Summary of Alberto Iannuzzo Thesis
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Deciphering the mechanisms responsible for the pathogenicity of CDC42 variants identified in autoinflammatory syndromes

Autoinflammatory syndromes (AIS) are rare inherited disorders caused by dysregulation of innate immunity, leading to uncontrolled systemic inflammation without infection. Many AIS result from mutations affecting key inflammatory pathways such as TNF, NF-κB, IL-1β, and inflammasomes. RHO family GTPases, including CDC42, regulate essential immune cell functions such as cytoskeletal organisation, migration, adhesion, polarity, and cell-cycle progression. Although abnormalities in RHO GTPase signalling have been linked to immunodeficiency and inflammatory diseases, their precise mechanisms remained unclear.

The aim of this PhD project was therefore to investigate how variants of CDC42 contribute to autoinflammatory disorders, with emphasis on cytoskeletal defects and signalling abnormalities.
Recent studies have identified several CDC42 mutations associated with severe AIS. C-terminal variants (R186C, C188Y, and 192C24) cause NOCARH syndrome, characterised by neonatal cytopenia, autoinflammation, rash, and hemophagocytosis. In contrast, the N-terminal Y64C mutation causes Takenouchi-Kosaki syndrome, featuring dysmorphic traits, neurodevelopmental delay, macrothrombocytopenia, and mild inflammation. Experimental results showed that the Y64C variant had normal cellular localisation and did not affect actin polymerisation or NF-κB signalling. Conversely, the C-terminal variants accumulated abnormally in the Golgi and nucleus, reduced actin polymerisation, and increased NF-κB phosphorylation and nuclear translocation. Importantly, actin depolymerisation alone did not trigger NF-κB activation, indicating that CDC42 mutations activate inflammatory signalling through independent mechanisms.
Further analyses focused on ER-Golgi trafficking, ER stress, STING activation, and type I interferon responses. The Golgi-trapped CDC42-R186C variant disrupted ER-Golgi transport, inducing ER stress and accumulation of STING in the Golgi due to defective COPI-mediated retrograde transport. This resulted in STING overactivation and increased expression of interferon-stimulated genes. Similar findings were observed with the 192C24 variant, whereas Y64C and C188Y, which do not accumulate in the Golgi, did not activate STING. A newly identified N-terminal T43I mutation did not affect localisation or interferon responses but caused hyperactivation of the Pyrin inflammasome, likely through enhanced CDC42 binding to Pyrin. These results suggest that CDC42 positively regulates the Pyrin inflammasome and that both CDC42 and RHOA participate in controlling Pyrin-mediated inflammation.
The study highlighted the importance of ER-Golgi trafficking defects and ER stress as drivers of immune dysregulation, similar to what occurs in diseases caused by mutations in COPI complex components such as COPA and COPZ1. Both CDC42- and COPA-related disorders show defective COPI-dependent retrograde transport, leading to ER stress and activation of NF-κB and type I interferon pathways. Elevated IFN-α levels in CDC42-R186C patients correlated with disease severity, and simultaneous activation of NF-κB, interferon, and Pyrin pathways was observed, suggesting that multiple inflammatory mechanisms contribute to pathology.
Overall, the work shows that different mutations in the same gene can produce distinct clinical phenotypes by affecting different cellular pathways. Golgi-trapped CDC42 variants activate both STING and Pyrin pathways, whereas T43I selectively activates Pyrin. In addition, mutations in different genes can lead to similar disease features, as seen with CDC42 and COPA variants. These findings emphasise the importance of precision medicine approaches that combine genetic diagnosis with functional studies to guide targeted therapies for autoinflammatory diseases caused by CDC42 mutations.

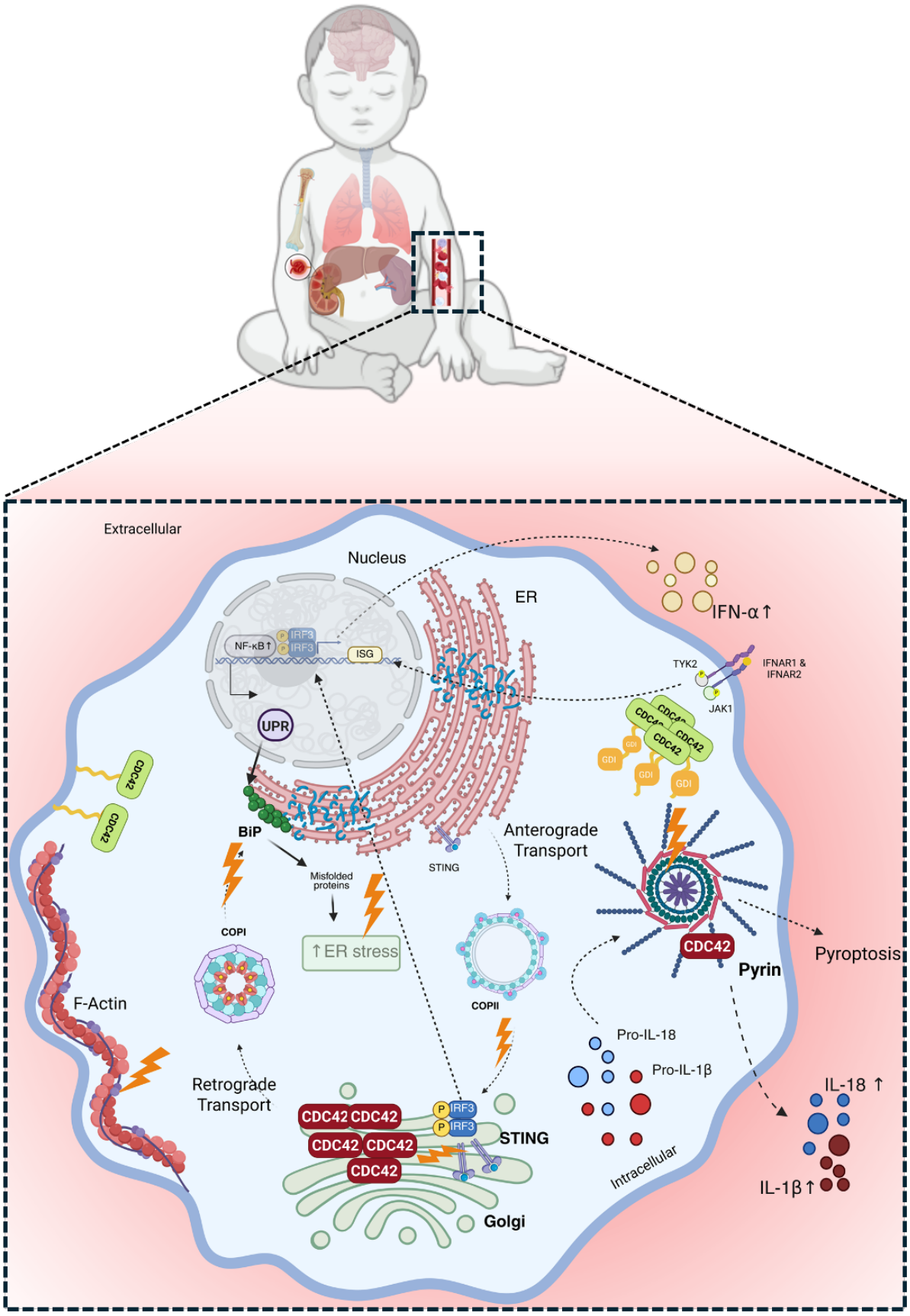
Extracellular
Nucleus
ER
IFN-α↑
NF-κB↑
IRF3
IRF3
ISG
TYK2
IFNAR1 &
IFNAR2
JAK1
UPR
GDI
CDC42
BiP
Misfolded
proteins
STING
Anterograde
Transport
COPI
↑ER stress
COPII
Pyroptosis
Pyrin
F-Actin
Pro-IL-18
Pro-IL-1β
Retrograde
Transport
IL-18 ↑
IL-1β↑
Intracellular
Golgi

Figure Legend

The patients analysed present a systemic inflammatory clinical phenotype, characterized by distinctive features including hemophagocytic lymphohistiocytosis (HLH) with hepatosplenomegaly, myelofibrosis, skin rashes, neurological and pulmonary involvement, often requiring hematopoietic stem cell transplantation (HSCT). This study focused on investigating the role of immune dysregulation and its contribution to the observed clinical phenotype. The figure illustrates the main cellular pathways involved, as described in the thesis. Specifically, it highlights how mutations affecting COPI and CDC42 impact retrograde and anterograde vesicular transport, type I interferon production, F-actin polymerization, and activation of the innate immune sensor Pyrin. Dysregulated signalling pathways are graphically indicated by a lightning bolt symbol. What we shown was that different mutations in the same gene can lead to heterogeneous phenotypes by deregulating distinct pathways. Clearly example is Golgi-trapped CDC42 variants hyperactivate both STING and pyrin, while the T43I mutation selectively hyperactivates pyrin. But mutations in different genes may also result in similar phenotypes, as observed with Golgi-trapped CDC42 variants and COPA. This complexity highlights the need for precision medicine approaches based on genetics and functional validation of pathogenic variants.