Early-onset autoimmune disease due to a heterozygous loss-of-function mutation in 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 STAT3 and 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 (Ingenuity Variant Analysis), we identified a single biologically plausible variant—a novel de novo heterozygous 2bp deletion in tumour necrosis factor-alpha-induced protein 3 (TNFAIP3, figure 1A). TNFAIP3 encodes the ubiquitin-editing enzyme A20, a negative regulator of the nuclear factor-κB (NF-κB) pathway. A20 removes K63-linked ubiquitin chains from key adaptor proteins, replacing them with K48-linked polyubiquitin chains, to trigger proteasomal degradation and termination of the NF-κB activation cascade. Polymorphisms in TNFAIP3 have been linked to the development of several autoimmune diseases in genome-wide association studies. A conditional knockout of A20 in immune cells leads to the development of autoimmunity in the mouse. However, autoimmune phenomena were not prominent in a recently described cohort of patients with
germline A20 haploinsufficiency, who instead presented with an autoinflammatory phenotype resembling Behçet’s disease. 9

In A20 fibroblasts. Initially, we stimulated these cells with TNF-α (10 ng/α) performed functional experiments in patient and control dermal (figure 1D). To address the consequence of this reduced A20 expression, we

| Parameters                  | Pretransplant | Post-transplant | Reference range |
|-----------------------------|---------------|-----------------|-----------------|
| Laboratory                  |               |                 |                 |
| Haemoglobin (g/dL)          | 9.8           | 12.4            | 13.5–17.5       |
| Leucocytes (10⁹/L)          | 1.88          | 3.47            | 150–450         |
| Lymphocytes (10⁹/L)         | 0.17          | 1.31            | 1.2–5.2         |
| Neutrophils (10⁹/L)         | 1.52*         | 1.79            | 1.8–8.0         |
| Monocytes (10⁹/L)           | 0.19          | 0.37            | 0.2–0.8         |
| Platelets (10⁹/L)           | 29            | 183             | 150–400         |
| CD3+ (cells/µL)             | 800           | 1914            | 800–3500        |
| CD8+ (cells/µL)             | 554           | 936             | 200–1200        |
| CD4+ (cells/µL)             | 238           | 920             | 400–1200        |
| CD56+ (cells/µL)            | 35            | 99              | 70–1200         |
| CD19+ (cells/µL)            | 138           | 99              | 200–600         |
| Activated T cells (HLA-DR+) | 55            | 25              | N/A             |
| CD4+ naive (%)              | Not detected  | 244             | N/A             |
| CD27+ IgD+ (naive) (%)      | 87            | 93              | 75.2–86.7       |
| CD27+ IgD+ (memory) (%)     | 9             | 4               | 4.6–10.2        |
| CD27+ IgD– class-switched (%) | 2           | 3               | 3.3–9.6         |
| IgM (g/L)                   | 0.55          | 0.25            | 0.50–1.90       |
| IgG (g/L)                   | 6.4           | 8.2             | 5.4–16.1        |
| IgA (g/L)                   | 0.92          | 0.33            | 0.80–2.80       |
| Tetanus (IU/mL)             | 0.93          | ND              | 0.1–1.0         |
| Haemophilus influenzae b (mg/ ml) | 1.8 | ND  | 1.0–20.0   |
| Pneumococcal (mg/mL)        | 10            | ND              | 20–200          |
| Anti-GAD antibody (IU/mL)   | >2000         | >2000           | 0–9.9           |
| Islet cell antibody         | Detected      | Detected        | N/A             |
| pANCA                       | Detected      | Detected        | N/A             |
| Clinical                    |               |                 |                 |
| FEV, (% predicted)          | 38            | 84              | 95–100          |

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

This case expands the clinical spectrum of A20 haploinsufficiency. 9 As A20 regulates multiple innate and adaptive signalling pathways, 1 it is logical that patients with inactivating mutations in A20 might manifest pathological features of autoimmunity 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.

Christopher J A Duncan,1 Emma Dinngan,1 Rachel Theobald,1 Angela Grainger,1 Andrew J Skelton,1 Radiful Hussain,2 Joseph D P Willet,1 David J Swan,3 Jonathan Coxhead,1 Matthew F Thomas,1 Julian Thomas,5 Veena Zamvar,6 Mary A Slatter,1 Andrew J Cant,1 Karin R Engelhardt,1 Sophie Hambleton1,7

1Primary Immunodeficiency Group, Institute for Cellular Medicine, Newcastle University, UK
2Bioinformatics Support Unit, Institute for Cellular Medicine, Newcastle University, UK
3Department of Paediatric Respiratory Medicine, Great North Children’s Hospital, Royal Victoria Infirmary, UK
4Department of Paediatric Gastroenterology, Great North Children’s Hospital, Royal Victoria Infirmary, UK
5Department of Paediatric Gastroenterology, Leeds General Infirmary, UK
6Department of Paediatric Immunology and Stem Cell Transplant Unit, Great North Children’s Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust, UK
7Correspondence to Prof Sophie Hambleton, Primary Immunodeficiency Group, Institute for Cellular Medicine, Newcastle University Medical School, Newcastle upon Tyne NE2 4HH, UK; sophie.hambleton@ncl.ac.uk

Acknowledgements The authors thank the patient and their family for their trust and assistance. They are grateful to colleagues in St. James’ Hospital, Leeds and Great North Children’s Hospital, Newcastle for providing clinical care. They thank R. Harry, N. Maney, J. Isaacs and A. Pratt for the kind gift of reagents.

Contributors CJAD, KRE and SH: designed research. MFT, JT, VZ, MAS, AJC and SH: clinical investigation and phenotyping; CJAD, ED, RT, AG, JPW 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.

Funding CJAD was supported by the British Medical Council, the British Infection Association and the UK National Institute for Health Research (NIHR). JPW, DJS, KRE and SH were supported by the Sir Jules Thorn Trust (12/JTA); AG was supported by the Bubble Foundation; AJS was supported by the MRC–Arthritis Research UK Centre for Integrated research into Musculoskeletal Ageing (CIMA) and NIHR Newcastle Biomedical Research Centre.

Competing interests None declared.

Patient consent Obtained.

Ethics approval NHS North East-Newcastle and North Tyneside 1 REC.

Provenance and peer review Not commissioned; externally peer reviewed.

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.
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.
REFERENCES
1. Catrysse L, Vereecke L, Beyaert R, et al. A20 in inflammation and autoimmunity. Trends Immunol 2014;35:22–31.
2. Wertz IE, Newton K, Seshasayee D, et al. Phosphorylation and linear ubiquitin direct A20 inhibition of inflammation. Nature 2015;528:370–5.
3. Graham RR, Cotsapas C, Davies L, et al. Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. Nat Genet 2008;40:1059–61.
4. Musone SL, Taylor KE, Lu TT, Tt L, et al. Multiple polymorphisms in the TNFAIP3 region are independently associated with systemic lupus erythematosus. Nat Genet 2008;40:1062–4.
5. Fung EY, Smyth DJ, Howson JM, et al. Analysis of 17 autoimmune disease-associated variants in type 1 diabetes identifies 6q23/TNFAIP3 as a susceptibility locus. Genes Immun 2009;10:188–91.
6. Lodolce JP, Kolodziej LE, Rhee L, et al. African-derived genetic polymorphisms in TNFAIP3 mediate risk for autoimmunity. J Immunol 2010;184:7001–9.
7. Musone SL, Taylor KE, Nithiam J, et al. Sequencing of TNFAIP3 and association of variants with multiple autoimmune diseases. Genes Immun 2011;12:176–82.
8. Tavares RM, Turer EE, Liu CL, et al. The ubiquitin modifying enzyme A20 restricts B cell survival and prevents autoimmunity. Immunity 2010;33:181–91.
9. Zhou Q, Wang H, Schwartz DM, et al. Loss-of-function mutations in TNFAIP3 leading to A20 haploinsufficiency cause an early-onset autoinflammatory disease. Nat Genet 2016;48:67–73.
10. Duncan CJ, Mohamad SM, Young DF, et al. Human IFNAR2 deficiency: Lessons for antiviral immunity. Sci Transl Med 2015;7:307ra154.