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SUPPLEMENTAL TABLES

Supplemental Table 1. Autoimmune regulator (*AIRE*) mutations and deletions detected in the American Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) patient cohort.

Coding DNA sequence	Protein sequence	Number of patients	Percent of patients	Previously described	*AIRE* exon
Homozygous for c.967_979del13	p.L323SfsX51	10	28.6%	Yes	8
Compound heterozygous for c.967_979del13					
and c.769C>T	p.R257X	9	25.7%	Yes	6
and c.190_226del37	p.S64TfsX71	3	8.6%	No	2
and c.1249_1250insC	p.L417PfsX7	2	5.7%	Yes	10
and c.1616C>T	p.P539L	1	2.9%	Yes	14
and c.522_523ins13	p.L175RfsX46	1	2.9%	No	4
and 1781-bp deletion of exons 1-4		1	2.9%	No	1-4
and wild-type *AIRE*		1	2.9%		
Homozygous for c.769C>T	p.R257X	1	2.9%		
Compound heterozygous for c.769C>T					
and c.789_789delC	p.A264LfsX114	1	2.9%	Yes	6
Homozygous c.328delC	p.R110fsX37	1	2.9%	No	3
Homozygous for wild-type *AIRE*		4	11.4%		
Total		**35**	**100%**		

Supplemental Table 2. Summary of autoantibody positivity in the 35 American Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) patients, their frequency in patients with or without the corresponding clinical manifestations, and their association with the time to development of the corresponding clinical manifestations.

AAB and corresponding manifestation*	AAB positivity in patients with the corresponding manifestation % (n)	AAB positivity in patients without the corresponding manifestation % (n)	AAB positivity in all 35 patients % (n)**	Association of AAB positivity with time to development of the corresponding disease (p-value¶)
IL-17A with CMC	40% (12/30)	20% (1/5)	37.1% (13/35)	0.3888
IL-17F with CMC	86.7% (26/30)	80% (4/5)	85.7% (30/35)	0.0382
IL-22 with CMC	86.7% (26/30)	80% (4/5)	85.7% (30/35)	0.0382
NALP5 with HP	46.9% (15/32)	0% (0/3)	42.9% (15/35)	0.1953
21-OH with AI	58.6% (17/29)	16.7 (1/6)	51.4% (18/35)	0.1729
SCC with AI	75.9% (22/29)	33.3% (2/6)	68.6% (24/35)	0.1048
21-OH and/or SCC with AI	79.3% (23/29)	33.3% (2/6)	71.4% (25/35)	0.0807
Anti-thyroglobulin and HT	25% (2/8)	3.7% (1/27)	8.6% (3/35)	0.7056
TPO and HT	25% (2/8)	7.4% (2/27)	11.4% (4/35)	0.9667
GAD65 and DM	100% (4/4)	51.6 (16/31)	57.1% (20/35)	0.5677
IA-2 and DM	75% (3/4)	3.2% (1/31)	11.4% (4/35)	<0.0001
SCC and hypogonadism	54.6% (6/11)	75% (18/24)	68.6% (24/35)	0.388
SCC and TF	66.7 (2/3)	81.8% (9/11)	78.6% (11/14)	0.7518
SCC and OF	75% (6/8)	53.9% (7/13)	61.9% (13/21)	0.4322
TPH and gastritis	94.4% (17/18)	64.7% (11/17)	80% (28/35)	0.0956
Intrinsic factor and B12 deficiency	100% (10/10)	24% (6/25)	45.7% (16/35)	0.0002
Anti-parietal cell and B12 deficiency	10% (1/10)	0% (0/25)	2.9% (1/35)	0.8289
TH and alopecia	66.7% (4/6)	48.3% (14/29)	51.4% (18/35)	0.3302
GAD65 and vitiligo	53.9% (7/13)	59.1% (13/22)	57.1% (20/35)	0.6233
GAD65 and ID	57.1% (16/28)	57.1% (4/7)	57.1% (20/35)	0.81
TPH and ID	92.9% (26/28)	28.6% (2/7)	80% (28/35)	0.0094
BPIFB1 and pneumonitis	85.7% (12/14)	14.3% (3/21)	42.9% (15/35)	0.0003
KCNRG and pneumonitis	28.6 (4/14)	0% (0/21)	11.4% (4/35)	0.0127
IFN-ω	N/A	N/A	97.1% (34/35)	N/A

IFN-α	N/A	N/A	33/35 (94.3%)	N/A
IFN-γ	N/A	N/A	0/35 (0%)	N/A
IL-1α	N/A	N/A	0/35 (0%)	N/A
IL-12p70	N/A	N/A	0/35 (0%)	N/A
GM-CSF	N/A	N/A	0/35 (0%)	N/A

* AAB, autoantibody; NALP5, NLR family and pyrin domain containing 5; 21-OH, 21-hydroxylase; SCC, side-chain cleavage enzyme; TPO, thyroid peroxidase; GAD65, glutamic acid decarboxylase 65; IA-2, tyrosine phosphatase-related islet antigen 2; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; BPIFB1, bactericidal/permeability-increasing fold-containing B1; KCNRG, potassium channel regulator; CMC, chronic mucocutaneous candidiasis; HP, hypoparathyroidism; AI, adrenal insufficiency; HT, hypothyroidism; DM, type-1 diabetes mellitus; TF, testicular failure; POF, primary ovarian failure; ID, intestinal dysfunction; N/A, not applicable.

** Assessment of AABs for ovarian failure and testicular failure were calculated in females (n=21) and males (n=14), respectively.

¶ P values were determined using log rank tests.

Supplemental Table 3. Distribution and prevalence of organ-specific non-endocrine manifestations in the 35 American Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) patients.

	Clinical manifestation						
Patient #	**Urticarial eryption**	**Hepatitis**	**Gastritis**	**Intestinal dysfunction**	**Pneumonitis**	**Sjogren's-like syndrome**	**Number of manifestations among the 6 non-endocrine manifestations**
1	No	No	Yes	Yes	Yes	Yes	4
2	Yes	No	No	No	Yes	No	2
3	Yes	No	No	Yes	Yes	No	3
4	Yes	Yes	Yes	Yes	Yes	No	5
5	No	Yes	Yes	Yes	No	Yes	4
6	No	No	No	Yes	No	No	1
7	Yes	No	No	No	No	No	1
8	Yes	Yes	No	No	Yes	No	3
9	Yes	Yes	No	Yes	No	No	3
10	Yes	Yes	Yes	Yes	No	Yes	5
11	Yes	No	Yes	Yes	Yes	Yes	5
12	Yes	No	Yes	Yes	No	No	3
13	Yes	Yes	Yes	Yes	Yes	Yes	6
14	No	No	No	Yes	No	No	1
15	No	No	No	No	No	No	0
16	Yes	No	No	Yes	No	No	2
17	Yes	Yes	Yes	Yes	Yes	No	5
18	Yes	No	Yes	Yes	No	Yes	4
19	No	No	No	Yes	No	Yes	2
20	Yes	Yes	Yes	Yes	Yes	Yes	6
21	No	No	Yes	Yes	No	No	2
22	No	No	No	No	No	No	0
23	No	No	No	No	No	Yes	1

24	No	Yes	Yes	Yes	No	No	3
25	Yes	Yes	Yes	Yes	Yes	Yes	6
26	Yes	No	Yes	Yes	No	No	3
27	Yes	Yes	No	Yes	Yes	No	4
28	Yes	Yes	No	Yes	No	Yes	4
29	Yes	Yes	Yes	Yes	Yes	Yes	6
30	Yes	Yes	No	Yes	No	No	3
31	No	No	Yes	Yes	Yes	Yes	4
32	Yes	Yes	Yes	Yes	Yes	Yes	6
33	No	No	No	No	No	No	0
34	Yes	No	No	Yes	No	Yes	3
35	Yes	No	No	Yes	No	No	2

Supplemental Table 4. Distribution of clinical manifestations appearing before a diagnostic dyad in the 28 patients in whom the diagnostic criteria did not develop as their first two manifestations.

Clinical Manifestation	Number of patients	Percent of patients
Urticarial eruption	22	78.6%
Intestinal dysfunction	15	53.6%
Enamel hypoplasia	12	42.9%
Sjögren's-like syndrome	7	25%
Pneumonitis	7	25%
Keratoconjuctivitis	4	14.3%
Hepatitis	4	14.3%
Nail dystrophy	4	14.3%
Vitiligo	2	7.1%
Hypothyroidism	2	7.1%
Alopecia	2	7.1%
Gastritis	1	3.6%
Growth hormone deficiency	1	3.6%
Type-1 diabetes	1	3.6%
Hypertension	1	3.6%

Supplemental Table 5. Clinical characteristics of intestinal dysfunction in the 15 children who developed it in early childhood before meeting a classic diagnostic dyad.

DEMOGRAPHICS			
Age (years)		**Gender**	
Mean	1.62	Female	9/15 (60%)
Range	0.17 – 5	Male	6/15 (40%)
TYPE OF INTESTINAL DYSFUNCTION			
Chronic diarrhea		8/15 (53.3%)	
Chronic constipation		2/15 (13.3%)	
Alternating pattern of both		5/15 (33.3%)	
ASSOCIATED SYMPTOMS			
Abdominal cramping/pain		12/15 (80%)	
Abdominal bloating/distention		11/15 (73.3%)	
Floating/"greasy" stool		11/15 (73.3%)	
Foul-smelling flatulence		11/15 (73.3%)	
DURATION OF SYMPTOMS			
1-5 years		3/15 (20%)	
>5 years		12/15 (80%)	
MAXIMAL NUMBER OF BOWEL MOVEMENTS PER DAY			
Chronic diarrhea (n=8)		**Alternating pattern (n=5)**	
3-4	1/8 (12.5%)	3-4	2/5 (40%)
5-10	3/8 (37.5%)	5-10	2/5 (40%)
>10	4/8 (50%)	>10	1/5 (20%)

Supplemental Table 6. Distribution of the first two consecutive manifestations among the 35 American Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) patients.

First two consecutive clinical manifestations	Number of patients	Percent of patients
CMC and hypoparathyroidism	7	20%
Urticarial eruption and CMC	6	17.1%
CMC and intestinal dysfunction	3	8.6%
Urticarial eruption and hypoparathyroidism	2	5.7%
Urticarial eruption and keratoconjuctivitis	2	5.7%
Urticarial eruption and intestinal dysfunction	2	5.7%
CMC and nail dystrophy	2	5.7%
Enamel hypoplasia and hypoparathyroidism	2	5.7%
Urticarial eruption and enamel hypoplasia	1	2.9%
Urticarial eruption and adrenal insufficiency	1	2.9%
Urticarial eruption and pneumonitis	1	2.9%
Hypertension and intestinal dysfunction	1	2.9%
CMC and Sjögren's-like syndrome	1	2.9%
CMC and enamel hypoplasia	1	2.9%
Hypoparathyroidism and hypothyroidism	1	2.9%
Intestinal dysfunction and Sjögren's-like syndrome	1	2.9%
Alopecia and nail dystrophy	1	2.9%
Within the classic triad	7	20%
Within the combined classic and adjunct triads	25	71.4%

Supplemental Table 7. Clinical characteristics of the urticarial eruption in the 22 patients who developed it in early childhood before meeting a classic diagnostic dyad.

DEMOGRAPHICS			
Age		**Gender**	
Mean	12 months	Female	16/22 (72.7%)
Range	3-36 months	Male	6/22 (27.3%)
APPEARANCE			
Type of rash		**Number of lesions**	
Macular	7/22 (31.8%)	<10	1/22 (4.5%)
Papular	5/22 (22.7%)	>10	21/22 (95.5%)
Maculopapular	10/22 (45.5%)		
Coloration		**Grouped or individual**	
Pink	6/22 (27.3%)	Grouped	7/22 (31.8%)
Red	6/22 (27.3%)	Individual	7/22 (31.8%)
Mixed	10/22 (45.5%)	Mixed	8/22 (36.4%)
Blanching rash			
Yes	6/22 (27.3%)		
No	16/22 (72.7%)		
ASSOCIATED SYMPTOMS			
Fever	5/22 (22.7%)	Irritability	4/22 (18.2%)
Rigors	2/22 (9.1%)	Pruritus	2/22 (9.1%)
AFFECTED AREAS			
First area affected		**Spread from initial location**	20/22 (90.9%)
Torso	11/22 (50.0%)		
Extremities	5/22 (22.7%)	**Areas of spread**	
Face	2/22 (9.1%)	Torso	7/20 (35%)
Combined	2/22 (9.1%)	Extremities	12/20 (60%)
Unknown	1/22 (4.5%)	Face	13/20 (65%)

DURATION			
Duration of 1st episode		**Self-limited resolution**	22/22 (100%)
≤ 1 week	6/22 (27.3%)		
1 week - 1 month	6/22 (27.3%)		
1-6 months	10/22 (45.5%)		
RECURRENCE			
Recurrence	16/22 (72.7%)	**Duration of 2nd episode**	
		≤ 1 week	4/16 (25.0%)
First area affected		1 week - 1 month	9/16 (56.3%)
Same as initial	15/16 (93.8%)	1-6 months	3/16 (18.8%)
Different from initial	1/16 (6.3%)		

Supplemental Table 8. Histological characteristics of the urticarial eruption in 3 children with Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) in whom skin biopsies were obtained.

Histological characteristic	Number of biopsies
Cellular composition	
Neutrophils	3/3
Monocytes/macrophages	3/3
Lymphocytes	3/3
Plasma cells	2/3
Eosinophils	1/3
Histological findings	
Karyorrhexis	3/3
Vacuolarization along epidermal interface	2/3
Basal membrane thickening	1/3
Vasculitis	0/3
Localization of inflammation	
Superficial	3/3
Perivascular	3/3
Interstitial	3/3
Panniculitis	2/3

Supplemental Table 9. Absolute numbers of lymphocyte subsets in the peripheral blood of Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) patients and healthy donors.

Cell subset	APECED patients (n=35¶)		Healthy donors (n=40)		p-value
	Absolute number of cell subset per µl of blood				
	Mean	Range	Mean	Range	
$CD3^+$ T cells	1691.6	393-2859	1399.6	529-2536	0.0289*
$CD4^+$ T cells	1090.4	274-1967	857.2	273-1808	0.0123*
Naïve $CD45RA^+$ $CD62L^+$ $CD4^+$ T cells	560.1	21-1230	428.5	99-1169	0.0271
Central memory $CD45RA^-$ $CD62L^+$ $CD4^+$ T cells	413	89-1112	379.7	122-729	0.9306
Effector memory $CD45RA^-$ $CD62L^-$ $CD4^+$ T cells	106.7	35-252	111.3	38-314	0.4610
Effector $CD45RA^+$ $CD62L^-$ $CD4^+$ T cells	10.6	0-120	7.5	0-90	0.7882
$CD8^+$ T cells	480.6	53-1280	456.5	143-854	0.9558
Naïve $CD45RA^+$ $CD62L^+$ $CD8^+$ T cells	276.5	13-1038	241.7	67-600	0.4082
Central memory $CD45RA^-$ $CD62L^+$ $CD8^+$ T cells	63.9	1-276	79	15-202	0.0347
Effector memory $CD45RA^-$ $CD62L^-$ $CD8^+$ T cells	80.2	8-554	74.8	15-175	0.4325
Effector $CD45RA^+$ $CD62L^-$ $CD8^+$ T cells	59.9	5-333	71.4	11-282	0.2815
CD4/CD8 ratio	3.13	0.5-14.4	2.16	0.61-6.1	0.0333
$CD20^+$ B cells	243.4	15-1216	179.6	39-480	0.8113
Immature $CD20^+$ $CD10^+$ B cells	50.2	3-304	48.7	8-170	0.1035
Immature $CD21^+$ $CD10^+$ B cells	34.1	0-256	30.7	1-111	0.0507
$CD20^+$ IgM^- $CD38^{hi}$ plasmablasts	0.9	0-7	0.15	0-3	0.025
Transitional $CD20^+$ IgM^+ $CD10^+$ B cells	56	2-291	42.3	6-142	0.3614
Transitional $CD20^+$ $CD38^+$ $CD10^+$ B cells	58.7	3-294	45.9	5-163	0.3461
Memory non-switched $CD20^+$ $CD27^+$ IgM^+ B cells	14.8	2-40	14.7	2-49	0.8202
Memory switched $CD20^+$ $CD27^+$ IgM^- B cells	13.7	0-52	15.3	1-53	0.1359
$CD19^+$ $CD21^{lo}$ $CD38^{lo}$ B cells	13.4	2.6-43.6	7.4	1.4-46	0.0007
NK cells	181.7	15-674	335.9	121-863	<0.0001
NKT cells	151.8	31-582	127.8	28-451	0.3590

* indicates use of an unpaired t-test. Otherwise, a Mann-Whitney test was performed. ¶n=35 except for B-cell analyses in which n=30.

Supplemental Table 10. Percent of lymphocyte subsets within corresponding lymphocytes in the blood of Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) patients and healthy donors

Cell subset	APECED patients (n=35¶)		Healthy donors (n=40)		p-value
	% of cell subset within corresponding lymphocytes				
	Mean	Range	Mean	Range	
$CD4^+$ T cells (within $CD3^+$ T cells)	65.3	28.2-92.2	61.2	32.7-81.7	0.1236*
$CD8^+$ T cells (within $CD3^+$ T cells)	27.4	6.3-54.9	37.6	15.8-66.4	<0.0001*
Naive $CD4^+$ T cells (within $CD4^+$ T cells)	50.1	7.7-77.6	43.9	20.7-70.2	0.0868*
Central memory $CD4^+$ T cells (within $CD4^+$ T cells)	38.1	19-67.3	41.9	22.1-62.7	0.1722*
Effector memory $CD4^+$ T cells (within $CD4^+$ T cells)	10.9	2.6-52.9	13.2	3.9-31.1	0.0295
Effector $CD4^+$ T cells (within $CD4^+$ T cells)	0.9	0-8.4	1.1	0-7.2	0.0322
Naive $CD8^+$ T cells (within $CD8^+$ T cells)	55.5	21.3-89.8	38.2	0-64	<0.0001*
Central memory $CD8^+$ T cells (within $CD8^+$ T cells)	13.5	1.9-42.1	12	0-24.2	0.6365
Effector memory $CD8^+$ T cells (within $CD8^+$ T cells)	17.4	3.1-53.4	39.1	18.1-100	<0.0001*
Effector $CD8^+$ T cells (within $CD8^+$ T cells)	13.6	2-51.4	10.6	0-30.8	0.4295
Immature $CD20^+$ $CD10^+$ B cells (within $CD20^+$ B cells)	23	0.5-68.4	26.6	14.9-44.8	0.0716
Immature $CD21^+$ $CD10^+$ B cells (within $CD20^+$ B cells)	11.7	0-63.2	15.9	2.2-39	0.0007
$CD20^+$ IgM^- $CD38^{hi}$ plasmablasts (within $CD20^+$ B cells)	0.3	0-3	0.1	0-0.9	0.0369
Transitional $CD20^+$ IgM^+ $CD10^+$ B cells (within $CD20^+$ B cells)	22.2	3-63.2	22.7	12.7-39	0.8527*
Transitional $CD20^+$ $CD38^+$ $CD10^+$ B cells (within B cells)	23.4	4.5-63.2	24.8	10.9-44.3	0.2405
Memory non-switched $CD20^+$ $CD27^+$ IgM^+ B cells (within $CD20^+$ B cells)	10.4	1.2-28.6	10.3	1.9-43.8	0.9366
Memory switched $CD20^+$ $CD27^+$ IgM^- B cells (within $CD20^+$ B cells)	7.1	0-21.6	9.1	1.4-21.1	0.0506
$CD19^+$ $CD21^{lo}$ $CD38^{lo}$ B cells (within $CD19^+$ B cells)	10.5	1.9-45.5	4.3	1-17.7	0.0004

* indicates use of an unpaired t-test. Otherwise, a Mann-Whitney test was performed. ¶n=35 except for B-cell analyses in which n=30.

Supplemental Table 11. Primers used for amplification and sequencing to identify autoimmune regulator (*AIRE*) mutations.

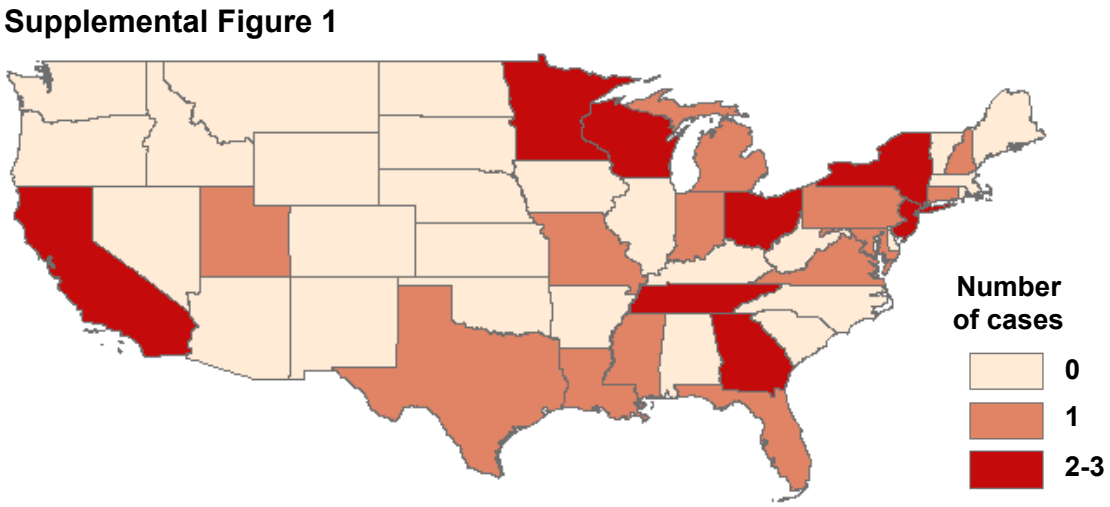
Primer ID	Primer sequence
AIRE_1F	AGGGGCTGCCAGTGTCC
AIRE_1R	TCCTCCTGGAACTTCCCC
AIRE_2F	ATGATGGAGATGGGCAGG
AIRE_2R	CAGCTGGGCTGAGCAGG
AIRE_3F	GATCCTAAGAGGCAAAGGGG
AIRE_3R	CTGGTCCAGTGTGTGGGTC
AIRE_4F	GGACTACCCAGCACTGGAC
AIRE_4R	ACAGGGTCTCAGAGGGCAG
AIRE_5F	GGCATAGAGTATGTGCTTGGG
AIRE_5R	GTGGTCCTCCTTCCATCTTG
AIRE_6-7F	ACTGCCAAGGCAGGTCC
AIRE_6-7R	AGGTAAAGGCAGAGGCAGC
AIRE_8F	GCTGAGGTCGGGAGAGACC
AIRE_8R	CTTCCCTTCAGGGTCAGTGG
AIRE_9F	CTGTGAAAAGACATGGTCGG
AIRE_9R	CAGGGGACAGACGGACAG
AIRE_10F	CACTGACTCCTGGGTGGTG
AIRE_10R	GTGAATTCATCCGCCCC
AIRE_11F	GGGTTCAGCTACATTTCCCC
AIRE_11R	GGTGGGGTGTAGGGTGTG
AIRE_12F	GAGGTGGCACTCCTGCTC
AIRE_12R	CCCTGAGATGTGCTCCCC
AIRE_13F	GAATTCCCATCTCAGTGTGG
AIRE_13R	CTGAGTTTCCACGGCTCAAG
AIRE_14F	TAACGATGGCCATGATTCTG
AIRE_14R	GGAAGGAGGTGTCCTTCTCAG
PCR cycling conditions: 94^{o}C for 2 min, [94^{o}C for 30s, 60^{o}C for 30s, 72^{o}C for 1 min](30 cycles), 4^{o}C hold *Betaine (5M)(Sigma Aldrich) was added to 1M (final) in the PCR reactions Sanger sequencing cycling conditions: 96^{o}C for 2 min, [96^{o}C for 10s, 60^{o}C for 4 min](30 cycles), 4^{o}C hold	

Supplemental Table 12. Second set of amplification and sequencing primers that were used for identifying autoimmune regulator (*AIRE*) mutations.

Amplification primers					
Exons	**F primer sequence**	**R primer sequence**	**Size**	**Annealing temperature**	**Notes**
1-3	CTGCGGGAGGCCCTGGCCCTGATTGG	GGAGGGGGCGGTCCAGTGCTGGGTAGTC	1656	68	+DMSO
4-5	GTGAAGTAGGCGGGCGGGTCTCATTTCCCTTTTAC	AGCGGCCCTGCTGTGCCCTGGTCAGAT	1609	68	+DMSO
6-7	ACTGGGGTGGGGGCGGGCTGGAGGAATG	GTGGAAGGCCCGAGGGCAGCCGTCACAG	1657	68	+DMSO
8-9	CGGGCTGGTGGGCGTCTGGGGGATTGTTAG	GACCGGGCTTGGGCATGGGGGACATAGTGCT	1999	68	+DMSO
10	CTAGGCTGGGCCACCCCCTCCTGTCCGTCTGT	GCCTTTCTCGCCTGGCCTCCCTCTCCTCCTGTTTC	820	68	+DMSO
11-12	GGGCACCGCCTTTCAGGAGACTCCCGCACTC	GGCTGGGGGTGAGACAACCTGGAGGGGTCAGAC	1406	68	+DMSO
13	GGCGGGGGTGGCATGGACCAGGCACTTT	GGGAGGGGCCGTGTGGGGGCTCTTGTTG	711	68	+DMSO
14	GCCTCGGGCCTCAGTTTCCCCACCTTTGACTTAG	GGATGAACCTTGGGGCCACCATGCTGAGTAAAATA	916	68	+DMSO

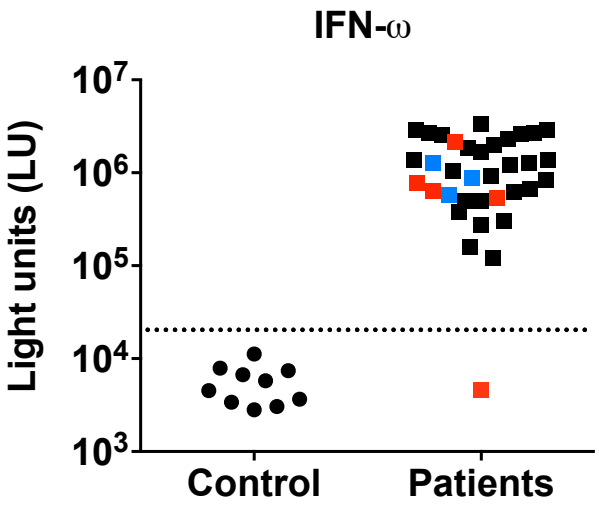
Sequencing primers					
Exons	**Primer sequence**	**Notes**	**Exons**	**Primer sequence**	**Notes**
1-2F	CTGCGGGAGGCCCTGGCCCTGATTGG	+DMSO	9R	GACCGGGCTTGGGCATGGGGGACATAGTGCT	+DMSO
2-3R	GTAAAAGGGAAATGAGACCCGCCCGCCTACTTC	+DMSO	10R	GCCTTTCTCGCCTGGCCTCCCTCTCCTCCTGTTTC	+DMSO
4R	TCTGGTGGTGCAGCTTAGGCCCTTGGAATGACACA	+DMSO	11R	GGAGTGTGGGGTGTGGGTGGGGTGTGGTTGTG	+DMSO
5F	GGGGGTGGGTCTGGTCATTGGTCATGCCTTCCTAT	+DMSO	12F	CAAACCCACCCAAACCCACCACTCCCACTCTCCA	+DMSO
6F	ACTGGGGTGGGGGCGGGCTGGAGGAATG	+DMSO	13R	GCTCAAGAGCAGTGGGGGCCGGCAGTCCTC	+DMSO
7R	CTCATTTAAACTATTACCATTGCGTATTATCAGGA	+DMSO	14F	GCCTCGGGCCTCAGTTTCCCCACCTTTGACTTAG	+DMSO
8F	GGGGAGTTCAGGTACCCAGAGATGCT	+DMSO	14R	GGGGTTGGGAGGGGAATGGGGAGTGAGTGTTTCAT	+DMSO

SUPPLEMENTAL FIGURES



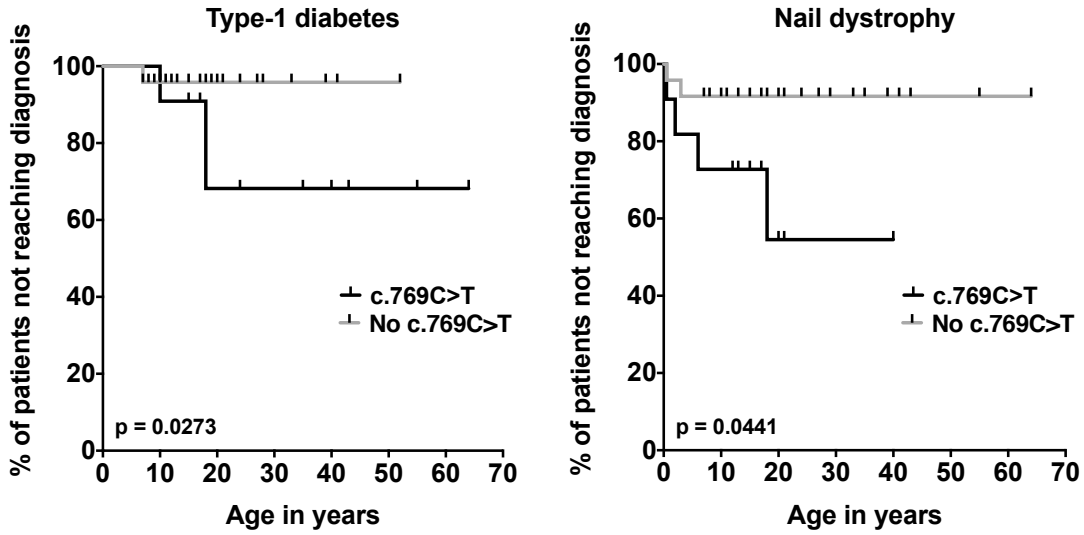
Supplemental Figure 1. Geographic distribution of the birthplace of Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) patients within the United States. Shown is the geographic distribution of the birthplace of the 33 US patients with enriched pockets noted in the northeast and upper midwest, parts of the south, and California.

Supplemental Figure 2



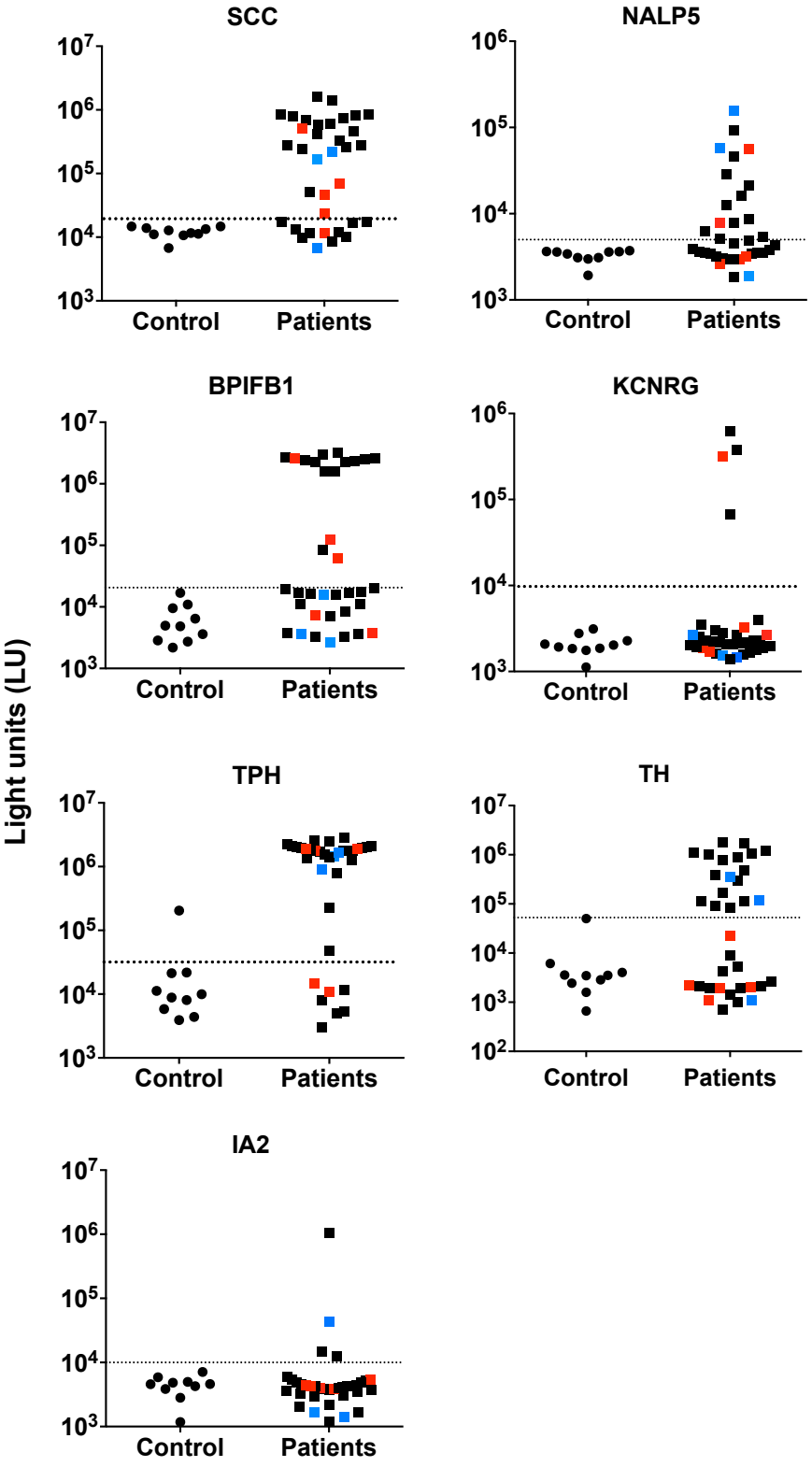
Supplemental Figure 2. Autoantibodies against interferon-omega are highly prevalent in American Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) patients. Shown is autoantibody immunoreactivity against interferon-omega expressed as light units (LU) using the Luciferase immunoprecipitation systems (LIPS) immunoassay. The dotted line represents the cut-off value for determining seropositivity. The 27 black squares correspond to the results obtained in the 27 APECED patients with biallelic *AIRE* mutations that include c.967_979del13 in heterozygocity (n =17) or homozygocity (n = 10), the 5 red squares correspond to the results obtained in the 5 APECED patients without biallelic *AIRE* mutations, and the 3 blue squares correspond to the results obtained in the 3 APECED patients with biallelic *AIRE* mutations other than c.967_979del13. n=35 patients; 10 healthy donors.

Supplemental Figure 3



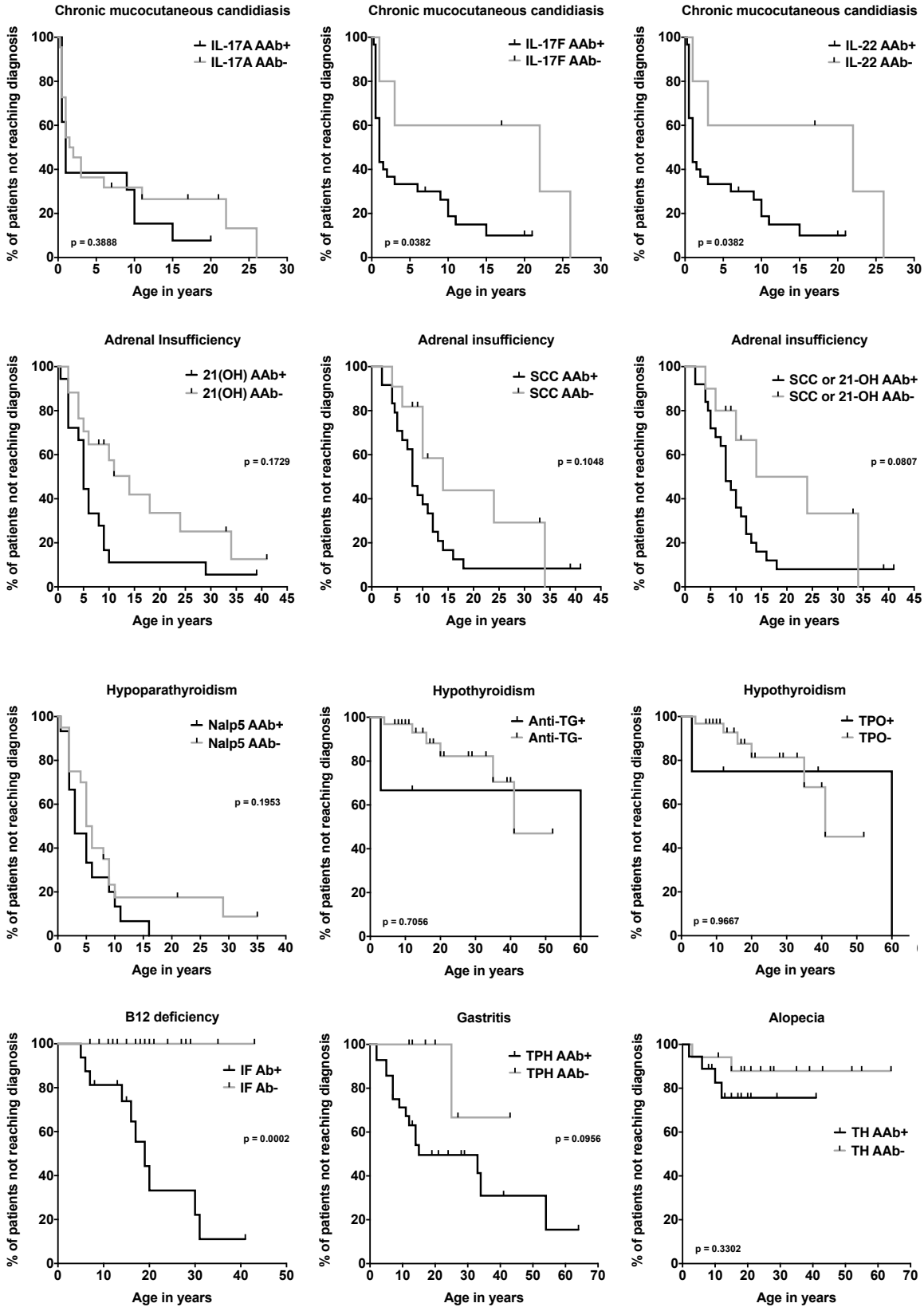
Supplemental Figure 3. Correlation between carriage of the c.769C>T allele and time to development of clinical manifestations in American Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) patients. Shown are Kaplan-Meier curves illustrating the correlation between carrying the c.769C>T allele and the time to development of type-1 diabetes and nail dystrophy. Analyses between carrying the c.769C>T allele and the time to development of any of the other 21 clinical manifestations did not reveal significant correlations (not shown).

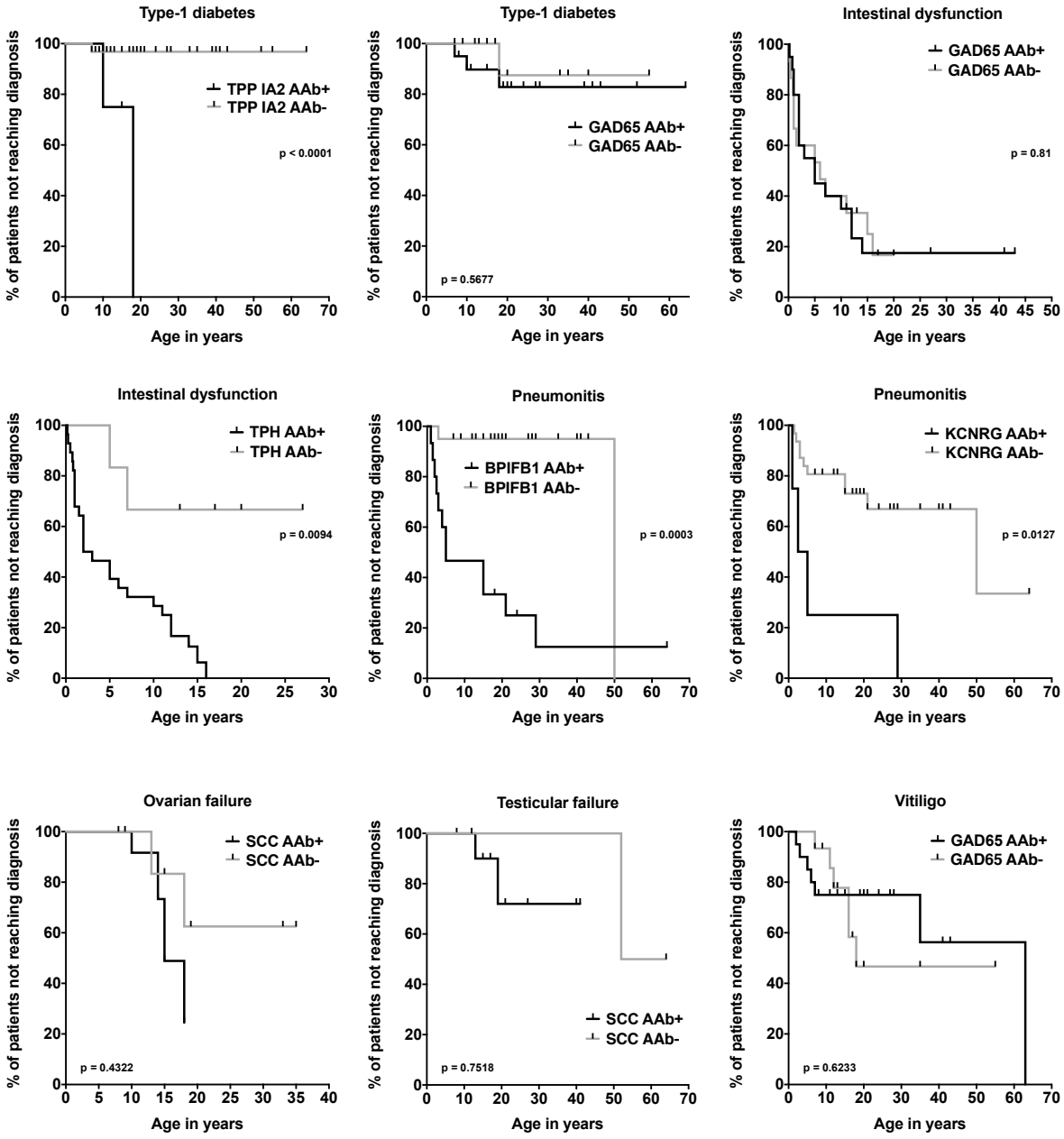
Supplemental Figure 4



Supplemental Figure 4. Autoantibody immunoreactivity against tissue autoantigens in the American Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) cohort. Shown is autoantibody immunoreactivity against the indicated autoantigens expressed as light units (LU) using the Luciferase immunoprecipitation systems (LIPS) immunoassay. The dotted line represents the cut-off value for determining seropositivity for the corresponding autoantibody. The 27 black squares correspond to the results obtained in the 27 APECED patients with biallelic *AIRE* mutations that include c.967_979del13 in heterozygocity (n =17) or homozygocity (n = 10), the 5 red squares correspond to the results obtained in the 5 APECED patients without biallelic *AIRE* mutations, and the 3 blue squares correspond to the results obtained in the 3 APECED patients with biallelic *AIRE* mutations other than c.967_979del13. SCC, side-chain cleavage enzyme; NALP5, NLR family and pyrin domain containing 5; BPIFB1, bactericidal/permeability-increasing fold-containing B1; KCNRG, potassium channel regulator; TPH, tryptophan hydroxylase; TH, tyrosine hydroxylase; IA-2, tyrosine phosphatase-related islet antigen 2. n=35 patients; 10 healthy donors.

Supplemental Figure 5

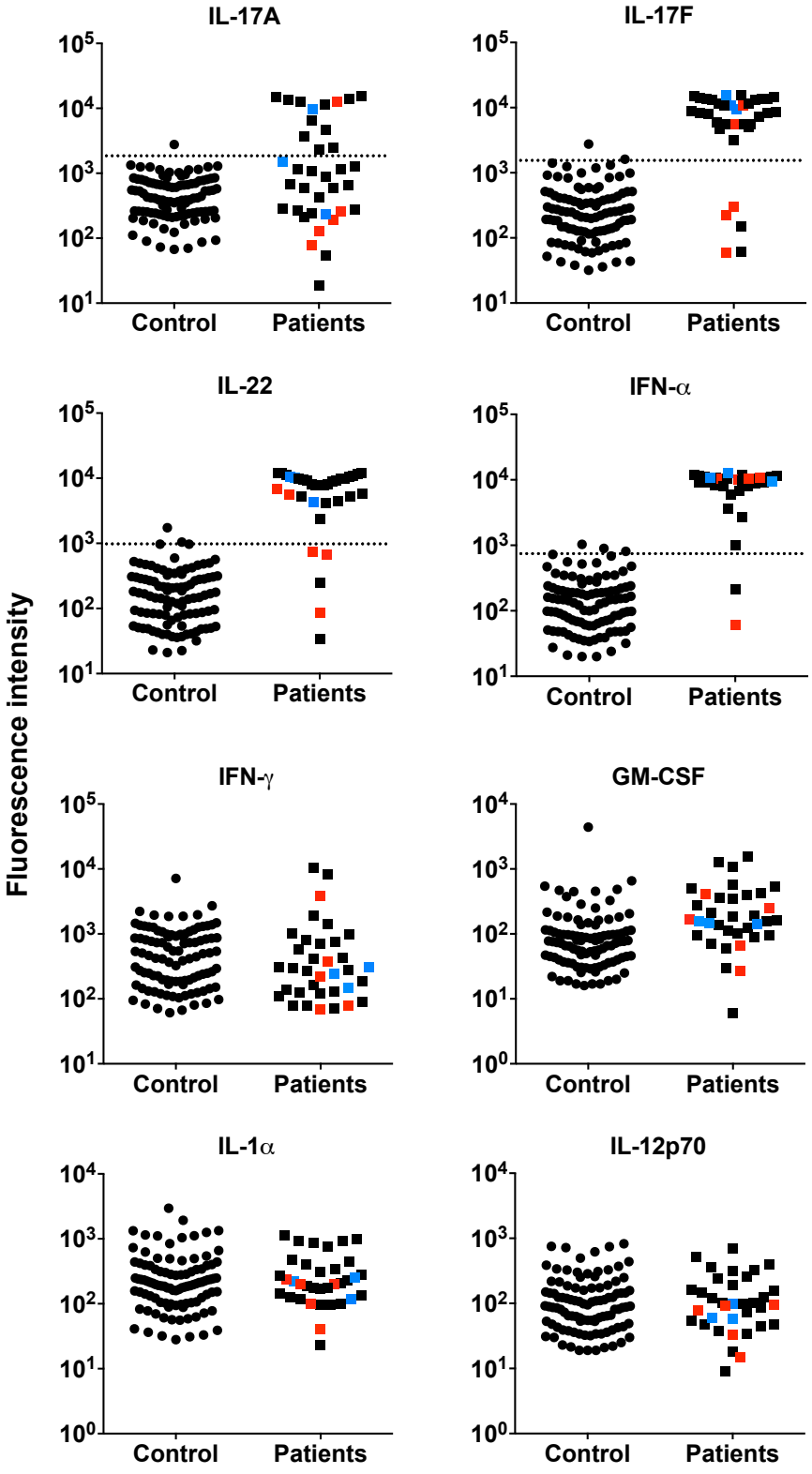




Supplemental Figure 5. Correlation between autoantibody immunoreactivity and the time to development of the corresponding clinical manifestations in the American Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) patients. Shown are Kaplan-Meier curves illustrating the correlation between the time to development of the indicated clinical manifestations with the presence or absence of the corresponding

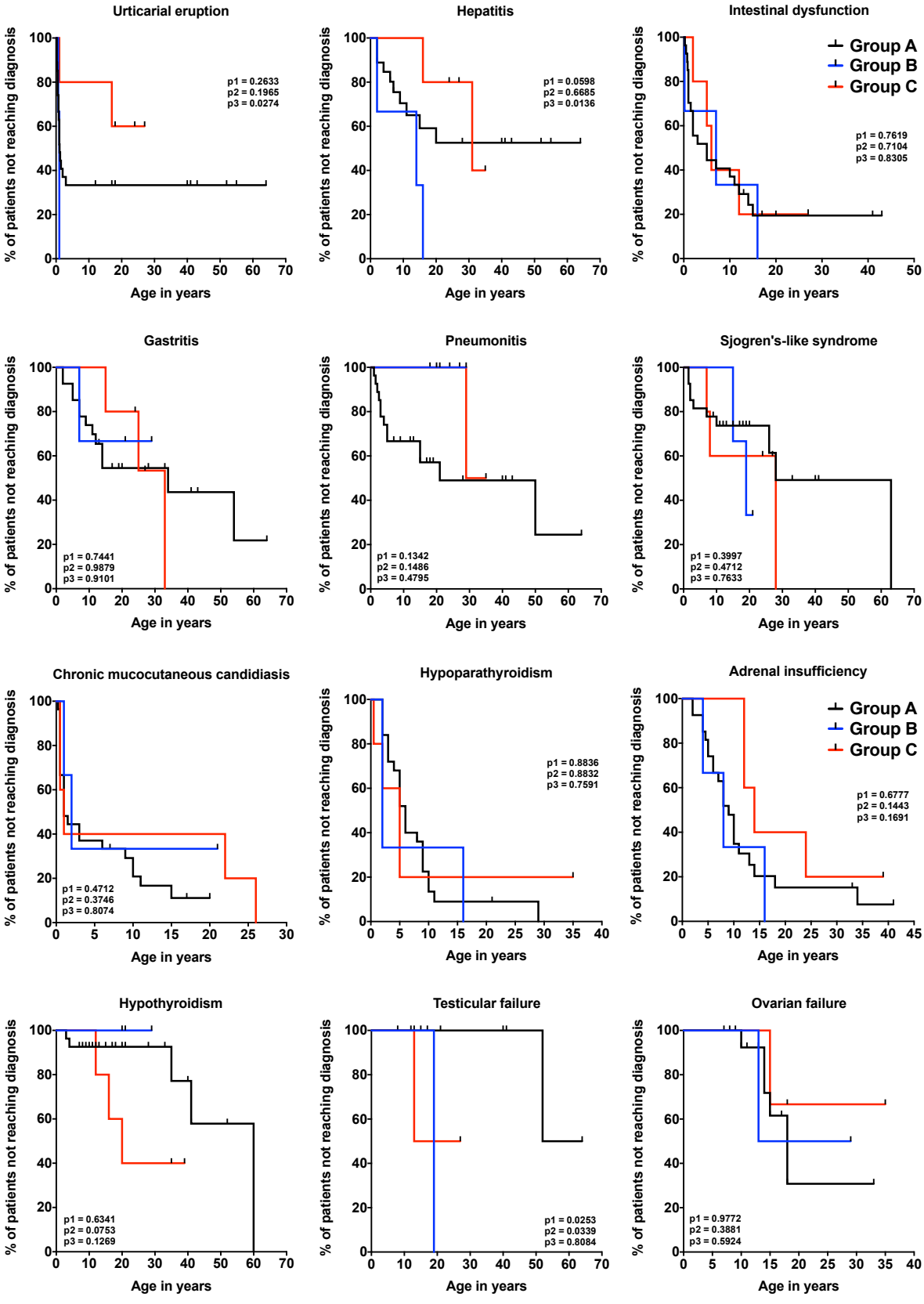
autoantibodies. AAB, autoantibody; NALP5, NLR family and pyrin domain containing 5; 21-OH, 21-hydroxylase; SCC, side-chain cleavage enzyme; TPO, thyroid peroxidase; TG, thyroglobulin; GAD65, glutamic acid decarboxylase 65; IA-2, tyrosine phosphatase-related islet antigen 2; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; BPIFB1, bactericidal/permeability-increasing fold-containing B1; KCNRG, potassium channel regulator; IF, intrinsic factor.

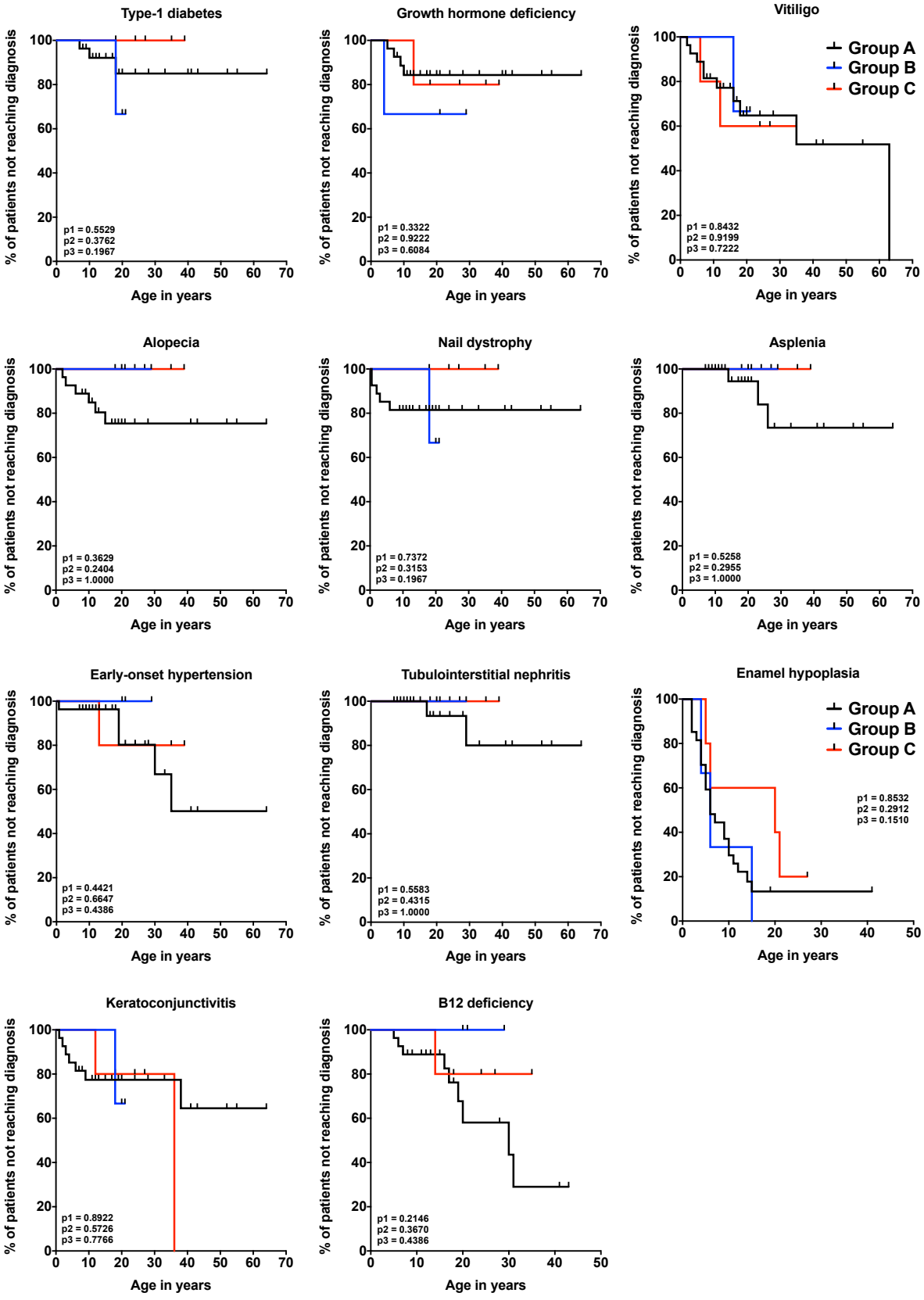
Supplemental Figure 6



Supplemental Figure 6. Anti-cytokine autoantibody immunoreactivity in the American Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) cohort. Shown is autoantibody immunoreactivity against the indicated cytokines expressed as fluorescence intensity using a particle-based multiplex assay. The dotted line represents the cut-off value for determining seropositivity for the corresponding autoantibody. The 27 black squares correspond to the results obtained in the 27 APECED patients with biallelic *AIRE* mutations that include c.967_979del13 in heterozygocity (n =17) or homozygocity (n = 10), the 5 red squares correspond to the results obtained in the 5 APECED patients without biallelic *AIRE* mutations, and the 3 blue squares correspond to the results obtained in the 3 APECED patients with biallelic *AIRE* mutations other than c.967_979del13. n=35 patients; 100 healthy donors.

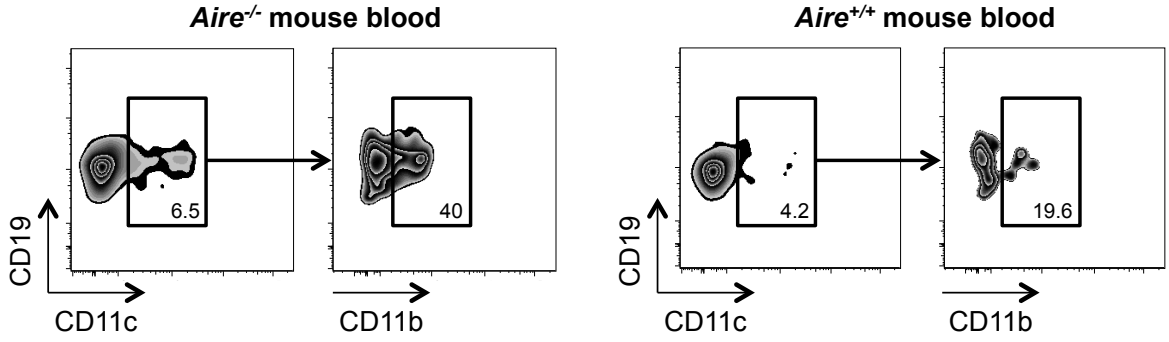
Supplemental Figure 7




Type-1 diabetes
% of patients not reaching diagnosis
Age in years
p1 = 0.5529
p2 = 0.3762
p3 = 0.1967
Growth hormone deficiency
p1 = 0.3322
p2 = 0.9222
p3 = 0.6084
Vitiligo
Group A
Group B
Group C
p1 = 0.8432
p2 = 0.9199
p3 = 0.7222
Alopecia
p1 = 0.3629
p2 = 0.2404
p3 = 1.0000
Nail dystrophy
p1 = 0.7372
p2 = 0.3153
p3 = 0.1967
Asplenia
p1 = 0.5258
p2 = 0.2955
p3 = 1.0000
Early-onset hypertension
p1 = 0.4421
p2 = 0.6647
p3 = 0.4386
Tubulointerstitial nephritis
p1 = 0.5583
p2 = 0.4315
p3 = 1.0000
Enamel hypoplasia
p1 = 0.8532
p2 = 0.2912
p3 = 0.1510
Keratoconjunctivitis
p1 = 0.8922
p2 = 0.5726
p3 = 0.7766
B12 deficiency
p1 = 0.2146
p2 = 0.3670
p3 = 0.4386

Supplemental Figure 7. Correlation between carriage of different *AIRE* mutations and the time to development of clinical manifestations in American Autoimmune polyendocrinopathy-Candidiasis-Ectodermal dystrophy (APECED) patients. Shown are Kaplan-Meier curves illustrating the correlation between carrying biallelic *AIRE* mutations that include c.967_979del13 in heterozygocity or homozygocity (Group A), or carrying biallelic *AIRE* mutations other than c.967_979del13 (Group B) or carrying no biallelic *AIRE* mutations (Group C) and the time to development of the 23 clinical manifestations. P1 corresponds to the *P* value that compares Group A with Group B, P2 corresponds to the *P* value that compares Group A with Group C, and P3 corresponds to the *P* value that compares Group B with Group C.

Supplemental Figure 8



Supplemental Figure 8. Gating strategy and representative FACS plots of CD11c$^+$CD11b$^+$ B cells in mouse peripheral blood. After initial FSC/SCC gating of peripheral blood cells (not shown), live CD45$^+$CD3$^-$NKp46$^-$CD19$^+$ B cells were selected (not shown) and CD11c$^+$CD11b$^+$ B cells were delineated. Shown are representative FACS plots of CD11c$^+$CD11b$^+$ B cells from *Aire*$^{-/-}$ (left panels) and *Aire*$^{+/+}$ mice (right panels), which show enrichment of these cells in Aire-deficient mouse peripheral blood.