Supplementary Information

Activating germline mutations in *STAT3* cause early-onset multi-organ autoimmune disease

Sarah E. Flanagan,[1,16] Emma Haapaniemi,[2,3,16] Mark A. Russell,[1,16] Richard Caswell,[1] Hana Lango Allen,[1] Elisa De Franco,[1] Timothy J. McDonald,[1] Hanna Rajala,[4,5] Anita Ramelius,[6,7] John Barton,[8] Kaarina Heiskanen,[3,9] Tarja Heiskanen-Kosma,[10] Merja Kajosaari,[9] Nuala P. Murphy,[11] Tatjana Milenkovic,[12] Mikko Seppänen,[13] Åke Lernmark,[6,7] Satu Mustjoki,[4,5] Timo Otonkoski,[3,9] Juha Kere,[2,3,14,15] Noel G. Morgan,[1] Sian Ellard[1] and Andrew T. Hattersley[1]

[1] Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, EX2 5DW, UK
[2] Folkhälsan Institute of Genetics, University of Helsinki, Helsinki, Finland
[3] Research Programs Unit, Molecular Neurology, University of Helsinki, Helsinki, Finland
[4] Hematology Research Unit Helsinki, Department of Hematology, University of Helsinki
[5] Helsinki University Central Hospital Cancer Center, Helsinki, Finland
[6] Department of Clinical Sciences, Lund University, Lund, Sweden
[7] CRC, Skåne University Hospital SUS, Malmö, Sweden
[8] Bristol Royal Hospital for Children, Upper Maudlin Street, Bristol, BS2 8BJ, UK
[9] Children's Hospital, Helsinki University Central Hospital, Helsinki, Finland
[10] Department of Pediatrics, Kuopio University Hospital, Helsinki, Finland
[11] Department of Diabetes and Endocrinology, Children's University Hospital, Temple St., Dublin 1, Ireland
[12] Department of Endocrinology, Institute for Mother and Child Health Care of Serbia 'Dr Vukan Cupic', Belgrade, Serbia
[13] Immunodeficiency Unit, Division of Infectious Diseases, Helsinki University Central Hospital, Helsinki, Finland
[14] Department of Biosciences and Nutrition, Karolinska Institutet, Hälsovägen 7, 14183 Huddinge, Sweden
[15] Center for Innovative Medicine, Karolinska Institutet, Hälsovägen 7, 14183 Huddinge, Sweden
[16] These authors contributed equally to this work

Table of Contents

Supplementary Table 1. Breakdown of variants identified by exome sequencing of individual 1

Individual 1	**substitutions**	**indels**
Total passing quality filters	18220	570
After dbSNP131 filtering	466	489
After 1000Genomes filtering	95	16
After excluding those in parents (*de novo*)	18	1
After excluding those outside of the coding regions and conserved splice sites (+/- 2bp)	15	0
After excluding non-synonymous and intronic variants	7	0
After manual inspection and exclusion of putative *de novo* variants present in a parent	1	0

Supplementary Table 2: Features of 25 individuals with multiple early onset-autoimmune disease sequenced for *STAT3* mutations

Patient	Early-onset diabetes	Autoimmune Enteropathy	Autoimmune pulmonary disease	Autoimmune thyroid dysfunction	Autoimmune joint disease	Dental anomalies	*STAT3* mutation identified
1	✓	✓		✓			p.T716M
2	✓	✓	✓			✓	p.K392R
3	✓				✓		p.N646K
4		✓				✓	p.K658N
5		✓	✓				No
6		✓	✓				No
7		✓		✓			No
8	✓			✓			No
9	✓			✓			No
10	✓	✓					No
11		✓		✓			No
12	✓	✓					No
13	✓	✓		✓			No
14	✓	✓		✓			No
15		✓		✓			No
16	✓	✓					No
17	✓	✓					No
18	✓			✓			No
19	✓		✓				No
20	✓	✓					No
21	✓	✓					No
22	✓	✓		✓			No
23	✓	✓					No
24	✓			✓			No
25	✓	✓		✓			No

Supplementary Table 3: Clinical characteristics of individuals with a *de novo STAT3* mutation

Table of clinical features	**PATIENT 1**	**PATIENT 2***	**PATIENT 3**	**PATIENT 4**	**PATIENT 5**
Mutation	p.Thr716Met (c.2147C>T)	p.Lys392Arg (c.1175A>G)	p.Asn646Lys (c.1938C>G)	p.Asn646Lys (c.1938C>G)	p.Lys658Asn (c.1974G>C)
Sex	Female	Female	Male	Male	Female
Current Age (yrs)	6	15	6	3	17
Birth weight (SDS)	-1.59	-5.81	-2.70	-1.59	-1
Growth (Height SDS)	-2.33	-6.63 Delayed puberty	-1.47	-2.05	-4.00 Delayed puberty
DIABETES insulin doses HbA1c					
Age at Diagnosis (Wks)	2	0	3	43	Not diabetic
GAD65 autoantibodies	35 RU (<34)	40.3 RU (<5.36)	5 (<34)	31.9 U/mL (<1.0)	Negative
I-A2 autoantibodies	0 (<5)	0.08 (<0.43)	0(<5)	9.5 U/mL (<5)	Negative
ZnTn8 autoantibodies	7 (<60)	Not tested	11(<60)	Not tested	Not tested
IAA autoantibodies	Not tested insulin treated	3224 RU(<1.56)	Not tested insulin treated	Not tested insulin treated	Negative
ICA	Negative	28 JDFU(<2.5)	Negative	Not tested	Negative
Pancreatic Exocrine Insufficiency	Fecal elastase 166mg/ g faeces (>200)	On enzyme replacement therapy- Low Fecal elastase	NO	NO	NO
T1D HLA Type†	High Risk DRB1*03 -DQB1*02/ DRB1*03 -DQB1*02	High Risk DRB1*04 - DQB1*0302	Low Risk DQA1*01-DQB1*05/ DQA1*01-DQB1*0602	Low Risk DQA1*03-B1*03:02/A1*01-B1*06:02	Low risk HLA-DQB *0302 negative
Current Insulin dose (U/Kg/day)	1.8	1.5	0.76	0.8	N/A
Current HbA1c (mmols/mol)	61	70	68	69	Not tested
ENTEROPATHY					
Type	Coeliac disease	Coeliac-disease	NO	NO	Autoimmune enteropathy
Onset of symptoms (months)	17 months	<12 months	N/A	N/A	<12 months
Coeliac HLA type	High Risk DR52-DR3-DQ2	High risk DQ2 & DQ8	Not tested	Not tested	Low risk DQ2 and DQ8 neg
Anti TtG autoantibodies	TtG IgA 20.47 U/mL (<10) TtG IgG > 200 U/mL (<10)	Not tested	<1.0 (<10)	2.8 (<7)	Not tested
Endomysial/gliadin autoantibodies	Not tested	Positive Endomysial & gliadin autoantibodies	Not tested	Not tested	Positive for gliadin autoantibodies
Responsive to gluten free diet	YES	YES	N/A	N/A	NO
OTHER AUTOIMMUNE FEATURES					
Eczema	NO	Mild atopic	YES	YES	Non-specific dematitis
Pulmonary manifestations	None	Desquamative interstitial pneumonitis	None	None	Asthma Recurrent URTI
Other features	Primary hypothyroidism (TPO antibodies 224 U/mL (<20). Lipoatrophy at sites of insulin injection	None	None	Juvenile arthritis, severe dry eyes	Progressive macular edema & vision loss. Kawasaki disease-like episodes
HEMATOLOGICAL FEATURES					
	NO	T-cell LGL leukemia.	Hb 10.0g/dl nd	NO	Lymphadenopathy

		Autoimmune cytopenias.	MCV 64.7fl (73-89) MCH 20.4pg (24-30)		& splenomegaly. Autoimmune cytopenias
DENTAL ANOMALIES					
	NO	Dentures	NO	NO	Delayed eruption of primary teeth
IMMUNODEFICIENCY					
Ig E	<2.0 (<100kU/L)	-	<2.0 (<100kU/L)	<2.0 (<100kU/L)	0.7 (3.9-10)
Eosinophils	1.3% (<6%)	-	0.30 x10^9 /L (<0.7x10^9/L)	0.28 x10^9 /L (0.08-1.1x10^9/L)	-
Infection susceptibility	NO	Recurrent bacterial LRTI, IVIG from 12 y/o	NO	NO	Recurrent bacterial URTI, IVIG from 12 y/o
Regulatory T cells (% of CD4+ T-cells)	NO	3.8 (5.1)[¤]	NO	NO	4.5 (5.1)[¤]
CD4+ T-cell IFNγ/TNFα production, unstimulated/stimulated (%)	NO	0.1/21.5 (0/8.0)[¤]	NO	NO	0.2/7.3 (0/8.0)[¤]

Table: TPO = Thyroid Peroxidase, TtG = Anti-Tissue trans glutaminase antibody, URTI=Upper Respiratory Tract Infections, LRTI=Lower Respiratory Tract Infections, IVIG = Intravenous Immunoglobulin Replacement Therapy, IFN-γ = Interferon-gamma, TNF-α =Tumor Necrosis Factor alpha. N/A = not applicable. Normal ranges are provided in parenthesis when appropriate. * Patient previously reported in[1]. †HLA typing was undertaken on patients 1-4 using previously described methods[2]. ¤Healthy control mean.

Supplementary Table 4: Clinical features of polyautoimmune disorders. Adapted from Gambineri and Torgerson (2012)[3] using additional published literature. Approximate % of patients affected with a condition are shown in brackets. Abbreviations APS1 (autoimmune polyendocrinopathy syndrome type I) IPEX (X-linked immunodysregulation, polyendocrinopathy, and enteropathy)

Disorder	**STAT3 polyautoimmunity**	**APS1**	**IPEX**	**CD25 deficiency**	**ITCH deficiency**
Gene	*STAT3*	*AIRE*	*FOXP3*	*IL2RA*	*ITCH*
Inheritance	Dominant	Recessive	Recessive	Recessive	Recessive
Number of patients reported	5 patients (5 families)	>150	>70	4 patients (4 families)	10 patients (1 family)
References	This paper	Peterson, & Peltonen (2005)[4]	d'Hennezel, *et al* (2012)[5] Wildin *et al* (2002)[6]	Bezrodnik, *et al* (2013)[7]	Lohr *et al* (2010)[8]
Common clinical features (present in >50% of patients	Type 1 diabetes (80%) Enteropathy (60%) Short stature (100%)	Hypoparathyroidism (80%) Adrenal insufficiency (88%) Mucocutaneous candidiasis (86%)	Enteropathy (100%) Eczematous Dermatitis (70%) Type 1 diabetes (67%)	Enteropathy (100%) Recurrent persistent viral infection(100%) Eczema (75%)	Developmental delay (100%) Macrocephaly (90%) Dysmorphic facies (90%) Chronic lung disease (90%) Hepatosplenomegaly (90%)
Additional clinical features	Autoimmune thyroid (40%) Juvenile Chromic Rheumatoid Arthritis (20%) Fibrotic lung disease (20%)	Gonadal insufficiency (45%) Type 1 Diabetes (13%) Pernicious anaemia (13%) Alopecia areata (45%) Vitiligo (15%) Autoimmune hepatitis (15%) Keratitis (28%)	Autoimmune thyroid(35%) Autoimmune haemolytic anaemia (32%), Thrombocytopenia (15%), Lympthadenopathy (8%)	Hypothyroidism (50%) Type 1 diabetes (25%) Alopecia Universalis (50%) Lympthadenoptathy (50%)	Hypothyroidism (40%) Autoimmune Hepatitis (30%) Enteropathy (20%) Type 1 diabetes (10%)
Other laboratory or diagnostic features	Normal eosinophil count Low-normal IgE	Autoantibodies to type I interferons (IFN-α or ω)	Eosinophilia Very elevated IgE FOXP3 expression in CD4+ T Cells low	IgE typically normal CD25 expression on T cells absent or low (flow cytometry)	

Supplementary Table 5: *STAT3* primers for Sanger sequencing

Exon	Forward Primer Sequences (M13 tailed) All primers start 5' TGTAAAACGACGGCCAGT	Reverse Primer Sequences (M13 tailed) All primers start 5' CAGGAAACAGCTATGACC
2	CCCCAGAGCATCTTTATCCC	CTCATTTTCCCCATCACCTG
3	GGGTTATAGCATCAGGTTTGC	AAGTATACAGAGCTTTGAGAAAGGG
4	TAGTAACGACCTCCCCTTCG	TCTGTTGGATTCTTTTGGTGG
5	TTCCCTTCCTCTTGTGATGG	CAAGAGAAGGCTCCCTGTTG
6	AACAGGGAGCCTTCTCTTGG	ATGACCAGGCTCCTTTGAGG
7	GGAGGTACGGGTCCTCAAAG	CAACTCCAGAGCAGGAACTTCT
8	ATTTCAGCGTCTTGTGGCAG	GCTAAATTTGAATATGGAAAAGTCC
9	TTTTCAGCATCCACCCAAC	GGAAAGAGAAGATGGGCTCAC
10	GGTAATTTAGCATCCTTGTCCC	ATGGCAACAAATTTCAACCC
11	GACAGCTTGGCCTATTTACCTG	TGTCCACAAAATGAAGATCTCTG
12	TGCGCTGATCAACTGTAACTG	ATTCCCACATCTCTGCTCCC
13	ATTCCCACATCTCTGCTCCC	TGCGCTGATCAACTGTAACTG
14	ATGGAAGAATCCAACAACGG	GTTCATGTCACTTTGGCCTG
15	TGCTGCTTAGACTGGTCTCG	CCCCTGTACGTAGCCTCTCA
16	CACTCCTCGCCTAGAGTTGG	GTCCTCGCTTGGTGGTG
17	AACATGCTGACCAACAATCC	GCCTTGCTCAGGAAAGAAAC
18F	AAATCCTCAGGCCCGTCTAC	CCTTCAAAGATGTGAAAGCTG
18R	CCTTCAAAGATGTGAAAGCTG	AAATCCTCAGGCCCGTCTAC
19	CTGAACTCTTGGTCCAGCG	AAAGCCCATGATGTACCTGG
20	GCTGGCAAGGGCTTCTC	AAGCAAACCAATCCTTCAGC
21	CACTACAATTCTTTCCCATAAGGAG	AACAGGGTGTTCAGGGTCTC
22	TAAATGAGGGCAGACAACCC	TCAAACTCTGGTCTCCAACAG
23	AGCCCCTGGGCTATGTTTAG	TCTCTTTTGGAAAGCAAAGCTC
24	TCCAGGGAGGAGGGTAAATC	AGCAGATCACCCACATTCAC

Supplementary Table 6: Primer sequences for site-directed mutagenesis

Mutation	Forward Primer	Reverse Primer
Polyautoimmune mutations:		
p.K392R	ATTCTGGGCACAAACACAAGAGTGATGAACATGGAAG	TCTTCCATGTTCATCACTCTTGTGTTTGTGCCCAGAATG
p.N646K	ACACAAAGCAGCAGCTGAAGAACATGTCATTTGCTG	AGCAAATGACATGTTCTTCAGCTGCTGCTTTGTG
p.K658N	TCATCATGGGCTATAACATCATGGATGCTACC	TGGTAGCATCCATGATGTTATAGCCCATGATG
p.T716M	ATCTGTGTGACACCAATGACCTGCAGCAATACC	TGGTATTGCTGCAGGTCATTGGTGTCACACAG
Hyper IgE mutations:		
p.R382W	AGCTCTCAGAGGATCCTGGAAATTTAACATTCTGGGC	TGCCCAGAATGTTAAATTTCCAGGATCCTCTGAGAGC
p.V637M	AGACCCAGATCCAGTCCATGGAACCATACACAAAG	TGCTTTGTGTATGGTTCCATGGACTGGATCTGGGTC

References

1 Otonkoski, T. *et al. Diabetologia* **43**, 1235-1238,(2000).
2 Sjoroos, M. et al *BioTechniques* **18**, 870-877 (1995).
3 Gambineri, E. & Torgerson, T. R. *et al CMLS* **69**, 49-58, (2012).
4 Peterson, P. & Peltonen, L.. *Journal of autoimmunity* **25 Suppl**, 49-55,(2005).
5 d'Hennezel, E. *et al Journal of medical genetics* **49**, 291-302, (2012).
6 Wildin, R. S. *et al Journal of medical genetics* **39**, 537-545 (2002).
7 Bezrodnik, L. *et al Clinical and experimental immunology* (2013).
8 Lohr, N. J. *et al. American journal of human genetics* **86**, 447-453 (2010).
9 Koskela, H. L. *et al. N Engl J Med* **366**, 1905-1913 (2012)