Author/s:
Fox, LC;Tan, M;Brown, AL;Arts, P;Thompson, E;Ryland, GL;Lickiss, J;Scott, HS;Poplawski, NK;Phillips, K;Came, NA;James, P;Ting, SB;Ritchie, DS;Szer, J;Hahn, CN;Schwarer, A;Blombery, P

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DR. LUCY C FOX (Orcid ID : 0000-0002-3855-8232)

PROF. HAMISH S SCOTT (Orcid ID : 0000-0002-5813-631X)

DR. PIERS ALEXANDER BLOMBERY (Orcid ID : 0000-0002-9902-0022)

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Corresponding author mail id: Lucy.fox@petermac.org

A synonymous *GATA2* variant underlying familial myeloid malignancy with striking intrafamilial phenotypic variability

Lucy C. Fox[1,2,3], Michelle Tan[4], Anna L. Brown[5,6,8,9], Peer Arts[5,6], Ella Thompson[1,3], Georgina L. Ryland[1], Jennifer Lickiss[1], Hamish S. Scott[5,6,7,8,9,10], Nicola K. Poplawski[8,11], Kerry Phillips[6,11], Neil A. Came[1,3], Paul James[3,12], Stephen B. Ting[4], David S. Ritchie[2,3], Jeff Szer[2,3], Christopher N. Hahn[5,6,8,9], Anthony Schwarer[4], Piers Blombery[1,2,3]

[1]Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, Australia

[2]Clinical Haematology at Peter MacCallum Cancer Centre and The Royal Melbourne Hospital, Melbourne, VIC, Australia

[3]Faculty of Medicine, Dentistry and Health Science, University of Melbourne, Parkville, Australia

[4]Eastern Health, Box Hill, Australia

[5]Centre for Cancer Biology, alliance between SA Pathology and University of South Australia, Adelaide, South Australia, Australia.

[6]Molecular Pathology Research, Genetics and Molecular Pathology, SA Pathology, Adelaide, South Australia, Australia.

[7]School of Biological Sciences, University of Adelaide, Adelaide, South Australia, Australia.

[8]Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia.

[9]School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, South Australia, Australia.

[10]Cancer Genomics Facility, Australian Cancer Research Foundation, Centre for Cancer Biology, SA Pathology, Adelaide, South Australia, Australia

[11]Adult Genetics Unit, Royal Adelaide Hospital, Adelaide, Australia

[12]Familial Cancer Centre, Peter MacCallum Cancer Centre, Melbourne, Australia

Heterozygous germline variants in the transcription factor GATA2 have been implicated in the multifaceted GATA2 deficiency syndrome. The original descriptions of pathogenic germline *GATA2* variants encompassed four overlapping phenotypes including (i) predisposition to myelodysplastic syndrome (MDS)/acute myeloid leukaemia (AML) (Hahn et al., 2011), (ii) monocytopenia and atypical mycobacterial infection (MonoMac) (Hsu et al., 2011), (iii) dendritic cell, monocyte, B and NK lymphocyte (DCML) deficiency (Dickinson et al., 2011) and (iv) lymphoedema with a predisposition to MDS and AML (Emberger Syndrome) (Ostergaard et al., 2011). The prevalence of myeloid malignancy in patients harbouring a pathogenic germline variant is approximately 75%, with a median age of onset of 20 years (Wlodarski et al., 2017). Whilst incomplete penetrance has been noted with *GATA2* mutations, in different kindred, the phenotypic features have been described to be similar and broadly conforming to the above phenotypes (Al Seraihi et al., 2018, Holme et al., 2012). However, intrafamilial phenotypic variability has also been reported with some pedigrees containing individuals displaying different combinations of the above mentioned phenotypes while harbouring the same pathogenic variant (Spinner et al., 2014). Here, we report a large pedigree with a synonymous germline *GATA2* variant presenting marked intrafamilial phenotypic variability which highlights several challenges and opportunities for improving the diagnosis of GATA2 deficiency syndrome.

A 19-year-old female (proband IV-4, Figure 1) presented with bacterial pneumonia requiring admission for intravenous antibiotics. She had previously been noted to have fluctuating cytopenias but had largely remained healthy during childhood. Bone marrow biopsy to investigate normocytic anaemia, neutropenia and monocytopenia demonstrated moderate hypocellularity for age with trilineage dysplasia. Metaphase karyotyping demonstrated a monosomy 7. Targeted amplicon next generation sequencing (NGS) focussing on somatic variants (Yannakou et al., 2018) was performed on her bone marrow aspirate and detected

an acquired mutation in *SETBP1* (p.Asp868Asn). She was diagnosed with MDS and commenced work up for allogeneic stem cell transplantation (alloSCT).

Her family history was significant for AML (Figure 1). Her father (III-4) was one of seven siblings and his brother (III-9) had died from AML aged 18 years. Subsequently, the proband's paternal aunt (III-10) was diagnosed with AML at age 16 and underwent alloSCT with the stem cell donor being the proband's father (III-4). She died from AML eight years after alloSCT, with the AML noted to have monosomy 7 in sex mismatched cells consistent with it being derived from donor cells (*i.e.* proband's father). Another paternal aunt (III-2) was well at age 59 with the exception of bilateral lower limb lymphoedema. Of her three children, one female (IV-1) had congenital bilateral arm and hand deformity (radial, ulnar and humeral hypoplasia, absence of multiple digits) and also experienced bilateral lower limb lymphoedema and cytopenias, and a son (IV-2) experienced hydrocele at age 18 and developed necrotising fasciitis following surgical repair. He had also been diagnosed with Autism Spectrum Disorder at age 13. The proband's brother (IV-6) was found to have moderate cytopenias and bone marrow biopsy was hypocellular with trilineage dysplasia sufficient to confer a diagnosis of MDS-multilineage dysplasia. Conventional karyotype in patient IV-6 was normal and there were no somatic mutations detected by targeted amplicon NGS. All other family members were well. The proband's paternal grandfather (II-1) died of cardiovascular disease in his 70s and was not known to have any haematological disease. His mother (I-2) (the proband's great grandmother) was known to have bilateral lower limb lymphoedema and her cause of death is unknown.

Given this pronounced family history, whole exome sequencing was performed on DNA extracted from peripheral blood mononuclear cells of the proband (IV-4) (Hahn et al., 2019, Blombery et al., 2020). A heterozygous synonymous variant in GATA2 (NM_032638.4:c.351C>G; p.(Thr117=)) was detected and confirmed to be germline by Sanger sequencing using DNA extracted from hair follicles; no other likely candidate variants were identified. RNA studies have previously demonstrated this variant introduces a cryptic splice donor site and results in an aberrantly spliced transcript with a 136 nucleotide internal deletion which in turn results in a frameshift and a putative truncated GATA2 protein (p.Val118Glnfs*55) (Wehr et al., 2018). This variant has previously been reported in a patient

with AML and a family history of MDS, and in an unrelated individual with immunodeficiency (Wehr et al., 2018).

The variant was considered causative of the proband's (IV-4) phenotype and testing identified an additional five family members with the *GATA2* variant (Figure 1). The proband (IV-4) underwent a matched unrelated donor alloSCT and remains well eighteen months later. Five of the six family members who carry the variant display varying degrees of monocyte, B cell and NK deficiencies (Table 1). The remaining carrier (IV-2) had a normal full blood examination but with the aforementioned history of hydrocele. The four carriers who to date have not demonstrated any signs of haematological malignancy are undergoing monitoring with six monthly full blood examination and annual peripheral blood cytogenetics and targeted amplicon NGS assessment, recognising the current lack of published data detailing germline GATA2 clonal evolution to strongly inform surveillance practice. All seven family members who tested negative for the *GATA2* variant show no signs of GATA2 deficiency syndrome.

This large pedigree presents several important features of germline *GATA2* mutation assessment and management. Firstly, the striking phenotypic variability observed across six genetically affected family members, with a kindred experiencing a wide range of GATA2 deficiency symptoms and signs, highlights the need to clinically evaluate each potential patient (and relatives of known carriers) for all features of the GATA2 deficiency spectrum. This marked phenotypic variability attributable to one germline variant is in keeping with previously described lack of correlation between *GATA2* mutation type and haematologic phenotype (Wlodarski et al., 2016). Secondly, the pathogenic variant in this case is synonymous, and which may not be identified with use of bioinformatics and curation filters that screen out such variants (typically used in broad sequencing approaches such as whole exome sequencing). Our pedigree, along with previous reports of this variant (Kozyra et al., 2017) supports routine curation of synonymous GATA2 variants when assessing for possible germline predisposition to haematological malignancy and/or immunodeficiency and exemplifies the need for provision of adequate clinical information and gene-specific expertise when curating variants. Thirdly, our pedigree describes donor-derived leukaemia with poor outcome, an increasingly appreciated occurrence and one of the important reasons to carefully examine for germline disease both clinically and in the laboratory prior to alloSCT

from a related donor (Galera et al., 2018). Finally, this pedigree highlights the challenges of counselling and monitoring these affected kindred, in whom the high prevalence rates and young median age of malignancy onset mandate close haematological follow up, but which must be balanced to avoid undue anxiety potentially over many decades.

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AL SERAIHI, A. F., RIO-MACHIN, A., TAWANA, K., BODOR, C., WANG, J., NAGANO, A., HEWARD, J. A., IQBAL, S., BEST, S., LEA, N., MCLORNAN, D., KOZYRA, E. J., WLODARSKI, M. W., NIEMEYER, C. M., SCOTT, H., HAHN, C., ELLISON, A., TUMMALA, H., CARDOSO, S. R., VULLIAMY, T., DOKAL, I., BUTLER, T., SMITH, M., CAVENAGH, J. & FITZGIBBON, J. 2018. GATA2 monoallelic expression underlies reduced penetrance in inherited GATA2-mutated MDS/AML. *Leukemia,* 32**,** 2502-2507.

BLOMBERY, P., FOX, L., RYLAND, G. L., THOMPSON, E. R., LICKISS, J., MCBEAN, M., YERNENI, S., HUGHES, D., GREENWAY, A., MECHINAUD, F., WOOD, E. M., LIESCHKE, G. J., SZER, J., BARBARO, P., ROY, J., WIGHT, J., LYNCH, E., MARTYN, M., GAFF, C. & RITCHIE, D. 2020. Utility of clinical comprehensive genomic characterisation for diagnostic categorisation in patients presenting with hypocellular bone marrow failure syndromes. *Haematologica*.

DICKINSON, R. E., GRIFFIN, H., BIGLEY, V., REYNARD, L. N., HUSSAIN, R., HANIFFA, M., LAKEY, J. H., RAHMAN, T., WANG, X. N., MCGOVERN, N., PAGAN, S., COOKSON, S., MCDONALD, D., CHUA, I., WALLIS, J., CANT, A., WRIGHT, M., KEAVNEY, B., CHINNERY, P. F., LOUGHLIN, J., HAMBLETON, S., SANTIBANEZ-KOREF, M. & COLLIN, M. 2011. Exome sequencing identifies GATA-2 mutation as the cause of dendritic cell, monocyte, B and NK lymphoid deficiency. *Blood,* 118**,** 2656-8.

GALERA, P., HSU, A. P., WANG, W., DROLL, S., CHEN, R., SCHWARTZ, J. R., KLCO, J. M., ARAI, S., MAESE, L., ZERBE, C., PARTA, M. J., YOUNG, N. S., HOLLAND, S. M., HICKSTEIN, D. D. & CALVO, K. R. 2018. Donor-derived MDS/AML in families with germline GATA2 mutation. *Blood,* 132**,** 1994-1998.

HAHN, C. N., BABIC, M., BRAUTIGAN, P. J., VENUGOPAL, P., PHILLIPS, K., DOBBINS, J., ARTS, P., WEE, A., SINGHAL, D., HIWASE, D. K., FENG, J., SCHREIBER, A. W., D'ANDREA, R. J., POPLAWSKI, N., BROWN, A. L. & SCOTT, H. S. 2019. Australian Familial Haematological Cancer Study - Findings from 15 Years of Aggregated Clinical, Genomic and Transcriptomic Data. *Blood,* 134**,** 1439-1439.

HAHN, C. N., CHONG, C. E., CARMICHAEL, C. L., WILKINS, E. J., BRAUTIGAN, P. J., LI, X. C., BABIC, M., LIN, M., CARMAGNAC, A., LEE, Y. K., KOK, C. H., GAGLIARDI, L., FRIEND, K. L., EKERT, P. G., BUTCHER, C. M., BROWN, A. L., LEWIS, I. D., TO, L. B., TIMMS, A. E., STOREK, J., MOORE, S., ALTREE, M., ESCHER, R., BARDY, P. G., SUTHERS, G. K., D'ANDREA, R. J., HORWITZ, M. S. & SCOTT, H. S. 2011. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. *Nat Genet,* 43**,** 1012-7.

HOLME, H., HOSSAIN, U., KIRWAN, M., WALNE, A., VULLIAMY, T. & DOKAL, I. 2012. Marked genetic heterogeneity in familial myelodysplasia/acute myeloid leukaemia. *Br J Haematol,* 158**,** 242-248.

HSU, A. P., SAMPAIO, E. P., KHAN, J., CALVO, K. R., LEMIEUX, J. E., PATEL, S. Y., FRUCHT, D. M., VINH, D. C., AUTH, R. D., FREEMAN, A. F., OLIVIER, K. N., UZEL, G., ZERBE, C. S., SPALDING, C., PITTALUGA, S., RAFFELD, M., KUHNS, D. B., DING, L., PAULSON, M. L., MARCIANO, B. E., GEA-BANACLOCHE, J. C., ORANGE, J. S., CUELLAR-RODRIGUEZ, J., HICKSTEIN, D. D. & HOLLAND, S. M. 2011. Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. *Blood,* 118**,** 2653-5.

KOZYRA, E. J., PASTOR LOYOLA, V., WEHR, C., SAHOO, S. S., VOSS, R., SZVETNIK, E. A., HIRABAYASHI, S., CATALA, A., HASLE, H., VAN DEN HEUVEL-EIBRINK, M. M., KALLAY, K., MASETTI, R., DE MOERLOOSE, B., SCHMUGGE, M., SMITH, O., USSOWICZ, M., STARY, J., MEJSTRIKOVA, E., PASAULIENĖ, R., BAUMANN, I., GÖHRING, G., SCHLEGELBERGER, B., SALZER, U., LÜBBERT, M., TROMPOUKI, E., NIEMEYER, C. M. & WLODARSKI, M. W. 2017. Systematic Assessment of GATA2 Genetic Variation Reveals the Presence of Novel Disease-Causing Synonymous Exonic Mutations. *Blood,* 130**,** 2449-2449.

OSTERGAARD, P., SIMPSON, M. A., CONNELL, F. C., STEWARD, C. G., BRICE, G., WOOLLARD, W. J., DAFOU, D., KILO, T., SMITHSON, S., LUNT, P., MURDAY, V. A., HODGSON, S., KEENAN, R., PILZ, D. T., MARTINEZ-CORRAL, I., MAKINEN, T., MORTIMER, P. S., JEFFERY, S., TREMBATH, R. C. & MANSOUR, S. 2011. Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). *Nat Genet,* 43**,** 929-31.

SPINNER, M. A., SANCHEZ, L. A., HSU, A. P., SHAW, P. A., ZERBE, C. S., CALVO, K. R., ARTHUR, D. C., GU, W., GOULD, C. M., BREWER, C. C., COWEN, E. W., FREEMAN, A. F., OLIVIER, K. N., UZEL, G., ZELAZNY, A. M., DAUB, J. R., SPALDING, C. D., CLAYPOOL, R. J., GIRI, N. K., ALTER, B. P., MACE, E. M., ORANGE, J. S., CUELLAR-RODRIGUEZ, J., HICKSTEIN, D. D. & HOLLAND, S. M. 2014. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood,* 123**,** 809-21.

WEHR, C., GROTIUS, K., CASADEI, S., BLECKMANN, D., BODE, S. F. N., FRYE, B. C., SEIDL, M., GULSUNER, S., KING, M. C., PERCIVAL, M. B., PRITCHARD, C. C., WALSH, T., WU, D., KEEL, S. & SALZER, U. 2018. A novel disease-causing synonymous exonic mutation in GATA2 affecting RNA splicing. *Blood*.

WLODARSKI, M. W., COLLIN, M. & HORWITZ, M. S. 2017. GATA2 deficiency and related myeloid neoplasms. *Semin Hematol,* 54**,** 81-86.

WLODARSKI, M. W., HIRABAYASHI, S., PASTOR, V., STARY, J., HASLE, H., MASETTI, R., DWORZAK, M., SCHMUGGE, M., VAN DEN HEUVEL-EIBRINK, M., USSOWICZ, M., DE MOERLOOSE, B., CATALA, A., SMITH, O. P., SEDLACEK, P., LANKESTER, A. C., ZECCA, M., BORDON, V., MATTHES-MARTIN, S., ABRAHAMSSON, J., KUHL, J. S., SYKORA, K. W., ALBERT, M. H., PRZYCHODZIEN, B., MACIEJEWSKI, J. P., SCHWARZ, S., GOHRING, G., SCHLEGELBERGER, B., CSEH, A., NOELLKE, P., YOSHIMI, A., LOCATELLI, F., BAUMANN, I., STRAHM, B. & NIEMEYER, C. M. 2016. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. *Blood,* 127**,** 1387-97; quiz 1518.

YANNAKOU, C. K., JONES, K., RYLAND, G. L., THOMPSON, E. R., REID, G., MCBEAN, M., TRAINER, A., WESTERMAN, D. & BLOMBERY, P. 2018. Incidental detection of germline variants of potential clinical significance by massively parallel sequencing in haematological malignancies. *J Clin Pathol,* 71**,** 84-87.

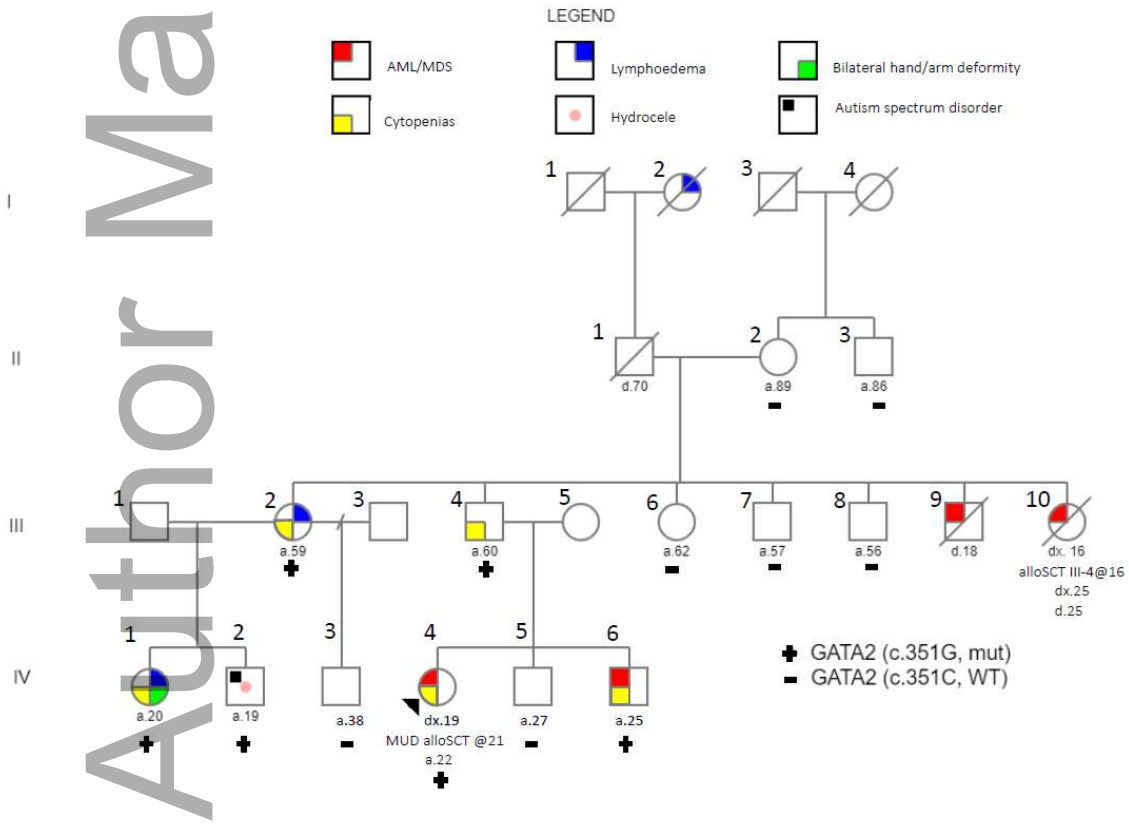


Figure 1: Pedigree of kindred harbouring GATA2 variant

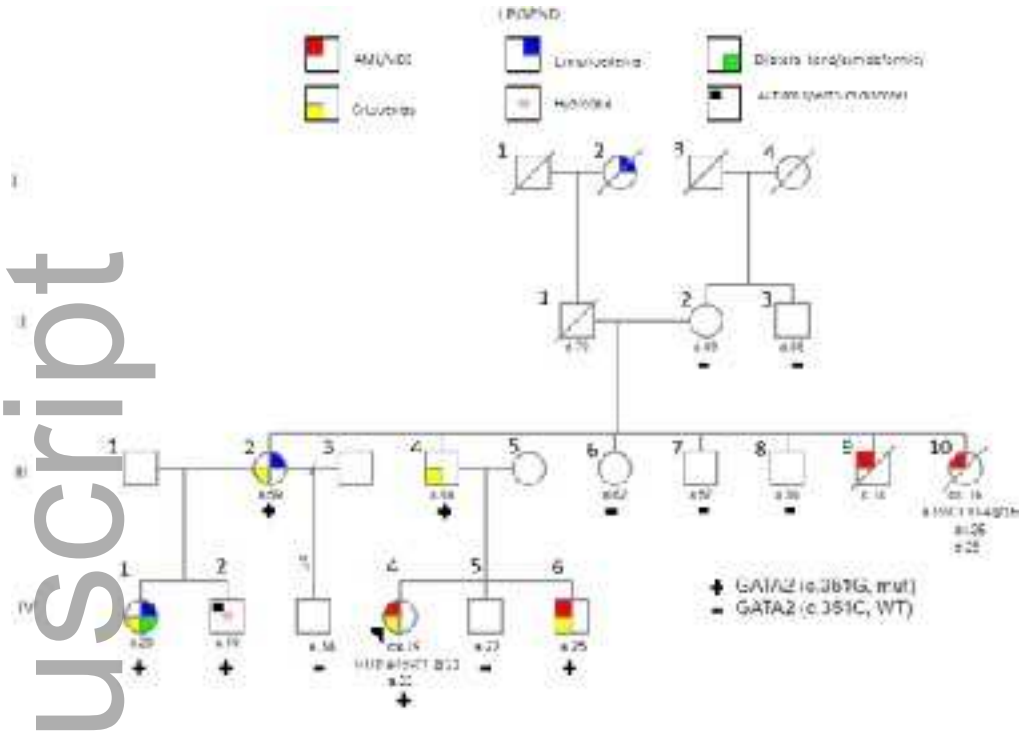
Pedigree diagram where squares represent males and circles represent females. Strikethrough represents family member deceased. Family members tested for GATA2 variant

NM_032638.4:c.351C>G; p.(Thr117=) are annotated with '+' for mutation detected and '-' for not detected (wild type). Arrowhead indicates proband, mutated (mut), wild type (WT), diagnosis (dx), current age (a), age at death (if known) (d), matched unrelated donor (MUD), allogeneic stem cell transplant (alloSCT).

Table 1: Clinical characteristics of the 6 family members harbouring the GATA2 c.351C>G

			Full Blood Examination									Lymphocyte subsets				
Pedigree	**Age**	**Phenotype**	**Haemoglobin Female [115-165g/L] Male [130-170g/L]**	**White Cell Count [4-12 x10^9/L]**	**Neutrophils [2-8 x10^9/L]**	**Lymphocytes [1.0-3.5 x10^9/L]**	**Monocytes [0.2-1.0 x10^9/L]**	**Platelets [150-450 x10^9/L]**	**Bone marrow aspirate & trephine**	**Cytogenetics (sample type)**	**Targeted amplicon next generation sequencing (sample type)**	**CD3+ T cells absolute [0.6-2.5 x10^9/L]**	**CD4+ T cells absolute [0.45-1.70 x10^9/L]**	**CD8+ T cells absolute [0.2-1.15 x10^9/L]**	**CD19+ B cells absolute [0.07-0.55 x10^9/L]**	**CD3- 16/56+ NK cells absolute [0.07-0.70 x10^9/L]**
IV-4	19	Myelodysplastic Syndrome	**101L**	**3.2L**	**1.1L**	1.1	**0.05L**	156	Moderately hypocellular, trilineage dysplasia with occasional small, hypolobated megakaryocytes	Monosomy 7 (BMA)	*SETBP1* (BMA)	1.47	0.64	0.76	**0.09L**	**0.03L**
IV-1	19	Severe bilateral arm/hand deformity, Lymphoedema	137	8.5	5.7	2.5	**0.00L**	**148L**	Not done	Not done	NVD (PB)	2.43	0.91	**1.26H**	**0.06L**	**0.03L**
IV-2	18	Hydrocele, necrotising fasciitis, Autism Spectrum Disorder	150	11.5	7.8	2.6	0.70	230	Not done	No mitoses, no monosomy 7 detected by FISH (PB)	NVD (PB)	2.02	0.76	0.86	0.08	0.07
IV-6	25	Myelodysplastic Syndrome	142	**2.6L**	**1.7L**	**0.8L**	**0.00L**	**70L**	Mildly hypocellular with multilineage dysplasia including	46, XY (BMA)	NVD (BMA)	0.77	**0.26L**	0.37	**0.02L**	**0.02L**

									multiple small, hypolobate megakaryocytes							
III-2	59	Lymphoedema	142	10.6	7.6	2.1	0.40	248	Not done	No mitoses, no monosomy 7 detected by FISH (PB)	NVD (PB)	1.44	0.97	0.4	0.08	**0.05L**
III-4	60	Nil	153	9.4	7.0	1.5	0.60	212	Not done	Not done	NVD (PB)	1.4	1.07	0.32	0.09	**0.03L**
Bone marrow aspirate (BMA), Peripheral blood (PB), Fluorescence In-situ Hybridisation (FISH), No variants detected (NVD), **BOLD** text indicates value outside normal reference range																



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