# Self-reverting mutations partially correct the blood phenotype in a Diamond Blackfan anemia patient

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**Self-reverting mutations partially correct the blood phenotype in a Diamond Blackfan Anemia patient**

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This file contains Materials and Methods, Supplementary Figures as well as the Supplementary References.

**Methods:**

**Ethics:** Experiments were carried out under ethical approval by the Peter MacCallum Cancer Centre Ethics committee (HREC 13-185) and the Australian Familial Haematological Cancer Study (REC1542/12/2015) approved by the Women's and Children's Hospital Human Research Ethics Committee in accordance with the Declaration of Helsinki.

**Generation of lymphoblastoid cell lines (LCLs):** Epstein Barr virus-transformed lymphoblastoid cell-lines (LCLs) were generated from PBMNCs isolated from the trio and two siblings, and three healthy donors. LCLs were cultured in RPMI-1640 with HEPES (Gibco Invitrogen) supplemented with 10% FBS (Sigma Aldrich) at 37$^{o}$C with 5% $CO_2$.

**Analysis of SBDS protein expression:** LCLs were washed once with PBS and pelleted at 500 x g for 5 minutes. Cell pellets were lysed in SDS lysis buffer (20 mM HEPES pH 7.9, 0.5 mM EDTA and 2% (w/v) SDS) and protein concentration quantified using the BioRad $D_C$ protein assay as per manufacturers' instructions. Protein samples (50 µg) were then mixed with Laemmli sample buffer[1] containing 8% β-mercaptoethanol, and heated at 95$^{o}$C for 5 minutes. Protein samples were then electrophoresed on Novex 4-20% Tris Glycine SDS-PAGE gels (ThermoFisher Scientific), and transferred onto PVDF membrane (Immobilon-P, Merck Millipore) using the BioRad Trans-Blot semi-dry transfer cell using standard settings. Membranes were then blocked in 5% (w/v) skim milk (Diploma Brand, Fonterra Food Services) in Tris buffered saline (TBS) pH 7.6 containing 0.05% (v/v) Tween 20 (TBST). Membranes were then subsequently immunoblotted with primary and

secondary HRP-conjugated antibodies (all prepared in 5% skim milk in TBST), and visualized using enhanced chemiluminescence (Western Lighting Plus ECL Kit, Perkin Elmer) on Hyperfilm (GE Life Sciences). SBDS antibody [EPR7820] (ab128946) was purchased from Abcam and the β-actin antibody was purchased from MP Biosciences (691002). Secondary antibodies (goat-α-mouse and goat-α-rabbit were purchased from BioRad (172-1011 and 170-6515, respectively).

**Analysis of *SBDS*, *RPS26* and *RPL41* mRNA expression (including RNA isolation and cDNA synthesis):** LCLs were washed once with PBS and pelleted at 500 x g for 5 minutes and RNA extracted using the Qiagen miRNeasy mini kit as per manufacturer's instructions. Isolated RNA (2 µg) was then subjected to RQ1 DNase treatment (Promega) for 30 minutes at 37$^{o}$C, followed by reverse transcription using the Superscript III kit (ThermoFisher Scientific) with random primers (Promega). cDNA was then stored at -20$^{o}$C prior to analysis. Taqman Fast Advanced Master Mix (#4444557) and Taqman primer/probe sets (SBDS, Hs04188846_m1; RPS26, Hs00955682_g1; RPL41, Hs00606029_g1; B2M, Hs00984230_m1) were utilized for real-time PCR quantitation of SBDS expression (available from Applied Biosystems, ThermoFisher Scientific). cDNA isolated as described above, was assayed using the aforementioned reagents on the Applied Biosystems StepOnePlus real-time PCR instrument as per the manufacturers' instructions using the instrument default cycling conditions. Data was analysed using the 7000 SDS 1.1 RQ Software (Applied Biosystems) where relative quantification of gene expression was performed (normalized to B2M expression).

**Targeted Next Generation Sequencing (NGS):** Trio gDNA was subjected to targeted next generation sequencing (NGS) of 94 genes and 284 SNPs associated with predisposition to cancer on the TruSight Cancer Panel (Illumina). gDNA from the proband (peripheral blood {PB}, hair) was also analysed on the 54 gene TruSight Myeloid panel. The MiSeq Reporter package was used to call variants and annotation was provided through our ACRF Cancer Genomics Facility custom pipeline, which takes into consideration pathogenicity/oncogenicity predictions (CADD>10, Polyphen 2, SIFT, Mutation Taster, GERP > 2, COSMIC parameters including specific-mutation and gene frequency), population minor allele frequencies (1000 GP, ESP, ExAC), OMIM, Gene Ontology and various parameters designed to

filter out systematic errors. Variants detected in PB and hair were analysed with VariantGrid (in-house analysis software) to identify somatic variants.

**Whole Genome Sequencing:** Genomic DNA samples from PB of the trio (proband, father and mother) were subjected to whole genome sequencing (WGS) by Illumina (San Diego, California, USA) with >30x coverage for more than 90% of the genome. Sequencing, alignment to the hg19 reference genome and variant calling were performed by Illumina using the Isaac software[2]. The single read spanning the 184kb deletion was only detected after re-mapping reads with BWA-MEM[3]. Variant annotation and filtration was also carried out by Illumina. Based on the reported family history, variants were evaluated based on autosomal recessive inheritance, *de novo* inheritance, x-linked inheritance, as well as dominant inheritance (based on literature reports of reduced penetrance for DBA). All variants meeting any of the above inheritance patterns, and found in any gene associated with DBA, short stature, anemia, or ribosomal protein were evaluated. Clinical interpretation was performed using the American College of Medical Genetics and Genomics guidelines for evaluation and classification of genetic variant information. In addition to this, clinical interpretation was performed for all single nucleotide variants, insertions up to 3 nucleotides in length, and deletions up to 11 nucleotides in length, in a subset of genes recommended by the American College of Medical Genetics and Genomics (ACMG). All calls within the subset of genes located in the Gene list appendix were evaluated for evidence of clinical importance including: allele frequency in population studies (dbSNP, 1000 Genomes, *etc*.), evidence in the scientific literature for likely causation of the condition, and consideration of the likely biological implications of the variant based on its expected characteristics.

**SNP array:** gDNA from the PB of the trio, and hair, bone marrow and lymphoblastoid cell lines (LCLs) of the proband were also analysed by high density CytoSNP 850K BeadArray (Illumina).

**Erythroid colony assays:** Erythroid burst forming units from PBMNCs were grown in MethoCult (#H4230, Stem Cell Technologies) in the presence of SCF (50 ng/ml), IL-3 (20 ng/ml) and EPO (2 U/ml) for 14 days. Colonies were scored and picked. Whole genome amplification was performed on colony genomic DNA using the

GenomePlex Complete WGA Kit (WGA2, Sigma Aldrich). Sanger sequencing was performed across informative selected SNPs on chromosome 12, to genotype the colonies for cnLOH events.

**Supplementary Table 1. Hematological parameters of the proband.** Blood parameters of the proband from age 9 months to present.

| | | **Ref range** | **19.01.2009** | **26.05.2009** | **2.06.2009** | **30.06.2009** | **03.07.2009** | **26.10.2009** | **18.11.2009** | **21.12.2009** | **12.01.2010** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age/Time-point** | **months** | | **9** | **13** | **13** | **14** | **14** | **18** | **18** | **20** | **20** |
| Red Blood Cells | x 10^12/L | 3.6 - 5.0 | 2.31 | 3.57 | 3.01 | 3.15 | 2.93 | 2.59 | 2.9 | 2.66 | 2.97 |
| Haemoglobin | g/L | 100 - 140 | **71** | **99** | **88** | **85** | **77** | **77** | **88** | **78** | **87** |
| Absolute Retic Count | x10^9/L | 25 - 100 | - | **4.3** | **13** | **4.1** | - | - | - | - | - |
| Packed cell volume | L/L | 0.32 - 0.4 | 0.2 | 0.3 | 0.26 | 0.26 | 0.24 | 0.21 | 0.24 | 0.22 | 0.24 |
| Mean Cell Volume | fL | 78 - 90 | 88 | 85 | 86 | 82 | 81 | 81 | 84 | 81 | 80 |
| MCH | pg | 24 - 34 | 31 | 28 | 29 | 27 | 26 | 30 | 30 | 29 | 29 |
| MCHC | g/L | 310 - 360 | 349 | 328 | 339 | 327 | 325 | 364 | 359 | 362 | 363 |
| White cell count | x10^9/L | 6 - 12 | 8.64 | 7.94 | 10.8 | 9.61 | 8.49 | 6.4 | 7.1 | 9 | 7.4 |
| Neutrophils | x10^9/L | 1 - 6.1 | 1.81 | 3.81 | 6.8 | 3.65 | 2.72 | 1.79 | 1.99 | 3.96 | 2.66 |
| Lymphocytes | x10^9/L | 2.1 - 8.3 | 5.62 | 3.57 | 3.46 | 4.9 | 4.92 | 3.84 | 4.4 | 4.32 | 4.04 |
| Monocytes | x10^9/L | 0.1 - 1.00 | 0.69 | 0.48 | 0.43 | 0.86 | 0.68 | 0.58 | 0.5 | 0.54 | 0.52 |
| Eosinophils | x10^9/L | 0.05 - 0.7 | 0.52 | 0.08 | 0.11 | 0.19 | 0.17 | 0.13 | 0.14 | 0.18 | 0.14 |
| Basophils | x10^9/L | | 0 | 0.08 | 0 | 0 | 0 | 0.06 | 0.07 | 0 | 0.04 |

| | | Ref range | 15.01.2010 | 10.05.2010 | 20.05.2010 | 07.06.2010 | 21.06.2010 | 06.07.2010 | 29.11.2010 | 06.12.2010 | 20.12.2010 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age/Time-point** | **months** | | **20** | **24** | **25** | **25** | **26** | **26** | **31** | **31** | **32** |
| Red Blood Cells | x 10^12/L | 3.6 - 5.0 | 2.63 | 2.4 | 3.65 | 2.62 | 3.86 | 2.61 | 4.68 | 4.09 | 3.14 |
| Haemoglobin | g/L | 100 - 140 | **76** | **74** | 112 | **79** | 118 | **79** | 129 | 113 | **90** |
| Absolute Retic Count | x10^9/L | 25 - 100 | - | **3.1** | - | - | - | - | 29 | **18** | 34.2 |
| Packed cell volume | L/L | 0.32 - 0.4 | 0.21 | 0.2 | 0.31 | 0.22 | 0.32 | 0.22 | 0.36 | 0.31 | 0.24 |
| Mean Cell Volume | fL | 78 - 90 | 80 | 85 | 84 | 82 | 84 | 82 | 77 | 76 | 77 |
| MCH | pg | 24 - 34 | 29 | 31 | 31 | 30 | 31 | 30 | 28 | 28 | 29 |
| MCHC | g/L | 310 - 360 | 362 | 367 | 365 | 367 | 364 | 366 | 359 | 360 | 371 |
| White cell count | x10^9/L | 6 - 12 | 6.1 | 6 | 7.2 | 8.3 | 6.7 | 5.9 | 5.7 | 4.8 | 4.2 |
| Neutrophils | x10^9/L | 1 - 6.1 | 1.95 | 1.44 | 3.31 | 2.32 | 1.27 | 1.53 | 1.71 | 0.77 | 1.22 |
| Lymphocytes | x10^9/L | 2.1 - 8.3 | 3.54 | 4.2 | 3.38 | 5.31 | 4.82 | 4.01 | 3.65 | 3.74 | 2.65 |
| Monocytes | x10^9/L | 0.1 - 1.00 | 0.49 | 0.24 | 0.36 | 0.42 | 0.34 | 0.18 | 0.29 | 0.24 | 0.25 |
| Eosinophils | x10^9/L | 0.05 - 0.7 | 0.06 | 0.06 | 0.07 | 0.25 | 0.27 | 0.12 | 0.06 | 0.05 | 0.08 |
| Basophils | x10^9/L | | 0.06 | 0 | 0 | 0 | 0 | 0.06 | 0 | 0 | 0 |

| | | Ref range | 17.01.2011 | 14.02.2011 | 9.05.2011 | 23.05.2011 | 18.06.2011 | 30.06.2011 | 29.07.2011 | 8.12.2011 | 19.12.2011 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age/Time-point** | **months** | | **32** | **33** | **36** | **37** | **37** | **38** | **39** | **43** | **43** |
| Red Blood Cells | x 10^12/L | 3.6 - 5.0 | 3.02 | 3.08 | 2.98 | 2.3 | 2.93 | 2.33 | 2.48 | 2.79 | 2.7 |
| Haemoglobin | g/L | 100 - 140 | **91** | **90** | **96** | **78** | 101 | **80** | **85** | 101 | 100 |
| Absolute Retic Count | x10^9/L | 25 - 100 | 40.5 | 62.2 | 54.2 | 53.1 | 54.8 | 91.6 | 67.7 | 63.3 | 81.3 |
| Packed cell volume | L/L | 0.32 - 0.4 | 0.25 | 0.25 | 0.27 | 0.22 | 0.28 | 0.23 | 0.24 | 0.28 | 0.27 |
| Mean Cell Volume | fL | 78 - 90 | 84 | 81 | 91 | 94 | 95 | 98 | 95 | 99 | 101 |
| MCH | pg | 24 - 34 | 30 | 29 | 32 | 34 | 34 | 34 | 34 | 36 | 37 |
| MCHC | g/L | 310 - 360 | 357 | 364 | 355 | 358 | 361 | 349 | 362 | 365 | 366 |
| White cell count | x10^9/L | 6 - 12 | 5 | 4.4 | 5.8 | 4.7 | 6.2 | 5.4 | 5.2 | 6.6 | 4.9 |
| Neutrophils | x10^9/L | 1 - 6.1 | 1.5 | 0.97 | 2.32 | 2.02 | 3.91 | 1.78 | 1.61 | 2.18 | 1.03 |
| Lymphocytes | x10^9/L | 2.1 - 8.3 | 3.15 | 2.9 | 3.13 | 2.21 | 1.86 | 3.13 | 3.07 | 3.96 | 3.63 |
| Monocytes | x10^9/L | 0.1 - 1.00 | 0.25 | 0.35 | 0.23 | 0.33 | 0.43 | 0.32 | 0.31 | 0.26 | 0.2 |
| Eosinophils | x10^9/L | 0.05 - 0.7 | 0.05 | 0.13 | 0.12 | 0.09 | 0 | 0.11 | 0.16 | 0.2 | 0.05 |
| Basophils | x10^9/L | | 0 | 0 | 0 | 0.05 | 0 | 0.05 | 0.05 | 0 | 0 |

| | | Ref range | 3.01.2012 | 17.01.2012 | 1.02.2012 | 29.06.2012 | 5.07.2012 | 12.07.2012 | 19.07.2012 | 26.07.2012 | 27.11.2012 | 27.11.2012 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age/Time-point** | **months** | | **44** | **44** | **45** | **50** | **50** | **50** | **50** | **51** | **55** | **55** |
| Red Blood Cells | x 10^12/L | 3.6 - 5.0 | 2.43 | 2.45 | 2.38 | 2.34 | 2.25 | 2.29 | 2.51 | 2.46 | 2.28 | 2.08 |
| Haemoglobin | g/L | 100 - 140 | **93** | **96** | **95** | **90** | **87** | **85** | **91** | **88** | **83** | **81** |
| Absolute Retic Count | x10^9/L | 25 - 100 | 78 | 81.3 | 72.8 | 90.6 | 60.1 | - | 60 | 66 | 41 | 54.3 |
| Packed cell volume | L/L | 0.32 - 0.4 | 0.26 | 0.27 | 0.26 | 0.24 | 0.24 | 0.24 | 0.26 | 0.25 | 0.23 | 0.22 |
| Mean Cell Volume | fL | 78 - 90 | 105 | 109 | 111 | 105 | 108 | 106 | 104 | 103 | 102 | 107 |
| MCH | pg | 24 - 34 | 38 | 39 | 40 | 38 | 39 | 37 | 36 | 36 | 36 | 39 |
| MCHC | g/L | 310 - 360 | 362 | 361 | 361 | 367 | 359 | 353 | - | - | 358 | 366 |
| White cell count | x10^9/L | 6 - 12 | 6.9 | 4.7 | 3.5 | 5.2 | 6.3 | 5.8 | 6.92 | 5.61 | 3.63 | 3.6 |
| Neutrophils | x10^9/L | 1 - 6.1 | 3.24 | 1.46 | 1.12 | 2.18 | 3.4 | 1.86 | 3.52 | 1.61 | 0.85 | 0.79 |
| Lymphocytes | x10^9/L | 2.1 - 8.3 | 3.38 | 2.91 | 2 | 2.44 | 2.39 | 3.36 | 2.89 | 3.66 | 2.53 | 2.41 |
| Monocytes | x10^9/L | 0.1 - 1.00 | 0.28 | 0.19 | 0.21 | 0.31 | 0.25 | 0.35 | 0.26 | 0.17 | 0.11 | 0.22 |
| Eosinophils | x10^9/L | 0.05 - 0.7 | 0.07 | 0.14 | 0.14 | 0.21 | 0.25 | 0.23 | 0.24 | 0.16 | 0.13 | 0.18 |
| Basophils | x10^9/L | | 0 | 0 | 0.04 | 0.05 | 0 | 0 | 0.01 | 0.01 | 0.01 | 0.04 |

| | | Ref range | 22.01.2013 | 23.02.2013 | 20.05.2013 | 23.12.2013 | 25.12.2013 | 20.01.2014 | 26.02.2014 | 26.03.2014 | 10.04.2014 | 17.07.2014 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age/Time-point** | **months** | | **57** | **58** | **61** | **68** | **68** | **69** | **70** | **71** | **71** | **74** |
| Red Blood Cells | x 10^12/L | 3.6 - 5.0 | 2.74 | 2.53 | 2.37 | 1.97 | 2.93 | 2.71 | 2.39 | 2.19 | 2.31 | 2.2 |
| Haemoglobin | g/L | 100 - 140 | **95** | **88** | **83** | **72** | 102 | **91** | **91** | **85** | **91** | **79** |
| Absolute Retic Count | x10^9/L | 25 - 100 | 52 | 43 | 59 | 37.2 | - | 51 | 67.2 | 46.9 | 59.1 | 85.4 |
| Packed cell volume | L/L | 0.32 - 0.4 | 0.28 | 0.25 | 0.24 | 0.21 | 0.29 | 0.26 | 0.26 | 0.24 | 0.25 | 0.23 |
| Mean Cell Volume | fL | 78 - 90 | 102 | 100 | 100 | 107 | 99 | 97 | 107 | 107 | 108 | 104 |
| MCH | pg | 24 - 34 | 35 | 35 | 35 | 37 | 35 | 34 | 38 | 39 | 39 | 36 |
| MCHC | g/L | 310 - 360 | 341 | 349 | 352 | 342 | 351 | | 357 | 362 | 362 | 245 |
| White cell count | x10^9/L | 6 - 12 | 4.49 | 4.25 | 3.95 | 3.5 | 10 | 3.23 | 4.1 | 3.5 | 3.7 | 4.1 |
| Neutrophils | x10^9/L | 1 - 6.1 | 1.27 | 2.19 | 1.32 | 0.91 | 8.8 | 0.78 | 2.09 | 1.23 | 1.18 | 1.72 |
| Lymphocytes | x10^9/L | 2.1 - 8.3 | 2.85 | 1.85 | 2.13 | 2.1 | 0.9 | 2.25 | 1.56 | 1.93 | 2.48 | 2.05 |
| Monocytes | x10^9/L | 0.1 - 1.00 | 0.13 | 0.14 | 0.1 | 0.18 | 0.3 | 0.1 | 0.25 | 0.21 | 0 | 0.12 |
| Eosinophils | x10^9/L | 0.05 - 0.7 | 0.23 | 0.06 | 0.4 | 0.28 | 0 | 0.09 | 0.16 | 0.11 | 0.04 | 0.2 |
| Basophils | x10^9/L | | 0.01 | 0.01 | 0 | 0.04 | 0 | 0.01 | 0.04 | 0.04 | 0 | 0 |

| | | Ref range | 01.12.2014 | 10.12.2014 | 22.12.2014 | 24.02.2015 | 17.03.2015 | 14.04.2015 | 12.05.2015 | 25.05.2015 | 07.07.2015 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age/Time-point** | **months** | | **79** | **79** | **80** | **82** | **82** | **83** | **84** | **85** | **86** |
| Red Blood Cells | x 10^12/L | 3.6 - 5.0 | 2.6 | 2.24 | 2.16 | 2.3 | 1.94 | 2.61 | 2.37 | 2.12 | 2.59 |
| Haemoglobin | g/L | 100 - 140 | **90** | **85** | **82** | **87** | **75** | **91** | **89** | **80** | **98** |
| Absolute Retic Count | x10^9/L | 25 - 100 | 83 | 61.2 | - | 66 | 61.3 | 64.7 | 59 | 57 | - |
| Packed cell volume | L/L | 0.32 - 0.4 | 0.27 | 0.24 | 0.23 | 0.24 | 0.21 | 0.26 | 0.24 | 0.22 | 0.27 |
| Mean Cell Volume | fL | 78 - 90 | 102 | 105 | 108 | 105 | 109 | 98 | 102 | 104 | 104 |
| MCH | pg | 24 - 34 | 37 | 38 | 38 | 38 | 39 | 35 | 38 | 38 | 38 |
| MCHC | g/L | 310 - 360 | | 360 | 349 | 359 | 355 | 357 | 367 | 361 | 365 |
| White cell count | x10^9/L | 6 - 12 | 3.73 | 5 | 4.8 | 3.3 | 3.7 | 3.5 | 3.7 | 5.1 | 3.3 |
| Neutrophils | x10^9/L | 1 - 6.1 | 1.64 | 1.95 | 2.11 | 1.35 | 1.74 | 1.26 | 1.81 | 2.87 | 1.19 |
| Lymphocytes | x10^9/L | 2.1 - 8.3 | 1.73 | 2.4 | 2.16 | 1.39 | 1.53 | 1.88 | 1.63 | 1.88 | 1.75 |
| Monocytes | x10^9/L | 0.1 - 1.00 | 0.14 | 0.25 | 0.19 | 0.3 | 0.17 | 0.19 | 0.26 | 0.24 | 0.23 |
| Eosinophils | x10^9/L | 0.05 - 0.7 | 0.21 | 0.35 | 0.29 | 0.26 | 0.24 | 0.14 | 0 | 0.07 | 0.13 |
| Basophils | x10^9/L | | 0.01 | 0.05 | 0.05 | 0.03 | 0.03 | 0.02 | 0 | 0.04 | 0 |

| | | Ref range | 26.11.2015 | 04.12.2015 | 07.12.2015 | 11.01.2016 | 15.02.2016 | 20.06.2016 | 01.07.2016 | 13.07.2016 | 18.07.2016 | 01.08.2016 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Age/Time-point** | **months** | | **91** | **91** | **91** | **92** | **93** | **98** | **98** | **98** | **98** | **99** |
| Red Blood Cells | x 10^12/L | 3.6 - 5.0 | 2.3 | 1.93 | 2.1 | 1.91 | 2.52 | 2.39 | 2.36 | 2.28 | 2.52 | 2.57 |
| Haemoglobin | g/L | 100 - 140 | **74** | **66** | **70** | **64** | **85** | **90** | **89** | **84** | **97** | **95** |
| Absolute Retic Count | x10^9/L | 25 - 100 | 34 | 33.4 | - | 54.2 | 67.8 | 68.1 | - | 62 | 75.1 | 57 |
| Packed cell volume | L/L | 0.32 - 0.4 | 0.21 | 0.18 | 0.2 | 0.18 | 0.24 | 0.25 | 0.25 | 0.24 | 0.28 | 0.28 |
| Mean Cell Volume | fL | 78 - 90 | 90 | 95 | 95 | 96 | 95 | 104 | 107 | 105 | 111 | 107 |
| MCH | pg | 24 - 34 | 32 | 34 | 34 | 34 | 34 | 38 | 38 | 37 | 38 | 37 |
| MCHC | g/L | 310 - 360 | 359 | 359 | 355 | 352 | 357 | 363 | 352 | 350 | 344 | 345 |
| White cell count | x10^9/L | 6 - 12 | 4.12 | 3.7 | 3.2 | 3 | 3.2 | 4.6 | 4.3 | 2.74 | 3.7 | 3.03 |
| Neutrophils | x10^9/L | 1 - 6.1 | 1.99 | 1.85 | 2.24 | 1.2 | 1.63 | 2.58 | 2.01 | 1.43 | 2.11 | 1.31 |
| Lymphocytes | x10^9/L | 2.1 - 8.3 | 1.91 | 1.48 | 0.77 | 1.5 | 1.25 | 1.61 | 1.98 | 1.12 | 1.18 | 1.55 |
| Monocytes | x10^9/L | 0.1 - 1.00 | 0.14 | 0.19 | 0.1 | 0.15 | 0.19 | 0.28 | 0.2 | 0.09 | 0.26 | 0.1 |
| Eosinophils | x10^9/L | 0.05 - 0.7 | 0.06 | 0.15 | 0.06 | 0.12 | 0.1 | 0.09 | 0.1 | 0.09 | 0.11 | 0.06 |
| Basophils | x10^9/L | | 0.02 | 0.04 | 0.03 | 0.03 | 0.03 | 0.05 | 0.01 | 0.01 | 0.04 | 0.01 |

**Supplementary Table 2. Genes affected by germline mutations or deletions.** Functions and phenotypes associated with mutated/deleted genes[4-12] [http://www.ncbi.nlm.nih.gov/gene]

| Gene | Function | KO mouse | Human disease |
|---|---|---|---|
| **SBDS** | •Promotes release of EIF6 from the pre-60S ribosome which is required for the formation of a mature 80S functional ribosome | Early embryonic lethality in homozygous knockouts. Heterozygous knockouts appear normal | Shwachman Diamond Syndrome (autosomal recessive disorder) |
| | | | Heterozygous mutations associated with acquired aplastic anemia |
| **PMEL** | •Integral membrane protein exclusively expressed in pigment cells that synthesize eumelanins | Fully viable, fertile. No obvious developmental defects | |
| | | Mutations – partial occurrence of grey hair | |
| **CDK2** | •Cdk2/cyclin E complexes phosphorylate the retinoblastoma protein and drive cells through the G1/S transition into the S phase. | Viable but both males and females are sterile | |
| | •Cdk2 associates with cyclin A, which itself is essential for cell proliferation during early embryonic development. | | |
| **RAB5B** | •Localized to the early endosomal compartment | | |
| | •Implicated in the regulation of the plasma membrane to early endosomes transport | | |
| **SUOX** | •homodimeric protein localized to the intermembrane space of mitochondria. | | Sulfocysteinuria (autosomal recessive disorder) |

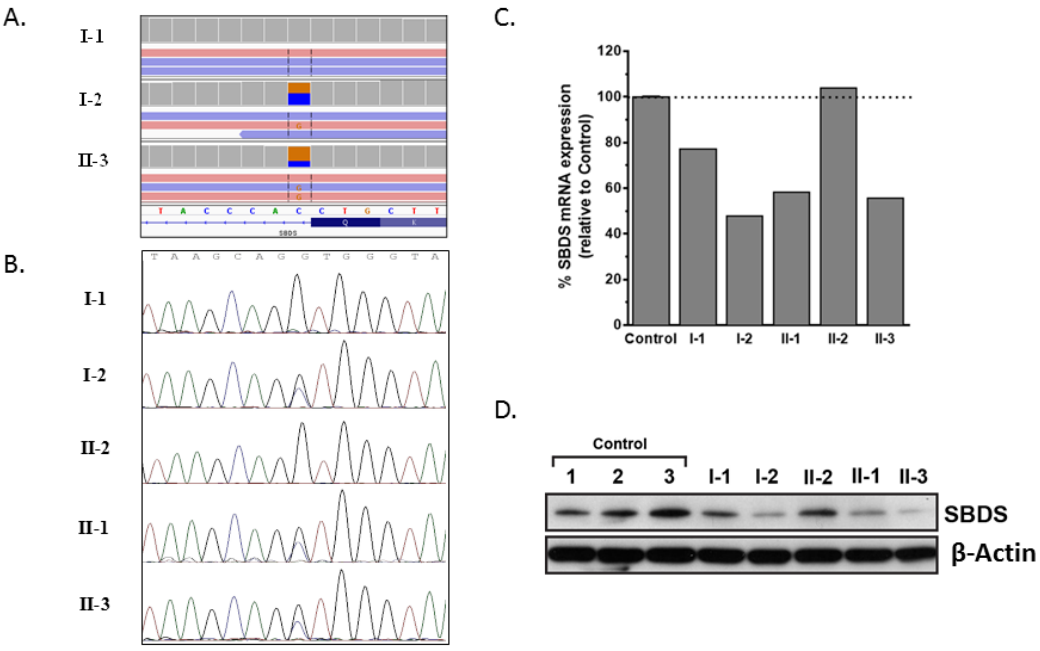
| | | | |
|---|---|---|---|
| | •Deficiency results in neurological abnormalities - often fatal at an early age | | Variant associated with increased risk of ALL (Not convincing) |
| **IKZF4** | •Transcriptional repressor - Interacts with SPI1 and MITF to repress transcription of the CTSK and ACP5 promoters via recruitment of corepressors SIN3A and CTBP2. | Viable and fertile. With no noted autoimmune phenotype. Normal Treg numbers and phenotypes | |
| | • May be involved in central and peripheral nervous system development. | | |
| **RPS26** | •ribosomal protein that is a component of the 40S subunit | | Diamond Blackfan Anemia (autosomal dominant disorder) |
| **ERBB3** | •Kinase-impaired HER receptor tyrosine kinase family member | Null mice die in utero at day 13.5 -18 due to cardiac cushion abnormalities | Lethal congenital contracture syndrome type 2 (autosomal recessive disorder) |
| | •Heterodimerizes with ERBB2 upon ligand binding to promote signaling | | |
| **PA2G4** | •Role in a ERBB3-regulated signal transduction pathway. | Knockout mice 30% smaller than hets and WT until puberty | |
| | • involved in growth regulation. | | |
| | • Corepressor of AR | | |
| | • Binds RNA. | | |
| | • May be involved in regulation of intermediate and late steps of rRNA processing. | | |
| | • May be involved in ribosome assembly. | | |

| | | | |
|---|---|---|---|
| | • Mediates cap-independent translation of specific viral IRESs (internal ribosomal entry site) (By similarity). | | |
| **RPL41** | •encodes a protein component of the large ribosomal subunit | | |
| | •a basic (positively charged) peptide consisting of only 25 amino acids | | |
| | •RPL41 deletion was detected in 59% of tumor cell lines by fluorescence in situ hybridization analyses and RPL41 down-regulation in 75% of primary breast cancers by real-time quantitative reverse transcription-polymerase chain reaction. These studies suggest a tumor suppression role for RPL41. | | |
| **ZC3H10** | •Inhibits anchorage independent growth in soft agar. Expression inversely correlates with breast cancer progression, hence suggesting a tumor suppressor function | | |
| **ESYT1** | •Stimulates formation of ER-PM junctions in a Ca2+-dependent manner | | |
| | •Promotes recruitment of the phosphatidylinositol transfer protein (PITP) Nir2 and phospholipid incorporation into the PM. | | |

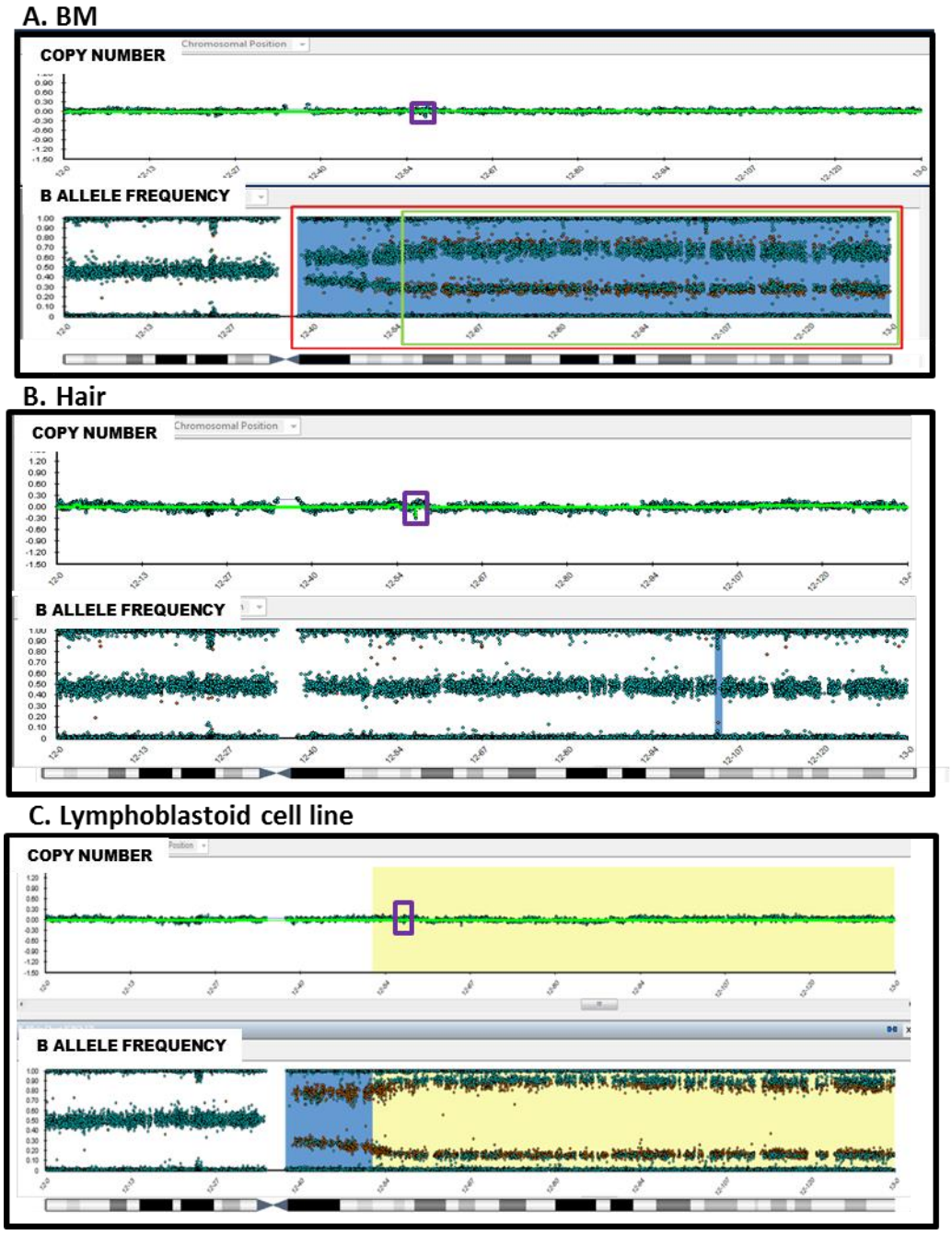
**Supplementary Table 3. Allelic imbalance in affected child across chr 12q.**
Analysis of reads across bases where child is heterozygous in the targeted NGS (TruSight Cancer) panel, confirmed allelic imbalance with an over-representation of the paternal allele in peripheral blood. Paternal allele is depicted in bold in column showing the proband's genotype.

| Chromosomal position | I-1 (Father) Nucleotide (percentage) | I-2 (Mother) Nucleotide (percentage) | II-3 (Proband) Nucleotide (percentage) |
|---|---|---|---|
| chr12:51155663 | C (100) | C/T (51/49) | **C**/T (**68**/32) |
| chr12:53273962 | T (100) | T/A (46/54) | **T**/A (**64**/36) |
| chr12:58142854 | A (99) | C (99) | **A**/C (**62**/38) |
| chr12:58144665 | T (99) | C (100) | **T**/C (**68**/32) |
| chr12:58145156 | C (99) | T (99) | **C**/T (**71**/28) |

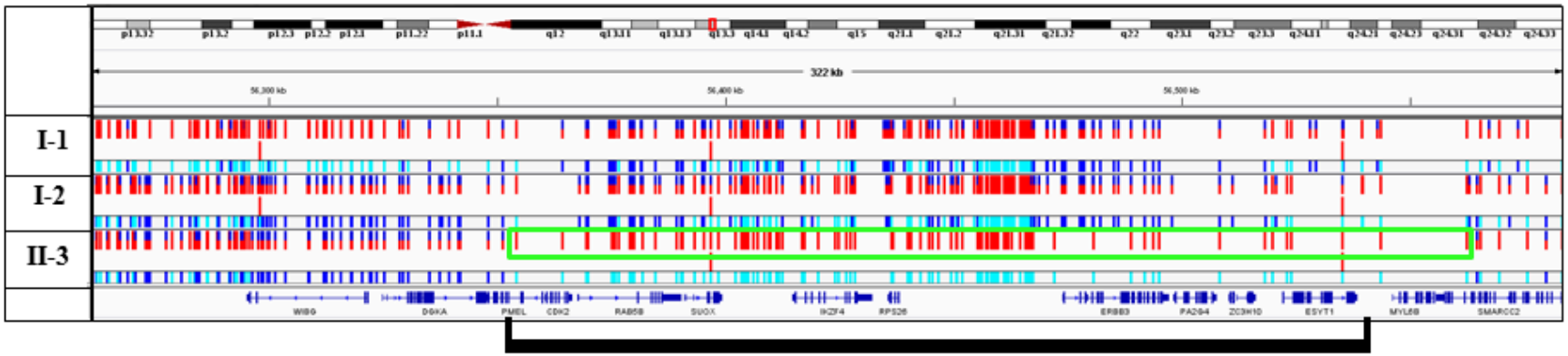
**Supplementary Figure Legends:**




**Supplementary Figure 1. Mutation in splice donor site in SBDS.** WGS identified a c.258+1G>C splice donor site mutation in the proband and mother (A). This mutation was confirmed by Sanger sequencing in the proband, mother and brother (B) Quantitative RT-PCR (C) and western blot analysis (D) reveal lower expression of SBDS in individuals with the mutated allele compared to normal controls.




**Supplementary Figure 2. Deletion in chromosome 12q found in BM and hair of the proband but regions of copy neutral loss of heterozygosity (cnLOH) restricted to BM and LCL.** (A) SNP microarray confirms 184 kb deletion encompassing 11 genes on chr 12q in BM (purple box) and reveals cnLOH comprising at least two regions of chr 12q (denoted by green and red boxes). (B) 184 kb deletion confirmed to be germline by SNP microarray on DNA from hair. (C) cnLOH events confirmed in larger proportions in LCL when compared to BM.

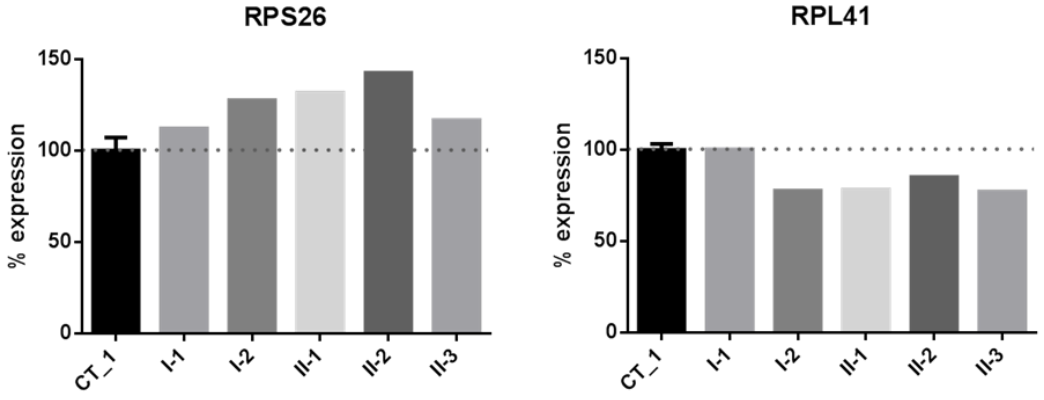



**Supplementary Figure 3. Loss of heterozygosity within 184 kb deletion in proband.** Region of loss of heterozygosity (green box) encompasses 184 kb deletion (black bar) in the proband (II-3). The parents are heterozygous across this region. Alternative alleles are depicted by blue and red bars.

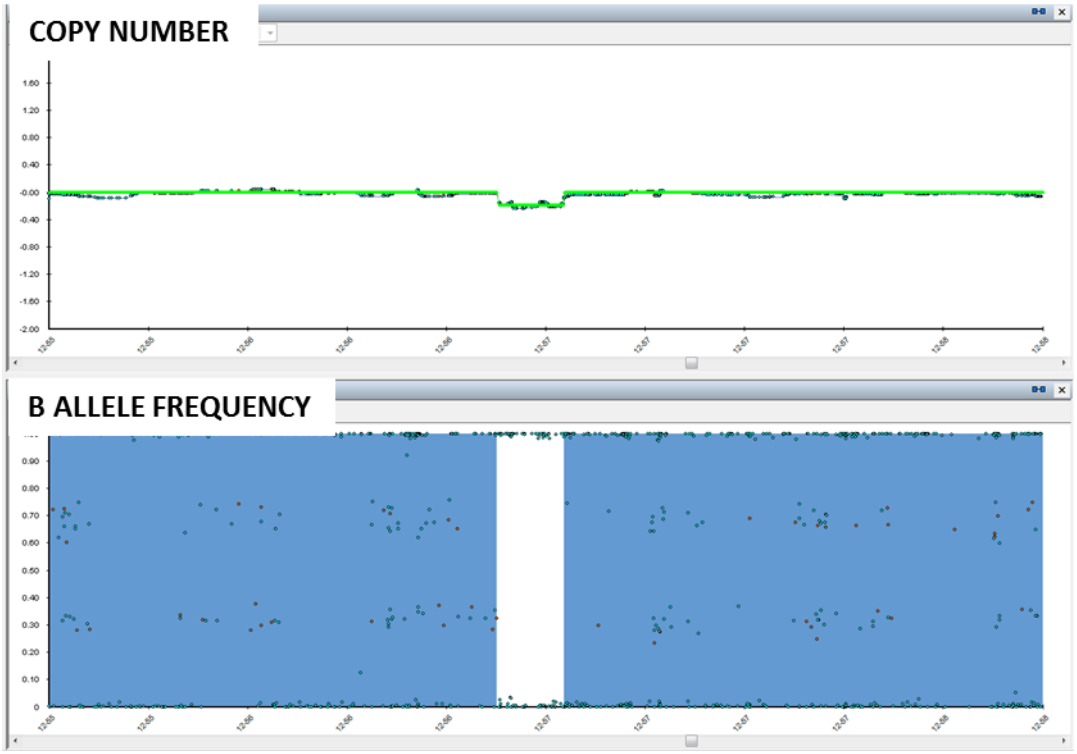
```
AluY      GAGGCTGAGGCAGGAGAATGGCGTGAACCCGGGAGGCAGAGCTTGCAGTGAGCCGAGATT
Sanger    GAGGCTGAGGCAGGAGAATGGCGTGAACCCGGGAGGCAGAGCTTGCAGTGAGCCGAGATC
AluSc     GAGGCTGAAACAGGAGAGTCGCTTGAACCAGGGAGGCGGAGGTTGCAGTGAGCCAAGATG
          ********  ******* * ** ****** ******* *** ************ ****

AluY      GCGCCACTGCACTCCAGCCTGGGCGACAGAGCGAGACTCCGTCTCAAAAAAGAAAAAAAA
Sanger    GCGCCACTGTACTCCAGCCTGGGCGACACAGCGAGACTCCATCTCAAAAAAAAAAAA---
AluSc     GCGCCACTGCACTCTAGCCTGGGCGACACAGCGAAACTCCATCTCAAAAAAAAAAAA---
          ********* **** ************* ***** ***** ********** *****
```

**Supplementary Figure 4. Breakpoints of the 184 kb deletion lie within Alu repeats with high sequence similarity.** 184 kb deletion is likely the result of homologous recombination event between Alu repeats – AluY (56354900-56355009) and AluSc8 (56539460-56539754) which show 88% sequence identity. The breakpoint has been narrowed down to a region of 56 bp using Sanger sequencing (underlined). Regions that are identical to AluY (green) and AluSc8 (blue) are highlighted.




**Supplementary Figure 5. Proband exhibits similar levels of RPS26 and RPL41 expression as unaffected controls.** Quantitative RT-PCR for RPS26 and RPL41 revealed similar expression levels in the proband (II-3) and the other family members as well as normal unrelated controls. CT_1, average of 3 unrelated individuals.




**Supplementary Figure 6. Loss of heterozygosity seen within the 184 kb deleted region.** Copy number chart shows germline 184 kb deletion and the B allele frequency chart shows corresponding to region of loss of heterzygosity as well as the surrounding regions of cnLOH.

Supplementary References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