Autism, asthma, inflammation, and the hygiene hypothesis

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Summary   Inflammation and the genes, molecules, and biological pathways that lead to inflammatory processes influence many important and disparate biological processes and disease states that are quite often not generally considered classical inflammatory or autoimmune disorders. These include development, reproduction, aging, tumor development and tumor rejection, cardiovascular pathologies, metabolic disorders, as well as neurological and psychiatric disorders. This paper compares parallel aspects of autism and inflammatory disorders with an emphasis on asthma. These comparisons include epidemiological, morphometric, molecular, and genetic aspects of both disease types, contributing to a hypothesis of autism in the context of the immune based hygiene hypothesis. This hypothesis is meant to address the apparent rise in the prevalence of autism in the population.
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Introduction

Autism is an enigmatic childhood disorder of unknown origin. It is characterized by developmental, language, and social deficits, ranging in severity from patients with profound deficits to individuals that are high functioning. Although the underlying etiological basis of autism has eluded researchers, the genetic heritability of autism is quite strong [1]. Specifically what genes are involved and how they contribute to the disease phenotype is unclear.

Many theories regarding the biological basis of autism have been suggested, including neurodevel-opmental, exposure to environmental toxins, particularly to mercury [2], and immune [3] hypotheses. More recently, theories of hyper-systemizing and assortative mating [4,5] and hyper-dopamine [6] have been proposed. At this time there is little definitive evidence to support any single theory of the fundamental biological nature of autism.

Numerous reports have described imbalances in immune and inflammatory processes in autistic patients, including aberrations in antibody levels, cytokines, and cellular subsets [7–12]. Additionally, recent reports have described an increased frequency of HLA-A2 [13] and HLA-DR4 [14] antigens in autism. Interestingly, epidemiological studies have provided evidence for the association of asthma and allergies [15] or autoimmune disorders in families with autistic children [16–19]. The ex-

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act significance of immune abnormalities and the relationship of infections, immunizations, allergies, inflammation, or other aspects of immune response to disease etiology are unclear and controversial. Alterations of immune and inflammatory processes in autism have recently been reviewed [3,20—24].

One of the challenges in the early study of the molecular basis of classical autoimmune disorders was the attempt to establish the relevance of highly variable and fluctuating immune serum proteins and cell populations to disease etiology. That is, are fluctuations in any set of cytokines, immune mediators, or T cell populations, causative or are they epiphenomena due to peripheral effects of target tissue destruction, transient common infections, or more importantly, are they echoes of long ago infections. There is an ever present ''which came first, the chicken or the egg'' nature in the study of highly variable immune mediators. Are oligoclonal antibody bands found in the CSF of multiple sclerosis patients [25] related to the etiology of the disease or are they end stage phenomena? Do alterations in cytokines from a patient with systemic lupus erythematosus have a role in disease etiology or are they late stage responses to tissue destruction brought on by other mechanisms? Similarly, are immune aberrations in autism disease causing or are they epiphenomena?

Other comparisons of autism to asthma and autoimmune/inflammatory disorders

In addition to imbalances in immune molecular mediators, there are other seemingly unrelated parallels in the study of immune and inflammatory disorders as compared to autism that, when viewed collectively, may provide additional support for shared aspects of disease etiology between immune and inflammatory disorders and autism. These include; sex bias, birth order, age-of-onset, neonatal head circumference, increasing prevalence in the population, rural versus urban disease comparisons, and shared molecular and genetic markers.

Disease onset and sex bias

In asthma and in autism presentation is in early childhood. Both disease types have an age of onset in early childhood; 2—4 years for children with autistic disorder [26] and 3—6 for wheeze and asthma [27]. In addition, both autism and asthma display a skewed sex bias toward boys. This bias is approximately 4:1 boys to girls in autism [1] and approximately 2:1 in asthma [28,29]. It is well known that in most adult autoimmune and inflammatory disorders, including asthma, there is a predominance of adult women with the diagnosis. However, less well known is that prior to puberty this skewing is toward boys [30] . This male bias prior to puberty may be true in other immune mediated disorders as well such as multiple sclerosis [31], Type 1 diabetes, and thyroiditis.

Birth order

Some studies have shown birth order to be relevant in atopic disorders as well as autism. In both cases, being first born may carry a greater risk for disease than later births. In a large study of 11, 371 Italian young men those with no siblings had the highest level of serum IgE sensitization. An inverse association was observed between number of siblings at time of testing and prevalence of high atopy $P < 0.0001$ [32]. Similar findings have been shown in for atopic disease in Crete [33], asthma, eczema-urticaria and hay fever in Scotland [34], asthma with allergic rhinitis in Denmark [35] and asthma, allergy, and eczema in the Netherlands [36]. These observations are thought to be related to increased transmission of childhood infections due to a growing family size in the context of the hygiene hypothesis (see below). Similarly, the risk of autism has been shown in some cases to be related to birth order in the same direction as asthma and atopic disorders, with risk decreasing with a greater number of older siblings in the United States [37—39], Western Australia [40] and England [41].

Increased neonatal head circumference

Increased neonatal head circumference has been found in both autism and asthma. Increases in neonatal head circumference have been associated with asthma and atopy. In particular, head circumference has been associated with elevated serum IgE levels and hay fever disorders [42—45]. Increased neonatal head circumference or macrocephaly is a robust finding in autism with the largest effect between the ages of 2—5 [46—51]. This brain size difference is largely back to normal by adolescence. The biological basis for this increase is unknown although genetic, infectious, and inflammatory mechanisms have been proposed [50] (see PTEN below).

Increase in prevalence in the population: parallel ''epidemics''

Both autism and asthma have had reports of apparent increases in the population over the last 30 years. Numerous studies show general increases in prevalence in both asthma [52,53] and autism [54–58], at roughly similar rates over the last 30 years. In both disease types this has been often referred to as an ''epidemic'' [54,59]. In both disease types this apparent increase is controversial. Changes in diagnostic classifications and access to health care resources have confounded the interpretation of prevalence estimates in the study of asthma and autism. Significant increases in disease prevalence over a short time in evolutionary terms suggest that purely genetic mechanisms may not be solely responsible [60]. Given the strong heritability of autism, changing environmental modifiers in the context of the background genetics of autism may be important over the past 30 years. There have been similar increases in the prevalence in classical autoimmune diseases over the same time span as well, including Type 1 diabetes [61,60,62].

Rural vs urban disease distribution

Both autism and asthma appear to show uneven geographical distributions in disease prevalence. Differential susceptibility or resistance to asthma and allergies is found in urban environments versus rural or farm environments [63–65]. Although the exact mechanistic basis of the difference is not known, this distribution pattern of disease is thought to have an inverse relationship to infection and is central to the hygiene hypothesis (see below).

The geographical distribution of autism is less clear although there is evidence that there may be an urban versus rural distribution. This has been found in epidemiological studies from multiple countries including Denmark [66], the United States [67], England [55,68] and Japan [69]. Interestingly, in studies of autism that analyzed numerous familial risk factors, a major risk factor for autism was increasing degree of urbanization [55,68]. In a study from the US, the urban versus rural distribution was attributable to mercury exposure in the environment, however this may reflect an industrialized versus rural pattern as well [67].

The Inuit of northern Canada may provide an interesting population case study. This isolated rural population exists in crowded living conditions, with high levels of mercury and other environmental toxins in the diet [70]. However, autism is essentially non-existent in the Inuit. In a recent report Fombonne et al. state; No case of autism has ever been reported in an Inuit child in the past 15 years [71] . In parallel, asthma and atopic disorders are uncommon in Inuit children, even with very high rates of lower respiratory infections prior to age 2 and particularly high rates of childhood smoking (31.9%) [72].

Molecular and genetic markers shared with inflammatory/autoimmune diseases

Like many common human disorders, autism, asthma, and autoimmune disorders have been studied using genetic linkage and genetic association approaches. The chromosomal regions identified in linkage studies and the specific variants of genes identified in genetic association studies are quite often not unique to any one disorder. Many, if not most, genes in the human genome have broad based effects influencing different cells and tissues at different times of development under the influence of different environmental modifiers. In the context of common human disease, important regulatory genes may effect disease susceptibility differently when found in combination with different disease associated alleles [73].

ADRB2, beta(2)-adrenergic receptor

The gene for the beta(2)-adrenergic receptor encodes a member of the G protein-coupled receptor superfamily and is expressed on epithelial and endothelial cells of the lung, mast cells, as well as airway smooth muscle cells. ADRB2 activation is thought to work through increased intracellular cAMP levels [74]. Polymorphisms in ADRB2, including the Glu27 allele, have been studied in multiple disease states including hypertension [75], atopic dermatitis [76], Graves disease [77], rheumatoid arthritis [78,79], obesity [80] and in particular, asthma [81]. ADRB2 is of major interest in asthma as it may be involved in lung function as well as response to beta(2)-adrenergic agonists [82,83]. ADRB2 polymorphisms may not influence asthma incidence or prevalence but may influence persistence of asthmatic symptoms [84].

Importantly, the Glu27 allele of ADRB2 has recently been associated with autism in twins [85] as well as in the AGRE autism cohort [86].

PTEN-phosphatase and tensin homolog

PTEN, phosphatase and tensin homolog, is central to phosphoinositide metabolism as an important

regulatory checkpoint in the PI3K/ATK signaling pathway, effecting multiple downstream processes including immune function, cell growth, cell survival, and differentiation [87–89]. PTEN has been shown to play a role in lymphocyte proliferation, systemic autoimmunity, and autoimmune disease [87,88], as well as in benign tumors of the gastrointestinal tract. In relation to disease, PTEN has been implicated in bronchial asthma and allergic inflammation [90].

Interestingly, PTEN has been implicated in macrocephaly (OMIM # #153480) and Cowden disease (OMIM #158350). PTEN has been implicated in autism as well, in particular, within a subset of autistic individuals with macrocephaly [91,92]. A recent report described a patient with a PTEN mutation having autistic features, macrocephaly as well as nodular lymphoid hyperplasia of the small and large intestinal mucosa [93]. Moreover, a mouse model with specific deletions of PTEN in selected neuronal cell types resulted in macrocephaly, changes in social interactions, and increased responses to sensory stimuli, suggesting a model for autistic spectrum disorder [94].

MET – met proto-oncogene

The proto-oncogene MET, also known as hepatocyte growth factor receptor, encodes a tyrosine-kinase receptor which has been shown to have pleiotropic effects, in myocardial infarction, ischemia, angiogenesis, and importantly in cancer progression. Recently, polymorphic variants that result in reduced expression of MET has been genetically associated with autism [95]. MET also has been shown to effect the immune system [96], in particular it suppresses immune dendritic cell function [97]. In addition, c-MET and its ligand HGF have been shown to be involved in multiple neuronal processes including synaptic plasticity in the hippocampus [98], development of cortical pyramidal dendrites [99] and synaptic organization [100].

Genome wide scans

Genome wide scans are genetic linkage studies that use evenly spaced polymorphic markers that span the entire human genome in an attempt to link disease phenotypes to specific regions in the human genome. In a comparison of genome wide linkage studies between autoimmune and inflammatory disorders and similar studies in autism and Tourette syndrome, overlap of polymorphic markers were found to be statistically significant ($P = 0.01$) in chromosomal regions originally independently

identified in autism and Tourette's, or in autoimmune and inflammatory disorders. http://www.grc.nia.nih.gov/branches/rrb/dna/pubs/cgoatad.pdf This comparison was performed using the approach originally taken for autoimmune disorders [101]. Fig. 1 shows sixteen selected regions of the genome where this marker overlap occurs. A more comprehensive listing of marker overlap between autoimmune/inflammatory disorders and autism and Tourette syndrome can be found here: http://www.grc.nia.nih.gov/branches/rrb/dna/atsmap.htm Moreover, a subset of these markers found to be statistically significant in both disease classes is not due to simple coincidental overlap of genetic regions, but includes 144 identical polymorphic markers originally found to be statistically significant in both autism and autoimmune or inflammatory disorders, including asthma. For example, in the chromosomal region 17q25.3, the polymorphic marker D17S784 has been independently linked to psoriasis [102], Crohn's disease [103], Tourette syndrome [104], and autism [105]. A listing of markers independently found in both disease classes can be found here: https://www.quickbase.com/db/8qsiujvy.

Summary of disease comparisons

The epidemiological, morphometric, molecular, and genetic comparisons between autism and inflammatory disorders stated above highlight multiple lines of evidence in addition to humoral and cellular immune abnormalities with the goal to strengthen an etiological relationship between autism and autoimmune and inflammatory disorders. It is not suggested that these comparisons support any direct link between these disorders. However, these shared observations between autism and inflammatory disorders are used in support of the development of a hypothesis for the apparent rise in the prevalence of autism using the framework of the immune hygiene hypotheses.

The hygiene hypothesis

The hygiene hypothesis is a widely held theory of the etiology of asthma and atopic disorders which builds on observations of rural versus urban distribution of disease. It suggests that cleaner environmental conditions in westernized countries, as compared to developing countries, play a role in the increase of the prevalence of these disorders in western countries [106]. Moreover, low levels
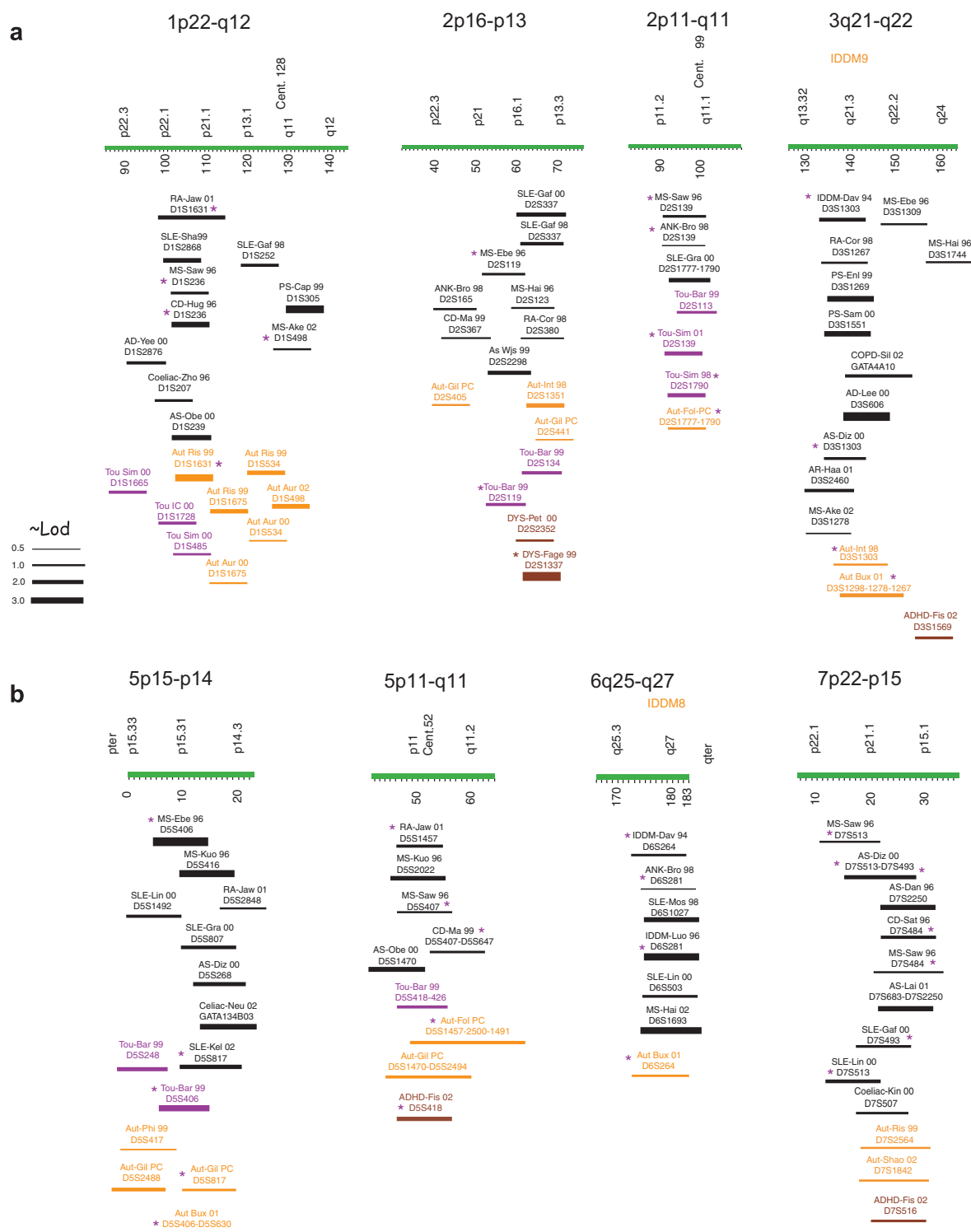
Figure 1 Selected clusters of linkage data from Autism, Tourette syndrome, and Autoimmune/Inflammatory disorders. All polymorphic markers come from independent genetic linkage whole genome scans. Each marker is positioned on a common reference map based on the LDB gmaps. Chromosome band and centimorgan position are shown above and below respectively for each chromosomal region. Polymorphic markers from autoimmune and inflammatory disorders are in black. Markers from autism are in orange. Markers from Tourette syndrome are in purple. All markers are arbitrarily assigned a 10-centimorgan interval at the peak marker reported. Line weight is proportional to LOD score of *P*-value. References and other information for all polymorphic markers can be found here: https://www.quickbase.com/db/8jp3dz49?a=q&qid=1.

Fig. 1 (continued)

of asthma and allergies are found with early exposure to cats [107,108], being raised in a farm environment [109] larger family size [110,35] day-care attendance [111] and birth order [32—36].

Risk for asthma and atopy may be due to a lack of early immune challenge of the post-natal immune system by microbial or parasitic infection possibly including environmental saprophytes and gut commensal organisms, relative to the developing innate immune system [112]. Alteration in the immune repertoire early in thymic development may lead to the establishment of immune hyper-

sensitivity ultimately leading to inflammatory pathology.

In certain ways, the hygiene hypothesis is counterintuitive, in that less clean polluted environments were once thought to cause asthma. Moreover, it is common practice in western society to ''protect'' children from bacteria and microorganisms through isolation indoors and through overuse of antibacterial soaps. This practice may be harmful in not allowing robust immune challenge in early neo-natal development.

The hygiene hypothesis is not without criticism. The changes in the prevalence of atopic disorders may have more complex etiologies with regard to overall microbial load or helminth infection in the general population [113–116] rather than with simple notions of personal or community hygiene practices.

Autism and the hygiene hypothesis

As compared above, similarities between autism, asthma, and inflammatory disorders raise the possibilities of shared mechanisms between these disease types. These include altered immune function in both types of disorders, a similar sex bias at diagnosis, similar birth order relationships, unexplained increased neonatal head circumference, a similar increase in prevalence rates during the last quarter century, a possible rural–urban distribution of the diagnosis with disease being more prevalent in urban environments, and shared molecular and genetic factors between autism and asthma. This adds multiple lines of evidence that mechanisms important in the etiology of immune and inflammatory processes may contribute to the etiology of autism.

It is proposed here that the hygiene hypothesis, a viable theory in the etiology of asthma, should be considered in the etiology of autism. Underlying factors important in the hygiene hypothesis, whether they are truly related to hygiene practices or to overall microbial or parasitic load, thought to be relevant to the increase in asthma and atopy, may contribute to the rise in the incidence of autism as well. Altered patterns of infant immune stimulation may hypersensitize the early immune system not toward allergic sensitivity and bronchial hypersensitivity but to inflammatory or cytokine responses affecting brain structure and function leading to autism. It is well documented that immune cytokines play an important role in normal brain development as well as pathological injury in early brain development [117,118]. It is hypothesized that immune pathways altered by hygiene practices in western society may effect brain structure or function contributing to the development of autism.

Acknowledgements

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