FAS mutations are an uncommon cause of immune thrombocytopenia in children and adults without additional features of immunodeficiency

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Primary immune thrombocytopenic purpura (ITP) is an autoantibody-mediated bleeding disorder characterised by thrombocytopenia resulting from accelerated platelet destruction and impaired platelet production without evidence of other haematological, immunological or infectious abnormalities (Cines et al, 2009). Secondary ITP is diagnosed when there is an underlying precipitation of the ITP. The frequency of different aetiologies vary by age, gender and geographic location but no good estimates exist of incidence or prevalence of these underlying diseases. Secondary ITP is estimated to encompass 20% of cases of ITP (Arnold et al, 2017; Cines et al, 2009). Management of secondary ITP cases have usually addressed the underlying disease, e.g. common variable immunodeficiency (CVID) and ITP, but large numbers of patients with ITP have not been studied for various secondary causes. The general consensus is to not further investigate ITP unless there are indications of underlying conditions on examination or by history, such as identification of splenomegaly or history of infections (Provan et al, 2010). It is therefore not known whether ITP can be the dominant feature of genetic disease. This may be most relevant in Evans syndrome, where autoimmune haemolytic anaemia (AIHA) and/or neutropenia are features. One report initially suggested that many cases of Evans syndrome (approximately 50%) are secondary to mutations in the FAS gene and Autoimmune LymphoProliferative Syndrome (ALPS) (Teachey et al, 2005); however, this has not been confirmed.

We therefore screened a cohort of patients with presumed primary ITP for mutations in the genes most commonly associated with secondary ITP in primary immunodeficiencies: WAS (Wiskott-Aldrich Syndrome, WAS), FAS (ALPS) and TNFRSF13B (also termed TACI) (CVID). We chose these 3 for different reasons. The X-linked thrombocytopenia form of
WAS (XLT) strongly resembles ITP and does not always have a history of immunodeficiency disease, such as infection or eczema, although it is restricted to males. In the past, numerous cases of XLT have been mistaken for ITP (Medina et al, 2017). We chose \textit{TNFRSF13B} because hypogammaglobulinemia secondary to \textit{TNFRSF13B} mutations has been linked with autoimmune cytopenias and cases of hypogammaglobulinaemia (CVID) may not have a history of infections (Zhang et al, 2007). Further, diagnosis of ITP and/or AIHA may precede infection susceptibility by years and sometimes even decades. Finally, since the availability of molecular testing for ALPS, it has never been clarified if there are non-classical forms, e.g. without hepatosplenomegaly and adenopathy. The latter has been suggested in cases of Evans syndrome (Price et al, 2014). This study was not independently funded so only certain molecular studies could be performed; other molecular abnormalities, such as \textit{STAT3} mutations, should also be explored.

In order to investigate the \textit{FAS}, \textit{TNFRSF13B} and \textit{WAS} mutation spectrum in ITP, we sequenced coding regions and exon/intron boundaries of the \textit{FAS} and \textit{WAS} genes in 130 adults with persistent or chronic primary ITP. They were all cases of primary ITP except 10, which were chosen specifically as cases of Evans syndrome. ITP was defined as an isolated platelet count $<100 \times 10^9/l$, with exclusion of other clinical causes of thrombocytopenia (screened for human immunodeficiency virus, Hepatitis B, Hepatitis C, autoimmune diseases and bone marrow failure). One hundred patients were recruited from New York Presbyterian Hospital and the three genes were Sanger sequenced (primers available upon request). The remainder of patients were attending Hammersmith hospital. For these patients, \textit{FAS}, \textit{TNFRSF13B} and \textit{WAS} sequencing data was obtained from whole exome sequencing data.
The study was approved by the Weill Cornell Medicine and Hammersmith Hospital Institutional Review Boards as part of larger studies on the pathophysiology of ITP. All cases screened had consented to the study.

Two nonsynonymous heterozygous variants in *FAS* were identified in these patients. One patient was homozygous for the rs56006128 variant (Genome Aggregation Database [gnomAD; https://gnomad.broadinstitute.org] minor allele frequency [MAF] = 0.002). This has been classified as variant of uncertain significance by ClinVar (https://www.ncbi.nlm.nih.gov/clinvar). This patient presented with moderate thrombocytopenia (platelet count 15–80 x 10^9/l) when aged 16 years together with intermittent lymphadenopathy. He has required treatment with steroids on only one occasion (with good response) because of a platelet count of 15 x 10^9/L. The second variant, c.595T>C (p.Cys199Arg), rs753487267 (gnomadAD MAF = 0.000004063), is located in an intracellular part of the FAS receptor, but outside the death domain. FAS mutations in this region were shown to have a dominant negative effect and result in an aberrant signaling (Price et al, 2014). The patient with the p.Cys199Arg mutation had some features of ALPS with slightly increased a/b-double negative T cells (2.9%) and reduced CD27+ B cells (0.5%). She also had increased numbers of activated CD4 and CD8 cells with increased HLA-DR and CD57 expression (Table 1). Of note, she had no history of hepatosplenomegaly or lymphadenopathy. Clinically, she has had a complete response of her cytopenias to rituximab. No potentially pathogenic variants were identified in *TNFRSF13B* or *WAS* in any of the tested 130 patients.

Even though ITP is common in patients with ALPS and CVID, occurring in up to 40% of
patients in the former and 15% in the latter (Feuille et al, 2018; Rao, 2015) this study of 130 ITP patients identified only two potentially functional mutations in the FAS gene and none in TNFRSF13B or WAS. The lack of findings in the latter genes may be partly due to screening of patients with symptoms potentially suggestive of ALPS, WAS or CVID out of the ITP populations at our two Platelet Disorder centres by routine testing (CVID) and clinical features (ALPS and WAS). Both centres often tested for immunoglobulin levels and platelet size.

The focus on FAS is particularly appropriate in view of recently published work showing that an important part of the pathophysiology of ITP may be failure of auto-reactive lymphocytes to undergo apoptosis (Boggio et al, 2017). Furthermore, in view of the association of FAS mutations with ITP as part of Evans syndrome, we deliberately included a total of 10 such patients, none of whom had FAS mutations. Given that ITP is a heterogeneous disease and additional disease-causing gene variants are being discovered, the results provided here suggest that a much larger cohort should be analysed for other immune single nucleotide variants for a more robust analysis of genetic causes of ITP.

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Table 1. Immunological profile of the patient with the p.Cys199Arg mutation

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<thead>
<tr>
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<th>Patient</th>
<th>Normal range</th>
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<tbody>
<tr>
<td>Alpha-beta DNT (%)</td>
<td>2.90</td>
<td>0.1–0.9</td>
</tr>
<tr>
<td>Alpha-beta DNT (9×10⁹/l)</td>
<td>91</td>
<td>2–17</td>
</tr>
<tr>
<td>CD8/CD57 (%)</td>
<td>22</td>
<td>&lt;15.8</td>
</tr>
<tr>
<td>CD8/CD57 (9×10⁹/l)</td>
<td>722</td>
<td>&lt;239</td>
</tr>
<tr>
<td>CD3/HLA-DR (9×10⁹/l)</td>
<td>361</td>
<td>&lt;291</td>
</tr>
<tr>
<td>CD4/HLA-DR (9×10⁹/l)</td>
<td>147</td>
<td>&lt;85</td>
</tr>
<tr>
<td>CD8/HLA-DR (9×10⁹/l)</td>
<td>263</td>
<td>&lt;131</td>
</tr>
<tr>
<td>CD20/CD27 (% of B cells)</td>
<td>0.50</td>
<td>0.7–6.3</td>
</tr>
</tbody>
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DNT, double negative T cells.