To the Editor

WISKOTT ALDRICH SYNDROME-2 CAUSED BY NOVEL WISKOTT ALDRICH SYNDROME PROTEIN - INTERACTING PROTEIN (WIP) DEFICIENCY IS ASSOCIATED WITH JUVENILE MYELOMONOCYTIC LEUKAEMIA – A CASE REPORT

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Wiskott Aldrich Syndrome (WAS, OMIM #301000) is a rare X linked disorder, affecting 1 in 50 000 and 1 in 250 000 live births, characterised by a triad of micro thrombocytopenia, eczema and immunodeficiency with a predisposition towards autoimmunity and lymphoproliferative disorders [1]. The WAS gene encodes the WAS protein (WASP), which is a key member of the WASP family proteins, which are cytoplasmic actin cytoskeletal regulatory proteins with diverse functions. A related disorder with a different molecular mechanism and inheritance pattern is Wiskott-Aldrich Syndrome-2 (WAS2, OMIM #614493). WAS2 is an extremely rare recessive disorder caused by biallelic pathogenic variants in the WIPF1 gene on chromosome 2, that encodes a protein binding partner called WASP-interacting protein (WIP), also known as WAS/WAS ligand interacting protein family member 1 (WIPF1) [2]. Pathogenesis of WAS2 is thought to be related to premature degradation of WASP, since WIP is the stabilising protein to WASP. Mutations in WIPF1 are extremely rare and have so far been reported in only three kindreds of 6 patients [3]. WIP deficiency, though expected to present similarly to classic WAS, confers a phenotype with slight differences. We report the clinical features of the seventh case of WAS2 related to WIP deficiency. We extend the phenotype of WIP to include transient, Juvenile myelomonocytic leukaemia (JMML), which has previously been described a rare presentation of WAS, but never in WAS2.

A 4week old male infant born to consanguineous parents of Libyan origin, was referred with bloody vomiting and was found to have isolated thrombocytopenia. Blood smear confirmed thrombocytopenia and platelets were small (Figure S1). Notable family history included a male sibling that sadly died aged 7 months with recurrent infections and bleeding tendency. With the micro thrombocytopenia and a family history with X linked inheritance, a provisional diagnosis of Wiskott Aldrich syndrome was made. Flow cytometric studies for WASP showed absent WASp and immunoblot revealed presence of severely reduced amounts of WASP in the cytoplasmic lysate. Single gene sequencing and analysis of the WAS gene, however, did not identify any plausible causative variants. Further, genetic testing by next generation sequencing of a panel of genes associated with primary immunodeficiency was arranged and this identified a homozygous pathogenic WIPF1 variant, c.354dupT p. (Asn119Ter). Parental testing confirmed the variants were inherited from heterozygous, carrier parents. The WIPF1 variant was assessed as a class 5 pathogenic variant using American College of Medical Genetics (ACMG) (Table S1).

The infant was worked up for allogeneic stem cell transplant in view of the transfusion dependent thrombocytopenia and underlying life limiting condition. On follow up, at 60 days of life, he developed signs of myeloproliferative disorder with gross hepatosplenomegaly, leucocytosis with a total leucocyte count of 30×10^9/L alongside monocytosis with a monocyte count of 5×10^9/L and a leuko-erythroblastic blood picture (Figure S2) with circulating myelocytes and erythroid precursors (manual white cell differential of the blood film showed 37% neutrophils,17% monocytes,20% lymphocytes, 4% eosinophils, 22% myelocytes). The haemoglobin F percentage was raised for age (71% at 2 months of age). The bone marrow was hypercellular with expanded left shifted myelopoiesis and a M:E ratio of 15:1 and blasts less than 5%. The karyotype was normal and the conventional cytogenetic analyses did not show any clonal abnormalities and FISH was negative for BCR-ABL1 rearrangement. The molecular mutations for JMML like NRAS, KRAS, CBL, PTPN11, NF1 were negative. There were no clinical features of Neurofibromatosis type 1. A diagnosis of JMML was established as all four of category I criteria and two of the category III criteria of 2016 WHO diagnostic criteria for JMML were met as (Table S2) [4]. The patient also developed hepatopathy with transaminitis. At 6 months of age, the myeloproliferative

features subsided with normalisation of white cell count and the monocyte count, although hepatosplenomegaly persisted.

He deteriorated with respiratory illness requiring oxygen support. Respiratory secretions were negative for viruses by PCR testing. Development of eczema of the scalp with erythematous, crusty lesions were also apparent. He had a matched sibling donor haematopoietic stem cell transplant with Fludarabine, Treosulphan, Thiotepa and Alemtuzumab conditioning and with Cyclosporin as graft versus host disease (GVHD) prophylaxis, at 7 months of age. The patient engrafted after 11 days and became transfusion independent 25 days after transplant. He had a mild steroid responsive gut GVHD which settled down quickly. The patient remains GVHD free and donor cell engrafted, now two years after haematopoietic stem cell transplant (HCT) and is fully donor immune-reconstituted and all infection and GVHD prophylaxis has been stopped.

WASP is a multidomain protein which plays a key role in cell signalling and cytoskeletal reorganisation. In resting cells, WASP remains in an autoinhibited state in the cytoplasm, stabilised by WIP [2]. WASP -WIP complex stabilises WASP, thereby preventing its degradation. WIP is also crucial in recruiting the WASP-WIP complex to the Zap-70 when there is TCR ligation and aids to localise WASP at the immune synapse, through which, defects in T cell proliferation in WASP - WIP related disorders can be explained. WIP deficiency also imparts defects in chemotaxis and natural killer cell mediated cytotoxicity.

WIP is encoded by WIPF1 gene, located in Chromosome 2. WAS2 is an autosomal recessive disorder leading to premature degradation of WASP, causing clinical features overlapping with WAS. However, the clinical phenotype does not entirely mirror as that seen in Classic WAS.

WIPF1 mutation is a novel mutation described in only 3 kindreds with 6 patients, to the best of our knowledge [3]. Early onset of immunodeficiency with viral and bacterial infections were apparent in all previously reported with WAS2 related WIP deficiency [3]. All patients had major viral respiratory illness requiring hospitalisation ranging from a requirement of oxygen supplementation to ventilatory support in severe cases. All seven patients reported with WAS2 in the literature had moderate to severe thrombocytopenia. In contrast to patient with WAS, intriguingly, patients with WAS2 appear to have normal sized platelets. Hepatosplenomegaly is also a prominent feature of WAS2. Our patient represents the second patient to also have hepatopathy [3]. This is the first reported cases of JMML in association with WAS2.

Though WASP is expressed in all haematopoietic cells, the absence of WASP mainly affects thrombopoiesis and lymphopoiesis with myelopoiesis and erythropoiesis being relatively spared. However, there are seven previous reports of JMML in children with classic WAS [6]. There is no apparent association between WASP and the RAS/MAPK signalling pathway. There is a need for further research to identify new roles for WASP in transcriptional regulation in haemopoiesis, which might throw light on the pathogenesis of JMML in WAS. The association with JMML has not been reported in literature in association with WIPF1 mutation.

The dysregulation in RAS/MAPK signalling caused by mutations of NRAS, KRAS, PTPN11 and CBL and NF1 can be found in 80-90% of the cases of JMML [5]. However, in the remaining 10-20% of patients without a molecular mutation, the diagnosis is largely dependent on a combination of laboratory and clinical parameters, as stringently assigned by the 2016 WHO criteria. It has been well recommended to consider Wiskott-Aldrich syndrome in male infants with JMML, where none of the 5 canonical causative molecular mutations of JMML can be identified [5]. With increasing association of JMML in WAS and WIP related WAS disorders, further research is needed to explain the pathogenesis of JMML in WASP-WIP complex mutations. In children presenting with features of JMML, we recommend a strong degree of clinical suspicion of and encourage investigations to actively look for WAS and WAS2, particularly when the classic driver mutations of JMML are not identified.

Authorship contribution

The first draft of the manuscript was written by Srividhya Senthil and it was reviewed by Adrian J. Thrasher, Kimberly C. Gilmour and Robert F. Wynn. All of the authors read and approved the final manuscript. The corresponding author for the manuscript is Srividhya Senthil.

Declarations

The authors declare no competing interests.

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