Final report – Job Research Foundation – 2019-2022

Defining the mechanism of *STAT3* dominance underlying Job's syndrome

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Dissecting the mechanism of STAT3 dominance underlying Job's Syndrome

Project Summary

The autosomal dominant (AD) form of Job's syndrome is caused by mono-allelic mutations in *STAT3*, whereas the autosomal recessive (AR) form is caused by bi-allelic mutations in *ZNF341*. Both genes encode transcription factors. STAT3 normally resides in the cytoplasm and translocates into the nucleus, upon activation, as a phosphorylated dimer that binds promoters containing a specific DNA motif, whereas ZNF341 dimers reside in the nucleus, where they constitutively bind promoters containing another DNA motif. ZNF341 controls the expression of various genes including *STAT3* itself, especially by STAT3-activating cytokines (auto-induction). ZNF341 deficiency thus underlies a drastic reduction of STAT3 transcription, expression, activation, and activity. **Diminished levels of STAT3 activity therefore define the unifying mechanism underlying Job's syndrome**. However, only very few predicted pathogenic mutations in *STAT3* have been experimentally proven to be loss-of-function, and even fewer have been proven to be dominant by a mechanism of negative dominance (*n=5*). Moreover, recent reports of *STAT3* alleles predicted to be loss-of-expression paradoxically suggest that Job's syndrome might also occur by haplo-insufficiency. **Overall, more than ten years after the discovery of *STAT3* mutations, the biochemical basis of Job's syndrome surprisingly remains largely unknown**. What is the relationship between the levels of STAT3 expression vs. STAT3 activity in the patients' heterozygous cells? Do the biological and clinical features of Job's syndrome manifest when the levels of STAT3 activity are at 50% (haplo-insufficiency) or at 25% (negative dominance)? To address these fundamental questions of considerable clinical relevance, **we will test the hypothesis that all types of *STAT3* pathogenic mutations operate by negative dominance, including those predicted to be loss-of-expression, and that there is no haplo-insufficiency at the *STAT3* locus.** We will test this general hypothesis by tackling the following three specific aims: **(1)** We will study the dominance and its mechanism for all *STAT3* variations reported in public and Job's databases, as well as an engineered complete deletion of the *STAT3* locus. We will do so by transfecting recipient epithelial cell lines that constitutively lack STAT3, as well as in heterozygous B cells and T cells upon stimulation with CD40L+IL21 and CD2/CD3/CD28; **(2)** We will test the basal and inducible expression of STAT3 and it activity in cells heterozygous and homozygous for ZNF341 mutations, compared with cells from healthy controls and patients with heterozygous *STAT3* mutations. We will use various cells, such as primary T cells, primary B cells, and primary fibroblasts; and **(3)** We will measure the negative selection that operates at the human *STAT3* locus by combining various computational scores. We will compare *STAT3* with genes known to underlie primary immunodeficiencies that are dominant by haplo-insufficiency vs. those that operate through negative-dominance. **Collectively, our approach will decipher the biochemical basis of Job's syndrome**, facilitating genetic diagnosis and counseling, as well as paving the way for the study of the cellular basis of the biological and clinical manifestations of the disease, and the development of novel preventive or therapeutic approaches.

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Aim 1: Mechanism of STAT3 dominance in Job's syndrome

During the 3 last years, we investigated the functional impact of all mutations associated with Hyper IgE syndrome (HIES), and the impact of the most common variants reported in the general population. We showed that all STAT3 mutations underlying an autosomal dominant (AD) form of HIES, even mutations creating a premature stop codon, do so by negative dominance, as opposed to haplo-insufficiency. The 'stop mutations' are dominant-negative via one of three gene products: (i) the C-truncated protein itself, (ii) an N-truncated protein due to re-initiation of translation, or (iii) an isoform generated by alternative splicing. This is consistent with Yoshi Minegishi's breakthrough and classic 2007 paper in *Nature* (https://nature.com/articles/nature06096). We also found that 7 of the 150 mutations are not loss-of-function and dominant negative in our assay. It could suggest that these mutations have been erroneously associated to HIES. The clinical implications are clear and STAT3 mutations must be biochemically tested thoroughly, including for negative dominance to reach a diagnosis of HIES. The biological implications are also clear and indicate that half of STAT3 is apparently enough, while lower amounts dangerously reduce fitness. Overall, our work provides an overarching view of STAT3 variants in patients with HIES and the general population and establishes that negative dominance is the only mechanism of dominance underlying AD-HIES in patients heterozygous for pathogenic STAT3 variants. All these data are now published in *Journal of Experimental Medicine* in the August 2021 issue.

Asano et al. J. Exp. Med. (2021) 218 (8); https://doi.org/10.1084/jem.20202592

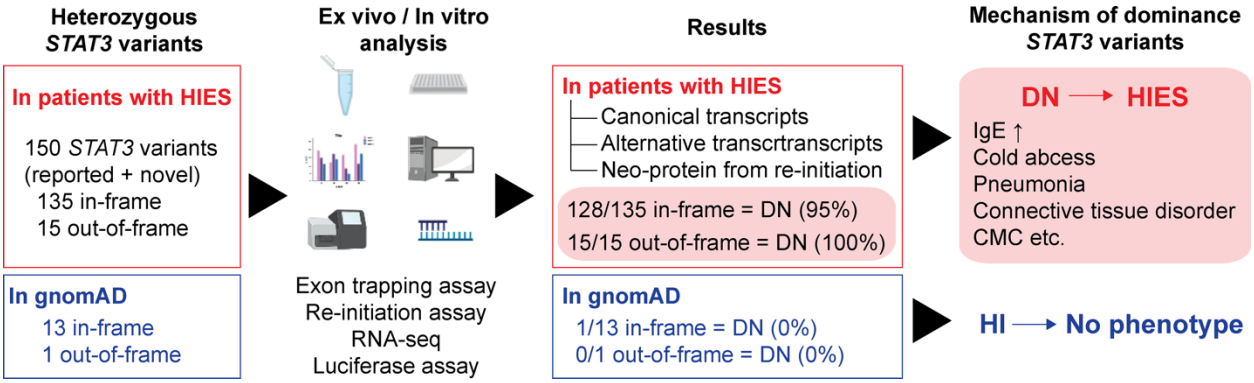

Figure 1: Graphical abstract published in "Human STAT3 variants underlie autosomal dominant hyper-IgE syndrome by negative dominance"

Aim 2: Autosomal dominant IL6ST deficiency: A new genetic etiology of Job's syndrome

In our Aim 2, we initially planned to provide a detailed analysis of the levels of STAT3 associated with clinical phenotypes in HIES patients. We have reoriented our efforts following an important discovery related to HIES. Indeed, we recently identified a novel genetic etiology of autosomal dominant HIES in patients without mutations in *STAT3* or *ZNF341*. We found twelve patients from eight unrelated kindreds with AD HIES carrying heterozygous *IL6ST* mutations. *IL6ST* encodes GP130, the common co-receptor to all "IL-6 family" cytokine members, notably including IL-6, IL-

11, IL-27, LIF and OSM. Biallelic *IL6ST* autosomal recessive (AR) mutations were previously reported in only two patients with AR HIES (Schwerd et al. 2017; Shahin et al. 2019). In our study, we identified eight different truncating mutations of GP130, one of which was recurrent. Intriguingly, the mutant alleles encode GP130 receptors with the transmembrane domain but lack both the recycling motif and all four STAT3-recruiting tyrosine residues. Upon overexpression, the mutant proteins accumulate at the cell surface and are loss-of-function and dominant negative for cellular responses to IL-6, IL-11, LIF, and OSM. Moreover, the patients' heterozygous leukocytes and fibroblasts respond poorly to IL-6 and IL-11 but retain a normal response to LIF and OSM. Consistently, patients with *STAT3* and *IL6ST* mutations display infectious and allergic manifestations of AR IL-6R deficiency, some of the skeletal abnormalities of AR IL-11R deficiency, but no common features with Stüve Wiedemann syndrome (AR LIF-R deficiency). Dominant negative *STAT3* and *IL6ST* mutations thus appear to underlie clinical phenocopies through impairment of the IL-6 and IL-11 response pathways. These data are now published in *Journal of Experimental Medicine* in the March 2020 issue.

Béziat et al. *J Exp Med* (2020) 217 (6): e20191804 ; https://doi.org/10.1084/jem.20191804

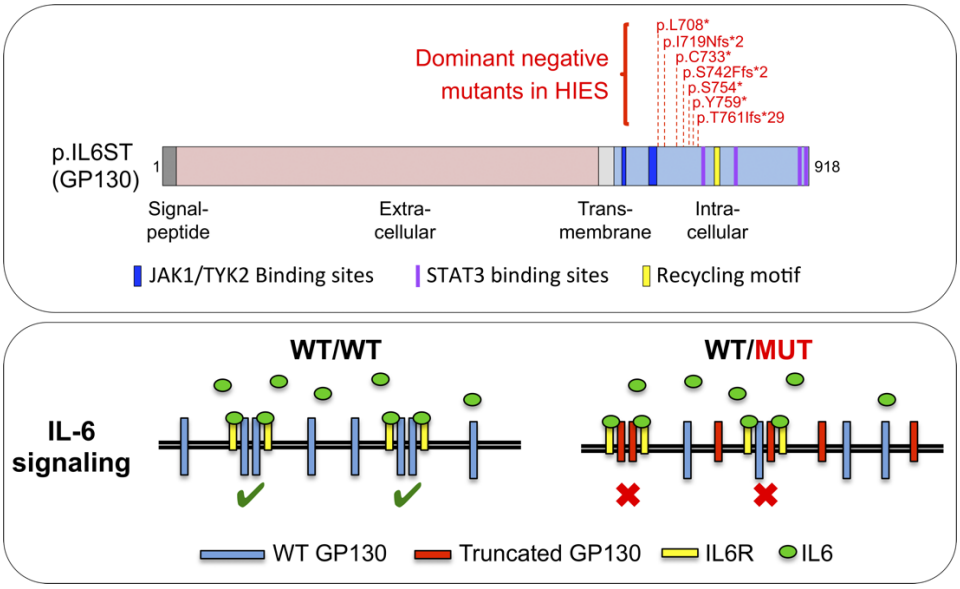

Figure 2: Graphical abstract published in "*Dominant-negative mutations in human IL6ST underlie hyper-IgE syndrome"*

Negative (or purifying) selection is the natural process by which deleterious alleles are selectively purged from the population (Loewe 2008). Variants of human genes that cause severe diseases are expected to be the primary targets of negative selection, particularly for diseases affecting heterozygous individuals. Nevertheless, the extent to which negative selection affects known human disease-causing genes and the factors determining its strength remain largely unknown,

Aim 3: Study population genetics of STAT3

particularly because our knowledge of the severity, mode, and mechanism of inheritance of human diseases remains incomplete (Bustamante et al. 2005). We developed a new measurement, consensus negative selection (CoNeS), to take into account information from both interspecies (the *ƒ* parameter from SnIPRE (Eilertson et al. 2012), lofTool (Fadista et al. 2017) and evoTol (Rackham et al. 2015)) and intraspecies (RVIS (Petrovski et al. 2013)), LOEUF (Karczewski et al. 2019), pLI (Lek et al. 2016) and SIS (Aggarwala and Voight 2016)) statistics measuring the strength of negative selection. CoNeS was obtained through a standardized (i.e. mean of 0 and SD of 1) projection of these seven methods on the first principal component, which captures 81.2% of the total variance. We found that genes underlying autosomal dominant (AD) or X-linked Inborn Error of Immunities (IEI) are under stronger negative selection than those underlying autosomal recessive (AR) IEI, which are under no stronger selection than genes not known to be disease-causing. However, we find that genes with mutations causing AR IEI, which are lethal before reproductive maturity and that display complete penetrance, are under stronger negative selection than other genes underlying AR IEI. We also found that genes underlying AD IEI by haploinsufficiency are under stronger negative selection than genes underlying AD IEI through other mechanisms. STAT3 has a CoNeS of -1.94, with only 1.45% of the genes being under stronger negative selection. Only three genes underlying IEI are under stronger negative selection: ACTB, CHD7 and KMT2D with CoNeS of -2.16, -2.08 and -2.94 respectively. All three of these genes underly autosomal dominant immunodeficiencies that operate by haploinsufficiency. These data are now published in *Proc Natl Acad Sci USA* in the January 2021 issue.

Rapaport *et al.* PNAS (2021) 118 (3): e2001248118; https://doi.org/10.1073/pnas.2001248118

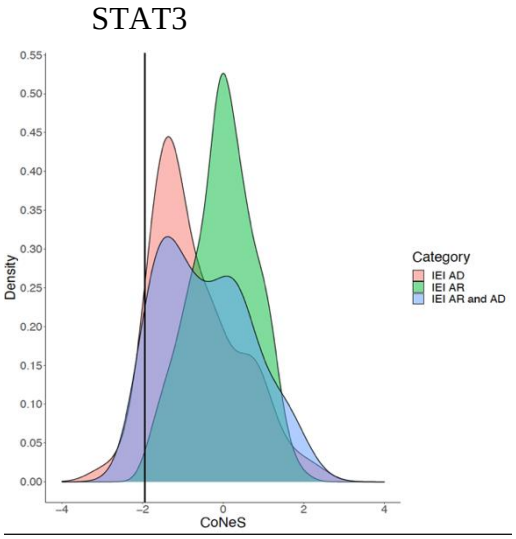

Figure 3: CoNeS analysis. CoNeS is our custom-built score integrating known negative selection scores through a principal-component projection. The mean value is 0 and low values (negative values) correspond to strong negative selection. The vertical black bar indicates STAT3 CoNeS.

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