Detection of Acquired *GATA1* Mutations in Children with Transient Leukaemia or Myeloid Leukaemia in Down Syndrome

Bristol Genetics Laboratory

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Clinical Background

- 5-30% of Down Syndrome children are born with Transient Leukaemia of Down Syndrome (TL-DS). Many cases resolve without treatment but TL-DS results in early death in 15-23%
- TL-DS is characterised by increased circulating megakaryoblasts (blasts). TL-DS cells spread throughout the body
- New guidelines (2017)[1] aim to introduce standardised care and improve diagnosis. Blasts are extremely sensitive to chemotherapy and TL-DS is successfully treated with low doses
- 20-23% of survivors will develop Acute Myeloid Leukaemia of Down Syndrome (ML-DS) in the first 4 years of life
- TL-DS is driven by pathogenic variants in the haematopoietic transcription factor gene *GATA1* on the X chromosome, and is ***only*** seen in conjunction with Trisomy 21
- Diagnosis of TL-DS requires *GATA1* sequence analysis. Detection of all clinically relevant mutations requires high sensitivity (alleles present at <5%)

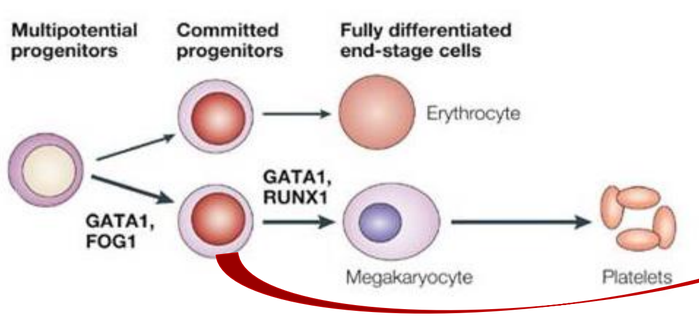
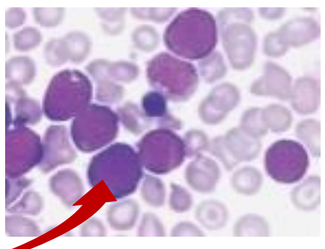


Image adapted from: Hitzler & Zipursky, (2005)



Typical appearance of blasts in peripheral blood

Fig. 1

Disease Model

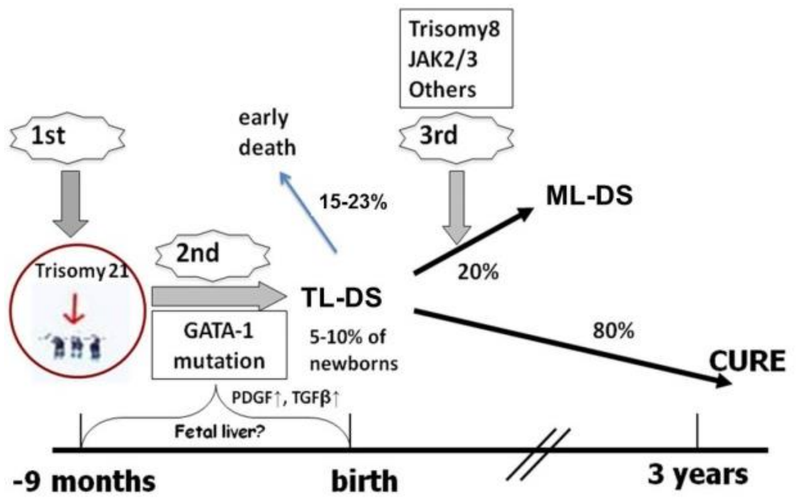


Fig. 2

~750 DS babies born each year in the UK

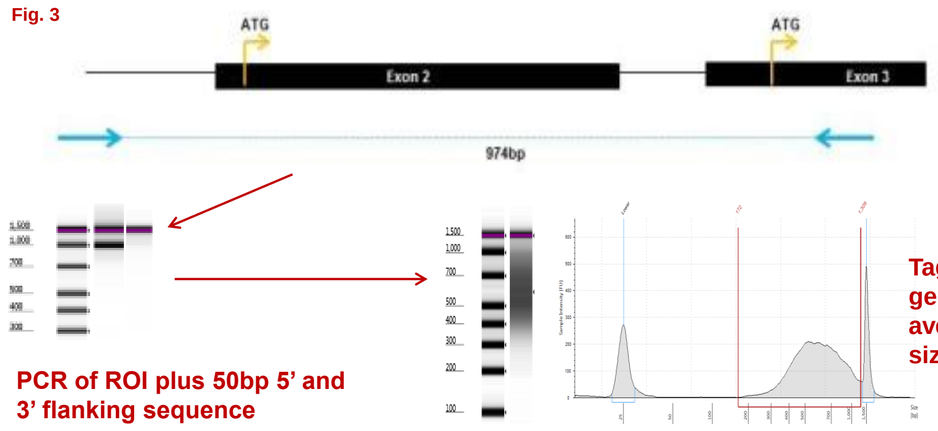
Image adapted from: Down Syndrome, Subrata Dey (ed), 2013.

- Disease development is a multi-step process: 1st hit is T21, *GATA1* mutation is the 2nd hit leading to TL-DS. If additional oncogenic mutations (e.g. *JAK2*) occur this drives development of ML-DS (3rd hit)
- 'Silent TL-DS' patients are asymptomatic but harbour *GATA1* mutation(s) and are at risk of progression to ML-DS
- All cases of TL-DS and ML-DS have an acquired pathogenic N-terminal variant resulting in a truncated GATA1 protein (GATA1s) lacking an activation domain
- *GATA1* mutations ≥100,000x more frequent in DS. Multiple clones are common (up to 6 have been observed). Minor clones can give rise to ML-DS
- High frequency mutagenesis results from constant transcription and replication of *GATA1* in the abnormal T21 fetal liver

Experimental Design and Validation

- Alford *et al* described *GATA1* truncating mutations in 226 patients (Fig. 4). Introduction of a stop codon is the most common effect (76%) others impact on splicing (12%) and start codon (4%)
- 97.5% TL-DS causing mutations in exon 2. The remainder are in the first part of exon 3
- Illumina Nextera XT kit used for library preparation with 1ng of cleaned-up PCR product (Fig. 3)
- Libraries were pooled and sequenced on an Illumina MiSeq, bioinformatic analysis used an in-house somatic pipeline
- Control samples reflecting the spectrum of variants were obtained from research labs performing TL-DS/ML-DS *GATA1* testing and used to validate the assay (including 7 analysed blind). Samples from 2 referrals to the lab were also tested (Fig. 5)

Fig. 3



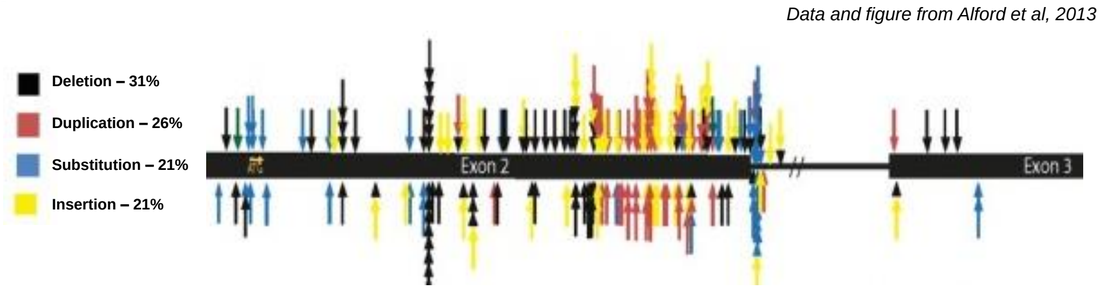
PCR of ROI plus 50bp 5' and 3' flanking sequence

Tagmentation to generate library with average fragment size ~550bp

Reported GATA1 Variants

Fig. 4

Data and figure from Alford et al, 2013



Variants Detected

Missense Variant abolishing start codon
Frameshift Variants introducing PTC
Splicing Variants leading to exon skipping
In-frame deletion (2nd variant identified in BGL patient)

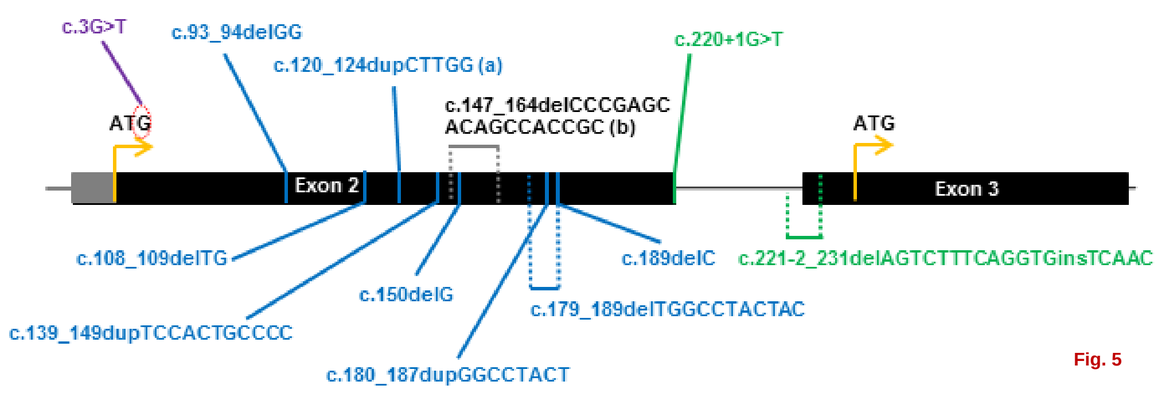


Fig. 5

Assay Validation: Control Samples

Coverage: Average = 47,360x
Variant Allele Frequencies detected: 1.3% - 74.2%
Analytical Sensitivity: 100% (variants scored >1% VAF detected)
Analytical Specificity: 100% (no pathogenic variants detected in wild type samples)

Desired limit for sensitivity of variant detection is 1% VAF. Further analysis and validation is required to confirm this. An additional variant reported to be at 0.01% VAF was not detected. Pathogenic variants were confirmed with the lab of origin.

Variants detected are representative of the mutation spectrum.

Case Study

Referral for 5 week old infant:
Transient Abnormal Myelopoiesis (TAM) in patient who has trisomy 21. Low platelets and abnormal blood. For GATA1 mutation analysis.

A deletion which disrupts the reading frame and introduces a premature termination codon was detected in exon 2 at 11.7% VAF. This specific variant had not previously been reported in the literature, but is consistent with pathogenic variants in TL-DS patients.

This confirmed a diagnosis of TL-DS in this patient.

Appropriate treatment (Cytarabine) was enabled and clinical monitoring for progression to ML-DS advised.

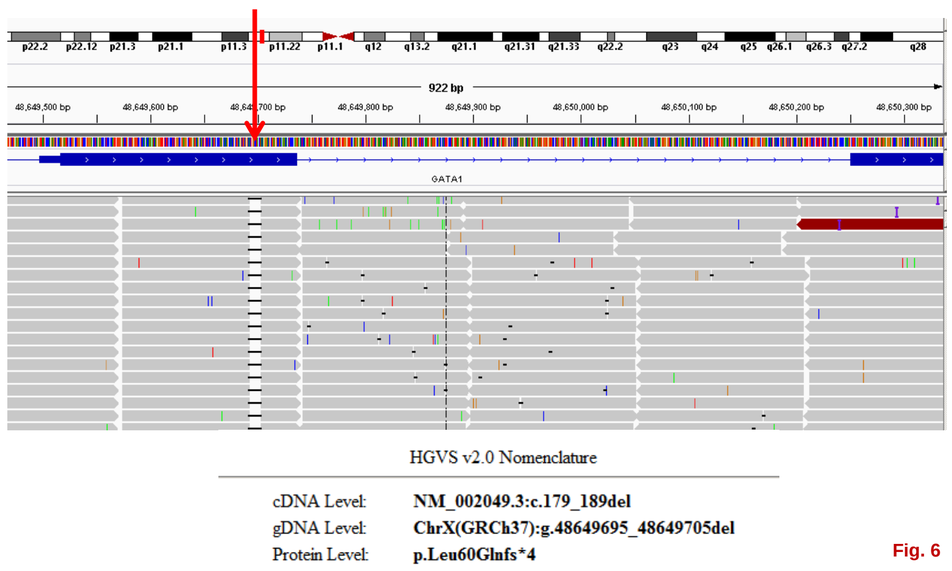
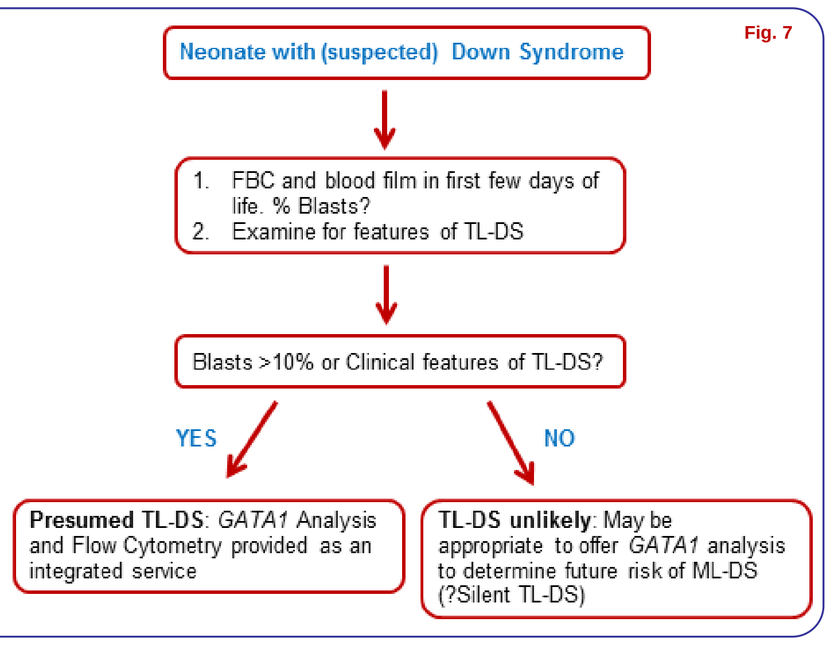


Fig. 6

The Testing Pathway

- This assay will provide the first routine and sensitive NHS test for *GATA1* variants
- A value of 10% blasts life identifies all neonates with clinical features of TL-DS for *GATA1* testing
- An integrated flow cytometry and *GATA1* molecular testing pathway is planned at BGL
- The clinical utility of offering testing for ?Silent TL-DS (blasts <10%) is unknown. This would require sensitive detection of unknown variants
- Monitoring of disease burden with repeat *GATA1* molecular testing is recommended for TL-DS patients. This requires sensitive detection of a known variant(s)
- Numbers for testing are currently unpredictable, this assay can be scaled up or down and and/or pooled with other Illumina libraries

Fig. 7



Further Work

- Establish sensitivity of assay and coverage limitations
- Validation of assay as a screening tool in ?Silent TL-DS if clinically relevant
- Assessment of assay and RQ-PCR for monitoring a variety of *GATA1* variants
- Development of a website landing page for referrals planned with information and downloadable form
- This aims to be a UK-wide service. There is a need to reach out to clinicians from various specialisms involved in the care of DS infants. This is proposed to be achieved via clinician networks and attendance at conferences

Acknowledgements

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1: DRAFT DOCUMENT British Committee for Standards in Haematology. Tunstall *et al,* (2017). Guidelines for the investigation and management of Transient Leukaemia of Down Syndrome (TL-DS). 2: Roberts, *et al, (*2013) *Blood*, 122(24), 3908–17. 3: Alford *et al*, (2016), *Blood* 118(8), 2222–2238. 4: Hitzler & Zipursky, (2005) *Nature Reviews C*ancer 5, 11-20 5: (Book) *Down Syndrome*, Dey (ed), (2013).