ONLINE REPOSITORY TEXT

METHODS

Subjects and blood samples

Blood samples were obtained from subjects after written informed consent according a research protocol approved by the University of Utah Institutional Review Board. A retrospective review of records was completed.

DNA isolation and gene sequencing

DNA was extracted from whole blood using Gentra Puregene chemistry (Qiagen, Valencia, CA). Genomic DNA from unaffected family members was PCR amplified at the RAG1 exon 2 locus using High Fidelity Phusion DNA Polymerase (New England BioLabs, Ipswich, MA) and the following primers: forward 5'-TGACTTGTTTTCATT GTTCTCAGG-3', reverse 5'-TTGAGTTTCCCTCTGGGTTG-3'. Sanger sequencing was performed using standard methods (Applied Biosystems Capillary Sequencer) with the amplicons and the following primers: 5'- CAACATCTTC TGTCGCTGAC-3' and 5'-GAATCCCTCT GCCAGTACAG -3'.

Autoantibody microarray

Human serum samples obtained from family members with RAG1 deficiency were probed against an autoantibody protein microarray. Age-matched healthy controls and a lupus positive control were also probed. The microarray is a HydroGel slide printed with a selection of 76 glomerular, nuclear and other autoantigens as well as six control proteins in duplicates. The array is probed with serum samples, developed with Cy3 fluorescently labeled anti-IgG and Cy5 labeled anti-IgM, then scanned at 635nm and 570 nm fluorescence, respectively. The mean fluorescence intensity is converted into an optimized dataset

based on the normal control. Data was analyzed for clustering and visualized by MultiExperiment Viewer (MeV), Cluster 3.0 and MapleTree v.0.2.3.2.

Anticytokine autoantibody assays

Patient and control plasmas at 1:100 dilution were screened for autoantibodies using a bioplex assay against 9 cytokines. Normal PBMCs in the presence of patient or normal plasma (10% concentration) were stimulated for 15 minutes with either IFN-α or IFN-γ. Cells were fixed and permeabilized and evaluated by flow cytometry for presence of either IFN-α or IFN-γ induced phosphoSTAT-1.

Population genetic disease estimate

RAG1 and *RAG2* mutations known to be associated with congenital immune deficiency disorders were procured from the Human Gene Mutation Database (HGMD) v.2012.3. Variants included missense mutations, nonsense mutations, insertions and deletions. *RAG1* and *RAG2* variants in the 1000 Genomes database were identified. The 1000 Genomes database is a comprehensive database of genomes obtained from multiple populations around the world, and it identifies 98% of alleles whose frequency is >1% in these populations (E10). Lower frequency alleles are also identified in these populations. Variants present in both databases were intersected, resulting in only 2 disease-causing variants present in both HGMD and the European populations of the 1000 Genomes database. Allele frequencies of the European populations were then estimated from the 1000 Genomes database. Calculations were made focusing on the European 1000 Genomes populations for two reasons: 1) The case report involves a family of European descent, and 2) there is a strong bias for European populations in existing mutation data sets such as HGMD. The references associated with these variants were manually reviewed to ensure they were correctly associated with a primary immunodeficiency disease.

REFERENCES

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FIGURE LEGENDS

Figure E1. Heatmaps. Yellow indicates fold increase in signal compared to healthy controls (mean+1SD).

Figure E2. Anticytokine autoantibody evaluation. A. Patient and control plasmas were screened for autoantibodies against 9 cytokines. Patient data represents one plasma sample from patient P1, and two plasma samples from P2. B. Normal peripheral blood mononuclear cells in the presence of patient (pre-transplant) or normal plasma were stimulated for 15 minutes with either IFNα or IFNγ. Cells were fixed and permeabilized and evaluated by flow cytometry for presence of either IFNα- or IFNγ-induced phosphoSTAT-1.

REPOSITORY TABLES

Table E1. Clinical laboratory evaluation of patients with RAG1 deficiency.

	Patient 1 (II.1) 3.5 years	Reference range (age-adjusted)	**Patient 2 (II.4) 2 years**	Reference range (age-adjusted)
Lymphocytes cells/μl (% of total lymphocytes)	CD3+ 691 (47%) CD4+ 458 (31%) CD4+CD45RA+ 46 (3%) CD4+CD45RO+ 427 (30%) CD8+ 187 (13%) NK cells 278 (19%) CD19+ B cells 464 **(32%H)**	684-2170 (58-87%) 381-1469 (32-62%) 44-869 (3-28%) 92-1040 (16-46%) 196-1060 (4-27%) 76-570 (4-27%) 116-613 (5-23%)	CD3+ **972** (62%) L CD4+ **380 (24%)** L CD4+CD45RA+ **47 (3%)** L CD4+CD45RO+ 395 (25%) CD8+ **232 (15%)** L NK cells 213 (14%) CD19+ B cells **360** (23%) L	1460-5440 (53-81%) 1020-3600 (31-54%) 200-3400 (15-70%) 50-1500 (5-30%) 570-2230 (16-38%) 80-340 (3-19%) 430-3300 (11-45%)
Lymphocyte mitogen proliferation	Low proliferation to PHA. Normal proliferation to Con A and PWM.		Normal proliferation to PHA, Con A, and PWM	
Immunoglobulin levels (mg/dL)	IgG 1135 IgM 90 IgA 98 IgE 2		IgG 973 IgA 44 IgM 70 IgE 1 (obtained at 11months)	
Additional laboratory data	T cell spectratyping: Mild skewing for clonal T cell population			

Abnormal results in **bold**. Con A=concanvalin A; H=high; L=low; PHA=phytohemagglutinin; PWM=pokeweed mitogen.

Table E2. *RAG1* mutation genotypes in the affected family.

Correlating protein change and references of previously reported cases with the same variant are listed.

Full references are available in the Online Repository Text.

***RAG1* mutation**	**Exon**	**Protein**	**References**
c.1420C>T	2	p.Arg474Cys	*Villa et al., 2001*(E1) *Sobacchi et al., 2006*(E2) *Gruber et al., 2009*(E3) *Kuijpers et al., 2011*(E4) *Asai et al., 2011*(E5) *Kwan et al.,2013*(E6)
c.2949delA	2	p.Lys983AsnfsX9	Novel mutation

Table E3. Patient 2 IgG autoantibody values above 2.0
(Fold increase compared to mean of controls +1SD)

Antigen	Patient 2	Mother	Patient 1	Control	Lupus
ds RNA	15.18	2.80	1.22	0.86	297.86
Collagen I	2.77	1.52	1.26	0.93	0.00
SS-A/SS-B	2.57	4.48	0.60	0.66	6.37
Heparin	2.23	2.74	0.27	1.10	8.05
H1	2.18	1.05	0.35	0.48	52.57

Table E4. Mother IgG autoantibody values above 2.0
(Fold increase compared to mean of controls +1SD)

Antigen	Mother	Patient 1	Patient 2	Control	Lupus
TPO	14.86	0.76	0.82	0.88	1.81
PL-7	13.96	0.52	0.40	0.68	2.38
TTG	9.40	0.62	0.58	0.65	2.31
M2 Antigen	8.92	0.64	0.39	0.85	4.31
Cardolipin	7.71	0.11	0.35	0.82	1.79
Ro/SS-A(60KDa)	7.63	0.41	0.21	0.37	1.64
U1-snRNP-C	7.36	0.61	0.52	0.87	2.51
LC1	6.95	0.66	0.51	0.74	3.38
CENP-A	6.69	0.73	0.53	0.87	19.30
GBM	6.26	0.57	0.31	0.86	3.36
L-Thyroxine	6.14	0.00	3.21	0.00	0.00
Thyroglobulin	6.11	0.63	0.23	0.51	1.37
CENP-B	6.01	0.60	0.36	0.95	2.25
KU (P70/P80)	5.91	0.59	0.36	0.70	4.15
U1-snRNP-BB'	4.94	0.47	0.61	0.40	3.24
Scl-70	4.86	0.53	0.36	0.80	1.42
C1q	4.57	0.73	0.41	0.90	4.05
SS-A/SS-B	4.48	2.57	0.60	0.66	6.37
Entaktin EDTA	4.28	0.81	0.45	0.60	2.33
Collagen VI	4.14	0.56	0.47	0.72	21.46
Ro/SSA (52kd)	3.47	0.62	0.54	0.75	2.33
PL-12	3.15	0.49	0.37	0.80	2.61
Proteoglycan	3.09	0.37	0.21	0.54	0.80
Intrinsic Factor	3.05	0.45	0.25	1.01	1.71
DGPS	2.81	0.91	0.93	1.00	11.17
ds RNA	2.80	15.18	1.22	0.86	297.86
Heparin	2.74	2.23	0.27	1.10	8.05
B2-glycoprotein I	2.69	0.58	0.47	0.96	1.73
Myosin	2.61	0.48	0.99	0.74	1.35
LKM1	2.58	0.47	0.23	0.70	1.59
La/SS-B	2.48	0.20	0.11	1.04	0.85
myelin basic protein	2.40	0.87	0.66	0.82	1.70
Topoisomerase	2.39	0.52	0.23	0.00	1.27
SRP54	2.18	0.66	0.40	0.90	2.07
Glom Extract	2.17	0.44	0.31	0.59	10.92
Hemocyanin	2.08	0.41	0.30	0.64	2.11

Table E5. Patient 1 IgM autoantibody values above 2.0
(Fold increase compared to mean of controls +1SD)

Antigen	Patient 1	Mother	Patient 2	Control	Lupus
Collagen I	4.12	2.75	3.29	0.00	0.00
ds RNA	3.29	4.39	1.10	0.00	18.12

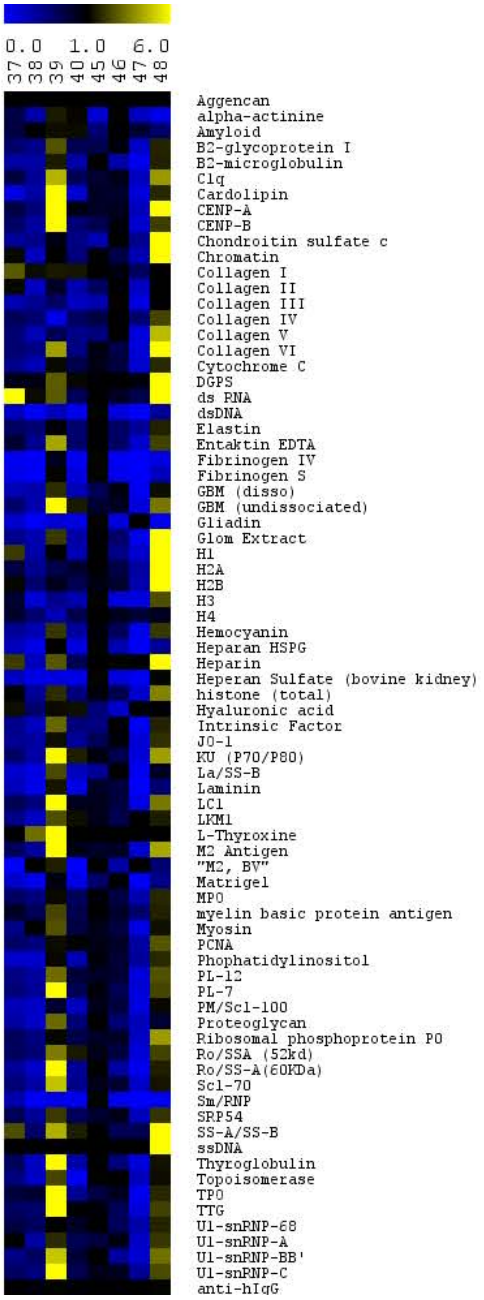
Table E6. Mother IgM autoantibody values above 2.0
(Fold increase compared to mean of controls +1SD)

Antigen	Mother	Patient 1	Patient 2	Control	Lupus
ds RNA	4.39	3.29	1.10	0.00	18.12
alpha-actinin	2.80	0.52	0.35	1.05	0.00
Collagen I	2.75	4.12	3.29	0.00	0.00
DGPS	2.57	0.86	0.29	0.71	3.43

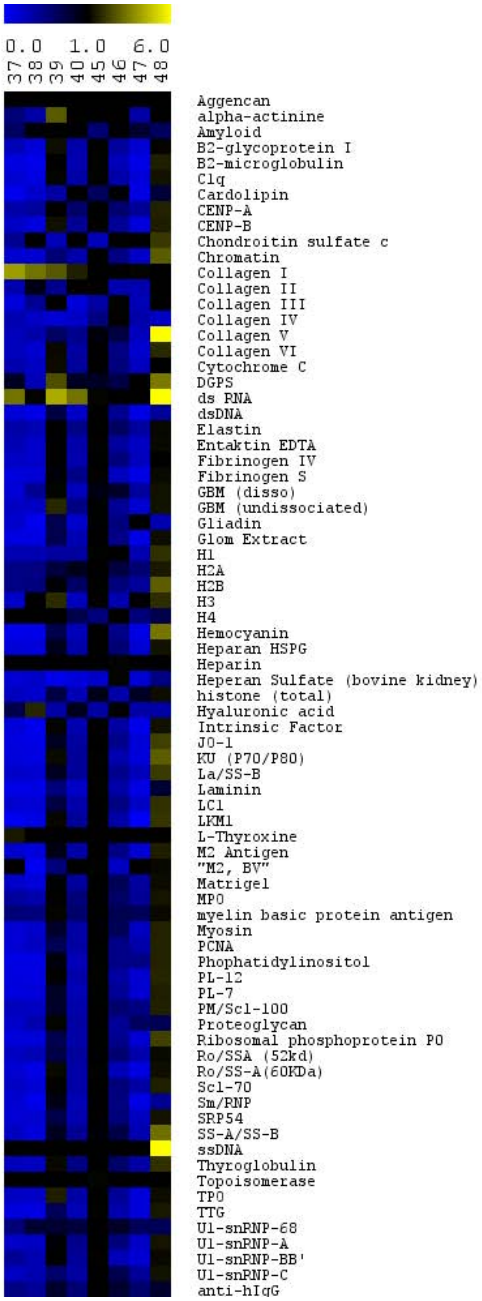
Table E7. Carrier rate of *RAG1* disease-associated variants in the European populations of the 1000 Genomes population database. Variants are known to be associated with immunodeficiency and were identified through HGMD. Positions are based on human genome reference NCBI build 37.3.

Chromosome	Gene	Base position	Variant	Allele frequency	Carrier frequency	References
11	RAG1	36596200	c.1346G>A (p.Arg449Lys)	0.00923	1:54	Sobacchi et al., 2006(E2) Bell et al., 2011(E7)
11	RAG1	36597870	c.3016A>G (p.Met1006Val)	0.00396	1:126	Sheehan et al., 2009(E8) Safaei et al., 2011(E9)

A. IgG autoantibody array



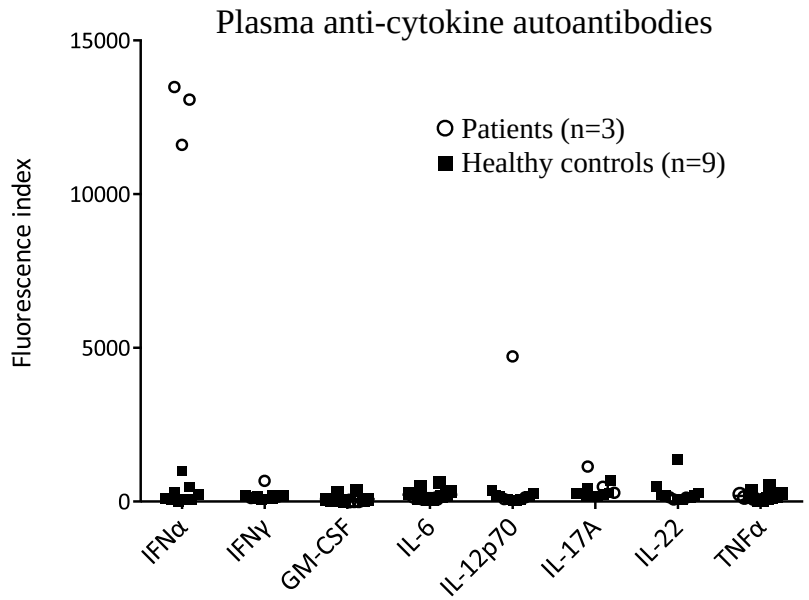
B. IgM autoantibody array



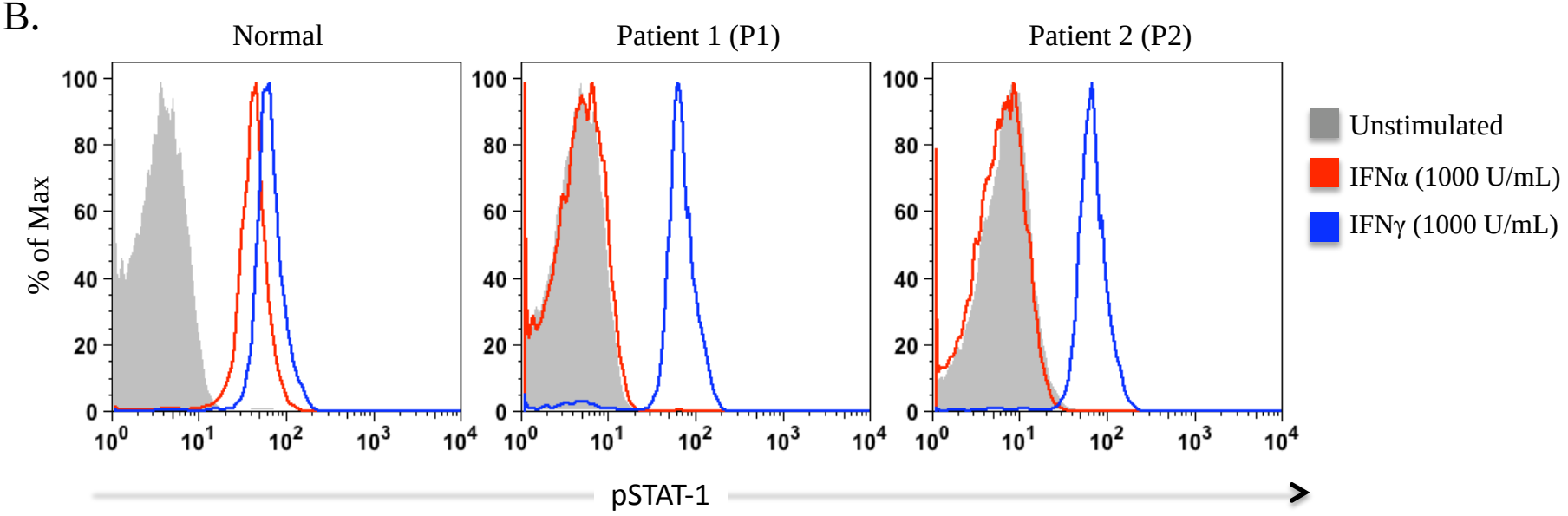
Array samples:

37. Patient 1 (II.1)
38. Patient 2 (II.4)
39. Mother (I.2)
40. Father (I.1)
45. 2 year old healthy control
46. Adult healthy control
47. Adult healthy control
48. Lupus patient

A.


Plasma anti-cytokine autoantibodies
Fluorescence index
15000
10000
5000
0
Patients (n=3)
Healthy controls (n=9)
IFNα
IFNγ
GM-CSF
IL-6
IL-12p70
IL-17A
IL-22
TNFα

B.


Normal
Patient 1 (P1)
Patient 2 (P2)
% of Max
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
pSTAT-1
Unstimulated
IFNα (1000 U/mL)
IFNγ (1000 U/mL)