

## **Mature Analysis Of The Prospective Oxford Downs Cohort Study: Timing and Clinical Impact Of *GATA1* mutations in Downs NewBorns And The Lessons For Management Of Down NewBorns.**

**Authors: Irene, Neha, RN, Laure, Marlen, Alison, Catherine Paresh**

Children with Down syndrome (DS) have a high risk of developing a unique form of AML known as myeloid leukaemia of DS (ML-DS) defined by the presence of one or more acquired N-terminal truncating mutations in the hematopoietic transcription factor gene *GATA1*. ML-DS is confined to children  $\leq 4$  y of age and preceded by a neonatal preleukemic syndrome, Transient Abnormal Myelopoiesis (TAM). Although the same *GATA1* mutations are present in ML-DS and TAM, indicating they are clonally-linked disorders, chemotherapy is essential to cure ML-DS whereas most cases of TAM spontaneously resolve without treatment.

To determine the natural history of *GATA1* mutations in DS and the true risk of subsequent leukemia conferred by *GATA1* mutations we established the Oxford DS Cohort Study that prospectively analyses serial clinical, hematological and *GATA1* mutational data from children with DS from birth to age 4y. All children had serial blood counts and smears and mutation analysis of exon 2/3 of *GATA1* by next-generation-sequencing (NGS). A diagnosis of TAM was made if peripheral blood (PB) blasts were  $>10\%$  and a *GATA1* mutation was detected. DS neonates with PB blasts  $\leq 10\%$  and a *GATA1* mutation were designated Silent TAM as our previous data on 18/80 DS neonates with blasts  $\leq 10\%$  showed that small mutant *GATA1* clones were clinically and hematologically undetectable [Ref: Roberts et al Blood 2013].

We have now studied 468 neonates (gestational age [GA] at birth 29-42 wks) with karyotypically-confirmed DS in the study. 130/471 (27.6%) had  $\geq 1$  *GATA1* mutation confirming the high frequency of *GATA1* mutations in DS neonates. Multiple *GATA1* clones (2-7) were detected in 19% of TAM and 21% of Silent TAM ( $p=ns$ ). A diagnosis of TAM was made in 55/471 (11.7%). 75/471 (15.9%) had Silent TAM and 341/471 had no *GATA1* mutations. Typical clinical findings of TAM (hepatomegaly, splenomegaly, skin rash and effusions) were uncommon in Silent TAM, affecting 7%, 1.3%, 1.3% and 3% neonates respectively compared to 40%, 29%, 17% and 10% of neonates with TAM ( $p<0.0001$ ). There were no specific hematological abnormalities in Silent TAM: median WBC and blast % were not increased:  $13.1 \times 10^9/L$  v  $14.5 \times 10^9/L$  and 4% v 4% (Silent TAM v no *GATA1* mutation) and no neonates with Silent TAM had leucocytosis (WBC  $>35 \times 10^9/L$ ) or anemia. There was a strong correlation ( $r=0.753$ ;  $p<0.0001$ ) between PB blast% and mutant *GATA1* clone size (assessed by VAF) with a greater mutant clone size in TAM v Silent TAM (median VAF 17.1% v 1.2%;  $p<0.0001$ ).

Unexpectedly, clone size was inversely proportional to GA ( $r=-0.2569$ ;  $p=0.004$ ). Despite similar median GA at delivery for all 3 groups, no *GATA1* mutations were detected before

31 wks GA and no cases of TAM before 33 wks; the highest frequency of *GATA1* mutations was seen at 34-35 wks GA (40%) reducing to 7% at  $\geq 40$  wks. This suggests *GATA1* mutations are likely to be acquired and expand during a narrow time window early in the 3rd trimester of fetal life. Consistent with this, *GATA1* mutations were not detected in 16 DS fetal liver samples (GA 8-19 wks) analysed over the same period. Similarly, in a case of TAM presenting *in utero* with fetal pericardial effusion and 96% PB blasts at 27 wks GA, the blast% and mutant *GATA1* VAF spontaneously fell progressively (blasts 19%; VAF 23% at birth at 34 wks GA; undetectable at 4 wks postnatal age).

*Outcome:* 7 neonates have developed ML-DS: 6/52 survivors with TAM; 1/71 survivors with Silent TAM and 0/328 survivors without neonatal *GATA1* mutations. 5 yr overall survival was the same in all 3 groups (95.1%, 95.8%, 95.1% for TAM, Silent TAM and no *GATA1* mutations;  $P_{\log\text{-rank}}=0.8994$ ) while 5 yr event-free survival was lower in TAM due to later ML-DS (89.2%, 92.8%, 95.1% in TAM, Silent TAM and neonates with no *GATA1* mutations;  $P_{\log\text{-rank}}=0.0262$ ). ML-DS was diagnosed at a median age of 14 m (range 3-18 m) and in all cases was preceded by a fall in platelet count. No single risk factor at birth specifically predicted for ML-DS although all 6 neonates with TAM who developed ML-DS had a mutant *GATA1* VAF of >15% and blasts >20% at birth. The DS neonate with Silent TAM had a VAF of 2.5% and blasts of 4%; this neonate was the only one of the cohort with a partial trisomy 21.

*Conclusion:* Acquired N-terminal mutations in *GATA1* are very frequent in neonates with DS and most probably occur in the 3rd trimester; in the majority of cases mutant *GATA1* clones are very small, clinically silent, resolve spontaneously and confer an extremely low risk of ML-DS.

Characters (no spaces): 3796