

Hiroshi M. Ueno, PhD^a
Teruhiko Kato, MSc^a
Hidenori Ohnishi, MD, PhD^b
Norio Kawamoto, MD, PhD^{b,c}
Zenichiro Kato, MD, PhD^{b,c}
Hideo Kaneko, MD, PhD^{b,d}
Naomi Kondo, MD, PhD^{b,e}
Taku Nakano, PhD^a

From ^aResearch and Development Department, Bean Stalk Snow Co Ltd, Kawagoe, Japan; ^bDepartment of Pediatrics, Graduate School of Medicine, Gifu University, ^cStructural Medicine, United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, ^dDepartment of Clinical Research, National Hospital Organization, Nagara Medical Center, and ^eHeisei College of Health Sciences, Gifu, Japan. E-mail: hiroshi-ueno@beanstalksnow.co.jp.

This study was supported in part by the Research and Development Program for New Bio-industry Initiatives of the Bio-oriented Technology Research Advancement Institution (BRAIN), Japan (grant no. 2005-2009). BRAIN made no substantial contribution to the study, except for the financial support.

Disclosure of potential conflict of interest: H. M. Ueno, T. Kato, and T. Nakano are employees of Bean Stalk Snow Co Ltd. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Sicherer SH, Sampson HA. Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol* 2014;133:291-307.e5.
2. Ueno HM, Kato T, Ohnishi H, Kawamoto N, Kato Z, Kaneko H, et al. T-cell epitope-containing hypoallergenic β -lactoglobulin for oral immunotherapy in milk allergy. *Pediatr Allergy Immunol* 2016;27:818-24.
3. Ruiter B, Trégoat V, M'Rabet L, Garssen J, Bruijnzeel-Koomen CA, Knol EF, et al. Characterization of T cell epitopes in α S1-casein in cow's milk allergic, atopic and non-atopic children. *Clin Exp Allergy* 2006;36:303-10.
4. Archila LD, Khan FS, Bhatnagar N, Robinson D, Farrington ML, Kwok WW. α S1-Casein elucidate major T-cell responses in cow's milk allergy. *J Allergy Clin Immunol* 2017;140:854-7.e6.
5. Matsuo H, Yokooji T, Taogoshi T. Common food allergens and their IgE-binding epitopes. *Allergol Int* 2015;64:332-43.
6. Farrell HM Jr, Jimenez-Flores R, Bleck GT, Brown EM, Butler JE, Creamer LK, et al. Nomenclature of the proteins of cows' milk—sixth revision. *J Dairy Sci* 2004;87:1641-74.
7. Yokooji T, Kurihara S, Murakami T, Chinuki Y, Takahashi H, Morita E, et al. Characterization of causative allergens for wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat proteins in facial soap. *Allergol Int* 2013;62:435-45.
8. Liu Y, Guo R. Aggregation properties of aqueous casein hydrolysate solutions at different pH. *Int Dairy J* 2008;18:1022-7.

Available online April 26, 2018.
<https://doi.org/10.1016/j.jaci.2018.04.005>

Screening for Wiskott-Aldrich syndrome by flow cytometry



To the Editor:

Wiskott-Aldrich syndrome (WAS) is a rare X-linked disease commonly associated with symptoms of immune deficiency. A diagnosis of WAS is suspected on the basis of clinical parameters including thrombocytopenia, eczema, recurring infections, and autoimmunity.¹ WAS protein (WASP) is exclusively expressed in hematopoietic lineages. Lowered levels are thought to be a feature of WAS.² However, the diagnostic accuracy of flow cytometric WASP measurement has not been determined, and optimal diagnostic cutoff values are lacking. We therefore reviewed results from 107 individuals with suspected WAS to calculate the diagnostic accuracy of a rapid whole-blood flow cytometry-based assay and found it reliable for predicting mutations in WAS.

WASP expression levels were determined in lymphocytes gated by CD45 versus side light scatter. A range of WASP protein expression was seen, from normal in healthy controls to those with reduced or poor WASP expression (Fig 1, A; see Fig E1, A-C, in this article's Online Repository at www.jacionline.org). Detailed methods can be found in this article's Online Repository at www.jacionline.org. The age at WASP staining ranged from 1 month to 32 years with a median of 1 year.

All 58 persons with normal WAS sequence displayed WASP expression at levels comparable to those in healthy controls (mean staining ratio, 0.94 ± 0.15) while 41 of 49 patients with a genetic abnormality were found with reduced staining ratios (mean staining ratio, 0.34 ± 0.28) (Mann-Whitney nonparametric test $P < .0001$). We further divided the patients with genetic abnormalities into 5 categories, that is, missense ($n = 30$), insertion ($n = 4$), deletion ($n = 8$), gross deletion ($n = 4$), and nonsense ($n = 3$) (see Fig E2, A, in this article's Online Repository at www.jacionline.org). Table E1 in this article's Online Repository at www.jacionline.org lists all patients in this study. Every patient with gross deletions had poor WASP expression. Eight patients from the other mutation categories had normal WASP staining despite having a variant in WAS. Three of these patients carried variants of unknown clinical significance (VUCS). Interestingly, the c.538C>A (p.H180N) variant (staining ratio, 0.98) has been implicated in patients with X-linked thrombocytopenia (SCV000053274).³ It was not possible to rule out mosaicism or the influence of maternal cells with the available data; however, no patient included here was observed to have bimodal WASP staining. In addition, of the 8 patients with normal WASP expression, 3 mutations were intronic and 3 located at the C-terminus. Because the WASP antibody recognizes an epitope in the region between aa.146-265, it may still bind proteins bearing missense or C-terminal-truncating mutations.⁴

Six patients with missense mutations displayed staining ratios between 0.3 and 0.5. This "intermediate" staining ratio was not observed in other mutation groups, possibly due to low sample numbers. All missense mutations were "probably damaging" on PolyPhen-2 except c.995T>C (p.V332A) (0.0, benign), a known VUCS with a 0.84 staining ratio. Fold stability change ($\Delta\Delta G$) predictions reported a range of slightly stabilizing to highly destabilizing effects (Fig 1, B).^{5,6} Certain damaging mutations might not influence the antibody epitope, leading to only slightly reduced antibody staining. Mutations that destabilize the protein could lead to degradation and poor detection, whereas stabilizing mutations could hinder protein function yet display normal levels of WASP staining. Highly accurate and reproducible staining is crucial for establishing diagnostic cutoff estimation. To evaluate staining consistency, we compared patients bearing similar WAS mutations. Here, the c.167C>T (p.A56V) mutation was found in 4 patients, c.356G>A (p.G119E) in 3, and c.559(+5) in 5. Results exhibited good reproducibility (see Fig E2, B). WAS scores were available for 28 of the 49 confirmed WAS cases (Table E1). Scores did not strongly correlate to WASP staining index or age of presentation (Fig 1, C; Fig E2, C and D).^{7,8} Interestingly, patients with autoimmunity (WAS score 5) were all low WASP expressers. No malignancies were reported in any of the 28 patients with available scores.

From the receiver-operating characteristic curve, when all entries were considered, a cutoff staining ratio of 0.60 returned a sensitivity and specificity of 82.6% and 100%, respectively.

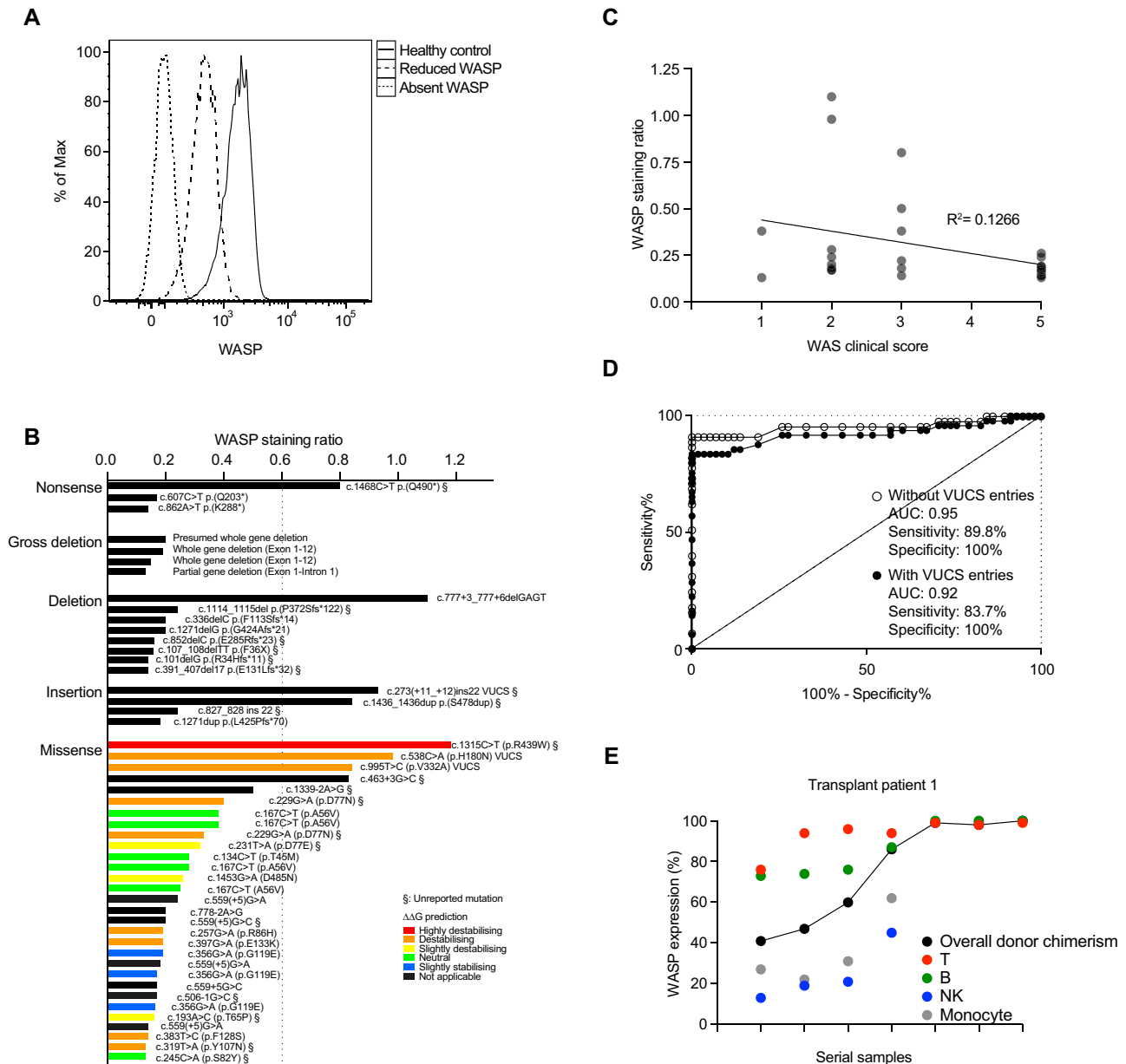


FIG 1. WASP staining accurately diagnoses WAS. **A**, Wide range of WASP staining intensity highlights the importance of assay standardization to ensure reproducible results. **B**, No clear genotype-phenotype correlation. **C**, Poor association between WASP staining and WAS clinical score. **D**, Receiver-operating characteristic curves with or without VUCS cases. **E**, Posttransplant whole-blood genetic chimerism levels correlate to WASP expression. AUC, Area under the curve.

Removing 3 VUCS entries improved the sensitivity and specificity to 89.1% and 100%, respectively (Fig 1, D). By removing VUCS entries, the overall accuracy of the test improved from 92.3% to 95.2%, validating the assay ability in predicting WAS genetic abnormalities. All patients with reduced WASP expression carried a WAS variant but patients suspicious of WAS expressing normal WASP should not be excluded from WAS sequencing because 8 of 49 patients (16.3%) with WAS variants expressed normal WASP. Normal WASP level is thus insufficient to rule out WAS.

WASP quantification is also useful for lineage-specific donor chimerism monitoring following allogeneic hematopoietic cell transplantation. Percent total blood genetic chimerism correlated

with specific leukocyte subset WASP chimerism in 2 patients studied longitudinally (Fig 1, E; Fig E2, E; see Table E2 in this article's Online Repository at www.jacionline.org). For each patient, sorting lymphocyte subsets before genotyping yielded very comparable specific subset genotype-phenotype results (Fig E2, F).

Future efforts to improve the accuracy of WAS screening diagnostics could include the development of a functional assay demonstrating defective actin remodeling during lymphocyte polarization.⁹ Such an assay might potentially also be useful in diagnosing X-linked thrombocytopenia and X-linked neutropenia, and would be especially useful for the evaluation of potential patients with WAS with novel mutations of unknown significance with unperturbed WASP staining. Patients with

residual WASP function may be associated with milder clinical phenotypes, and a functional assay that could demonstrate lowered but not defective function in this population may allow disease risk stratification as well as diagnosis via one assay.

In summary, we demonstrated the efficacy of a whole-blood flow cytometry–based staining protocol as a tool to quickly diagnose patients with WAS with up to 89% sensitivity and 100% specificity. However, WAS diagnosis cannot be excluded in clinically suspicious cases when WASP is present. Inclusion of clinical markers and other possible functional assays in a combined algorithm could improve the overall accuracy for diagnosing WAS.

Samuel C. C. Chiang, PhD^a

Sue M. Vergamini, BS^a

Ammar Husami, BS^b

Lisa Neumeier, MT^a

Kathryn Quinn, MS^a

Teresa Ellerhorst, BS^a

Linda Sheppard, BS^a

Carrie Gifford, MBA^a

David Buchbinder, MD^c

Avni Joshi, MD^d

Marianne Ifversen, MD, PhD^e

Gary I. Kleiner, MD, PhD^f

James B. Bussell, MD^g

Shanmuganathan Chandrakasan, MD^h

Robert D. Peseck, MDⁱ

Tamara C. Pozos, MD, PhD^j

Melissa J. Rose, DO^k

Amy M. Scurlock, MD^j

Kejian Zhang, MBA, MD^{b,l}

Yenan T. Bryceson, PhD^{m,n}

Jack Bleesing, MD, PhD^a

Rebecca A. Marsh, MD^a

From the Divisions of ^aBone Marrow Transplantation and Immune Deficiency and ^bHuman Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; ^cCHOC Children's Hospital, Orange, Calif; ^dthe Division of Pediatric Allergy and Immunology, Mayo Clinic Children's Center, Rochester, Minn; ^ethe Department of Pediatric and Adolescent Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; ^fthe Department of Pediatrics, University of Miami Miller School of Medicine, Miami, Fla; ^gWeill Cornell Medical College, New York, NY; ^hthe Division of Bone Marrow Transplant, Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Emory University School of Medicine, Atlanta, Ga; ⁱArkansas Children's Hospital and the University of Arkansas for Medical Sciences, Little Rock, Ark; ^jPediatric Infectious Diseases and Immunology, Children's Minnesota, Minneapolis and St Paul, Minn; ^kNationwide Children's Hospital and the Ohio State University College of Medicine, Columbus, Ohio; ^lCoyote Bioscience, Campbell, Calif; ^mthe Center for Hematology and Regenerative Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden; and ⁿBroegelmann Research Laboratory, Department of Clinical Sciences, University of Bergen, Bergen, Norway. E-mail: sam.chiang@cchmc.org.

Disclosure of potential conflict of interest: Revenue generated by the clinical testing performed in the Diagnostic Immunology Laboratory funds a proportion of the salaries for S. M. Vergamini, L. Neumeier, K. Quinn, T. Ellerhorst, L. Sheppard, C. Gifford, and J. Bleesing, and funds the research, development, and validation of clinical tests. The rest of the authors declare that they have no relevant conflicts of interests.

REFERENCES

1. Candotti F. Clinical manifestations and pathophysiological mechanisms of the Wiskott-Aldrich syndrome. *J Clin Immunol* 2018;38:13-27.
2. Jin Y, Mazza C, Christie JR, Giliani S, Fiorini M, Mella P, et al. Mutations of the Wiskott-Aldrich syndrome protein (WASP): hotspots, effect on transcription, and translation and phenotype/genotype correlation. *Blood* 2004;104:4010-9.
3. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res* 2016;44:D862-8.

4. Kawai S, Minegishi M, Ohashi Y, Sasahara Y, Kumaki S, Konno T, et al. Flow cytometric determination of intracytoplasmic Wiskott-Aldrich syndrome protein in peripheral blood lymphocyte subpopulations. *J Immunol Methods* 2002;260:195-205.
5. Quan L, Lv Q, Zhang YSTRUM. structure-based prediction of protein stability changes upon single-point mutation. *Bioinformatics* 2016;32:2936-46.
6. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7:248-9.
7. Zhu Q, Zhang M, Blaese RM, Derry JM, Junker A, Francke U, et al. The Wiskott-Aldrich syndrome and X-linked congenital thrombocytopenia are caused by mutations of the same gene. *Blood* 1995;86:3797-804.
8. Buchbinder D, Nugent DJ, Fillipovich AH. Wiskott-Aldrich syndrome: diagnosis, current management, and emerging treatments. *Appl Clin Genet* 2014;7:55-66.
9. Houmadi R, Guipouy D, Rey-Barroso J, Vasconcelos Z, Cornet J, Manghi M, et al. The Wiskott-Aldrich syndrome protein contributes to the assembly of the LFA-1 nanocluster belt at the lytic synapse. *Cell Rep* 2018;22:979-91.

Available online May 3, 2018.
<https://doi.org/10.1016/j.jaci.2018.04.017>

Intestinal microbial-derived sphingolipids are inversely associated with childhood food allergy



To the Editor:

Food allergy is a life-threatening disease that is common and increasing in prevalence, yet the factors leading to its development are poorly understood.¹ Microbial composition has been associated with risk of food allergy,² and integrative analysis of the human intestinal microbiome and metabolome could provide insights into mechanisms of microbial-associated pathogenic changes.³ Here, we performed a prospective, untargeted, integrative analysis of the intestinal bacterial microbiome and metabolome during infancy, testing associations with the development of clinical food allergy and sensitization to foods at age 3 years. Our goal was to identify microbial-associated metabolites that were associated with food allergy or sensitization.

For detailed methods, see this article's [Methods](#) section in the Online Repository at www.jacionline.org. Subjects were offspring of participants in the Vitamin D Antenatal Asthma Reduction Trial (NCT00920621),⁴ a multicenter randomized controlled trial of vitamin D supplementation in pregnancy to prevent asthma in offspring. The study protocol was approved by the institutional review boards at each center and all participants provided written informed consent. Food allergy and sensitization at age 3 years were based on parental questionnaire responses and serum specific IgE testing. Stool samples were collected between age 3 and 6 months from 333 subjects. Microbiome composition analysis by bacterial 16S rRNA sequencing and metabolomic analysis with ultraperformance LC/MS-MS were performed on stool samples from 12 children with food allergy (see [Table E1](#) in this article's Online Repository at www.jacionline.org), 32 with food sensitization, and 37 controls.

Subjects were well matched on baseline characteristics, with a few exceptions including age, solid food introduction at stool sample collection, and asthma/recurrent wheeze at age 3 years (see [Table E2](#) in this article's Online Repository at www.jacionline.org). Of several potential determinants of the intestinal microenvironment analyzed, only mode of delivery differed by phenotype, with a higher percentage of subjects born by Cesarean section

METHODS

Patient samples

Institutional review board approval was obtained for this study. All reported results of WASP testing performed at the Diagnostic Immunology Laboratory at Cincinnati Children's Hospital between June 2010 and October 2016 were retrieved. After removing incomplete or repeated entries, entries with a bimodal staining pattern in which mean channel fluorescence (MCF) cannot be correctly determined, and selecting only for entries with completed Sanger sequencing of *WAS*, 49 samples with genetically confirmed *WAS* mutations and 58 samples with no apparent mutation in *WAS* were collected. From these 107 samples, analyses of diagnostic accuracy were performed. All but 1 sample with a mutation in *WAS* were male. The only female sample was a heterozygous carrier with normal levels of WASP staining and therefore included in the control group for analysis.

WASP staining

Whole-blood samples from patients and healthy controls were surface stained for CD45 (BD Biosciences, La Jolla, Calif), fixed, and then permeabilized before being incubated with anti-WASP antibody (BD Biosciences, clone 5A5).^{E1} Cells were acquired on a FACS Canto II (BD Biosciences) and analyzed with FCS Express (v4, De Novo Software, Glendale, Calif). Results were expressed as the gated lymphocyte arithmetic MCF ratio of WASP from the patient against the lymphocyte MCF of WASP from a freshly drawn healthy control.

In samples analyzed for posttransplant engraftment screening, additional antibodies against CD3 (clone SK7), CD14 (MφP9), CD19 (SJ25C1), and CD56 (MY31) were added into the surface staining step. For this assay, results were expressed as the percentage WASP-positive cells for each leukocyte subpopulation.

Sequencing

Genomic DNA from peripheral whole blood was isolated followed by PCR-based amplification and bidirectional Sanger sequencing (Applied Biosystems, Foster City, Calif). Exons including intron boundaries of the *WAS* transcript NM_000377.1 were captured and sequenced. Mutations in untranslated exons,

regulatory, and further into introns were not sequenced. Large deletions involving 1 or more whole exons were detected using comparative genomic hybridization array using custom 180K array (Agilent Technologies, Inc, Santa Clara, Calif) and CytoSure (Oxford Gene Technology, Oxfordshire, United Kingdom). Single-nucleotide substitutions, small deletions, and insertions were analyzed using Sequencher software (GeneCodes, Ann Arbor, Mich). Variants were mapped to human genome reference sequence (hg19). Where variants fell below 1% occurrence in frequency, they were analyzed with gnomAD database (gnomad.broadinstitute.org), SIFT, MutationTaster, and PolyPhen-2 *in silico* predictions.^{E2-E4} Finally, all variants were classified into pathogenic, benign, or uncertain clinical significance (VUCS) on the basis of published evidence of function in literature of the specific variants and American College of Medical Genetics guidelines.^{E5} *In silico* prediction were not concordant, suggesting the need for improvements.

Statistics

Graphs, receiver operating characteristic curves, and accuracy calculations were created using Prism (v7, GraphPad, La Jolla, Calif). Optimal cutoff value was determined by the point on the receiver operating characteristic curve with the largest sensitivity + specificity combined value.

REFERENCES

- E1. Kawai S, Minegishi M, Ohashi Y, Sasahara Y, Kumaki S, Konno T, et al. Flow cytometric determination of intracytoplasmic Wiskott-Aldrich syndrome protein in peripheral blood lymphocyte subpopulations. *J Immunol Methods* 2002;260:195-205.
- E2. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009;4:1073-81.
- E3. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods* 2014;11:361-2.
- E4. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7:248-9.
- E5. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405-24.

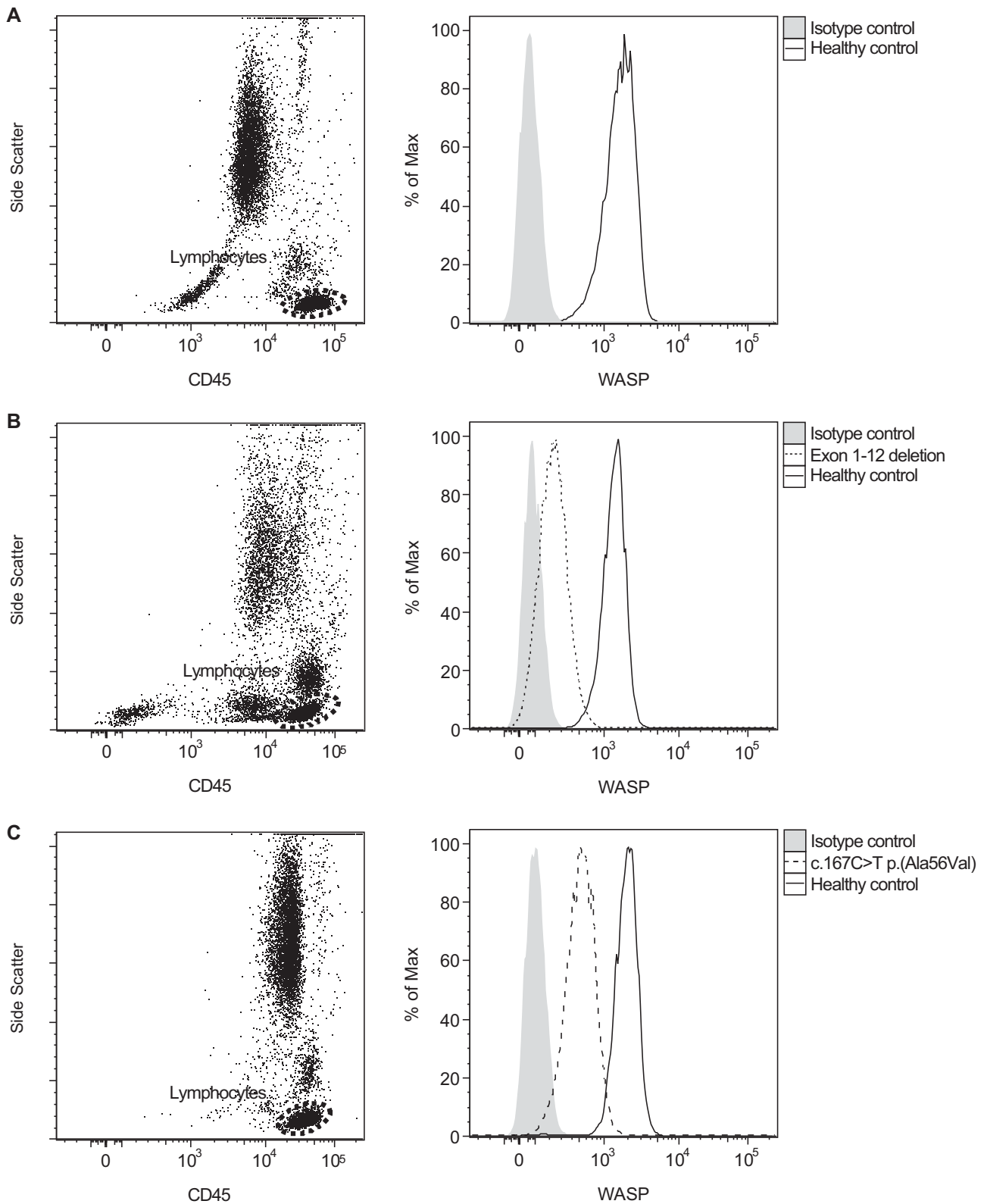


FIG E1. Gating protocol and WASP expression examples. Representative plots of **(A)** healthy control showing normal WASP expression. **B** and **C**, Patients with WAS with varying levels of WASP staining. Plots combined in [Fig 1, A](#).

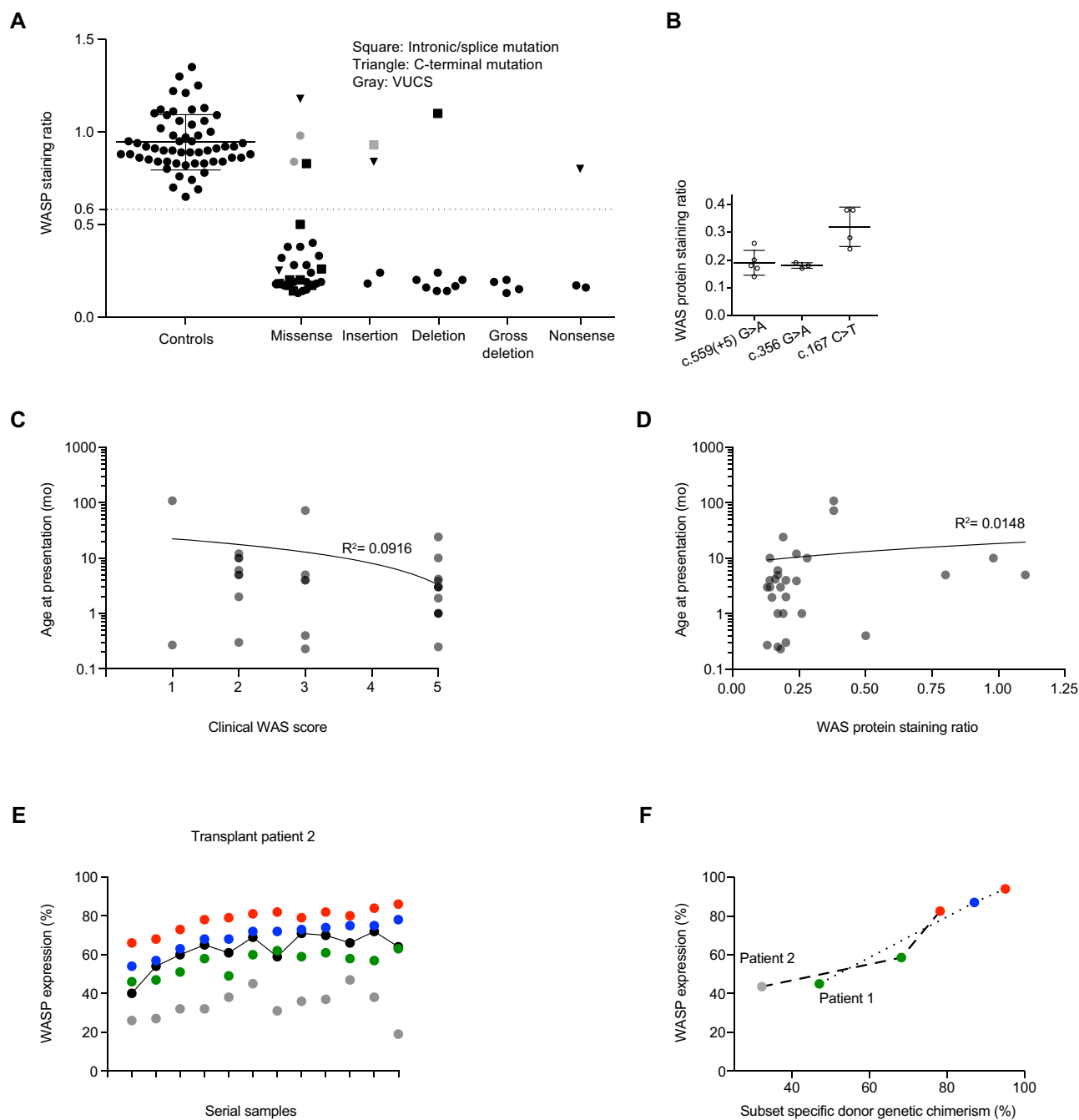


FIG E2. Further characterization of the WAS cohort. **A**, WASP staining index of 107 patients divided by genetic findings. **B**, Reproducible staining results when comparing patients with similar mutations. **C** and **D**, Poor association seen between WASP expression, WAS clinical score, and age of presentation. **E**, Posttransplant patient studied longitudinally showing correlated blood genetic chimerism and WASP levels. **F**, Isolated lymphocyte subsets show similar levels of genetic chimerism and WASP expression.

TABLE E1. WASP staining, WAS genetic evaluation, and WAS scores of patients included in this study

No.	WASP stain index	Presentation age	Variant reported	PMID	dbSNP frequency (%)	genomAD	SIFT	Mutation Taster score	Poly Phen-2	WAS score	Lowest platelet count $\pm$ 6 mo of WASP sampling	Eczema	Infections	Autoimmune disease
Missense/ intronic														
1	0.28	10 mo	c.134C>T p.(Thr45Met)	7753869, 19817875, 23160469 25525159	rs132630273	NA	0.01	Disease causing	1	2	8 K/mcL	Mild	Minor	None
2	0.28	NA	c.167C>T p.(Ala56Val)	7795648, 19817875, 25091438	rs132630269	NA	0.24	Disease causing	1	NA				
3	0.38	9 y	c.167C>T p.(Ala56Val)							2	86 K/mcL	Mild	None	None
4	0.24	NA	c.167C>T p.(Ala56Val)							NA				
5	0.38	6 y	c.167C>T p.(Ala56Val)							3	30 K/mcL	Persistent but manageable	Minor	None
6	0.16	NA	c.193A>C p.(Thr65Pro)	NA	NA	NA	0.14	Polymorphism	0.990	NA				
7	0.33	NA	c.229G>A p.(Asp77Asn)	NA	NA	NA	0	Disease causing	0.994	NA				
8	0.40	NA	c.229G>A p.(Asp77Asn)						NA					
9	0.32	NA	c.231T>A p.(Asp77Glu)	NA	NA	NA	0	Disease causing	0.999	NA				
10	0.13	1 wk	c.245C>A p.(Ser82Tyr)	NA	NA	NA	0	Disease causing	1	1	37 K/mcL	None	None	None
11	0.19	2 y	c.257G>A p.(Arg86His)	8069912, 19817875, 23160469,	rs132630268	NA	0.01	Disease causing	1	1	9 K/mcL	Persistent but manageable	None	HSP
12	0.13	NA	c.319T>A p.(Tyr107Asn)	NA	NA	NA	0.1	Polymorphism	1	NA				
13	0.18	3 mo	c.356G>A p.(Gly119Glu)	8931701, 19817875	NA	NA	0.34	Disease causing	1	5	33 K/mcL	Mild	Recurring	ITP
14	0.19	1 mo	c.356G>A p.(Gly119Glu)							5	25 K/mcL	Mild	Recurring	ITP
15	0.17	1 mo	c.356G>A p.(Gly119Glu)							5	32 K/mcL	Mild	Recurring	ITP
16	0.18	1 wk	c.383T>C p.(Phe128Ser)	8931701	NA	NA	0.01	Disease causing	1	3	51 K/mcL	Persistent but manageable	Recurring	None
17	0.19	NA	c.397G>A p.(Glu133Lys)	7753869, 19817875	NA	NA	0.04	Disease causing	1	NA				

(Continued)

TABLE E1. (Continued)

No.	WASP stain index	Presentation age	Variant reported	PMID	dbSNP frequency (%)	genomAD	SIFT	Mutation Taster score	Poly Phen-2	WAS score	Lowest platelet count $\pm$ 6 mo of WASP sampling	Eczema	Infections	Autoimmune disease
18	0.83	NA	c.463+3G>C p.?	NA	NA	0.0019	Predicted loss at donor site 3bp upstream (34.8%)			NA				
19	0.17	6 mo	c.506-1G>C p.?	Skip of exon 6 is likely, MaxEnt, NNSPLICE, and HSF algorithms predict loss of acceptor site (100%)						2	80 K/mcL	Mild	Recurring	None
20	0.98	NA	c.538C>A p.(His180Asn)	20173115, 24728327	NA	0.095	NA	NA	NA	2	180 K/mcL	None	Minor	None
21	0.17	5 mo	c.559+5G>C p.?	NA	ClinVar -Likely pathogenic		NA	NA	NA	2	21 K/mcL	Mild	None	None
22	0.20	4 mo	c.559+5G>C p.?							2	12 K/mcL	Persistent but manageable	Recurring	None
23	0.26	1 mo	c.559+5G>A p.?	9326235, 25525159	ClinVar -Likely pathogenic		NA	NA	NA	5	45 K/mcL	Mild	Minor	ITP
24	0.18	NA	c.559+5G>A p.?							NA				
25	0.14	10 mo	c.559+5G>A p.?							5	34 K/mcL	Mild	Minor	ITP
26	0.20	NA	c.778-2A>G p.?	8595430	NA	NA	NA	NA	NA	NA				
27	0.84	NA	c.995T>C p.(Val332Ala)	17400488, 22995991, 24728327, 24369837,	rs2737799	0.47	0.4	Polymorphism	0	NA				
28	1.18	NA	c.1315C>T p.(Arg439Trp)	NA	NA	NA	0.01	Disease causing	1	NA				

(Continued)

TABLE E1. (Continued)

No.	WASP stain index	Presentation age	Variant reported	PMID	dbSNP frequency (%)	genomAD	SIFT	Mutation Taster score	Poly Phen-2	WAS score	Lowest platelet count $\pm$ 6 mo of WASP sampling	Eczema	Infections	Autoimmune disease
29	0.50	NA	c.1339-2A>G p.?	Skip of exon 11 is likely. MaxEnt, NNSPLICE, and HSF algorithms predict loss of acceptor site (100%)						3	14 K/mcL	Persistent but manageable	None	None
30	0.25	NA	c.1453G>A p.(Asp485Asn)	11298372, 19817875,	RCV000 482823.1		0	Disease causing	1	NA				
Gross deletion														
31	0.13	3 mo	Partial gene hemizygous deletion Exon 1-Intron 1 by array CGH arr[hg19] Xp11.23 (48,533,049-48, 542,539)x0							5	20 K/mcL	None	Recurring	AIHA
32	0.15	NA	Full gene hemizygous deletion Exon 1-Exon 12. Confirmed by array CGH arr[hg19] Xp11.23 (48,533,302-48, 613,923)x0							5	15 K/mcL	Difficult to control	Recurring	ITP
33	0.19	NA	Full gene hemizygous deletion Exon 1-Exon 12. Confirmed by array CGH arr[hg19] Xp11.23 (48,533,302-48, 613,923)x0							NA				
34	0.20	2 mo	Presumed whole gene deletion.							2	2 K/mcL	None	Minor	None

(Continued)

TABLE E1. (Continued)

No.	WASP stain index	Presentation age	Variant reported	PMID	dbSNP frequency (%)	genomAD	SIFT	Mutation Taster score	Poly Phen-2	WAS score	Lowest platelet count $\pm$ 6 mo of WASP sampling	Eczema	Infections	Autoimmune disease
Insertion														
35	0.93	NA	c. 273(+11_+12) ins C		rs782793722	0.46	NA	NA	NA	NA				
36	0.24	NA	p.? c.827_828 ins22	NA	NA	NA	NA	NA	NA	2	40 K/mcL	Mild	Minor	None
37	0.18	NA	p.? c.1271dup	8528198	NA	NA	NA	NA	NA	NA				
38	0.84	NA	p.(Leu425Profs*70) c.1434_1436dup		NA	NA	NA	NA	NA	NA				
			p.(Ser478dup)											
Deletion														
39	0.14	4 mo	c.101delG	NA	NA	NA	NA	NA	NA	3	23 K/mcL	Persistent but manageable	None	None
			p.(Arg34Hisfs*11)											
40	0.16	4 mo	c.107_108delTT	NA	NA	NA	NA	NA	NA	5	1 K/mcL	Persistent but manageable	Minor	None
			p.(Phe36X)											
41	0.20	1 wk	c.336 delC	15284122	NA	NA	NA	NA	NA	2	16 K/mcL	Mild	None	None
			p.(Phe113Serfs*14)											
42	0.14	3 mo	c.391_407del17	NA	NA	NA	NA	NA	NA	5	17 K/cmL	Persistent but manageable	Minor	ITP, colitis
			p.(Glu131Leufs*32)											
43	1.1	5 mo	c.777+3_777 +6delGAGT, p.?	10737997	NA	NA	NA	NA	NA	2	5 K/mcL	Mild	Minor	None
44	0.16	NA	c.852delC	NA	NA	NA	NA	NA	NA	NA				
			p.(Glu285Argfs*23)											
45	0.24	4 mo	c.1114_1115del	NA	NA	NA	NA	NA	NA	5	6 K/mcL	None	Life threatening	ITP, IAHA
			p.(Pro372Serfs*122)											
46	0.20	NA	c.1271delG	7753869	NA	NA	NA	NA	NA	NA				
			p.(Gly424Alafs*21)											
Nonsense														
47	0.17	1 wk	c.607C>T	8682510	NA	NA	NA	NA	NA	5	45 K/mcL	Mild	Minor	Autoimmune neutropenia
			p.(Q203*)											
48	0.14	NA	c.862A>T	21488158, 25525159	NA	NA	NA	NA	NA	NA				
			p.(Lys288*)											
49	0.80	5 mo	c.1468C>T	NA	NA	NA	NA	NA	NA	3	18 K/mcL	Persistent but manageable	Minor	None
			p.(Gln490*)											

SIFT score [0-1]: Deleterious score ≤ 0.05 , Tolerated >0.05 .

PolyPhen-2 score [0-1]: Benign, 0-0.15; Probably damaging, 0.85-1.

dbSNP, Single Nucleotide Polymorphism database; genomAD, Genome Aggregation Database; NA, not available; SIFT, Sorting Intolerant from Tolerant.

TABLE E2. Genetic chimerism and WASP-positive leukocyte subset staining in transplanted patients

Transplant patient no.	Sample no.(chronological)	Overall donor genetic chimerism	Percentage of WASP-positive cells per subset			
			T cell	NK cells	B cells	Monocytes
1	1	86	94	87	45	62
	2	60	96	76	21	31
	3	41	76	73	13	27
	4	47	94	74	19	22
	5	99	99	100	99	99
	6	100	99	100	100	100
	7	98	98	100	99	100
2	1	40	66	46	54	26
	2	54	68	47	57	27
	3	60	73	51	63	32
	4	65	78	58	68	32
	5	61	79	49	68	38
	6	66	80	58	75	47
	7	69	81	60	72	45
	8	71	79	59	73	36
	9	72	84	57	75	38
	10	70	82	61	74	37
	11	59	82	62	72	31
	12	64	86	63	78	19