



**Karolinska  
Institutet**

**Institutionen för molekylär medicin och kirurgi**

# The Mechanism of Action of SOCS2 and its Role in Metabolism and Growth

**AKADEMISK AVHANDLING**

som för avläggande av medicine doktorsexamen vid Karolinska  
Institutet offentligen försvaras i Rockefeller, Nobels väg 11

**Fredagen den 14:e Juni, 2013, kl 09.00**

av

**Mattias Vesterlund**

Civilingenjör

*Huvudhandledare:*

Professor Amilcar Flores-Morales  
Köpenhamns Universitet  
Faculty of Health Sciences

*Fakultetsopponent:*

Associate Professor Mads Gyrd-Hansen  
Köpenhamns Universitet  
Faculty of Health Sciences

*Bihandledare:*

Professor Gunnar Norstedt  
Karolinska institutet  
Institutionen för molekylär medicin och kirurgi

*Betygsnämnd:*

Associate Professor Lars-Arne Haldosén  
Karolinska Institutet  
Institutionen för biovetenskaper och  
närlingslära

Professor Stefan Knapp  
Oxford University  
Nuffield Department of Medicine

Associate Professor Sergiu-Bogdan Catrina  
Karolinska Institutet  
Institutionen för molekylär medicin och  
kirurgi

Professor Claes-Göran Östenson  
Karolinska Institutet  
Institutionen för molekylär medicin och kirurgi

Associate Professor Anna Dimberg  
Uppsala Universitet  
Institutionen för immunologi, genetik och  
patologi

**Stockholm 2013**

## ABSTRACT

A well-known function of Growth Hormone (GH) is the regulation of postnatal longitudinal growth but it also affects other biological processes, for instance metabolism and inflammation. Actions of GH are tightly regulated at several levels and by several different factors and are initiated by GH binding to membrane bound GH receptors (GHR). The intracellular signaling of GH and other related hormones and cytokines is predominately mediated by the JAK-STAT pathway. This pathway is regulated in a negative feedback manner by the Suppressors of Cytokine Signaling (SOCS) family of proteins. One of the family members, SOCS2, is intimately tied to GH by virtue of the phenotype that results from its absence. SOCS2<sup>-/-</sup> mice are 40% larger than wild type littermates due to increased GH sensitivity.

Here, the molecular mechanism behind SOCS2s negative regulation of GH signaling, and its effects on metabolism and inflammation are described. We demonstrate that SOCS2 assembles a canonical E3 ubiquitin ligase complex with Elongin B, Elongin C, Cullin 5 and Rbx2 and that this complex has intrinsic E3 ligase activity *in vitro*. Overexpression of SOCS2 and its complex members leads to ubiquitination and proteasomal degradation of the GHR. We also outline the importance of the different domains of SOCS2, and demonstrate the necessity of the SOCS-box for proper SOCS2 activity. In a follow up study the claim that the naturally occurring Ser52Asn polymorphism of SOCS2 affects its activity and may contribute to acromegaly in humans was investigated. The Ser52Asn mutant was however found to be as efficient at regulating GH signaling as the wildtype and we conclude that it is unlikely to contribute to increased GH sensitivity. In Paper III the phenotype of SOCS2<sup>-/-</sup> mice under conditions of dietary stress is described. We report that SOCS2 deletion protects against high fat diet (HFD) induced hepatic steatosis but simultaneously leads to decreased insulin sensitivity. SOCS2<sup>-/-</sup> mice were found to have increased triglyceride output from the liver but also increased plasma levels of pro-inflammatory cytokines without apparent macrophage infiltration. *In vitro* examination of macrophages revealed increased phagocytic activity and cytokine production in the absence of SOCS2 and suggests a direct role for SOCS2 in the regulation of TLR4 signaling. Finally, the results of a screening effort to identify SOCS2-modulating, drug-like molecules are included. We have identified a prospective hit that binds to and inhibits SOCS2 activity *in vitro*. In summary, SOCS forms an E3 ligase complex which targets the GHR for degradation. This forms the molecular basis of its physiological actions. SOCS2<sup>-/-</sup> mice are protected from HFD induced hepatic steatosis but suffer from deteriorated insulin sensitivity related to increased inflammation.