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RESEARCH

Inflammatory Proteomic Analysis of 22q11.2 Deletion Syndrome

Valentina Frusone[1] · Kelly Maurer[2] · Beverly S. Emanuel[3] · Donna McDonald-McGinn[3,4] · Kathleen E. Sullivan[2]

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To the Editor

Chromosome 22q11.2 deletion syndrome (22q11.2DS) is the most common chromosome deletion syndrome and is the result of de novo non-allelic homologous meiotic recombination. Associated phenotypic features are multisystemic and are largely related to haplosufficiency for the 30 to 50 genes in the deleted region. The structural differences include but are not limited to heart, palate, and gastrointestinal tract. Over time, speech delays, cognitive deficits, ADHD, autism, and anxiety disorders may become apparent. Immunodeficiency is a central component and autoimmunity can be seen in 10–30%. The most typically identified compromise to host defense relates to diminished T cell production due to thymic hypoplasia. The consequences of the T cell lymphopenia include infections, atopy, and autoimmunity and biomarkers of autoimmunity and atopy have been identified, making risk stratification possible [1, 2]. Nevertheless, adults with 22q11.2DS have a range of medical issues which are not clearly referrable to the known structural differences. Psychosis, seizures, and other behavioral differences represent features not easily referrable to a structural anomaly. We undertook an unbiased proteomic analysis of adults with 22q11.2DS and unaffected relatives and controls to better understand the landscape that might contribute to the latter features. We utilized the Olink Target 96 Inflammation panel (Uppsala, Sweden) using 49 adult patients with 22q11.2DS and 62 healthy adult controls. Their clinical features are compiled in Supplemental Table 1. Samples were run in duplicate and normalization was performed according to company guidelines. Results are expressed as normalized protein expression (NPX) on a log2 scale.

We first evaluated between group effects to understand whether cytokines and other mediators were globally different in 22q11.2DS (Supplemental Fig. 1). There did appear to be a trend towards increased mediator expression in 22q11.2DS compared to controls. We further examined the mediators that were statistically significantly different between the two groups, after adjustment of the p avlues using the NPX method. No mediators were significantly decreased in patient samples (Fig. 1). Identification of high IL-4 was anticipated based on previous data [3].

We had a single patient with ITP (Supplemental Table 1) and that patient did not segregate separately from the other patients with 22q11.2DS. Another patient had autoimmune pancytopenia in the distant past but had recovered fully. Our frequency of hypothyroidism was comparable to other published series but our frequency of ITP was lower than in some published series which hampered efforts at analyzing associations of analytes with clinical subsets. Nevertheless, there was no single analyte difference associated with the patients who had hypothyroidism. Similarly, we assessed whether the three patients with clinically defined anxiety had altered expression of any mediators, particularly those related to neuronal survival. The three patients with anxiety, often a harbinger of psychosis, did not exhibit any differences from the other patients in this analysis.

The central finding of this study builds on findings of altered serum cytokines in 22q11.2DS. IP-10, IL-12 and IL-6 have been identified as increased in 22q11.2DS, the latter two particularly associated with psychosis or behavioral differences [4]. We have previously identified BAFF,

✉ Kathleen E. Sullivan
sullivank@chop.edu

[1] Drexel University, Philadelphia, PA 19104, USA

[2] Division of Allergy Immunology, ARC 1216-I CHOP Immunology, The Children's Hospital of Philadelphia, 3615 Civic Center Blvd., Philadelphia, PA 19104, USA

[3] Division of Human Genetics, The Children's Hospital of Philadelphia and Department of Pediatrics, Perelman School of Medicine of the University of Pennsylvania, Philadelphia, PA 19104, USA

[4] Division of Human Biology and Medical Genetics, Sapienza University, 00185 Rome, Italy

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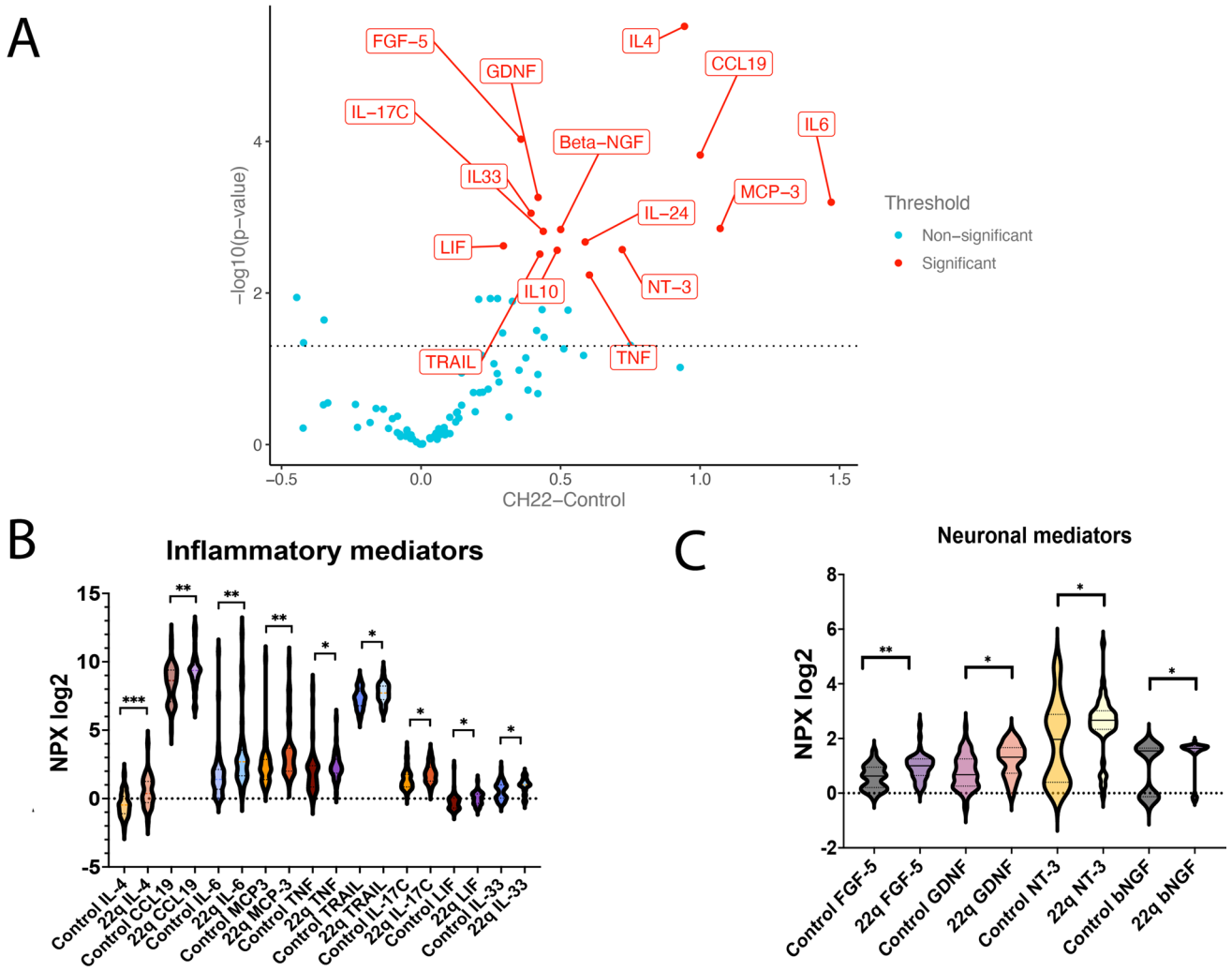

Fig. 1 Inflammatory mediators in patient and control serum. **A** Volcano plot demonstrating the statistically significant mediators. The log10 p value is given on the y-axis and the magnitude of the difference is on the x-axis. **B** Violin plots of patients (22q) and controls demonstrating the mediator NPX log2. **C** Violin plots of patients and controls demonstrating the mediator levels as NPX log2. This plot focused on the neurotrophic mediators. Adjusted *P* values are given above each pair of plots. Three asterisks indicate $p < 0.001$, two asterisks indicate $p < 0.01$ and one asterisk indicates $p < 0.05$. The median is given as a solid black line and the quartiles are given as dashed lines

IL-21, and IL-4 as increased [3, 5]. Of note, BAFF, IL-21 and IP-10 were not assayed on the Olink 96 Inflammation panel. Nevertheless, there has been a growing sense that the inflammatory cytokine milieu may contribute to some of the clinical features such as autoimmunity, inflammation, and perhaps even behavioral diseases. This study was larger than any other proteomic study performed to date and the large size and the unbiased survey of inflammatory ligands allowed us to identify clusters of proteins that may inform on the overall landscape in 22q11.2DS.

TNF, IL-6, MCP-3, CCL-19, IL-17C, and TRAIL are connected through their induction by bacterial products or TNF. Their roles are to regulate cell activation and inflammatory responses. While the elevations are mild, chronic inflammatory stimulation could have profound effects on immune function. Chronic inflammation can lead to a break in tolerance and alters cell physiology. Clinically, it is a component driving metabolic syndrome, cardiovascular disease and diabetes.

An unexpected finding was increased FGF5, GDNF, NT-3, and βNGF. FGF5 is best known for its role in hair growth but it is expressed by most neurons in the brain where it appears to regulate astrocyte homeostasis. High GDNF has been observed with anxiety. NT-3 has been identified in multiple studies as increased in serum with depression. βNGF is produced by the broadest range of cell types and receptors are correspondingly broadly expressed. In mice, βNGF is released from salivary glands

in states of conflict and stress. Exogenous administration of βNGF leads to hyperalgesia. The source of these mediators and what precise processes they impact is not fully known, however, chronic inflammation such as due to TNF and IL-6 can cause a breakdown in the blood brain barrier which has been proposed as a mechanism for psychosis. Inflammation has been documented in multiple ways as a contributor to psychosis, a concern in adults with 22q11.2DS.

IL-24, LIF and IL-4 do not easily connect to the other sets of mediators. Overexpression of LIF in mice leads to altered thymic and lymph node composition with expansion of B cells. IL-4 has been previously identified by us as increased in 22q11.2DS and tracked with homeostatic proliferation history. Indeed, clinical atopy is increased 22q11.2DS. IL-33 is also implicated in Th2 responses, consistent with the clinical phenotype.

This discovery approach represents the largest available unbiased dataset defining the landscape of inflammatory mediators in 22q11.2DS. Nevertheless, there are limitations to this study. Even with a large size, there was insufficient power to examine important clinical subsets. Among outlier patients, we did not detect any clinical themes, but a larger sample size might have revealed some commonalities. This dataset is also limited by a lack of mechanistic insights, a limitation that was anticipated given the survey nature of the design. The important contribution of this study is to confirm and expand a body of literature on cytokines in 22q11.2DS. To our knowledge, this is the first study to examine CNS-derived mediators and the finding of elevated FGF5, GDNF, NT-3, and βNGF is provocative. Whether these are downstream consequences of a CNS process, a response to variables we do not understand, or are true participants in some of the behavioral features in 22q11.2DS remains to be seen.

In summary, this study reveals important differences in inflammatory ligands in the serum of people who have 22q11.2DS compared to controls. These differences could be important in the clinical care of patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10875-024-01689-7.

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Author Contributions VF analyzed data and wrote a draft of the manuscript. KM and KES further analyzed data and KES wrote additional material and edited the final version. BE and DMM analyzed clinical information, provided clinical details, and were central to patient recruitment. All authors reviewed the manuscript.

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Data Availability Data are available upon request to the corresponding author.

Declarations

Competing Interests The authors declare no competing interests.

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