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Association of IL-12p70 and IL-6:IL-10 ratio with autism-related behaviors in 22q11.2 deletion syndrome: A preliminary report

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ABSTRACT

22q11.2 deletion syndrome (22q11DS) is a genetic disorder that conveys a significant risk for the development of social behavior disorders, including autism and schizophrenia. Also known as DiGeorge syndrome, 22q11DS is the second most common genetic disorder and is characterized by an elevated risk for immune dysfunction, up to 77% of individuals have an identifiable immune deficiency. We hypothesize that this immune dysfunction could contribute to the elevated risk of impaired social behavior seen in 22q11DS. The current study begins to elucidate these immune deficits and link them with the behavioral alterations associated with the disorder. Serum concentrations of a series of cytokines were examined, using a multiplex immunoassay, in sixteen individuals with 22q11DS and screened for autism-related behavior using the Autism Diagnostic Interview-Revised (ADI-R). This preliminary study examined correlations between specific immune proteins and each of the ADI-R algorithm scores (social, communication, and repetitive behavior). The inflammatory cytokine IL-1β, as well as the ratio between the inflammatory cytokine IL-6 and the anti-inflammatory cytokine IL-10, were correlated with social scores ($r = 0.851$, $p = 0.004$; $r = 0.580$, $p = 0.018$). In addition, the inflammatory cytokines interferon gamma and IL-12p70 were correlated with repetitive behaviors ($r = 0.795$, $p = 0.033$; $r = 0.774$, $p = 0.002$). Interestingly, IL-12 has been reported to be increased in autistic children. These data show a positive association between severity of autism-related behaviors and level of serum concentrations of inflammatory cytokines in individuals with 22q11DS, providing a basis for further inquiry.

1. Introduction

22q11.2 deletion syndrome (22q11DS) is the second most common childhood genetic disorder, behind Down's syndrome (~1:4000 births) (Oskarsdottir et al., 2004). 22q11DS, also known as DiGeorge syndrome and velo-cardio-facial syndrome, is characterized by a spontaneous 3MB hemizygous deletion on the long arm of chromosome 22 and results in a complex medical phenotype which may involve heart and palatal defects, facial dysmorphology, and thymic dysfunction (McDonald-McGinn et al., 1993).

22q11DS conveys a significant risk for the development of social behavior deficits, including autism-spectrum and schizophrenia-spectrum disorders. Research studies find that autism spectrum disorders occur in 14–45% of clinically-ascertained individuals with 22q11DS, as compared with >0.9% in the general population (McDonald-McGinn and Sullivan, 2011). Likewise, schizophrenia occurs in up to 33% of such individuals, which is 25–31 times higher than the general population (Drew et al., 2011). In fact, 22q11DS is the number one genetic risk factor for schizophrenia (Liu et al., 2002). Notably, *schizophrenia disorder 4* is one of the genes deleted from chromosome 22 in the syndrome.

There is evidence that immune disruptions lead to alterations in behavioral function and the emergence of neurodevelopmental and psychiatric disorders. Schizophrenia has been strongly linked to immune dysfunction. Indeed, some of the very first evidence linking prenatal immune function to neuropsychiatric outcomes was identified by researchers studying schizophrenia who found that the risk of disease was increased 7-fold for influenza exposure during the first trimester and that influenza exposure during early to mid-pregnancy increased the risk of schizophrenia 3-fold (Brown et al., 2004). Other maternal infections that have been linked to the development of schizophrenia include non-specific bacterial infections, Toxoplasma gondii, and herpes simplex virus type 2 (Sorensen et al., 2009; Mortensen et al., 2010; Pedersen et al., 2011). In addition, there is growing evidence for additional immune disturbances among schizophrenic patients. Higher concentrations of constitutively active and endotoxin-induced

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chemokines (i.e., monocyte chemotactic protein-1, macrophage inflammatory protein-1 alpha) and cytokines [i.e., interleukin (IL)-8, IL-18, and interferon gamma (INFγ)] are found in individuals with schizophrenia (Reale et al., 2011).

Similarly, in autism, there is evidence of altered immunity occurring both immediately after birth and throughout disease progression. There are multiple lines of evidence that suggest a role for immune dysfunction in autism, including neuroinflammation involving microglia, increased inflammatory cytokine and chemokine production in post-mortem brain tissue, systemic alterations of immune proteins, and reduced immunoglobulin (Ig) antibody production (Onore et al., 2012). Immune function may also relate to social behavioral outcomes (Onore et al., 2012). For example, increased plasma levels of cytokines including IL-1β, IL-6, IL-8 and IL-12p40 were observed in autistic children compared with age-matched controls, and this cytokine production was associated with more aberrant behaviors, especially in individuals with developmental and behavioral regression (Ashwood et al., 2011a). Moreover, significantly reduced plasma levels of IgG and IgM were found in children with autism compared with age-matched typically developing children and children with developmental disabilities other than autism, suggesting an underlying defect in immune function. The reduction in specific Ig levels correlated with behavioral severity. Specifically, patients with the highest scores in behavioral dysfunction exhibited the greatest reduction in peripheral blood concentrations of IgG and IgM (Heuer et al., 2008). Another study found that a 10-point difference in IgG concentrations conferred an increased risk for autism (Grether et al., 2010). Another line of evidence relevant to 22q11DS suggests an increase in the Th1/Th2 ratio of individuals with autism (Li et al., 2009). This increased pro-inflammatory state was associated with greater impairments in the core features of autism (Ashwood et al., 2011b). Together these studies suggest immune system dysfunction in autism.

A prominent feature of the medical phenotype for 22q11DS includes elevated risk of immune disorders, up to 77% of individuals have an identifiable immune dysfunction (McDonald-McGinn et al., 1993). The identified immune changes range from a primary T cell dysfunction to the presence of an autoimmune disease. Specifically, thymic hypoplasia in individuals with 22q11DS causes a reduced number of T cells at birth, and possibly the emergence of autoimmune disorders, allergies, and asthma, which can arise from an imbalance in T cell subsets. Of individuals with 22q11DS, 10% will develop autoimmune disorders (McDonald-McGinn and Sullivan, 2011), including Graves' disease, diabetes, and celiac disease. Moreover, 12% of individuals with 22q11DS have IgA deficiency (McDonald-McGinn et al., 1993). In addition, polyarticular juvenile rheumatoid arthritis occurs in children with 22q11DS at a frequency 20 times that of the general population, with an age of onset ranging from 17 months to five years (McDonald-McGinn et al., 1993). The source of these immune disturbances could be related to the deletion of several immune proteins whose DNA lies on the long end of chromosome 22, including interleukin 17 receptor A, immunoglobulin lambda-like polypeptide 1, and macrophage migration inhibitory factor. Despite multiple clinical studies that report a significant association between 22q11DS and immune-related disease, a systematic investigation of the immune profiles of individuals with 22q11DS, and their relationship with behavioral changes, has not previously been conducted.

In this preliminary study, we examined individuals with 22q11DS to identify genetically influenced immune disturbances. Cytokines that have been implicated in autism were measured in individuals with 22q11DS, using a multiplex immunoassay. Concentrations of the pro- inflammatory cytokines granulocyte macrophage colony-stimulating factor (GMcsf), INFγ, IL-12p70, IL-1β, IL-6, IL-8, and tumor necrosis factor-alpha (TNFα), and the anti-inflammatory cytokine IL-10 were examined. These immune proteins have not been studied in 22q11DS. In addition, autism-related behavior was examined as it relates to these cytokines.

2. Methods

2.1. Study population

Research participants were recruited in reverse-age order from a case registry of individuals diagnosed with 22q11DS, which is maintained at Children's Health Care of Atlanta. The current analysis is based on 16 individuals who participated in a study of neurocognitive and behavioral outcomes and who had also provided blood samples as part of a larger biological banking effort. All subjects had a confirmed diagnosis of 22q11DS by means of fluorescence in situ hybridization (FISH) for deletions on chromosome 22q11. The race/ethnicity distribution was as follows: 69% ($n = 11$) Caucasian, 19% ($n = 3$) African-American, 6% ($n = 1$) Hispanic, and 6% ($n = 1$) Asian. There were 9 (56%) males and 7 (44%) females, and the average age was 14 (SD = 1.98) with a range of 3–31 years.

2.2. Cytokine assessments

Cytokines were assessed using a multiplex immunoassay format (Meso Scale Discovery, Gaithersburg, Maryland; MS6000 Human ProInflammatory Ultra-Sensitive Kit) (Breen et al., 2011; Dabitao et al., 2011). Serum from 16 individuals with 22q11DS was collected to examine concentrations of the autism-related cytokines: GMcsf, INFγ, IL-12p70, IL-1β, IL-6, IL-8, TNFα and IL-10. Each sample was run in duplicate; including an 8-point standard curve, starting with 2500 pg/ml and continuing with a 4-fold dilution down to a zero concentration value. The lower limit of detection (LLOD) for each of these proteins is: 0.53, 1.4, 0.36, 0.27, 0.09, 0.50, and 0.21 pg/ml respectively. The recoveries of the assays were 98.8–102.5% for the measured cytokines. The calculated coefficient of variation (CV) for each protein was less than 15%; any duplicate that had a higher CV was removed from analysis.

2.3. Behavioral assessments

Autism-related behavior was measured using the Autism Diagnostic Interview-Revised (ADI-R). This semi-structured interview, designed to identify individuals with a probable diagnosis of autism (Lord et al., 1994; Risi et al., 2006), relies on detailed parental report, rather than direct observation, and is considered one of the gold-standard assessments for evaluating autism-related behaviors. Each reported behavior or symptom is rated according to a three- or four-point scale (0–2, or 0–3), with higher scores reflecting greater severity of autism-related behaviors. Current and past symptoms of autism are recorded. The ADI-R yields diagnostic algorithm scores, comprised primarily of the past symptom ratings, across three domains: 1) the "social" domain, which assesses qualitative abnormalities in reciprocal social interaction, 2) the "communication" domain which assesses qualitative abnormalities in communication, with different algorithms for verbal and nonverbal individuals, and 3) the "repetitive behavior" domain which assesses restricted, repetitive, and stereotyped patterns of behavior. Higher scores indicate greater social impairments, greater communication impairments, or the presence of more severe or more frequent repetitive behavior. Although the ADI-R algorithm scores were originally designed to generate categorical diagnostic information, (Lord et al., 1994; Risi et al., 2006), these scores also provide reliable continuous metrics; which represent the severity