Early-onset autoimmune disease due to a heterozygous loss-of-function mutation in \textit{TNFAIP3} (A20)

Rare Mendelian disorders increasingly contribute to our understanding of the genetic architecture of autoimmune disease and the key molecular pathways governing its pathogenesis. Early-onset autoimmune disease can arise through activating mutations in inflammatory signalling pathways or loss-of-function mutations in immunoregulatory proteins.

We investigated the molecular basis of complex autoimmunity—characterised by the onset of insulin-dependent diabetes, cytopenias, hepatitis, enteropathy and interstitial lung disease at age 10—in a 14-year-old boy of healthy non-consanguineous British parents. Immunological analysis revealed lymphopaenia with no naive T cells and a high proportion of activated T cells (table 1). Pathogenic variants in \textit{STAT3} and \textit{FOXP3} were excluded. The clinical course was refractory to intensive immunosuppression with prednisolone, sirolimus, tacrolimus, infliximab or rituximab, necessitating haematopoietic stem cell transplantation. Twenty-one months post-transplant, he is thriving off all immunosuppressive medication with complete remission of autoimmune disease (except diabetes).

Ethical approval was granted (ref: 10/H0906/22) and written informed consent provided prior to study commencement. By whole exome sequencing of peripheral blood genomic DNA (Illumina MiSeq) and downstream bioinformatic filtering (Ingenium Variant Analysis), we identified a single biologically possible \textit{nuity Variant Analysis), we identified a single biologically

We extended our analysis to inflammasome activation. Owing to the lack of leucocyte material, we were not able to distinguish from reported pathogenic A20 mutations, although

Here we provide novel validation of considerable existing evidence that implicates \textit{TNFAIP3} in autoimmune pathogenesis. This case expands the clinical spectrum of A20 haplosufficiency. As A20 regulates multiple innate and adaptive signalling pathways, it is logical that patients with inactivating mutations in A20 might manifest pathological features of autoimmunity

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pretransplant</th>
<th>Post-transplant</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>9.8</td>
<td>12.4</td>
<td>13.5–17.5</td>
</tr>
<tr>
<td>Leucocytes (10(^3)/L)</td>
<td>1.88</td>
<td>3.47</td>
<td>150–450</td>
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<tr>
<td>Lymphocytes (10(^3)/L)</td>
<td>0.17</td>
<td>1.31</td>
<td>1.2–5.2</td>
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<tr>
<td>Neutrophils (10(^3)/L)</td>
<td>1.52*</td>
<td>1.79</td>
<td>1.8–8.0</td>
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<tr>
<td>Monocytes (10(^3)/L)</td>
<td>0.19</td>
<td>0.37</td>
<td>0.2–0.8</td>
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<tr>
<td>Platelets (10(^3)/L)</td>
<td>29</td>
<td>183</td>
<td>150–400</td>
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<tr>
<td>CD3+ (cells/µL)</td>
<td>800</td>
<td>1914</td>
<td>800–3500</td>
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<td>CD8+ (cells/µL)</td>
<td>554</td>
<td>936</td>
<td>200–1200</td>
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<tr>
<td>CD4+ (cells/µL)</td>
<td>238</td>
<td>920</td>
<td>400–1200</td>
</tr>
<tr>
<td>CD56+ (cells/µL)</td>
<td>35</td>
<td>99</td>
<td>70–1200</td>
</tr>
<tr>
<td>CD19+ (cells/µL)</td>
<td>138</td>
<td>99</td>
<td>200–600</td>
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<tr>
<td>Activated T cells (HLA-DR+ %)</td>
<td>55</td>
<td>25</td>
<td>N/A</td>
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<tr>
<td>CD4+ naive (%)</td>
<td>Not detected</td>
<td>244</td>
<td>N/A</td>
</tr>
<tr>
<td>CD27– IgD+ (naive) (%)</td>
<td>87</td>
<td>93</td>
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<tr>
<td>CD27– IgD+ (memory) (%)</td>
<td>9</td>
<td>4</td>
<td>4.6–10.2</td>
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<tr>
<td>CD27+ IgD– (class-switched) (%)</td>
<td>2</td>
<td>3</td>
<td>3.3–9.6</td>
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<tr>
<td>IgM (g/L)</td>
<td>0.55</td>
<td>0.25</td>
<td>0.50–1.90</td>
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<tr>
<td>IgG (g/L)</td>
<td>6.4</td>
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<td>IgA (g/L)</td>
<td>0.92</td>
<td>0.33</td>
<td>0.80–2.80</td>
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<td>Haemophilus influenzae b (mg/mL)</td>
<td>0.93</td>
<td>ND</td>
<td>0.1–10</td>
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<tr>
<td>Pneumococcal (mg/mL)</td>
<td>10</td>
<td>ND</td>
<td>20–200</td>
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<tr>
<td>Anti-GAD antibody (IU/mL)</td>
<td>&gt;2000</td>
<td>&gt;2000</td>
<td>0–9.9</td>
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<td>Islet cell antibody</td>
<td>Detected</td>
<td>Detected</td>
<td>N/A</td>
</tr>
<tr>
<td>pANCA</td>
<td>Detected</td>
<td>Detected</td>
<td>N/A</td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
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<tr>
<td>FEV (%, predicted)</td>
<td>38</td>
<td>84</td>
<td>95–100</td>
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</table>

*Peripheral neutrophils were supported pretransplant by recombinant granulocyte colony stimulating factor. Post-transplant parameters were obtained at 18 months (FBC and T-cell indices, lung function) or 21 months post-H SCT (B cell and antibody indices). Post-H SCT antibody indices were measured during concomitant subcutaneous immunoglobulin supplementation. No other autoantibodies were detected pre-H SCT or post-H SCT.

FBC, full blood count; FEV, forced expiratory volume in 1 s; GAD, glutamic acid decarboxylase; HLA-DR, human leucocyte antigen–antigen D related; HSC T, haematopoietic stem cell transplantation; ND, not done; pANCA, perinuclear anti neutrophil cytoplasmic antibody.
Figure 1  

**TNFAIP3** variant identification and functional validation. (A) The family pedigree is shown (triangles are used to preserve the anonymity of healthy unaffected siblings). The first-born infant died as a result of prematurity. Whole exome sequencing data were filtered (Ingenuity Variant Analysis) by confidence (call quality ≥20; read depth ≥10; allele fraction ≥45%); frequency (ExAc allele frequency ≤0.01%); deleteriousness (nonsense/deleterious missense (SIFT/PolyPhen), splice-site disruption); genetic segregation (ie, present in patient and absent from 47 unrelated disease controls) and biological function (linked to phenotype), identifying a single heterozygous frameshift variant in **TNFAIP3** (c.1466_1467TGdel). (B) Variant confirmation by Sanger sequencing. (C) The c.1466_1467TGdel variant resulted in a frameshift and premature stop codon (V489Afs*7) in the second ZnF domain and is distinct from previously described mutations in the OTU and ZnF4 domains (blue). (D) V489Afs*7 reduced basal and TNF-induced A20 protein in patient (P) versus control (C1, C2) fibroblasts (immunoblot representative of n=4 independent experiments with n=4 controls). (E) Signalling responses downstream of TNF-α stimulation in patient fibroblasts were exaggerated and prolonged compared with control (immunoblot representative of n=4 independent experiments with n=4 controls). (F) RNA-seq analysis of transcriptional response to 6-hour TNF-α stimulation in patient and control fibroblasts (stimulations performed in triplicate in a single experiment). Top panel: displayed in red are significant (FDR-corrected p≤0.01) DE transcripts regulated ≥4-fold (≥2log₂-fold); middle panel: Venn diagram displaying all overlapping DE transcripts ≥2-fold (≥log₂-fold); Bottom panel: top 20 significant DE transcripts in patient (red bars) versus control (black bars), demonstrating many major NF-κB target genes. (G) Levels of IL-6 quantified by ELISA in supernatants from patient and control fibroblasts stimulated with TNF-α for 24 hours (mean±SD of average values from two independent experiments in patient and n=4 controls compared by one-sample t-test; **p=0.0015). DE, differentially expressed; FDR, false discovery rate; IL-6, interleukin 6; NF-κB, nuclear factor-κB; OTU, ovarian tumour; PolyPhen, polymorphism phenotyping; SIFT, Sorting Intolerant from Tolerant; TNF-α, tumour necrosis factor-alpha; TNFAIP3, tumour necrosis factor-alpha-induced protein 3; ZnF, zinc finger.

and/or autoinflammation. Finally, we report that correction of the molecular defect within the haematopoietic cell compartment could represent a viable treatment option for severe clinical manifestations.

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Contributors CJAD, KRE and SH: designed research. MFT, JT, VZ, MAS, AJC and SH: clinical investigation and phenotyping. CJAD, ED, RT, AG, JDPW and DJS: performed experiments. CJAD, ED, RT, AJS, KRE and SH: analysed and interpreted data. CJAD and AJS: performed statistical analysis; CJAD and SH: wrote the manuscript. All authors: reviewed the manuscript for intellectual content.

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Data sharing statement RNA-Seq data have been submitted to GEO (ref: GSE95078). Details of Sanger sequencing primers and the Ingenuity Variant Analysis bioinformatic filtering strategy are available on request.

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