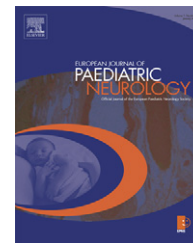




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Original article

Atopic features in early childhood autism

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ABSTRACT

Background: Autism is a developmental disorder of unknown etiology. Sensitivity to dietary and environmental antigens has been considered in its pathogenesis.

Aim: To examine immediate hypersensitivity in early childhood autism.

Methods: We investigated 30 autistic children (23 boys, seven girls 2–4 years old) for atopic history, serum IgG, IgA, IgM, IgE levels, and skin prick tests (SPT) with 12 common antigens. **Results:** Nine/30 autistic children (30%) and 1/39 (2.5%) age-matched neurological controls from the same hospital had a family history suggestive of atopy ($p < 0.005$). No patient in the autism and 28% in control group had symptoms of respiratory allergy (wheezing or asthma) ($p < 0.005$), and 6/30 (20%) autistic vs. 7/39 (17%) control children had history suggesting other allergic disorders ($p = ns$). Eleven/23 (47.8%) autistic children had at least one positive skin test, similar to age-matched population controls. Serum IgG, IgA, and IgM levels were within age-appropriate limits. Serum IgE was elevated in four patients (13.3%). Specific IgE levels were negative in four cases with multiple SPT positivity.

Conclusions: This study suggests allergic features based on history, skin tests, and serum IgE levels are not frequent in young autistic children despite family history. This discrepancy between predisposition and manifestation might imply immunological factors or environmental conditions.

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1. Introduction

Sensitivity to dietary and environmental antigens has been suggested among etiological factors in childhood autism. The presence of gastrointestinal symptoms and favorable behavioral changes observed in some patients after elimination of cow's milk and wheat from diet could support this theory.¹ A randomized trial indicates gluten- and casein-free diet may reduce some autistic traits.² The hypothesis of immunologi-

cal disturbances in autism is further supported by the observation of antibodies against brain tissue.³ HLA association⁴ and family history: allergic disorders in the family and maternal autoimmune diseases were found to be more common in autistic children, and the risk of autistic spectrum disorders were elevated in the presence of maternal asthma and allergy during pregnancy.^{5–7}

The clinical relevance of many laboratory and especially *in vitro* findings has been controversial, partly due to the

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limited number and heterogeneity of patient groups. In this study, we investigated the presence of allergic features in “pure” autism by history and examination in a standardized protocol.

2. Patients and methods

Thirty-two children with autism, 25 boys and seven girls, newly diagnosed according to Diagnostic and Statistical Manual (DSM-IV) criteria who were 2–4 years old and within the first 6 months of diagnosis were considered for the study. All patients were seen in the child psychiatry and child neurology clinics of Hacettepe University, Faculty of Medicine, underwent detailed physical and neurological examination, routine tests for aminoacidopathies, and chromosomal analysis and metabolic tests when indicated. Children born prematurely or with low birth weight, those with a neurological, metabolic or systemic disorder, dysmorphic features, or clinically manifest seizures were excluded.

Childhood Autism Rating Scale (CARS) was used as a numerical instrument, and scored as described previously and standardized for Turkey.⁸ CARS is an observational test containing 15 items, where a score of 30–36.5 indicates mild/moderate and 37–60, severe autism.

The study was approved by the Institutional Ethics Committee, and informed parental consent was obtained.

A standard questionnaire validated and currently used in the department of allergy of the same hospital was filled out by the physician in order to screen for allergic symptoms.⁹ The questions include the presence of respiratory, eye, gastrointestinal, and skin symptoms in the patient and family; their relation with seasonal, nutritional, and environmental changes, any autoimmune diseases in the family, and household conditions. For comparison, 39 age- and sex-matched hospital controls from the department of child neurology from the same area were also given the questionnaire. As done for the autistic group, they were not pre-selected for allergic history. Their clinical diagnoses were febrile convulsion, mild to moderate developmental delay, or epilepsy. None had a metabolic, hereditary disorder or a genetic syndrome. An allergist blinded to the groups examined the questionnaire and rated allergic features from 0 to 3 (0: no suggestive symptoms in family or patient, 1: symptoms in one or more family member, 2: symptoms in the patient, and 3: symptoms in both family and patient).

Serum IgG, IgA, and IgM levels were measured by rate nephelometry (Immagine Immunochemistry Systems, Beckman Coulter, Fullerton, USA). The tests measure the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen (Ig in the sample)–antibody reaction. The reference interval values for serum IgG, IgM, and IgA were established using an Array[®] 360 System and were verified on the Immagine Immunochemistry System. Sensitivity for IgA determination is 6.7 mg/dl, for IgG, 33.3 mg/dl, and for IgM, 4.2 mg/dl. Serum IgE levels were measured by fluoroenzymeimmunoassay (ImmunoCAP, Phadia, Sweden) and compared to previously established normal values of the laboratory (>100 IU/ml). Antigen-specific IgE was measured in children who had

positive skin prick tests (SPT) using fluoroenzymeimmunoassay kit (Phadia AB, Uppsala, Sweden).

SPT with a battery of 12 common allergens (Stallergene, France) were applied: wheat, cereal mix, hen's egg, cow's milk, tree mixture, composite, *Alternaria alternata*, *Cladosporium herbarum*, house dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), grass mix, cat fur, and aspergillus, along with positive control (histamine) and negative control (vehicle). The patients had not taken any antihistaminic drugs within 10 days prior to skin testing, as recommended prior to and verified on the day of testing. One drop of each SPT solution was placed on the back and penetrated with a separate lancet. Reactions to skin test solutions were measured after 20 min. The size of each wheal was documented as the mean of the longest diameter and the diameter perpendicular to it. A mean wheal diameter of at least 3 mm greater than the negative control was interpreted as positive.

3. Results

The questionnaire for allergic symptoms indicated familial atopy in 30% of autistic children and 2.5% of hospital controls ($p < 0.005$) (Table 1). Taken together, 3/30 children of the autism group (10%) and 15/39 of the hospital controls (61%) had a score of at least 1 ($p < 0.005$), and the rate of reported allergic symptoms was 6/30 vs. 7/39 (p :ns). Groups did not differ significantly in early-life environmental factors likely to affect allergic state: area of residence, day care attendance, breast feeding, and birth order. Parental autoimmune disease was present in two autism (vitiligo, psoriasis) and one control case (arthritis) (p :ns). CARS scores of the autism group were 33–50, mean 43.6, and median 44.5.

Of total 276 skin tests applied, 27 (9.7%) were positive, most commonly against aspergillus and grass antigens, followed by cat fur and *D. farinae*. Eleven/23 (47.8%) of children who received skin tests had a positive result with at least one antigen and five of them, with multiple antigens. House dust mite sensitivity was seen in three (13%), pollen, five (21.7%), and mold, in six (26%) children.

Table 1 – Parental questionnaire results

Symptoms	Autism group n = 30 (%)	Hospital controls n = 39 (%)
Urban residence	24 (80)	30 (77)
Attended day care	4 (13)	7 (18)
Breast-fed at least 4 months	25 (83)	36 (93)
First child in the family	16 (53)	16(41)
Familial atopy	9 (30)*	1 (2.5)
Bronchial asthma	0 (0)*	11 (28)
Other allergic disorders	6 (20)	7 (17)

* $p < 0.005$.

Serum IgG, IgA, and IgM were within age-appropriate limits according to laboratory standards. Serum IgE was elevated in 4/30 cases (13.3%), all associated with allergic symptoms in the patient or in a family member, or SPT positivity. Antigen-specific IgE tests done in four out of five children with multiple SPT positivity were negative.

4. Discussion

Many studies examined hypersensitivity or intolerance to environmental and food antigens in autism: however, their interpretation and comparison may be difficult due to methodological differences or anecdotal nature of the information. In addition, autistic spectrum disorders are a mixed group: inclusion of patients of various ages and clinical phenotypes can cause discrepancies, which we intended to avoid by studying newly diagnosed cases with idiopathic childhood autism in a narrow age range.

Previously published findings in autism include a cytokine profile of T helper type 2, or contrarily, type 1 predominance, IgE-mediated or non-IgE-mediated allergic reactions, high levels of IgA, IgG, or IgM against milk antigens, and altered cell-mediated immunity or TNF α production by T cells.^{10–12} Family history of allergy and maternal autoimmune disorders have been reported as more frequent in autistic children with gastrointestinal symptoms.^{13,14} Drugs having the potential to shift the T-cell response from Th2 to Th1, or to reduce Th2 cytokine expression have been of therapeutic benefit in autistic spectrum disorders in an open-label study.¹⁵ We intended to investigate allergic features mediated by lymphocytes of the Th2-type. The frequency of clinical signs and symptoms suggestive of allergy in our series (30%) falls within the range of 25–50% observed in Turkey.^{16,17} Similarly, serum Ig levels were not different from the control population. Lucarelli et al.¹⁸ found specific antibodies of the IgA, IgG, or IgM type against milk antigens to be elevated in children with autistic spectrum disorder, whereas another study showed no difference between the rates of elevated IgE and specific IgE levels or SPT positivity in patients (19.1%) and healthy siblings or general population.¹⁹ Because we could not apply SPT to our control group for ethical reasons, we compared the rate of skin test positivity to previous series: the rate of 47.8% in the autism group is higher than reported from Finland and UK (17–19.6% in 4–5-year-old children unselected for atopy), but not significantly different from normal 2–3-year-old Turkish children: sensitivity to house dust mite, pollens, and molds were found as 30.7%, 46.1%, and 26.9%, respectively, in healthy children, and 13.0%, 21.7%, and 26.0% in our autistic group ($p = ns$).^{20–22} SPT positivity and elevated serum IgE levels were not accompanied by elevated antigen-specific IgE. The lower sensitivity and limited correlation of serum allergen-specific IgE with allergic symptoms or skin test results has been described before. Whether the presence of specific IgE develops into an allergic disorder depends on an interaction of multiple factors including a family history, epitope-specificity and polyclonality of IgE, the balance between T regulatory cells and Th1/Th2 cells, or the polymorphisms of the high IgE affinity receptor.²³ These might represent issues to be investigated further in childhood autism.

Our results suggest the rate of atopic features in young autistic children is similar to or lower than the control population despite a higher frequency of familial atopic history. This finding may reflect a particular immune-response pattern in autistic patients, or the influence of acquired or environmental conditions.^{24,25} A reporting bias, i.e., lower awareness of parents about symptoms of allergy should also be considered but is unlikely because all symptoms in the questionnaire (coughing, wheezing, eye injection, etc.) rely on observation by parents and not on expression by the child. Although life-style conditions were reported as similar in our autism and control groups, certain factors such as peculiar eating habits, or contact with unusual objects might have been overlooked. Another limitation of this study is that our clinical cases might not represent the entire autistic population: our scores on CARS is in the moderate- to severe autism range. Comparison of allergic features between cases in the two extremes of the autistic spectrum would be useful to explain any possible cause-and-effect relationship, and longitudinal follow-up of cases will help to assess the nature, frequency, and age of development of any allergic disorders in this patient group.

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REFERENCES

- Knivsberg AM, Reichelt KL, Høien T, Nødland M. A randomised, controlled study of dietary intervention in autistic syndromes. *Nutr Neurosci* 2002;5:251–61.
- Millward C, Ferriter M, Calver S, Connell-Jones G. Gluten- and casein-free diets for autistic spectrum disorder. *Cochrane Database Syst Rev* 2004;2:CD003498.
- Singer HS, Morris CM, Williams PN, Yoon DY, Hong JJ, Zimmerman AW. Antibrain antibodies in children with autism and their unaffected siblings. *J Neuroimmunol* 2006;178:149–55.
- Lee LC, Zachary AA, Leffell MS, et al. HLA-DR4 in families with autism. *Pediatr Neurol* 2006;35:303–7.
- Comi AM, Zimmerman AW, Frye VH, Law PA, Peeden JN. Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. *J Child Neurol* 1999;14:388–94.
- Croen LA, Grether JK, Yoshida CK, Odouli R, Van de Water J. Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a casecontrol study. *Arch Pediatr Adolesc Med* 2005;159:151–7.
- Sweeten TL, Bowyer SL, Posey DJ, Halberstadt GM, McDougle CJ. Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. *Am J Psychiatry* 2003;160:1691–3.
- Sucuoglu B, Oktem F, Akkok F, Gokler B. Otistik çocukların değerlendirilmesinde kullanılan ölçeklere ilişkin bir çalışma. *3P Dergisi* 1996;4:6–12 [English Abstract].

9. Saraclar Y, Sekerel BE, Kalayci O, et al. Prevalence of asthma symptoms in school children in Ankara, Turkey. *Respir Med* 1998;**92**:203–7.
10. Gupta S, Aggarwal S, Rashanravan B, Lee T. Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. *J Neuroimmunol* 1998;**85**:106–9.
11. Jyonouchi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *J Neuroimmunol* 2001;**120**:170–9.
12. Jyonouchi H, Sun S, Itokazu N. Innate immunity associated with inflammatory responses and cytokine production against common dietary proteins in patients with autism spectrum disorder. *Neuropsychobiology* 2002;**46**:76–84.
13. Molloy CA, Morrow AL, Meinzen-Derr J, et al. Familial autoimmune thyroid disease as a risk factor for regression in children with autism spectrum disorder: a CPEA study. *J Autism Dev Disord* 2006;**36**:317–24.
14. Valicenti-McDermott M, McVicar K, Rapin I, Wershil BK, Cohen H, Shinnar S. Frequency of gastrointestinal symptoms in children with autistic spectrum disorders and association with family history of autoimmune disease. *J Dev Behav Pediatr* 2006;**27**(Suppl. 2):S128–36.
15. Boris M, Kaiser CC, Goldblatt A, et al. Effect of pioglitazone treatment on behavioral symptoms in autistic children. *J Neuroinflammation* 2007;**4**:3.
16. Saraclar Y, Yigit S, Adalioglu G, Tuncer A, Tuncbilek E. Prevalence of allergic diseases and influencing factors in primary-school children in the Ankara Region of Turkey. *J Asthma* 1997;**34**:23–30.
17. Anlar FY, Sancak R, Ozturk F. Childhood allergic disorders in Samsun, Turkey: discrepancy between reported and diagnosed. *Pediatr Allergy Immunol* 2006;**17**:635–8.
18. Lucarelli S, Frediani T, Zingoni AM, et al. Food allergy and infantile autism. *Panminerva Med* 1995;**37**:137–41.
19. Ono SJ. Molecular genetics of allergic diseases. *Annu Rev Immunol* 2000;**8**:347–66.
20. Arshad SH, Tariq SM, Matthews S, Hakim E. Sensitization to common allergens and its association with allergic disorders at age 4 years: a whole population birth cohort study. *Pediatrics* 2001;**108**:E33.
21. Emin O, Nermin G, Ulker O, Gökçay G. Skin sensitization to common allergens in Turkish wheezy children less than 3 years of age. *Asian Pac J Allergy Immunol* 2004;**22**:97–101.
22. Poysa L. Atopy in children with and without a family history of atopy. II. Skin reactivity. *Acta Paediatr Scand* 1989;**78**:902–6.
23. Bousquet J, Anto JM, Bachert C, et al. Factors responsible for differences between asymptomatic subjects and patients presenting an IgE sensitization to allergens. A GA2 LEN project. *Allergy* 2006;**61**:671–80.
24. Infante-Rivard C, Amre D, Gautrin D, Malo JL. Family size, day-care attendance, and breastfeeding in relation to the incidence of childhood asthma. *Am J Epidemiol* 2001;**153**:653–8.
25. McGeady SJ. Immunocompetence and allergy. *Pediatrics* 2004;**113**(Suppl. 4):1107–13.