccal swabs and fingernails may be contaminated with hematopoietic cells.[6] Unfortunately, even if the proper material is selected. NGS panels for germline variants differ with

Final outcome

- Clinical Genetics reviewed the patient – phenotype was not suggestive of Noonan's
- Skin sent - grew VERY SLOWLY
- Brother lined up as donor
  - Transplant initially put on hold until the skin fibroblast results were obtained
  - Patient was so ill, transplant went ahead
- About 1 month after SCT, DNA finally available from skin– no evidence of PTPN11 variant.
- Highly likely to be SOMATIC in origin
- When results are negative, can never conclusively say that patient is not a germline mosaic and results are due to site sampling differences

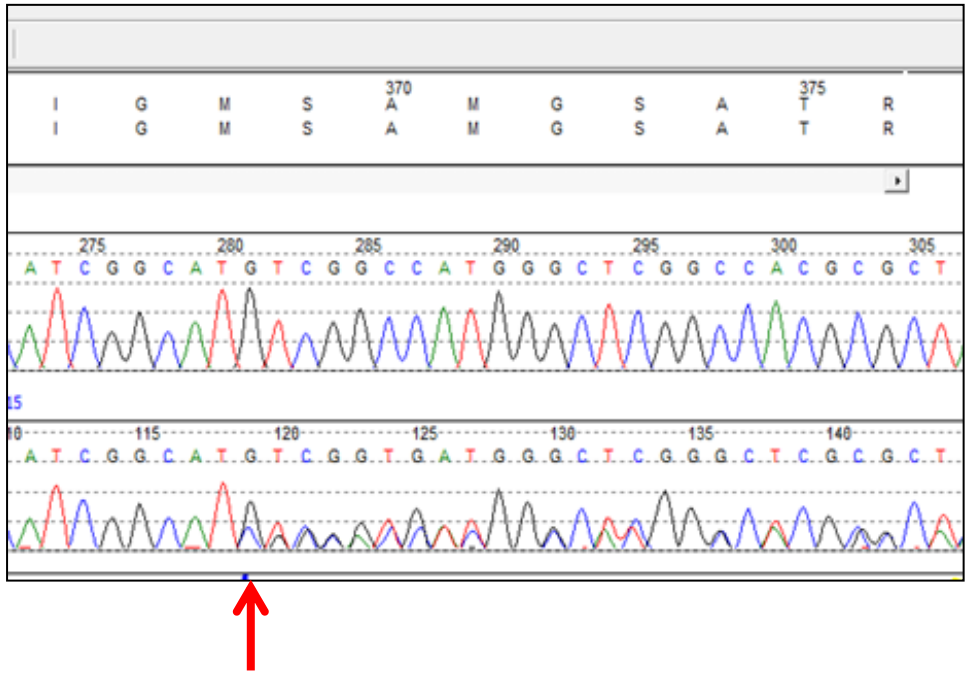
Case 2 Clinical details

- 52yr old male with high risk MDS
- Somatic testing in Glasgow identified *RUNX1* VUS with VAF suggestive of germline
- Patient being worked up for SCT, limited sibling donor options
- WMRGL received DNA extracted from buccal scrape to confirm germline origin

RUNX1 heterozygous in-frame duplication
c.1098_1103dup p.(Ile366_Gly367) by Sanger

Buccal scrapes not recommended source of germline material.

- Does not exclude a somatic origin
- Requested further sample for confirmation of origin: fresh blood for CD3+ T-cell isolation/skin punch biopsy.



Case 2 Follow-up

- DNA extracted :direct from skin: *RUNX1* c.1098_1103dup p.(Ile366_Gly367dup) ~50% VAF. Likely germline in origin
  - Germline classification reviewed using *RUNX1* ClinGen MM-VCEP guidelines
  - **PM6_Supporting only >> cold VUS**

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ClinGen Myeloid Malignancy Variant Curation Expert Panel recommendations for germline *RUNX1* variants

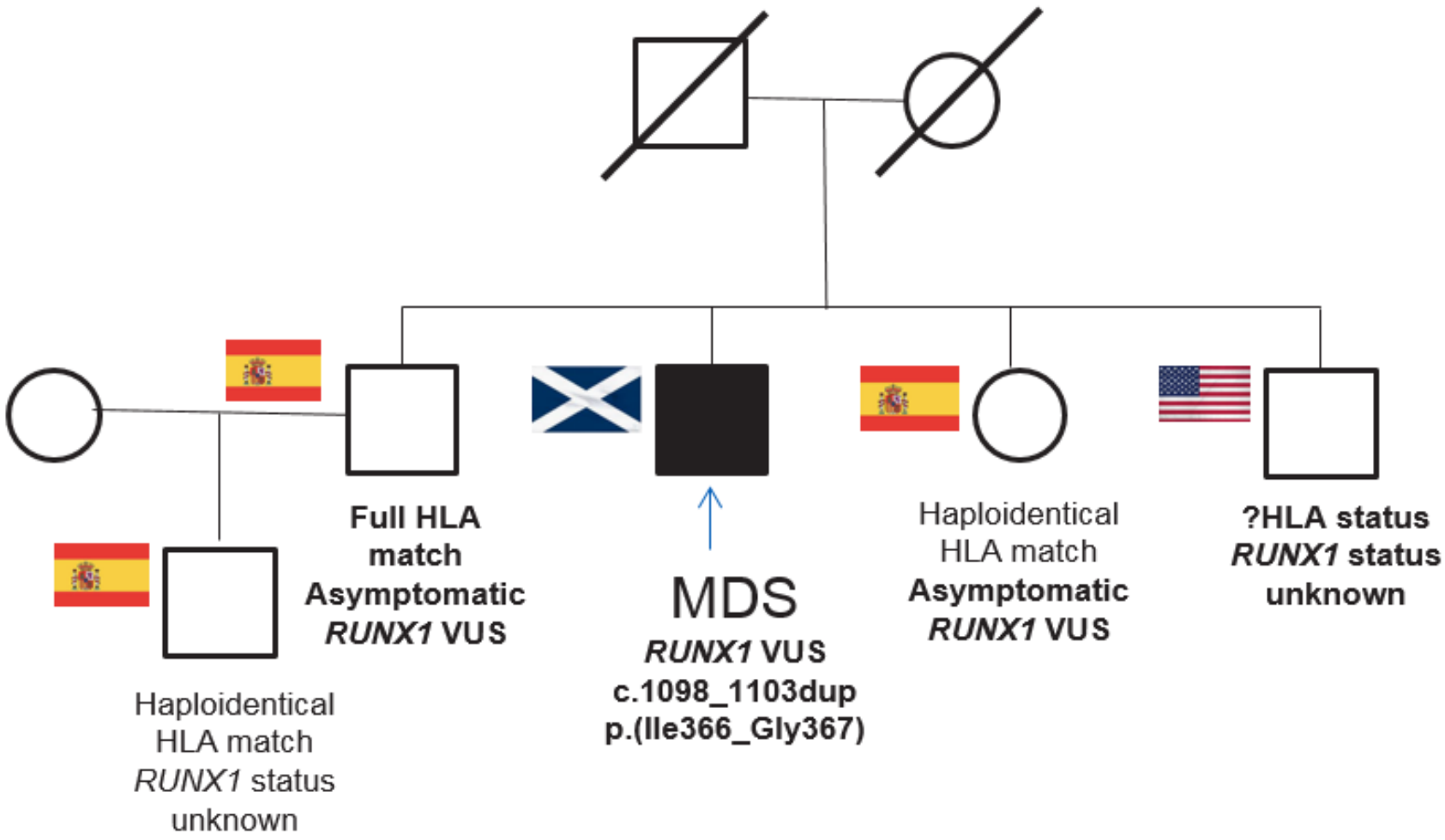
- Plan to transplant with HLA-matched sibling donor
  - **offered to test for the VUS in this potential donor**
  - Recommended referral to Clinical Genetics for counselling

Case 2 Family studies

- HLA-matched potential sibling donor 1 (67yrs age) gave consent for germline testing of *RUNX1*
  - **Heterozygous for c.1098_1103dup p.(Ile366_Gly367dup)**
  - Reiterated that we can't determine whether this is the causative variant in the index patient.
  - Strongly recommend referral to Clinical Genetics for counselling and segregation studies
- Haematological assessment of sibling donor: Normal FBC, no history of bleeding disorder. Platelet aggregation studies and genetic counselling to be arranged

- 2nd potential sibling donor (Haploidentical HLA match) consented to be tested: Also carried the *RUNX1* VUS ☹

Pedigree: Family in different geographic locations



Sometimes it is necessary to offer 'pre-symptomatic' testing for VUS

Introductions part 2: SEGLH experience

2016: Validated a rare inherited anaemia panel, mostly focused on red cell disorders, but with small amount of iBMF genes on the panel

2017: Large increase in iBMF genes tested, included panels for inherited neutropenia and thrombocytopenia.

Panel now includes genes that can acquire somatic mutations (!)

2016 BMF genes:

Bone Marrow Failure	
Gene	Transcript
DKC1	NM_001363.4
NHP2	NM_017838.3
NOP10	NM_018648.3
NT5C3A	NM_001002010.2
SBDS	NM_016038.2
TERC	NR_001566.1
TERT	NM_198253.2
TINF2	NM_001099274.1

Introductions part 2: SEGLH experience

2016: Started offering a rare inherited anaemia panel, mostly focused on red cell disorders, small amount of iBMF genes on the panel

2017: Large increase in iBMF genes tested, included panels for inherited neutropenia and thrombocytopenia.

Panel now includes genes that can acquire somatic mutations (!)

2018: In partnership with the LMH lab at KCH, first germline confirmation of variant on DNA extracted from skin biopsy undertaken.

2021: New panel iteration to fulfil GMS test directory, including the R347 Inherited predisposition to AML and R366 Inherited susceptibility to ALL panels.

2022: Work closely with LMH on local cases for germline confirmation, however receiving increasing number from across London with highly variable quality of referrals.

- Good local infrastructure with Haematology consultants and Clinical Genetics.

2021 (NHSE) genes:

Thrombocytopenia	ABCG5	NM_022436
Thrombocytopenia	ABCG8	NM_022437
Thrombocytopenia	ACTN1	NM_001130004
Thrombocytopenia	ADAMTS13	NM_139025
Thrombocytopenia	ANKRD26	NM_014915
Thrombocytopenia	AP3B1	NM_003664
Thrombocytopenia	CECR1 (ADA2)	NM_001282225
Thrombocytopenia	CYCS	NM_018947
Thrombocytopenia	ETV6	NM_001987
Thrombocytopenia	FLI1	NM_002017
Thrombocytopenia	FYB1	NM_001465
Thrombocytopenia	GATA1	NM_002049
Thrombocytopenia	GP1BA	NM_000173
Thrombocytopenia	GP1BB	NM_000407
Thrombocytopenia	GP9	NM_000174
Thrombocytopenia	IKZF1	NM_006060
Thrombocytopenia	LAT	NM_001014987
Thrombocytopenia	MECOM	NM_004991
Thrombocytopenia	MPL	NM_005373
Thrombocytopenia	MYH9	NM_002473
Thrombocytopenia	NBEAL2	NM_015175
Thrombocytopenia	RUNX1	NM_001754
Thrombocytopenia	SAMD9	NM_017654
Thrombocytopenia	SLFN14	NM_001129820
Thrombocytopenia	SRC	NM_005417
Thrombocytopenia	STIM1	NM_003156
Thrombocytopenia	THPO	NM_000460
Thrombocytopenia	TUBB1	NM_030773
Thrombocytopenia	WAS	NM_000377
Thrombocytopenia	WIPF1	NM_001077269
Neutropenia	ACKR1	NM_002036
Neutropenia	AP3B1	NM_003664
Neutropenia	CECR1 (ADA2)	NM_001282225
Neutropenia	CSF3R	NM_156039
Neutropenia	CXCR4	NM_003467
Neutropenia	ELANE	NM_001972
Neutropenia	FCGR3B	NM_001244753
Neutropenia	G6PC3	NM_138387
Neutropenia	GFI1	NM_005263
Neutropenia	HAX1	NM_006118
Neutropenia	JAGN1	NM_032492
Neutropenia	RAC2	NM_002872
Neutropenia	RMRP	NR_003051
Neutropenia	TAZ	NM_000116
Neutropenia	TCN2	NM_000355
Neutropenia	USB1	NM_024598
Neutropenia	VPS45	NM_007259
Neutropenia	WAS	NM_000377
Neutropenia	WIPF1	NM_001077269

Inherited bone marrow failure	ACD	NM_001082486
Inherited bone marrow failure	ANKRD26	NM_014915
Inherited bone marrow failure	CECR1 (ADA2)	NM_001282225
Inherited bone marrow failure	CTC1	NM_025099
Inherited bone marrow failure	CXCR4	NM_003467
Inherited bone marrow failure	DKC1	NM_001363
Inherited bone marrow failure	DNAJC21	NM_001012339
Inherited bone marrow failure	EFL1	NM_024580
Inherited bone marrow failure	ERCC6L2	NM_020207
Inherited bone marrow failure	G6PC3	NM_138387
Inherited bone marrow failure	GATA2	NM_032638
Inherited bone marrow failure	GFI1	NM_005263
Inherited bone marrow failure	HAX1	NM_006118
Inherited bone marrow failure	IKZF1	NM_006060
Inherited bone marrow failure	KIF23	NM_138555
Inherited bone marrow failure	KLF1	NM_006563
Inherited bone marrow failure	LAT	NM_001014987
Inherited bone marrow failure	MPL	NM_005373
Inherited bone marrow failure	MYSM1	NM_001085487
Inherited bone marrow failure	NBN	NM_002485
Inherited bone marrow failure	NHP2	NM_017838
Inherited bone marrow failure	NOP10	NM_018648
Inherited bone marrow failure	PARN	NM_002582
Inherited bone marrow failure	RTEL1	NM_001283009
Inherited bone marrow failure	RUNX1	NM_001754
Inherited bone marrow failure	SAMD9	NM_017654
Inherited bone marrow failure	SAMD9L	NM_152703
Inherited bone marrow failure	SBDS	NM_016038
Inherited bone marrow failure	SRP72	NM_006947
Inherited bone marrow failure	TCN2	NM_000355
Inherited bone marrow failure	TERC	NR_001566
Inherited bone marrow failure	TERT	NM_198253
Inherited bone marrow failure	TINF2	NM_001099274
Inherited bone marrow failure	USB1	NM_024598
Inherited bone marrow failure	VPS45	NM_007259
Inherited bone marrow failure	WRAP53	NM_018081
Inherited predisposition to AML	ACD	NM_001082486
Inherited predisposition to AML	ANKRD26	NM_014915
Inherited predisposition to AML	CEBPA	NM_004364
Inherited predisposition to AML	CHEK2	NM_007194
Inherited predisposition to AML	DDX41	NM_016222
Inherited predisposition to AML	ETV6	NM_001987
Inherited predisposition to AML	GATA2	NM_032638
Inherited predisposition to AML	RTEL1	NM_001283009
Inherited predisposition to AML	RUNX1	NM_001754
Inherited predisposition to AML	SAMD9	NM_017654
Inherited predisposition to AML	SRP72	NM_006947
Inherited predisposition to AML	TERC	NR_001566
Inherited predisposition to AML	TERT	NM_198253
Inherited predisposition to AML	TP53	NM_000546
Inherited predisposition to ALL	ETV6	NM_001987
Inherited predisposition to ALL	PAX5	NM_016734

SEGLH: Identification of potential germline variants using somatic panels – who to follow-up?

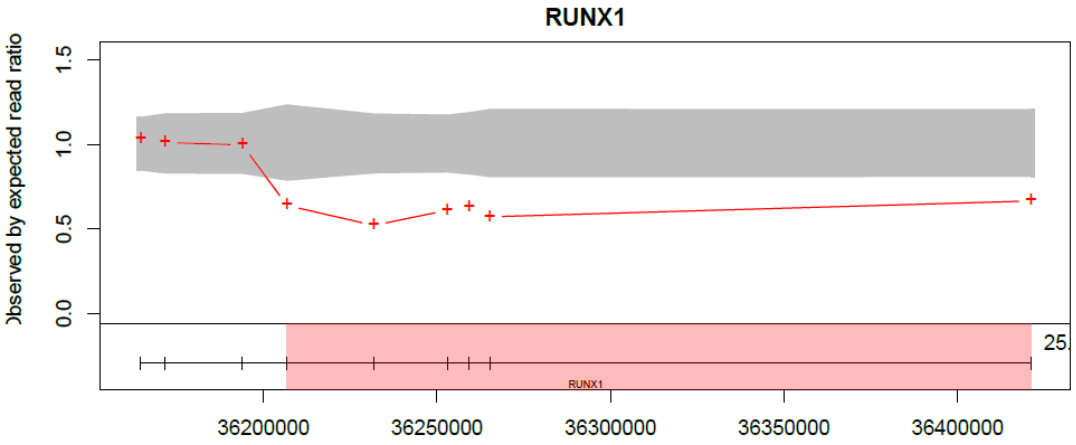
- As a receiving centre we have no influence on cases being offered germline confirmation and little ability to gate keep referrals.
- In theory only variants classified as pathogenic or likely pathogenic in the germline setting are highlighted on the report for potential follow-up.
- We undertake our own ACMG score of the variant.
- We also have very little influence on the sample type

Case 3

- 19y/o female, referred for constitutional testing, BMF and Thro
  - ?MAHA/MDS/aHUS
- Blood film:
  - Dysplastic neutrophils, myelocytes and occasional blast cells, known leucoerythroblastic picture

- BM: Hypercellular, moderate dyserythropoiesis, some dysgranulopoiesis, and very reduced megakaryopoiesis

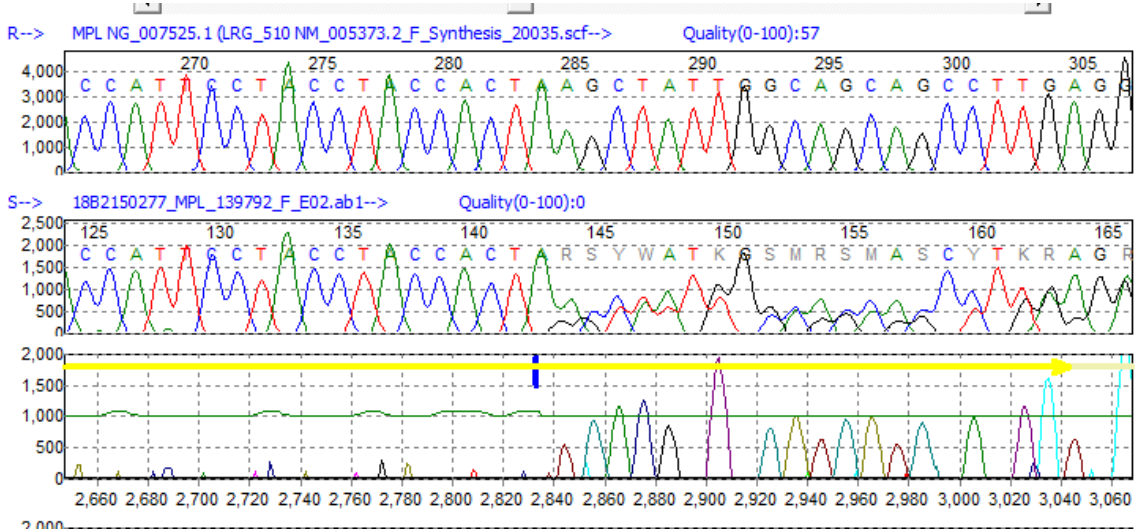
- NM_005373.2 MPL c.1888delA; p.(Ser630fs) in 39% of reads
  - ACMG class 4
- Partial RUNX1 gene deletion (reads ratio of 60%)



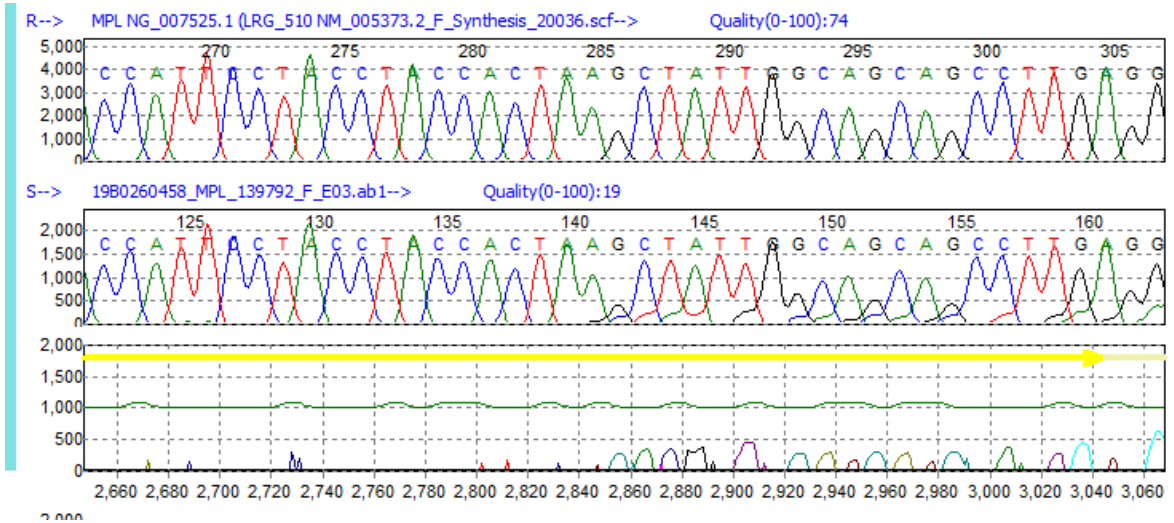
Case 3

- Sample received for germline confirmation
  - DNA extracted from uncultured fibroblasts
- Small yield sample to confirm both the MPL (Sanger) and RUNX1 (NGS) variants
- Sanger result showed presence of MPL variant at lower level than in blood

DNA extracted from blood



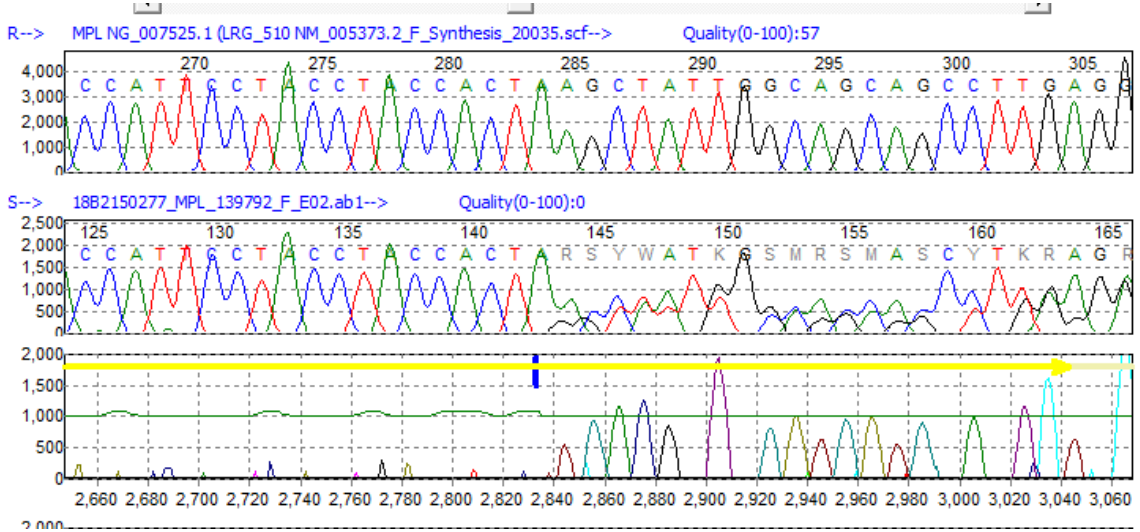
DNA extracted from uncultured fibs



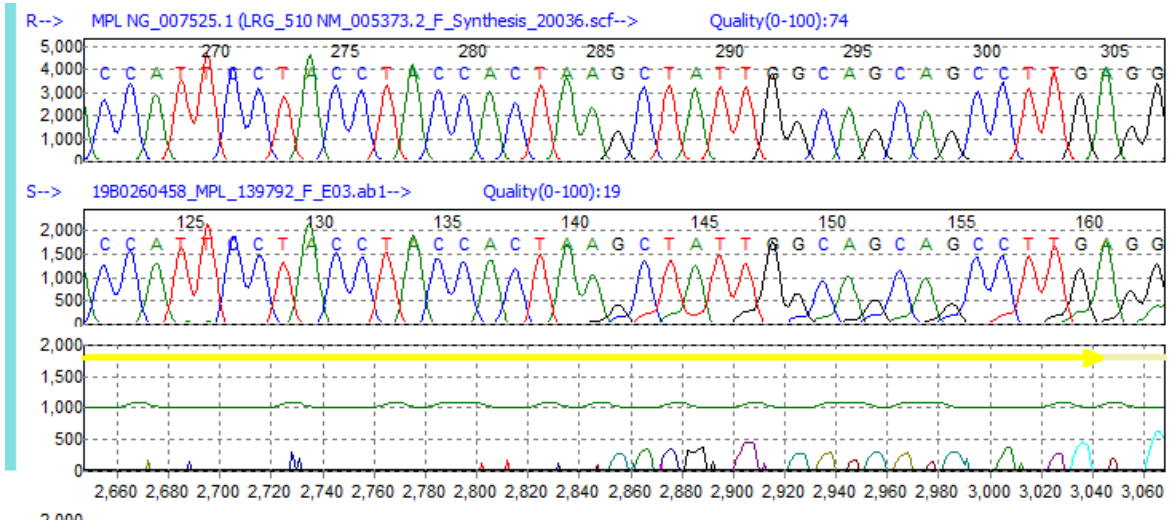
Case 3

- Reduced variant intensity seen
  - (Sanger is not a quantitative assay)
- This could be mosaicism or infiltration of leukocytes into the raw fibroblast sample.
- Sample insufficient for any further follow up testing so this was the only shot

DNA extracted from blood



DNA extracted from uncultured fibs



Case 3

- After MDT decision was to report the variant as likely somatic:

> These results show that the MPL variant can be detected in DNA extracted from XXXXXXX's skin biopsy. The variant is at a low level, which indicates that the variant is not present in a heterozygous state and is therefore not likely to be present in the germline.

- Sample insufficient for any further testing, this was the only shot
  - nature of the RUNX1 deletion not established

- Had this sample had been cultured, the uncertainty and testing limitation in this case would have been avoided
  - Facility for doing culturing on any scale doesn't exist in SEGLH

Additional concerns/Take home messages

- Inconstancy of variant interpretation can cause issues at many points in the pathway.
  - Patients are undergoing invasive skin biopsy unnecessarily.
  - Cascade family testing is being offered before ACMG germline classification and Clinical Genetics intervention.
  - Once a patient has been offered testing it becomes very hard to explain that familial testing could do more harm than good.
  - Late introduction of Clinical Genetics outside of a malignancy context raises questions of prenatal diagnosis and PGD.

- Effective management requires MDT approach: Clinical Scientists with expertise in both Haemato-Oncology and relevant inherited cancers/germline disorders; Haematologists; Clinical Geneticists

- Variants can be uploaded to Can-Var UK website

https://canvaruk.org/

CanVar-UK V2.0
Cancer Predisposition Gene Variant Database

Issues with tissues

WMRGL

- No control over sample type when DNA forwarded from other laboratories
- Nail clippings, buccal swabs and CD3+ T-cells may be contaminated
- Culturing of fibroblasts requires additional space, staff and resources
- Skin often fails to grow or takes time
- Constitutional (germline) mosaicism
- Deviation from ‘gold standard’ workflow required to deliver urgent results

SEGLH

- Repeated incorrect samples types sent (saliva still thought to be a different DNA source)
- No guidelines around testing remission samples
- Lack of significant facility for culturing fibroblasts
- Distance from DNA extractions creates referral delays

Infernal referrals

WMRGL

- No FH leukaemia (actually intended for R91 and R92) or lack of clinical details provided
- Pre-symptomatic testing and family studies requested for variants never confirmed to be germline in origin
- External requests for germline confirmation where VAF was <40%
- Lack of input from Clinical Genetics: inaccurate information provided regarding risks, issues with consent

SEGLH

- No clear guidance of when to test and not
- Increasing number of samples being received for non-cancer patients/genes
- Pathway transitioning through another lab can add legitimacy to a referral
- Inconsistent variant interpretation

What next (+/- consensus guidelines)?

- Inequitable access to germline experience by laboratories performing somatic testing.
  - Participation in relevant GenQA schemes and competence assessment for ACGS variant classification
- Formalise a communications network within **and** between GLHs for advise

- Inequity of access to testing cultured fibroblasts: issues with capacity, funding and expertise.

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SEGLH

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Comments/questions?