

Comment on Bocchini *et al* 2016

When half a glass of STAT3 is just not enough.

Arian D. J. Laurence¹ and Holm H. Uhlig^{1,2}

¹ Translational Gastroenterology Unit, Nuffield Department of Medicine, John Radcliffe Hospital, and ² Department of Paediatrics, Oxford University.

*In this issue of Blood, Bocchini et al report a novel mechanism by which STAT3 mutations result in an unstable protein and give rise to a reduction in STAT3 signalling, suggesting that pathogenic mutations do not always confer dominant negative effects via forming of non-functional STAT3 dimers but some may limit availability of total protein causing STAT3 haploinsufficiency*¹.

Job's or Hyper IgE syndrome (HIES) is a multisystem immuno-deficiency characterized by dermatitis, recurrent fungal and staphylococci infections of the skin and lungs together with disorders of connective tissue including joint hypermobility, bone fractures, retained primary teeth and craniosynostosis².

In 2007 pathogenic heterozygous mutations in the signal transducer and activator of transcription (STAT) 3 gene were described³. Those are found in approximately two thirds of the patients with classical HIES. The link with STAT3 can explain a number of characteristic features of HIES. HIES patients have a relative lack of interleukin (IL)-17 secreting T helper (Th17) cells⁴, which are implicated in defense against extracellular bacteria and fungi⁵. Loss of IL-6, IL-21 and IL-23 signaling contributes to immune defects whereas defective IL-11 signaling explains features of the skeletal phenotype⁶. Remarkably, the remaining STAT3 activity in multiple signaling cascades is sufficient to prevent additional pathology, i.e. to allow leukemia inhibitory factor signaling to achieve placental implantation and sufficient IL10 signalling prevents infantile enterocolitis. No homozygous loss-of-function mutations have been described in humans and homozygous STAT3 deficiency in mice is embryonically lethal⁷.

STAT protein dimers are recruited to cytokine receptor complexes and are activated by phosphorylation of their tyrosine residues. The phosphorylated protein dimers undergo a conformational change and translocate to the nucleus to bind target gene promoter and enhancer elements⁸ (**figure 1**). This activation by phosphorylation has led to the assumption that total STAT protein matters little to overall STAT signaling and that loss of a single STAT gene allele is unlikely to be of consequence.

The STAT3 mutations in HIES are exclusively heterozygous and tend to effect either the DNA binding or Src homology (SH) 2 domains of the protein. Mutations in the SH2 domain result in a protein dimer that is unable to be

adequately phosphorylated at the cytokine receptor complex and mutations in the DNA binding domain result in a protein dimer that is unable to efficiently bind target gene loci. As the active STAT transcription factor works as a hetero or homo-dimer this has given rise to the assumption that STAT3 mutations associated with HIES acts in a dominant negative manner. Measurements of lymphocytes from patients with HIES have demonstrated a 75% reduction in STAT3 activity in patients with mutations in the DNA binding region of STAT3 in keeping with the stoichiometric assumption that 75% of STAT3 dimers would contain a mutant copy of STAT3 either as homo- or heterodimer ⁴.

In their current work, Bocchini et al looked at 77 identified mutations of STAT3 linked with HIES and propose a novel mechanism by which HIES associated mutations restrict STAT3 signaling. Computer modeling suggested that 20 of these mutations would result in a structurally unstable mis-folded protein and a further 42 were predicted to impair both protein stability and function. They confirm that mutations, predicted to destabilize STAT3 protein, resulted in a shortened STAT3 protein half-life in EBV cell lines derived from HIES patients. Furthermore, activation of the heat shock pathway reduced STAT3 misfolding and was able to correct the functional impairment associated with many STAT3 mutations associated with HIES. One of the small molecules investigated, geranylgeranylacetone, induces the heat shock protein pathway and reduced STAT3 degradation. This compound has been used in Japan as an anti-ulcer drug, raising the possibility of its use in the treatment of some forms of HIES and other diseases associated with impaired STAT signaling.

Mutations that give rise to unstable proteins are hard to equate with a dominant negative effect assuming a model where mutated monomers are cleared leaving behind the stable wild type protein. The authors counter this assumption, although they only provide indirect evidence that the presence of a mutant allele results in a reduced stability of any associated wild type allele, as total STAT3 protein has a reduced half-life in EBV cell lines derived from patients. They suggest that as STAT3 exists as a dimer in the inactive un-phosphorylated form, clearance of the misfolded form results in a bystander reduction in any associated wild type STAT3. This again does not explain why the residual 25% of STAT3 that would be expected to exist as a wild type dimer does not simply remain and accumulate to maintain an adequate supply of protein.

An alternative hypothesis would conclude that despite the critical role played by tyrosine phosphorylation and DNA-binding for the activity of STAT3, the level of total protein plays another important contributory role by setting the threshold for available protein. According to this interpretation, some mutations that give rise to HIES cause a haplo-insufficiency disorder ⁹ rather than a dominant negative disorder. The distinction is not merely academic as agents that increase total STAT3 protein, as suggested by the authors, will only work if they give rise to functional protein. Thus the findings by Bocchini et al places some genetic forms of HIES into the growing spectrum of haploinsufficiency disorders that present with immune defects, ⁹ which has clear implications for the treatment of this immuno-deficiency.

References

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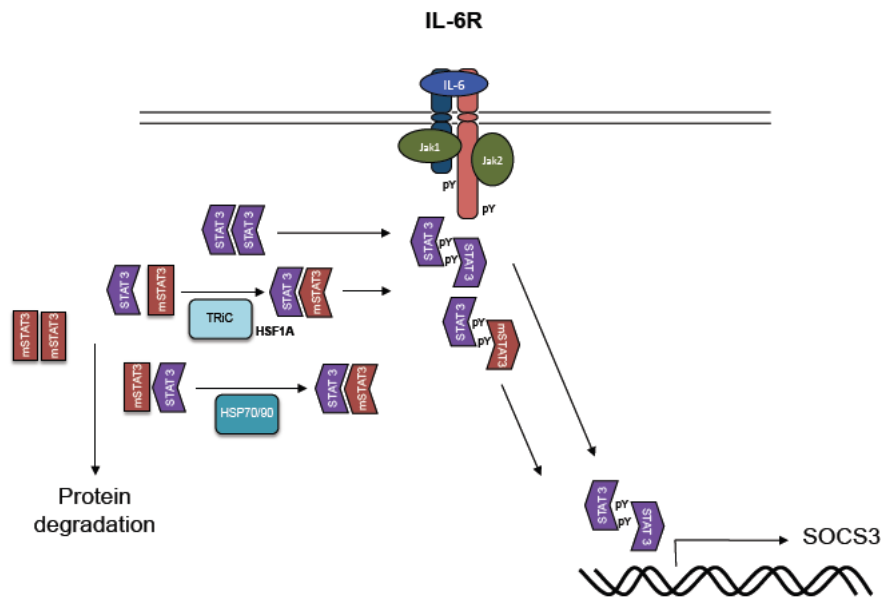


Figure 1: Wild type and mutant STAT3 are able to form homo and heterodimers. Wild type homodimers are phosphorylated down stream of cytokine receptor complexes and translocate to the nucleus where they act as transcription factors. In HIES some mutant copies of STAT3 result in misfolded protein that is cleared together with any associated wild type STAT3. Heat shock proteins are able to reverse this, restoring STAT3 function.