HHS Public Access

Author manuscript

J Allergy Clin Immunol. Author manuscript; available in PMC 2017 October 01.

Published in final edited form as:

J Allergy Clin Immunol. 2016 October ; 138(4): 1222–1224.e2. doi:10.1016/j.jaci.2016.05.007.

Distinct mutations at the same positions of STAT3 cause either Loss or Gain of Function

Prabha Chandrasekaran, Ph.D[a,b,*], **Ofer Zimmerman, M.D**[a,*], **Michelle Paulson, M.D**[a], **Elizabeth P. Sampaio, M.D, Ph.D**[a], **Alexandra F. Freeman, M.D**[a], **Kathryn J. Sowerwine, M.D**[a], **Darell Hurt, Ph.D**[c], **Julio C. Alcántara-Montiel, M.D, Ph.D**[d], **Amy P. Hsu, B.A**[a], and **Steven M. Holland, M.D**[a]

[a]Immunopathogenesis Section, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland, USA

[b]Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland, USA

[c]Bioinformatics and Computational Biosciences Branch (BCBB), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland, USA

[d]Department of Molecular Biomedicine, CINVESTAV-IPN, Mexico City, Mexico

Keywords

STAT3; HIES; Job's syndrome; Large Granular Lymphocytic leukemia (LGL); autoimmunity

To the Editor

Signal transducer and activator of transcription 3 (STAT3) directly or indirectly regulates the pathways of IL-6, IL-10, IL-11, IL-17, IL-23, G-CSF, M-CSF, and leptin, among others. *STAT3* has 24 exons, which are spliced into three isoforms, resulting in proteins of 722 or 770 amino acids[1]. Dominant negative (DN) *de novo* and inherited germline mutations in *STAT3* predominantly in the DNA Binding domain (DBD) and Src Homology 2 (SH2) domains of STAT3 cause Hyper-IgE (Job's or HIES) syndrome[2, 3]. Recently, somatic mutations in *STAT3* have been found in up to 40% of patients with large granular lymphocytic leukemia (LGLL)[4], so far all in the SH2 domain. More recently, germline gain of function (GOF) mutations in *STAT3* were shown to underlie multi-organ immune disease with immunodeficiency and lymphoproliferation[5]. *STAT3* mutations have also been

Corresponding Author: Steven Holland, M.D., CRC B3-4141, MSC 1684, Bethesda, Maryland 20892-1684, 301-402-7684 voice, 301-480-4507 fax, smh@nih.gov.

*Equal contributors

Disclosure of potential conflict of interest

The authors declare no potential conflict of interest.

identified in leukemia[6], lymphoproliferative disorders[7], aplastic anemia and autoimmune disorders[8, 9]. Dominant negative STAT3 mutations cause defective IL-6 and IL-10 signaling and diminished DNA-binding[2]. In contrast, GOF mutations are hypermorphic with higher DNA-binding, leading to aberrant STAT1 and STAT5 signaling and decreased Treg numbers[8].

Missense mutations and deletions have been reported affecting at least 89 different positions in *STAT3* (see Figure E1 in online repository). Most of the reported mutations have been in the DBD and the SH2 domains of *STAT3*, where both hypomorphic and hypermorphic mutations have been reported, with some positions supporting both hypomorphic and hypermorhphic functions, depending on the amino acid.

We identified six positions in the SH2 domain at which distinct point mutations have been reported to cause either HIES or lymphoproliferation/autoimmunity (Table 1). Polyphen-2 (Polymorphism Phenotyping v2) predictions showed that all of the substitutions were possibly or probably damaging, except the arginine substitution at position 647 which was predicted to be benign (Table 1). We functionally characterized the HIES mutations N647D and K658E and their leukemia and autoimmunity counterparts N647I and K658N. We transfected Cos-7 cells with plasmids encoding wild type (WT) or mutant alleles and stimulated them with Oncostatin M (OSM). Upon stimulation with OSM, the levels of phosphorylated STAT3 (Y705; pSTAT3) were higher for GOF N647I and K658N and lower for DN N647D and K658E alleles, compared to the WT *STAT3* transfected cells (Figure 1A, for methods see the online repository). Stimulation of transfected Cos-7 cells with various concentrations of IL-6 demonstrated a similarly clear demarcation between hypomorphic and hypermorphic *STAT3* alleles (Figure 1B). Further, transfected Cos-7 cells showed a dose-dependent response in the WT and GOF mutants while the response of the DN mutants was profoundly blunted (Figure 1B). Transfection of U3A cells was in agreement with the Cos-7 data of the STAT3 alleles (Figure 1C).

Three-dimensional protein structural analyses showed that the mutated amino acid substitutions radically changed the electrostatic potentials in the mutated positions. Despite the benign prediction by Polyphen-2 analysis, the DN mutation N647D changes the electrostatic potential to be much more negative, which might adversely affect DNA binding affinity (Figure 1D). In contrast, the GOF N647I mutation reshapes the binding pocket and removes the localized positive electrostatic potential. The DN missense mutation K658E alters both the local molecular surface shape and the local electrostatic potential from slightly positive to significantly negative, which would probably disrupt binding of STAT3 to DNA. In contrast, GOF K658N does not alter the molecular landscape nearly as much and the electrostatic potential shift is not nearly as strong as the K658E (Figure 1D).

These structural and functional analyses provide in vitro functional confirmation and molecular predictions of different nonsynonymous mutations in *STAT3* at exactly the same positions that can be either hypomorphic or hypermorphic. Therefore, gain and loss of function mutations in *STAT3* are not so much a function of the region of *STAT3* in which they occur as they are a function of their effect on local charge and DNA binding affinity. Presumably, similar variations occur in regions of STAT3 involved in protein-protein

interactions, as well. Rational classification of STAT3 mutations hence requires functional analyses in conjunction with the clinical diagnosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Declaration of all sources of funding: This research was supported by the Division of Intramural Research, NIAID, NIH and in part by the National Cancer Institute, National Institutes of Health (contract HHSN261200800001E), Bethesda, MD 20892. The content of this article are those of the authors and do not reflect the official policy of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

List of Abbreviations

DBD	DNA Binding Domain
DN	Dominant Negative
GOF	Gain of Function
HIES	Hyper-IgE syndrome
LGLL	Large Granular Lymphocytic Leukemia
OSM	Oncostatin M
SH2	Src Homology 2
STAT	Signal transducer and activator of transcription

References

1. Ren Z, Mao X, Mertens C, Krishnaraj R, Qin J, Mandal PK, et al. Crystal structure of unphosphorylated STAT3 core fragment. Biochemical and Biophysical Research Communications. 2008; 374:1–5. [PubMed: 18433722]
2. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 mutations in the hyper-IgE syndrome. N Engl J Med. 2007; 357:1608–19. [PubMed: 17881745]
3. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature. 2007; 448:1058–62. [PubMed: 17676033]
4. Koskela HL, Eldfors S, Ellonen P, van Adrichem AJ, Kuusanmäki H, Andersson EI, et al. Somatic STAT3 mutations in large granular lymphocytic leukemia. N Engl J Med. 2012; 366:1905–13. [PubMed: 22591296]
5. Flanagan SE, Haapaniemi E, Russell MA, Caswell R, Lango Allen H, De Franco E, et al. Activating germline mutations in STAT3 cause early-onset multi-organ autoimmune disease. Nat Genet. 2014; 46:812–4. [PubMed: 25038750]
6. Ohgami, RS.; Ma, L.; Merker, JD.; Martinez, B.; Zehnder, JL.; Arber, DA. Leukemia. England: 2013. STAT3 mutations are frequent in CD30+ T-cell lymphomas and T-cell large granular lymphocytic leukemia; p. 2244-7.
7. Jerez A, Clemente MJ, Makishima H, Koskela H, LeBlanc F, Peng Ng K, et al. STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia. Blood. 2012; 120:3048–57. [PubMed: 22859607]

8. Milner JD, Vogel TP, Forbes L, Ma CA, Stray-Pedersen A, Niemela JE, et al. Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. Blood. 2015; 125:591–9. [PubMed: 25359994]

9. Haapaniemi EM, Kaustio M, Rajala HLM, van Adrichem AJ, Kainulainen L, Glumoff V, et al. Autoimmunity, hypogammaglobulinemia, lymphoproliferation, and mycobacterial disease in patients with activating mutations in STAT3. Blood. 2015; 125:639–48. [PubMed: 25349174]

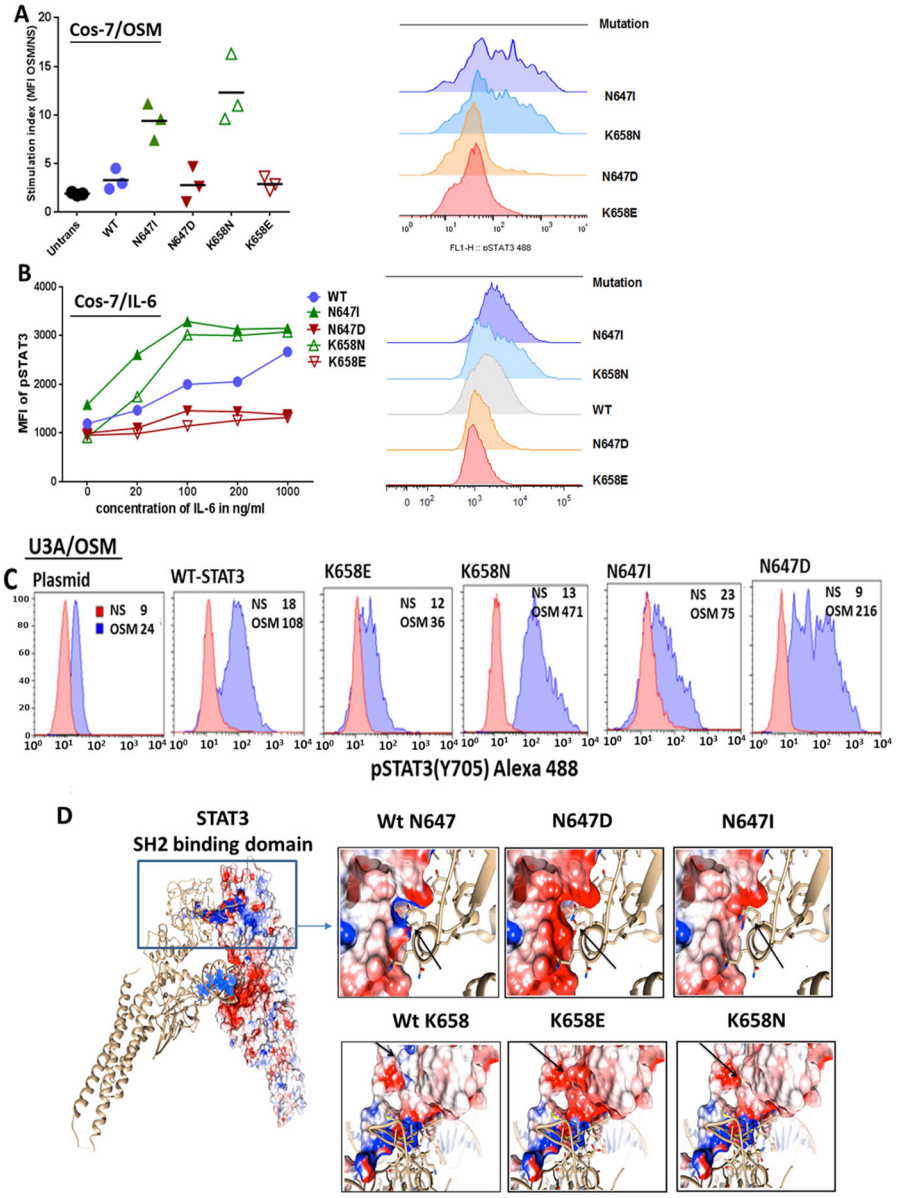

Figure 1. Functional characterization of STAT3 mutations

Wild type (WT) or mutant *STAT3* allele transfected Cos-7 (A&B) or U3A (C) cells were assessed for pY(705)STAT3 by flow cytometry after stimulation with Oncostatin M or varying concentrations of IL-6. **(D)** Shows the electrostatic potential near the WT or mutated STAT3 residues and their DNA binding. Negative charge is shown in red, positive in blue.

Table 1

Gain of function and dominant negative mutations occurring in at the same positions in STAT3 protein with initial citations. The PolyPhen-2 scores and their predictions indicate the effects of single residue changes in the structure and function of STAT3.

Mutation*	Disease	PolyPhen-2	
		Score	Prediction
S614G [E1]	HIES	0.994	Probably damaging
S614R [E2]	T-LGL	1.000	
G617E [E3]	HIES	1.000	Probably damaging
G617V [E1]	HIES	0.998	
G617R [E4]	DLBCL	1.000	
G618D [E5]	HIES	0.892	Possibly damaging
G618R [E2]	NK-LGL	0.983	
N647D [E6]	HIES	0.037	Benign
N647I [E7]	LGLL	0.395	
Y657C [E6]	HIES	1.000	Probably damaging
Y657S [E8]	HIES	0.999	
Y657N [E9]	HIES	0.999	
Y657ins [E10]	γδ T lymphoma		
Y657dup [E11]	T-LGL		
Y657_M660dup [E12]	IHCA, CHC1021T		
Y657_K658insY [E7]	LGLL		
K658M [E2]	T-LGL	0.994	Probably damaging
K658N [E7]	LGLL/autoimmunity	0.990	
K658Y [E12]	IHCA; CHC379T	0.956	Possibly damaging
K658E [E13]	HIES	0.926	

HIES-Hyper-IgE syndrome, DLBCL-Diffuse Large B cell Lymphoma, LGLL- Large granular lymphocytic leukemia, T-LGL- T-cell large granular lymphocytic leukemia, NK-LGL- Natural killer cell large granular lymphocytic leukemia; IHCA- Inflammatory hepatocellular adenomas.

*see online repository (Supplementary Text) for the references.