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INFLAMMASOME BUT NOT IFN-I/III RESPONSE IS ALTERED IN CHILDREN WITH LONG COVID

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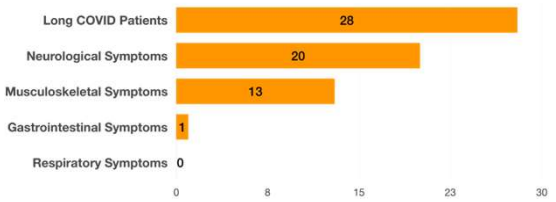
BACKGROUND

Severe COVID-19 is less common in children than in adults. Increasing evidence show that distinct immune-pathological changes can persist weeks or months after SARS-CoV-2 infection, leading to "Long COVID (LC)".
In this context, interferon (IFN) response and inflammation-related miRNAs/genes in peripheral blood mononuclear cells (PBMCs) could have an important role. Hence, we investigated the systemic type I/III interferon (IFN-I/III) and inflammation response in PBMCs of children with and without LC symptoms.

METHODS

Blood samples were collected from children attending Umberto I hospital of Rome, within 3-6 months after a SARS-CoV-2 positive test and from control children. RNA was extracted from PBMCs for determining the levels of IFN-I (IFN-α2a, -β, -ε and -ω), IFN-III (IFN-λ1-3), NLR family pyrin domain containing 3 (NLRP3) and interleukin 1 beta (IL-1β) genes, and miR-141 expression by quantitative RealTime-PCR assays, normalized to housekeeping β-glucuronidase (GUS) gene and RNU6B, respectively.

Total Patients n = 74	SARS-CoV-2 recovered Patients		Healthy Controls n = 18
	Patients with Long COVID symptoms n = 28	Patients without Long COVID symptoms n = 28	
Age, mean (SD)	12.5 (3.0)	11.8 (3.0)	10.5 (3.1)
Male (n = 44)	13 (46.42 %)	15 (53.57 %)	16 (88.88 %)
Female (n = 30)	15 (53.57 %)	13 (46.42 %)	2 (11.11 %)



SARS-CoV-2 might be responsible for dysregulation of IFN-I/III response 3/6 months after infection

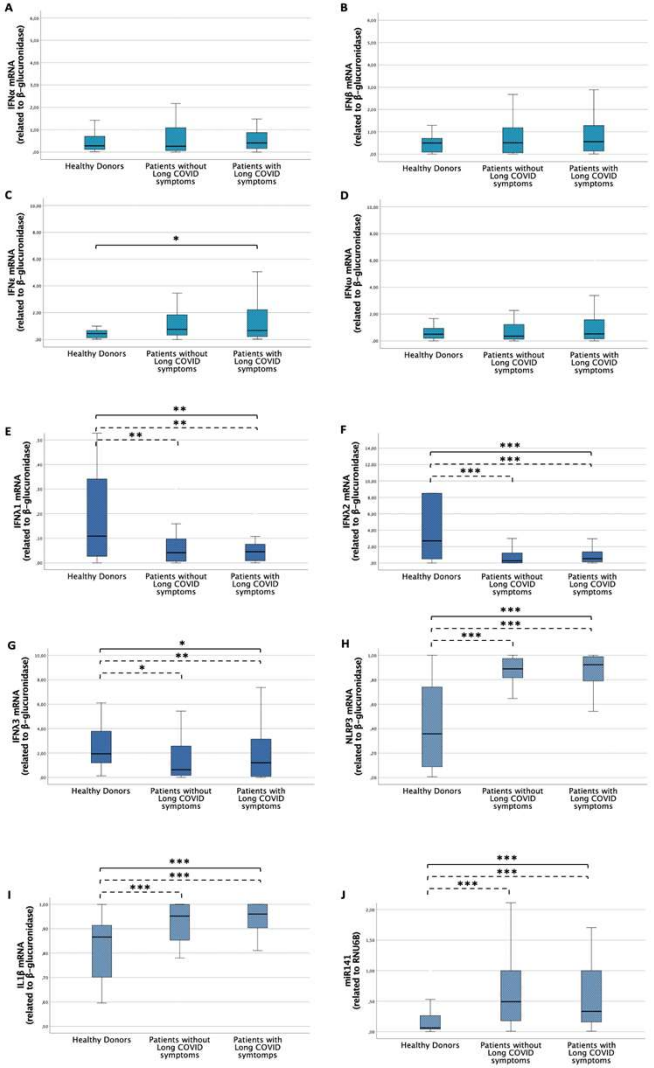
Prolonged inflammatory state could lead to LC symptoms in children

RESULTS

28 participants (M 12.5y SD 3.0) with LC symptoms, 28 participants (M 11.8y SD 3.0) without LC symptoms and 18 children without previous SARS-CoV-2 infection (M 10.5y SD 3.1) were enrolled. Comparing the three study groups, we found reduced levels of IFN-λ1, IFN-λ2 and IFN-λ3 ($p=0.006$, $p<0.001$, $p=0.012$, respectively; Kruskal–Wallis (KW) test) mRNA in patients with previous SARS-CoV-2 infection as opposed to control group, whereas transcript levels of IFN-ε ($p= 0.019$; KW test) were increased in the former as compared to the latter group; as well, remaining IFN-I genes analyzed showed a tendency to be up-regulated. As far as NLRP3 and IL-1β levels was concerned, these genes were increased in LC patients ($p<0.001$ for both genes; KW test). Additionally, miR-141, which has been reported to regulate inflammasome response, was overexpressed in LC patients ($p<0.001$; Mann–Whitney test).

CONCLUSIONS

These results showed decreased levels of IFN-III mRNAs and an overexpression of IFN-ε in children after 3-6 months of SARS-CoV-2 infection regardless of development of LC symptoms, suggesting that SARS-CoV-2 might be responsible for dysregulation of IFN response through unknown mechanisms (e.g. epigenetic modifications). According to recent investigations, miR-141 regulates NLRP3 expression and ultimately inflammasome. In this regard, we found an overexpression of miR-141, NLRP3 and IL-1beta mRNAs in COVID-19 convalescent children and even more in LC children as opposed to healthy controls, questioning that a prolonged activation of inflammasome pathways could be associated with the development of LC syndrome.



Expression level of genes encoding IFN-α2a (A), IFN-β (B), IFN-ε (C), IFN-ω (D), IFN-λ1(E), IFN-λ2 (F), IFN-λ3 (G), NLRP3 (H), IL1β (I) and miR141 (J) in healthy donors (n=18), patients without Long COVID symptoms (n=28) and patients with Long COVID (n=28). Statistical analysis were performed using Kruskal-Wallis (continuous line) or Mann-Whitney tests (dashed line) (*p<.05, **p<.01, ***p<.001).