Origins of *STIL-TAL1* fusion genes in children who later developed paediatric T-cell acute lymphoblastic leukaemia; an investigation of neonatal blood spots.

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Abbreviations

ALL	Acute Lymphoblastic Leukaemia
MEM	Minimal Essential Medium
NBS	Neonatal Blood Spot
NRAS gene	NRAS proto-oncogene, GTPase gene
PCR	Polymerase Chain Reaction
STIL gene	SCL/TAL1 Interrupting Locus gene
TAL1 gene	T-cell Acute Leukaemia gene
WBC	White Blood Cells

Abstract

STIL-TAL1 fusion genes are present in approximately 11-28% of children with paediatric T-ALL but the developmental timing of the rearrangement is still unknown. To investigate whether the fusion gene can be detected in, neonatal blood spots (NBSs) from paediatric patients diagnosed with T-cell ALL, we have analysed DNA from 38 paediatric T-ALL patients by nested PCR and electrophoresis. The *STIL-TAL1* fusion gene was not detected in neonatal blood spots from any of the 38 T-ALL patients suggesting that *STIL-TAL1* fusion genes are most probably postnatal events in paediatric T-ALL.

Introduction

The acute lymphoblastic leukaemia's (ALLs) are a heterogeneous group of diseases that arise in B-lineage or T-lineage lymphocyte progenitor cells and possess chromosomal alterations that play a key role in initiation of paediatric ALL. Detection of these alterations at time of diagnosis is important and can be used for risk stratification [1].

Through studies of neonatal blood spots and monozygotic twins some childhood ALLs can be traced back to a prenatal origin, starting *in utero* with a preleukaemic clone, followed by the acquisition of postnatal genomic losses or gains subsequent to the prenatal cytogenetic aberration [2–9]. T-cell acute lymphoblastic leukaemia (T-ALL) is a rare form of paediatric ALL, accounting for about 15% of acute lymphatic leukaemia's.

Approximately 11-27 % of children with T-ALL present with the genetic aberration *STIL-TAL1* at diagnosis [10,11]. *STIL-TAL1* fusion is exclusively found in T-ALLs and based on single cell phylogenomics, is considered to be an early or initiating event in T-ALL [12].

Several deletions resulting in *STIL-TAL1* gene fusion have been described yet two of them, type I and type II, appear to be most common and represent 95% of total *STIL-TAL1* cases and occur in 10-19% and 1-3% of paediatric T-ALL patients respectively [11,13,14]. Both Type I and II deletions use the same cryptic heptamer RSS at the 5' break in the *STIL* gene but can use either one of two 3' RSS (only 1.7kb apart) found in the 5' region of *TAL1*.

Material and methods:

Sample population

Children with T-ALL were randomly identified from the Nordic Society of Paediatric Haematology and Oncology (NOPHO) register. The birthdates of the children were linked to the Swedish Medical Birth register to gain access to the personal codes of mothers and

children, necessary for NBS identification. NBS were then collected from 38 paediatric patients diagnosed with T-ALL between the years 1998 – 2006. Ages at diagnosis ranged between 1- 17 years and mean and median age were 8 and 9 years respectively. The material included samples from 32 males and 6 females. Other clinical and cytogenetic data are available in the supplementary file (Table S1 and S2).

NBS DNA extraction and amplification

Four punches, each 3mm in diameter were harvested from NBSs, estimated to contain about 180,000 leukocytes and 120,000 lymphocytes. The median storage time for the NBS was 9.9 years (range 2.6 - 22.8). DNA extraction was carried out by the modified minimal essential medium (MEM) method, [15,16].

NBS DNA was whole genome amplified (WGA) using the REPLI-g Mini Kit from QIAGEN. The *NRAS* gene was used as an integrity control for PCR amplification of NBS material.

STIL-TAL1 type I and II fusions were analysed independently by nested PCR based on a protocol described by Breit *et al* 1993 [13].

Detection sensitivities of these PCR assays was tested in a dilution series of diagnostic DNA from two patients with type I *STIL-TAL* rearrangements (Dr Giovanni Cazzaniga, Italy) and products were sequenced (supplementary data) using the BigDye® Terminator v3.1 kit (Applied Biosystems).

This study was approved by the Regional ethical review board in Stockholm.

Results:

The *NRAS* control gene was detected in all WGA NBS samples indicating presence of DNA (Figure 1A, Supplementary Figure S1A and S1B) and *STIL-TAL1* type I positive control

samples were clearly visible (Figure 1B, Supplementary Figure S2). *STIL-TAL1* fusion genes were identified out to 10^{-4} in positive controls mixed into control DNA, but could not be detected in any of the 38 patients analysed (Figure 1C, Supplementary Figures S2 and S3).

Discussion:

Neonatal blood spots are unique material for which the primary purpose is to examine for several serious inherited diseases demanding early treatment. All of the blood-spot samples in this series were negative for both *STIL-TAL* fusion and likely reflect a postnatal origin for this deletion. Alternatively, the results could be indicative of a prenatal origin with insufficient leukaemic or pre-leukaemic cells in the spot or reflect an inadequate sensitivity in the PCR assay. Given the sensitivity of our assays in the current study, we have no reason to exclude that these cases are genuinely postnatal in origin. However, positive blood spots may well contain very small numbers of positive cells or copies of fusion gene sequences [2,17] and therefore it is inevitable that some blood spot screens will produce false negative results. In a previous study [8], we were also unable to detect *STIL-TAL* deletions in blood spots of infant T-ALL cases and together with the results of the current study, *STIL-TAL1* appears to be a postnatal event. To our knowledge, the present study is the largest screening of *STIL-TAL1* in NBSs from paediatric T-ALL patients. As the prognostic value of *STIL-TAL1* is unclear, analysis of this genetic aberration is not routinely performed in the clinic; therefore it is uncertain how many of the patients in our material had a *STIL-TAL1* aberration at diagnosis. Nevertheless, *STIL-TAL1* aberrations occur in 11 - 27% of paediatric T-ALLs [10,11,13], therefore 4 - 10 of the 38 children included in this study would be expected to be positive for the *STIL-TAL1* when diagnosed.

The male to female ratio in this study differed from previous studies showing an overall higher prevalence of males (5:1). However, in studies by Mansur *et al* 2009 and D'Angio *et al* 2015, *STIL-TAL1* was reported to be more common in males than in females, which

theoretically would increase the likelihood of positive cases in our material [10,11]. Similarly, high levels of WBCs at diagnosis in patients positive for *STIL-TAL1,* have been reported by several studies [10,11,18,19]. In our material a total of eighteen patients had WBC >100 x 10^9/L, which further indicates that this to be a suitable patient group for *STIL-TAL1* analysis. Finally, our material included five children diagnosed with T-ALL before three years of age, an age group where *STIL-TAL1* appears to be rare [8,18]. However, If *STIL-TAL1* was a prenatal initiating event in T-ALL, there should be a high possibility that at least one of the NBS of these young patients would have been positive for the fusion. Our overall conclusion is that *STIL-TAL1* fusions, both type I and II, have post-natal origins in paediatric T-ALL.

References

1. Pui C-H, Robison Leslie L, Look AT. Acute Lymphoblastic Leukaemia. *Lancet*. 2008;371:1030-1043.

2. Gale KB, Ford a M, Repp R, et al. Backtracking leukemia to birth: identification of clonotypic gene fusion sequences in neonatal blood spots. *Proc Natl Acad Sci U S A*. 1997;94(December):13950-13954. doi:10.1073/pnas.94.25.13950.

3. Maia AT, Ford AM, Reza Jalali G, et al. Molecular tracking of leukemogenesis in a triplet pregnancy. *Blood*. 2001;98:478-482. doi:10.1182/blood.V98.2.478.

4. McHale CM, Wiemels JL, Zhang L, et al. Prenatal origin of TEL-AML1-positive acute lymphoblastic leukemia in children born in California. *Genes Chromosom Cancer*. 2003;37(July 2002):36-43. doi:10.1002/gcc.10199.

5. Ma Y, Dobbins SE, Sherborne AL, et al. Developmental timing of mutations revealed by whole-genome sequencing of twins with acute lymphoblastic leukemia. *Proc Natl Acad Sci U S A*. 2013;110:7429-7433. doi:10.1073/pnas.1221099110.

6. Cazzaniga G, Delft FW Van, Nigro L Lo, et al. Developmental origins and impact of BCR-ABL1 fusion and IKZF1 deletions in monozygotic twins with Ph + acute lymphoblastic leukemia Developmental origins and impact of BCR-ABL1 fusion and IKZF1 deletions in monozygotic twins with Ph ♀ acute lymphoblastic . 2014;118(20):5559-5564. doi:10.1182/blood-2011-07-366542.

7. Ford a M, Pombo-de-Oliveira MS, McCarthy KP, et al. Monoclonal origin of concordant T-cell malignancy in identical twins. *Blood*. 1997;89(1):281-285.

8. Mansur MB, van Delft FW, Colman SM, et al. Distinctive genotypes in infants with T-cell acute lymphoblastic leukaemia. *Br J Haematol*. 2015;171(4):574-584. doi:10.1111/bjh.13613.

9. Fasching K, Panzer S, Haas O a, Marschalek R, Gadner H, Panzer-Grümayer ER. Presence of clone-specific antigen receptor gene rearrangements at birth indicates an in utero origin of diverse types of early childhood acute lymphoblastic leukemia. *Blood*. 2000;95(8):2722-2724.

10. Mansur MB, Emerenciano M, Brewer L, et al. SIL-TAL1 fusion gene negative impact in T-cell acute lymphoblastic leukemia outcome. *Leuk Lymphoma*. 2009;50(8):1318-1325. doi:10.1080/10428190903040014.

11. D'Angio M, Valsecchi M., Testi A., et al. Clinical features and outcome of SIL/TAL1-positive T-cell acute lymphoblastic leukemia in children and adolescents: a 10-year experience of the AIEOP group. *Haematologica*. 2015:344-347.

12. Furness CL, Mansur MB, Weston VJ, et al. The subclonal complexity of STIL-TAL1+ T-cell acute lymphoblastic leukaemia. *Leuk 2018*. 2018:1. doi:10.1038/s41375-018-0046-8.

13. Breit TM, Mol EJ, Wolvers-Tettero IL, Ludwig WD, van Wering ER, van Dongen JJ. Site-specific deletions involving the tal-1 and sil genes are restricted to cells of the T cell receptor alpha/beta lineage: T cell receptor delta gene deletion mechanism affects multiple genes. *J Exp Med*. 1993;177(4):965-977. doi:10.1084/jem.177.4.965.

14. Carlotti E, Pettenella F, Amaru R, et al. Molecular characterization of a new recombination of the SIL / TAL-1 locus in a child with T-cell acute lymphoblastic leukaemia. 2002:1011-1018.

15. Barbi M, Binda S, Primache V, et al. Diagnosis of congenital cytomegalovirus infection by detection of viral DNA in dried blood spots. *J Clin Virol*. 1996;35(1):206-209. doi:10.1016/j.jcv.2005.08.010.

16. Priftakis P, Dalianis T, Carstensen J, et al. Human polyomavirus DNA is not detected in Guthrie cards (dried blood spots) from children who developed acute lymphoblastic leukemia. *Med Pediatr Oncol*. 2003;40(4):219-223. doi:10.1002/mpo.10246.

17. Wiemels JL, Ford a M, Van Wering ER, Postma A, Greaves M. Protracted and variable latency of acute lymphoblastic leukemia after TEL-AML1 gene fusion in utero. *Blood*. 1999;94(3):1057-1062. http://www.ncbi.nlm.nih.gov/pubmed/10419898.

18. Cavé H, Suciu S. Clinical significance of HOX11L2 expression linked to t (5; 14)(q35; q32), of HOX11 expression, and of SIL-TAL fusion in childhood T-cell malignancies: results of EORTC studies 58881 and 58951. *Blood*. 2004;103(2):442-450. doi:10.1182/blood-2003-05-1495.Supported.

19. Ballerini P, Landman-Parker J, Cayuela JM, et al. Impact of genotype on survival of children with T-cell acute lymphoblastic leukemia treated according to the French protocol FRALLE-93: The effect of TLX3/HOX11L2 gene expression on outcome. *Haematologica*. 2008;93(11):1658-1665. doi:10.3324/haematol.13291.

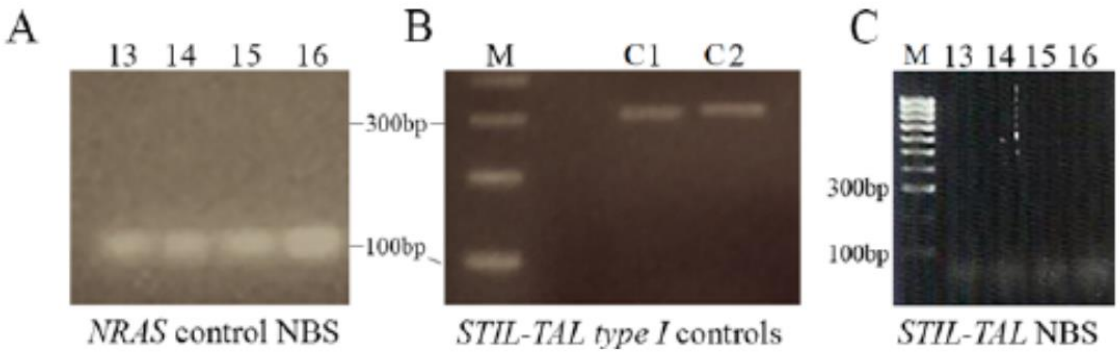

Figure 1.

STIL-TAL fusion does not backtrack to birth. A) *NRAS* integrity control PCR on four blood spot DNAs after WGA. B) Two control DNAs isolated from T-ALL patient diagnostic material after type I *STIL-TAL* assay PCR. C) Four NBS DNAs from children later diagnosed with T-ALL were assessed as PCR negative for type I *STIL-TAL* fusion.

Supplementary data legend

The supplementary file includes: Clinical characteristics and karyotype from the studied patients (Table S1 and S2). Primer sequences used for *STIL-TAL1* and *NRAS* analysis. Integrity control analysis and 38 NBS positive for *NRAS* (Figure S1), Sensitivity assay for detection of *STIL-TAL1* type I deletions (Figure S2), Absence of *STIL-TAL1* type II deletions in the 38 studied patients (Figure S3)