First rapport of Autosomal inborn error and Autoantibodies neutralizing type I IFN immunity in Guatemala patients with life-threatening COVID-19.

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Acute Respiratory Infections (ARI), especially influenza and COVID-19 pneumonia, are the significant causes of death in children under five years and elderly in Guatemala. Our findings, as a part of the COVID Human Genetic Effort (CHGE consortium, www.covidhge.com), are the first to underdiagnosed primary immunodeficiencies (PIDs) associated with patients admitted to Intensive Care Units (ICU) suffering ARIs or SARS-COV-2 in Guatemala.

Primary immunodeficiencies of type I IFN immunity, including autosomal recessive IRF7 and IFNAR1, autosomal dominant TLR3, and X-linked recessive TLR7, have been identified to cause life-threatening COVID-19. In contrast, autosomal (CHGE) recessive IRF7 and IRF9 and autosomal dominant TLR3 deficiencies have been identified to cause life-threatening influenza pneumonia. Moreover, autosomal recessive MDA5 deficiency has been determined to cause life-threatening rhinovirus pneumonia. Overall, PIDs of type I IFN immunity led to increased susceptibility to severe respiratory viral infections in otherwise healthy individuals. Therefore, **we hypothesize that the life-threatening cases of respiratory viral infections in children and elderly men in Guatemala are caused by under-diagnosed PIDs.**

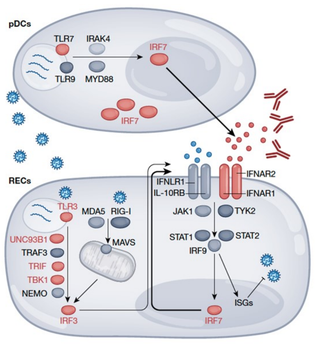


Fig. 1 Inborn errors of type I IFN immunity and auto-antibodies neutralizing type I IFN underlie life-threatening COVID-19 pneumonia by interfering with type I IFN immunity in tissue-resident RCs and blood plasmacytoid dendritic cells[1].

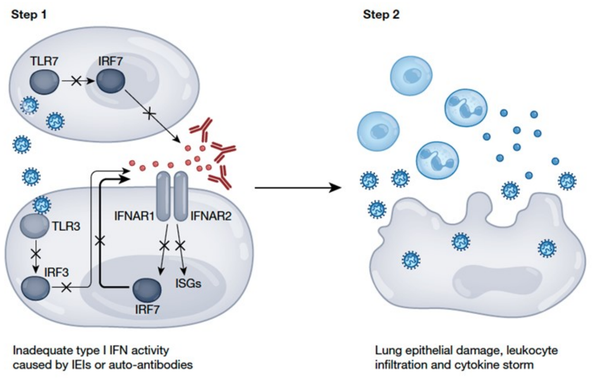


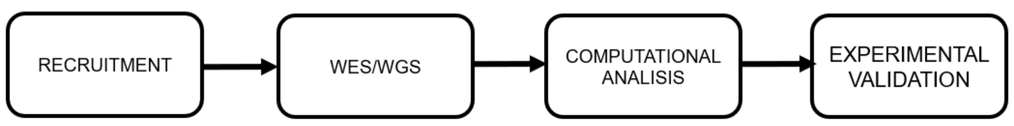
Fig. 2 Inborn errors of type I IFN immunity and auto-antibodies neutralizing type I IFNs underlie life-threatening COVID-19 pneumonia by facilitating the spread of the virus during the first few days of infection, triggering secondary leukocytic inflammation[1].

Specific Aims:

In collaboration with the CHGE, we use molecular diagnosis for known PID genes in the type I IFN pathway to guide the clinical treatment, including vaccination, intravenous immune globulin (IVIG), and genetic counseling. Our three-step strategy consists of

1. Recruit patients with life-threatening respiratory viral infections and perform a clinical investigation of the phenotypes,
2. Perform candidate gene sequencing to diagnose PIDs of the type I IFN pathways.
3. Perform Whole-Exome Sequencing (WES) with international collaborators to identify novel PIDs.

Experimental Approach:



Susceptible group:

1. SARS-CoV-2 patients diagnosed by PCR analysis

Patients diagnosed with Pneumonia, MIS-C, Long COVID, COVID Toes, Guillain-Barré, Encephalitis, etc.

Resistant Group

1. Individuals exposed to SARS-CoV-2
2. Remain seronegative.

1. Genetic homogeneity:
   Rare variants enriched in susceptible or resistant group

   Genetic heterogeneity
   Rare, deleterious variants
   Single case or multiple cases in the family

Results

We recruit patients through two hospitals with ICU centers in Guatemala. We have collected detailed clinical data and collected blood, plasma, and DNA from all the patients.

Autoantibodies (auto-Abs) neutralizing type I IFNs

In collaboration with our colleagues from Paris [a] [2], we searched for auto-Abs against IFN-α2 and -ω by establishing novel, sensitive, and robust assays to detect circulating IgG Auto-Abs. Our group used Gyros technology, a high-throughput automated enzyme-linked immunosorbent assay (ELISA) capable of detecting an extensive range of auto-Ab levels.

We have found that the Auto-Abs neutralizing high concentrations (10 ng/mL, in plasma diluted 1 to 10) of IFN-α and -ω are present in about 11% of our patients with critical COVID-19 pneumonia.

Patient	IFNα 10	IFN-β 10	IFN-ω 10	IFNα 100	IFN-ω 100
54-year-old male					
21HSD002G					
21HSD003G					
21HSD004G					
21HSD005G					
21HSD006G					
67-year-old Female					
21HSD008G					
21HSD009G					
21HSD010G					
21HSD011G					
21HSD012G					
21HSD013G					
21HSD014G					
21HSD015G					
21HSD016G					
21HSD017G					
21HSD018G					
21HSD019G					
21HSD020G					
21HSD021G					
21HSD022G					
21HSD023G					
21HSD024G					
21HSD025G					
21HSD026G					
21HSD027G					
21HSD028G					
21HSD029G					
21HSD031G					
21HSD032G					
21HSD033G					
21HSD034G					
21HSD035G					
21HSD036G					
21HSD037G					

High
Intermediate
low
Underdetectable

Fig. 3 ELISA results for auto-Abs against the 13 IFNα forms, IFN-ω and IFN-β, in the 37 patients with life-threatening COVID-19 and auto-Abs neutralizing 100 pg/mL IFN-α2 and auto-Abs neutralizing 10 ng/mL IFN-α2. (Induction (positive if <15)

Whole-Exome Sequencing (WES) [a] [3]

We run the Principal Component Analysis (PCA) with an Oblimin rotation. In an initial inspection, we selected a total of 10 components. A comparison of these components between inpatients with inborn and without inborn errors is carried out. Out of these components, the only ones that had a different behavior in both groups were components 5 and 6.

Table 1. Genes used in the Principal Component Analysis (PCA), most common genes in our WES dataset.

Gene	Without Inborn Error (count)	With Inborn Error (count)
ARSD	219	62
SLC9B1P1	132	11
ZNF417	71	9
ATXN3	70	11
ATXN1	34	6
PLCH2	18	5
VPS13B	18	7
FOXO6	62	8
MUC6	42	8
MUC16	29	7
RHBG	31	5
ARHGAP9	2	6
CYP2D6	1	9

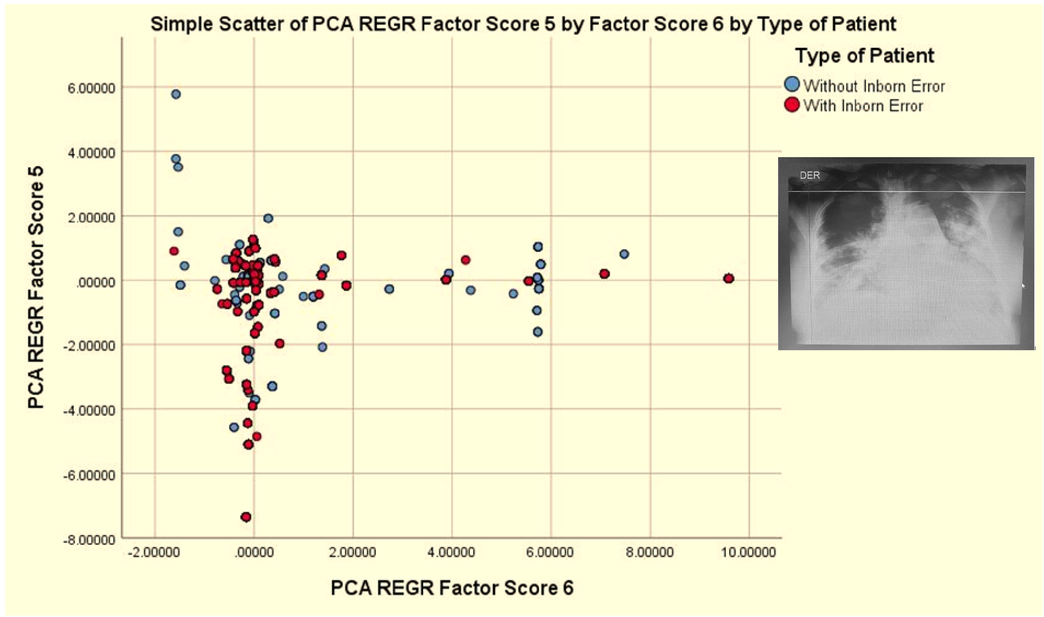


Fig. 4 For component 5, the gene involved is ARSD, along with stop gained and function with reference C to altering T. It seems more common for patients with inborn error to have this type of mutation in this gene. In component 6, patients without inborn error are more likely to have a mutation on gene SLC9B1P1, without a specific function with reference T and altering A or G. However, the difference could be more significant, suggesting more analysis needs to conclude about the genetic alterations that patients with the inborn error may yield and whether the previously mentioned genes impact the inborn error.

Our results will help diagnose PIDs in Guatemala patients, provide therapeutic options for these patients, and raise awareness of PIDs.

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