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EVALUATION OF SELECTED ASPECTS OF THE HYGIENE HYPOTHESIS AND THEIR EFFECT ON THE INCIDENCE OF ALLERGY

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Abstract
Objectives: The development of allergic conditions is largely dependent on the interactions between genetic (individual genetic predisposition) and environmental factors (exposure to risk factors). The aim of this study was an attempt to assess the influence of selected elements of the hygiene theory in the development of allergic diseases such as allergic rhinitis and asthma. **Material and Methods:** The study group consisted of 5518 women and 3868 men. The method that was used was the European Community Respiratory Health Survey II and International Study of Asthma and Allergies in Childhood questionnaire validated and adapted to Central and Eastern European conditions. The project was conducted in 8 urban areas (Gdańsk, Wrocław, Poznań, Katowice, Kraków, Lublin, Białystok, Warsaw) and 1 rural area (Krasnystaw county). This study had 2 stages; the first stage involved grouping the 22 500 respondents based on their questionnaire responses with the use of a Personal Digital Assistant (PDA); the second stage involved 7000 subjects, who underwent additional assessments: skin prick tests (birch, grasses/cereals, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, molds [set I: *Botrytis cinerea*, *Cladosporium herbarum*, *Alternaria tenuis*, *Curvularia lunata*, *Fusarium moniliforme*, *Helminthosporium*], molds [set II: *Aspergillus fumigatus*, *Mucor mucedo*, *Penicillium notatum*, *Pullularia pullulans*, *Rhizopus nigricans*, *Serpula lacrymans*], cat, dog, molds *Cladosporium herbarum*, *Alternaria tenuis*) and spirometry tests. **Results:** The age at which children attend the nursery school is critical to the development of allergic diseases; in allergic rhinitis, the risk of an IgE-dependent reaction is 2 times higher in the second than in the first year of life ($p = 0.00147$, $p < 0.05$), while in asthma, having a large number of siblings increases the risk of developing obstructive disease by almost 6 times ($p = 0.00316$, $p < 0.05$). **Conclusions:** The hygiene theory is particularly applicable and can explain the relationship of selected habits in the development of allergic diseases. Int J Occup Med Environ Health. 2023;36(1):69–83

Key words:
asthma, allergy, allergic rhinitis, hygiene hypothesis, European Community Respiratory Health Survey II, International Study of Asthma and Allergies in Childhood

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INTRODUCTION

Allergic conditions constitute a significant public health concern and are important from the point of view of clinical practice. Nearly 40% of Poland's population has been estimated to suffer from an allergic condition. Twenty-five percent of the general Polish population has been diagnosed with allergic rhinitis (AR); 5% with asthma, including 12% manifesting asthma symptoms; and 4% with atopic dermatitis. Moreover, 6–8% of children and 1–2% of adults are affected with a food allergy [1]. Literature reports indicate many potential causative factors (including environmental factors, lifestyle choices, diet, lack of vitamin D_3 supplementation and genetic predisposition) that may contribute to or exacerbate adaptive IgE-mediated reactions [2–5]. During the 1980s, a causal relationship between the incidence of allergic conditions and the number of children in the family and exposure to bacterial, viral, or parasitic infections has been observed [6]. These factors were shown to affect the immune response in a way that lowered the risk of developing allergies in the future [7]. This phenomenon has been explained by the hygiene hypothesis, which is based on the importance of well-balanced helper T-cell type 1 and 2 (Th_1 and Th_2) population sizes. Soon after birth, when the baby is naturally exposed to pathogens, Th_2 cells play an important role in maintaining T-cell population balance, whereas an absence of antigen stimulation (sterile living conditions) predisposes to allergy development later in life. The factors playing a decisive role in this protective effect, particularly in the case of obstructive respiratory conditions, including asthma, are the type of infection, duration of exposure, and the presence of other (e.g. genetic) factors.

The purpose of this study was to assess selected aspects of the hygiene hypothesis that explain the effect of multiple children in a family, the fact of attending a nursery/kindergarten, exposure to farm animals, and drinking non-pasteurized milk during childhood on the diagnosis rates of AR and asthma. The hygiene theory is already 30 years old and many studies have confirmed the effect of certain environmental factors on the development of allergic diseases. It was the authors' intention to use the results of a study involving a fairly large and varied group of adults and children to confirm the hygiene hypothesis and to emphasise the need to verify the hypothesis, as certain results of this research contradict the hygiene theory.

MATERIAL AND METHODS

This study was conducted in individuals randomly selected by the Polish Ministry of the Interior and Administration from the PESEL (Polish citizen identification number) database. The study method involved the use of questionnaires adapted for Central and Eastern Europe based on the European Community Respiratory Health Survey II (ECRHS II) [8] and International Study of Asthma and Allergies in Childhood (ISAAC) [9], which had been used as part of a larger project, titled the Implementation of a System for the Prevention and Early Detection of Allergic Diseases in Poland (No. 6 PO5 2005 C/06572) [10]. The project was conducted in 8 urban areas (Gdańsk, Wrocław, Poznań, Katowice, Kraków, Lublin, Białystok, Warsaw) and 1 rural area (Krasnystaw county).

This study had 2 stages. The first stage involved grouping the 22 500 respondents based on their questionnaire responses with the use of a personal digital assistant (PDA). The second stage involved 7000 subjects who underwent additional assessments in an outpatient setting: skin prick testing (birch; wormwood; grasses/cereals; *Dermatophagoides pteronyssinus* and *Dermatophagoides farina*; molds [set I: *Botrytis cinerea*, *Cladosporium herbarum*, *Alternaria tenuis*, *Curvularia lunata*, *Fusarium moniliforme*, *Helminthosporium*]; molds [set II: *Aspergillus fumigatus*, *Mucor mucedo*, *Penicillium notatum*, *Pullularia pullulans*, *Rhizopus nigricans*, *Serpula lacrymans*]; cat; dog molds *Cladosporium herbarum*, *Alternaria tenuis*) with a control solution of histamine.

Clinical diagnoses of AR and asthma were verified based on the Allergic Rhinitis and its Impact for Asthma (ARIA) and Global Initiative for Asthma (GINA) diagnostic criteria, respectively. Due to the variety of questionnaire items assessing the symptoms of AR (questions: v176 "Do you have any nasal allergies, including hay fever?"; v178 "Have you ever had a problem with sneezing, or a runny or blocked nose when you did not have fever, a cold, or the flu?"; v179 "Have you ever had a problem with sneezing or a runny or blocked nose when you did not have a cold or the flu in the last 12 months?") and the symptoms of asthma (v136 "Have you had wheezing or whistling in your chest at any time in the last 12 months?"; v137 "Have you been at all breathless when the wheezing noise was present?"; v138 "Have you had this wheezing or whistling when you did not have a cold?"; v139 "Have you woken up with a feeling of tightness in your chest at any time in the last 12 months?"; v140 "Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last 12 months?"; v141 "Have you had an attack of shortness of breath that came on following strenuous activity at any time in the last 12 months?"; v142 "Have you been woken up by an attack of shortness of breath at any time in the last 12 months?") the questionnaire-based and declared responses (specificity and sensitivity) were analyzed with respect to clinical diagnosis of AR or asthma.

The questionnaire item found to effectively verify and diagnose AR was v176: "Do you have any nasal allergies, including hay fever?" Correspondingly, the factor found to be the most consistent with the diagnosis of asthma was wheezing, with the odds ratio (OR) in respondents with wheezing 5 times as high as in those who denied wheezing. Therefore, the item considered in further analysis was the one characterized by the highest chances of accurately assessing the analyzed parameter, i.e., item v136 "Have you had wheezing or whistling in your chest at any time in the last 12 months?" (Table 1).

The research areas were selected intentionally rather than on a random basis, while the selection of responders at the centers was randomized. The random selection algorithm was designed to ensure that the sample was representative of the population. The selection process was based on the PESEL resource which is a Polish government-managed database of national identification numbers and contains details such as the forename, surname, registered home address, age and sex of each person. The questionnaire data were acquired using the computer assisted personal interviewing (CAPI). Personal digital assistants commonly known as palmtop computers, were used to make the survey takers' work much easier, as the software was designed to use automated question filtering. The patients were diagnosed only and exclusively on the basis of validated ECRHS and ISSAC questionnaires. This is normal in the case of such epidemiological studies. Other criteria for the clinical diagnosis of AR included those based on the international ARIA and GINA guidelines. Additionally, skin prick tests and spirometry tests were also used to verify the diagnosis made by the allergist and/or the pulmonologist.

The study population was homogeneous in terms of age, with the mean (M) age of women 31.9 years and the age of men M = 31.3 years. Overall, women constituted 59% (N = 5518) whereas men constituted 41% (N = 3868) of the study population. Respondents from urban areas (N = 8337) constituted 89% of the study population, whereas respondents from rural areas (living in Krasnystaw county N = 1049) constituted a considerably smaller proportion (11%).

Among respondents diagnosed with a single allergic condition the most numerous were those with AR, followed by those with asthma; the conditions in these patients have been shown to be largely hereditary (Table 2). The predisposition to allergies was decidedly more commonly inherited from the mother, which was also observed in siblings (Table 3). For comparison, as a control group the

Table 1. Logistic regression analysis results for allergic rhinitis and asthma in 2 stage study conducted in 8 urban areas and areas (Gdańsk, Wrocław, Poznań, Katowice, Kraków, Lublin, Białystok, Warsaw) and 1 rural area (Krasnystaw county), Poland

Questionnaire item	Allergic rhinitis				Asthma			
	OR	95% CI	p	AUC	OR	95% CI	p	AUC
v176	**4.45**	**3.75–5.28**	**<0.001**	**0.67**				
v178	4.06	3.38–4.88	<0.001	0.67				
v179	1.74	1.3–2.33	<0.001	0.54				
v136					**5.16**	**3.92–6.78**	**<0.001**	**0.66**
v137					1.72	1.12–2.63	0.014	0.56
v138					2.34	1.49–3.65	<0.001	0.60
v139					3.67	2.66–5.07	<0.001	0.58
v140					3.1	2.17–4.41	<0.001	0.56
v141					3.24	2.46–4.26	<0.001	0.62
v142					4.87	3.37–7.03	<0.001	0.57

AUC – area under the curve.
v176 "Do you have any nasal allergies, including hay fever?"; v178 "Have you ever had a problem with sneezing, or a runny or blocked nose when you did not have a fever, a cold or the flu?"; v179 "Have you had a problem with sneezing or a runny or blocked nose when you did not have a cold or the flu in the last 12 months?"; v136 "Have you had wheezing or whistling in your chest at any time in the last 12 months?"; v137 "Have you been at all breathless when the wheezing noise was present?"; v138 "Have you had this wheezing or whistling when you did not have a cold?"; v139 "Have you woken up with a feeling of tightness in your chest at any time in the last 12 months?"; v140 "Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last 12 months?"; v141 "Have you had an attack of shortness of breath that came on following strenuous activity at any time in the last 12 months?"; v142 "Have you been woken up by an attack of shortness of breath at any time in the last 12 months?"
Bolded are the most significant results.

Table 2. Study group characteristics in 2 stage study conducted in 8 urban areas and areas (Gdańsk, Wrocław, Poznań, Katowice, Kraków, Lublin, Białystok, Warsaw) and 1 rural area (Krasnystaw county), Poland

Variable	Participants (N = 9386)					
	with allergic rhinitis (N = 6713)		with asthma (N = 2508)		control group (N = 2403)	
	n	%	n	%	n	%
Age						
6–7 years	1705	25.4	868	34.6	699	29.1
13–14 years	1630	24.3	479	19.1	652	27.1
20–44 years	3378	50.3	1161	46.3	1052	43.8
Sex						
female	3538	52.7	1272	50.7	1324	55.1
male	3175	47.3	1236	49.3	1079	44.9
Area						
urban	6243	93.0	2320	92.5	2107	87.7
rural	470	7.0	188	7.5	296	12.3

Table 3. Genetic predisposition and incidence of allergies in 2 stage study conducted in 8 urban areas and areas (Gdańsk, Wrocław, Poznań, Katowice, Kraków Lublin, Białystok, Warsaw) and 1 rural area (Krasnystaw county), Poland

Genetic predisposition	Participants (N = 9386)													
	age						sex				area			
	6–7 years (N = 2534)		13–14 years (N = 2347)		20–44 years (N = 4505)		female (N = 5518)		male (N = 3868)		urban (N = 8337)		rural (N = 1049)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Allergic rhinitis														
mother	411	**24.1**	408	**25**	449	13.3	686	19.4	584	18.4	1,217	19.5	50	10.6
father	343	**20.1**	248	15.2	250	7.4	410	11.6	429	13.5	818	13.1	23	4.9
sibling(s)	390	**22.9**	432	**26.5**	540	16	715	**20.2**	648	**20.4**	1,311	**21**	51	10.9
grandparents														
maternal	189	11.1	192	11.8	95	2.8	219	6.2	254	8	462	7.4	15	3.2
paternal	111	6.5	77	4.7	61	1.8	127	3.6	117	3.7	237	3.8	11	2.3
Asthma														
mother	199	**22.9**	119	**24.8**	145	12.5	224	17.6	239	19.3	450	19.4	14	7.4
father	155	17.9	70	14.6	75	6.5	155	12.2	145	11.7	290	12.5	11	5.9
sibling(s)	222	**25.6**	136	**28.4**	163	14	254	20	267	**21.6**	496	**21.4**	24	12.8
grandparents														
maternal	100	11.5	60	12.5	35	3	86	6.8	108	8.7	188	8.1	6	3.2
paternal	65	7.5	20	4.2	22	1.9	52	4.1	54	4.4	104	4.5	2	1.1
Control group														
mother	102	14.6	87	13.3	89	8.5	173	13.1	104	9.6	255	12.1	22	7.4
father	74	10.6	54	8.3	33	3.1	87	6.6	74	6.9	150	7.1	11	3.7
sibling(s)	126	18	123	18.9	130	12.4	211	15.9	169	15.7	339	16.1	39	13.2
grandparents														
maternal	57	8.2	33	5.1	17	1.6	68	5.1	40	3.7	103	4.9	3	1
paternal	22	3.1	18	2.8	11	1	29	2.2	22	2	44	2.1	6	2

Bolded are the most significant results.

authors consider subjects without no diagnosed any allergy diseases. The differences in the fractions in Table 3 did not significantly differentiate the groups AR and asthma the control group ($p > 0.05$, prop.test).

This study was approved by the Medical University of Warsaw Institutional Review Board (KB/206/2005) and by the Chief Inspector of Personal Data Protection. Statistical analyses included frequency analysis, testing for equality of proportions (prop.test function in R), and a logistic regression model for the individual variables, with the use of the odds ratio and 95% confidence interval (CI) (Tables 1, 4, 5). The area under the curve (AUC) was also presented to assess for goodness of fit between the models and the data (Table 1). Analyses were performed with the use of R. The threshold of statistical significance was adopted at $p < 0.05$.

Table 4. Positive skin-prick test rates in 2 stage study conducted in 8 urban areas and areas (Gdańsk, Wrocław, Poznań, Katowice, Kraków, Lublin, Białystok, Warsaw) and 1 rural area (Krasnystaw county), Poland

Allergen	Participants with positive skin-prick test rate (N = 4002)																	
	6–7 years (N = 1129)						13–14 years (N = 1080)						20–44 years (N = 1793)					
	≥3 mm		3–5 mm		≥6 mm		≥3 mm		3–5 mm		≥6 mm		≥3 mm		3–5 mm		≥6 mm	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Urban area																		
birch	23	3.5	8	1.2	5	0.8	37	5.7	15	2.3	13	2.0	49	7.5	30	4.6	33	5.0
grasses / cereals	29	4.4	23	3.5	8	1.2	43	6.6	39	6.0	40	3.4	54	8.3	40	6.1	56	8.6
wormwood	32	4.9	6	0.9	2	0.3	60	9.2	11	1.7	1	0.2	77	11.8	41	6.3	12	1.8
Dermatophagoides pteronyssinus	54	8.3	29	4.5	10	1.5	57	8.7	44	6.7	32	4.9	119	18.2	53	8.1	41	6.3
molds (set I)	22	3.4	8	1.2	2	0.3	54	8.3	8	1.2	4	0.6	64	9.8	10	1.5	5	0.8
molds (set II)	19	2.9	0	0	0	0	24	3.7	2	0.3	0	0	56	8.6	5	0.8	1	0.2
dog	31	4.7	1	0.2	1	0.2	34	5.2	5	0.8	0	0	80	12.2	5	0.8	2	0.3
cat	27	4.1	8	1.2	5	0.8	44	6.7	23	3.5	8	1.2	71	10.9	30	4.6	16	2.4
Dermatophagoides farinae	54	8.3	28	4.3	7	1.1	55	8.4	42	6.4	23	3.5	100	15.3	45	6.9	29	4.4
hazel	21	3.2	13	2.0	1	0.2	35	5.4	18	2.8	3	0.5	47	7.2	25	3.8	30	4.6
alder	20	3.1	7	1.1	1	0.2	35	5.4	14	2.1	3	0.5	54	8.3	33	5.0	14	2.1
rye	24	3.7	17	2.6	4	0.6	49	7.5	19	2.9	13	2.0	58	8.9	38	5.8	40	6.1
narrow leaf plantain	21	3.2	0	0	0	0	30	4.6	7	1.1	0	0	70	10.7	13	2.0	2	0.3
Cladosporium herbarum	11	1.7	1	0.2	0	0	20	3.1	0	0	0	0	49	7.5	4	0.6	0	0
Alternaria tenuis	25	3.8	8	1.2	2	0.3	27	4.1	15	2.3	4	0.6	44	6.7	8	1.2	2	0.3
Rural area																		
birch	2	2.8	0	0	0	0	1	1.4	1	1.4	0	0	5	7.0	0	0	0	0
grasses / cereals	1	1.4	1	1.4	0	0	7	9.9	0	0	1	1.4	8	11.3	2	2.8	3	4.2
wormwood	4	5.6	0	0	0	0	7	9.0	0	0	0	0	6	8.5	0	0	0	0
Dermatophagoides pteronyssinus	6	8.5	2	2.8	0	0	9	12.7	4	5.6	4	5.6	13	18.3	5	7	0	0
molds (set I)	3	4.2	0	0	0	0	6	8.5	0	0	0	0	10	14.1	0	0	0	0
molds (set II)	3	4.2	0	0	0	0	7	9.9	0	0	0	0	7	9.9	0	0	0	0
dog	6	8.5	0	0	0	0	7	9.9	0	0	0	0	9	12.7	0	0	0	0

cat	2	2.8	1	1.4	0	0	3	4.2	1	1.4	0	0	5	7.0	0	0	0	0
Dermatophagoides farinae	4	5.6	0	0	0	0	11	15.2	2	2.8	1	1.4	14	19.7	3	4.2	0	0
hazel	4	5.6	0	0	0	0	6	8.5	1	1.4	0	0	4	5.6	1	1.4	0	0
alder	3	4.2	0	0	0	0	2	2.8	1	1.4	0	0	6	8.5	1	1.4	0	0
rye	3	4.2	0	0	0	0	3	4.1	0	0	1	1.4	4	5.6	1	1.4	2	2.8
narrow leaf plantain	4	5.6	0	0	0	0	4	5.6	0	0	0	0	8	11.3	1	1.4	0	0
Cladosporium herbarum	1	1.4	0	0	0	0	3	4.2	0	0	0	0	4	5.6	0	0	0	0
Alternaria tenuis	4	5.6	0	0	0	0	0	0	1	1.4	0	0	5	7.0	0	0	0	0

Set I – *Botrytis cinerea, Cladosporium herbarum, Alternaria tenuis, Curvularia lunata, Fusarium moniliforme, Helminthosporium;* set II – *Aspergillus fumigatus, Mucor mucedo, Penicillium notatum, Pullularia pullulans, Rhizopus nigricans, Serpula lacrymans.*

Table 5. Selected aspects of the hygiene hypothesis and allergic rhinitis, asthma and in the control group in 2 stage study conducted in 8 urban areas and areas (Gdańsk, Wrocław, Poznań, Katowice, Kraków Lublin, Białystok, Warsaw) and 1 rural area (Krasnystaw county), Poland

Item	Participants (N = 9386)													
	age						sex				area			
	6–7 years (N = 2534)		13–14 years (N = 2347)		20–44 years (N = 4505)		female (N = 5518)		male (N = 3868)		urban (N = 8337)		rural (N = 1049)	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Allergic rhinitis														
v204														
2/1	n.s.	n.s.	**2.05**	**1.32–3.19**	0.57	0.42–0.78	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
3/1	n.s.	n.s.	n.s.	n.s.	0.69	0.53–0.89	0.75	0.59–0.95	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
4/1	n.s.	n.s.	n.s.	n.s.	0.71	0.54–0.93	0.75	0.58–0.97	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
5/1	n.s.	n.s.	n.s.	n.s.	0.63	0.48–0.83	0.73	0.57–0.94	n.s.	n.s.	0.82	0.67–1	n.s.	n.s.
6/1	n.s.	n.s.	n.s.	n.s.	0.5	0.39–0.65	0.58	0.46–0.73	0.72	0.54–0.97	0.72	0.60–0.86	n.s.	n.s.
7/1	n.s.	n.s.	n.s.	n.s.	0.49	0.37–0.64	0.61	0.46–0.80	0.66	0.47–0.92	0.66	0.53–0.82	n.s.	n.s.
v205														
1/0	0.83	0.72–0.95	0.78	0.68–0.89	n.s.	n.s.	0.84	0.76–0.93	0.88	0.80–0.97	0.87	0.80–0.94	n.s.	n.s.
2/0	0.62	0.49–0.78	n.s.	n.s.	0.78	1/0	0.82	0.71–0.94	0.67	0.57–0.78	0.79	0.70–0.89	n.s.	n.s.
3/0	0.55	0.36–0.85	0.59	0.41–0.84	0.66	0.54–0.80	0.65	0.52–0.81	0.58	0.45–0.75	0.64	0.54–0.76	n.s.	n.s.
4/0	0.41	0.18–0.95	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
5/0	n.s.	n.s.	n.s.	n.s.	0.51	0.28–0.94	0.37	0.18–0.76	n.s.	n.s.	0.49	0.27–0.90	n.s	n.s.

Table 5. Selected aspects of the hygiene hypothesis and allergic rhinitis, asthma and in the control group – cont.

Item	Participants (N = 9386)													
	age						sex				area			
	6–7 years (N = 2534)		13–14 years (N = 2347)		20–44 years (N = 4505)		female (N = 5518)		male (N = 3868)		urban (N = 8337)		rural (N = 1049)	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Allergic rhinitis – cont.														
v463														
1/0	n.s.	n.s.	n.s.	n.s.	0.92	0.83–1	0.84	0.76–0.93	0.8	0.73–0.88	0.83	0.77–0.90	n.s.	n.s.
2/0	n.s.	n.s.	n.s.	n.s.	0.76	0.66–0.87	0.7	0.61–0.80	0.68	0.59–0.78	0.73	0.66–0.81	0.65	0.48–0.89
3/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.62	0.49–0.78	0.8	0.67–0.95	n.s.	n.s.
6/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.14	0.03–0.60	0.29	0.10–0.85	0.14	0.04–0.45	n.s.	n.s.
7/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.23	0.07–0.78	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
v464														
1/0	n.s.	n.s.	n.s.	n.s.	0.9	0.82–0.99	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.81	0.68–0.97	n.s.	n.s.	n.s.	n.s.
5/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1.88	1.02–3.45
5/1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.42	0.18–0.96	n.s.	n.s.
v466	n.s.	n.s.	n.s.	n.s.	0.72	0.56–0.92	n.s.	n.s.	0.64	0.48–0.85	0.75	0.6–0.94	n.s.	n.s.
v467	n.s.	n.s.	n.s.	n.s.	0.7	0.55–0.91	0.76	0.58–1	0.71	0.54–0.94	0.73	0.58–0.91	n.s.	n.s.
v616_														
002	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
003	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
v618_002	n.s.	n.s.	n.s.	n.s.	0.19	0.05–0.79	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Asthma														
v204														
2/1	n.s.	n.s.	n.s.	n.s.	0.47	0.31–0.71	0.6	0.41–0.87	n.s.	n.s.	0.71	0.54–0.93	n.s	n.s.
3/1	n.s.	n.s.	n.s.	n.s.	0.53	0.38–0.74	0.64	0.47–0.88	n.s.	n.s.	0.72	0.57–0.91	n.s.	n.s.
4/1	n.s.	n.s.	n.s.	n.s.	0.46	0.33–0.64	0.64	0.47–0.88	n.s.	n.s.	0.68	0.54–0.86	n.s.	n.s.
5/1	n.s.	n.s.	n.s.	n.s.	0.53	0.38–0.74	0.59	0.42–0.82	0.66	0.45–0.98	0.66	0.51–0.85	n.s.	n.s.

6/1	n.s.	n.s.	n.s.	n.s.	0.48	0.35–0.66	0.53	0.39–0.71	0.67	0.46–0.97	0.64	0.51–0.81	n.s.	n.s.
7/1	n.s.	n.s.	n.s.	n.s.	0.53	0.38–0.74	n.s.	n.s.	0.59	0.38–0.91	0.67	0.51–0.88	n.s.	n.s.
v205														
1/0	n.s.	n.s.	n.s.	n.s.	0.83	0.72–0.95	0.81	0.71–0.93	n.s.	n.s.	0.86	0.78–0.95	n.s.	n.s.
7/0	n.s.	n.s.	n.s.	n.s.	**6.5**	**1.89–22.34**	**5.2**	**1.57–17.19**	n.s.	n.s.	n.s.	n.s.	**4.73**	**1.15–19.4**
v463														
2/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.79	0.65–0.96	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
4/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1.54	1.04–2.28	n.s.	n.s.	1.61	1.13–2.29	n.s.	n.s.
5/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.12	0.02–0.87	n.s.	n.s.	n.s.	n.s.
v464														
1/0	n.s.	n.s.	n.s.	n.s.	0.85	0.74–0.97	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
3/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.6	0.38–0.94	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
v466	**1.9**	**1.04–3.48**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
v467	**1.85**	**1.01–3.38**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.
v618_														
002	n.s.	n.s.	**3.03**	**1.14–8.06**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.
003	n.s.	n.s.	**2.21**	**1.02–4.79**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.
Control group														
v205														
1/0	1.28	1.01–1.62	1.3	1.03–1.64	n.s.	n.s.	1.2	1.01–1.43	n.s.	n.s.	1.21	1.05–1.39	n.s.	n.s.
2/0	1.57	1.04–2.37	1.52	1.05–2.21	n.s.	n.s.	1.32	1.02–1.70	n.s.	n.s.	1.24	1.00–1.54	n.s.	n.s.
3/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1.55	1.03–2.34	n.s.	n.s.	1.52	1.07–2.16	n.s.	n.s.
v463														
1/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1.16	1.01–1.33	n.s.	n.s.
2/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1.36	1.05–1.75	n.s.	n.s.	1.3.	1.07–1.58	n.s.	n.s.
3/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**1.8**	**1.12–2.88**	**2.41**	**1.21–4.79**	**2.55**	**1.21–5.37**
4/0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**3.11**	**1.39–6.95**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
v464 6/0	n.s.	n.s.	n.s.	n.s.	**2.53**	**1.2–5.33**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
v466	n.s.	n.s.	1.71	1.27–2.3	1.39	1.17–1.66	1.3	1.09–1.55	1.46	1.19–1.78	1.4	1.21–1.63	n.s.	n.s.
v467	n.s.	n.s.	1.63	1.21–2.2	1.46	1.22–1.75	1.33	1.12–1.58	1.43	1.18–1.74	1.41	1.21–1.64	n.s.	n.s.

Table 5. Selected aspects of the hygiene hypothesis and allergic rhinitis, asthma and in the control group – cont.

Item	Participants (N = 9386)													
	age						sex				area			
	6–7 years (N = 2534)		13–14 years (N = 2347)		20–44 years (N = 4505)		female (N = 5518)		male (N = 3868)		urban (N = 8337)		rural (N = 1049)	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Control group														
v616_														
002	0.55	0.37–0.8	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
003	n.s.	n.s.	1.47	1.11–1.94	n.s.	n.s.	1.33	1.05–1.69	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
v620_														
002	n.s.	n.s.	n.s.	n.s.	2.77	1.08–7.1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
003	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1.23	1.04–1.47	n.s.	n.s.	1.22	1.06–1.4	n.s.	n.s.

v204 "At what age did you first go to school, kindergarten, or nursery?"; v205 "How many other children used to sleep in the same room with you before you were 5 years old?" (applicable for single-family housing); v463 "How many OLDER brothers or sisters do you have?"; v464 "How many YOUNGER brothers or sisters do you have?"; v466 "Were you regularly (at least once a week) in contact with farm animals (i.e. cattle, pigs, goats, sheep, or poultry) before the age of 1 year?"; v467 "Was your mother regularly (at least once a week) in contact with farm animals (i.e. cattle, pigs, goats, sheep, or poultry) during the pregnancy?"; v616_002 "Did you use these formulations up to (a total of) 7 days a year before the age of 1 year?"; v616_003 "Did you use these formulations up to (a total of) 7 days a year between the age of 1 and 3 years?"; v618_002 "Did you use to consume SOURED MILK/KEFIR at least 3 times a week before the age of 3 years?"; v618_003 "Did you use to consume SOURED MILK/KEFIR at least 3 times a week between the age of 3 and 7 years?"; v620_002 "Did you use to consume YOGHURT at least 3 times a week before the age of 3 years?"; v620_003 "Did you use to consume YOGHURT at least 3 times a week between the age of 3 and 7 years?"
For v204 OR was calculated with respect level 1, for v205, v463 and v464 OR was calculated with respect level 0.
Bolded are the most significant results.

RESULTS

Positive skin-prick-test rates in the study population

Skin prick testing produced considerably greater reactions in the urban area subpopulation, and the allergens producing the most pronounced reactions were (in decreasing order): house dust mite *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, grass, wormwood, cat, and dog allergens. The subgroups with the highest positive reaction rates in the skin prick test were adolescents (aged 13–14 years) and adults (Table 5).

The aspects of the hygiene hypothesis relevant to allergic rhinitis and asthma

The risk of developing AR in the subgroup of 13–14-year-olds was 2 times higher in those individuals who had attended a nursery at the age of 2 years rather than at 1 year ($p = 0.00147$, $p < 0.05$). Conversely, the risk of developing AR in the adult subgroup was 2 times lower if they had attended a nursery at the age of 2 years ($p = 0.000415$, $p < 0.05$). The greater the number of persons living in 1 household, particularly before the age of 5 years, the lower the risk of developing AR. Having numerous siblings has a protective effect against developing AR; the same protective effect was observed in households with a large number of people. Other analyzed factors, including exposure to livestock or non-pasteurized milk consumption, were also shown to have a preventive effect in AR. Interestingly, all the associations described above were most prominent in the subgroup of urban area residents (Table 5).

Conversely, in those diagnosed with asthma (particularly in women residing in rural areas), coming from a family with multiple siblings was observed to increase by over 5-fold the risk of developing an obstructive respiratory condition ($p = 0.00316$) (Table 5). The fact alone of being from a family with a greater number of children and being sent to kindergarten early reduced the risk of developing asthma. The risk of asthma was nearly 2 times higher in those respondents who had been exposed to livestock in their childhood. Moreover, dietary supplementation with kefir or non-pasteurized milk (up to the age of 7 years) had no protective effect against developing asthma later in life.

DISCUSSION

Over the last 15 years, there has been a rapid increase in the incidence of allergic conditions worldwide, particularly in highly developed countries. In Poland, nearly 40% of the population has been diagnosed with at least 1 allergic condition [1]. The development of allergic conditions is largely dependent on the interactions between genetic (individual genetic predisposition) and environmental factors (exposure to risk factors). The genetic makeup may increase the risk of developing allergic conditions, with environmental factors affecting gene expression and modifying allergic inflammatory response. The effect of the environment on allergy development is multifactorial. One important factor is exposure to inhalational and food allergens, which reveal the inborn predisposition to allergy development and initiate an immune response. Infectious pathogens exhibit a dual effect on allergy development, by sometimes inducing allergies and sometimes – in different circumstances – reducing the risk of allergy. Nonspecific environmental factors, such as cigarette smoke, exhaust fumes, and dust pollution facilitate allergy development [11].

Already in the 1980s, there were attempts to establish the cause of the increased prevalence of allergies. In 1989, David Strachan reported that individuals from families with many children who were raised along with their older siblings showed a lower risk of developing AR and eczema. Based on this observation, Strachan concluded that increased incidence of allergic conditions is associated with lifestyle changes, reduced family size, better living conditions, and increased hygiene levels. This gave rise to the hygiene hypothesis in the development

of allergic conditions [12]. The hypothesis is based on the physiological dominance of Th_2 cell activity during the fetal life. This is conducive to allergy development; however, the subsequent natural microbiological stimulation that leads to Th_1 cell activation has a protective role, since it restores the Th_1/Th_2 balance. Living in nearly sterile conditions hampers that natural Th_1 stimulation, thus contributing to allergy development [11]. It is now known that the course of the immune response to infectious agents depends on the nature of those agents, duration and extent of exposure, and the involvement of toll-like and NOD-like receptors [11,13].

This study assessed selected environmental factors and their effect on allergy development. The authors found the most commonly sensitizing allergens to be house dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), grass and wormwood pollens, and allergens of domestic animals (dogs and cats). The hygiene hypothesis is based on the assumption that exposure to inhalational allergens is an important risk factor for allergy development. Over its natural course, an allergic condition usually begins in the form of sensitization to indoor allergens, even as early as in the first year of life, with pollen sensitization developing somewhat later [11]. Miller [14] studied and confirmed the significant role of sensitization to house dust mites in the development of allergic conditions, including the phenomenon of cross-reactivity. Exposure to house dust mite allergens showed the strongest relationship with the development of asthma, with the highest rates of allergy to *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* observed among those affected with asthma [9,10,15,16]. Experts from the European Academy of Allergy and Clinical Immunology (EAACI) emphasize the important role of house dust mite sensitization in the pathogenesis of asthma and recommend the use of specific immunotherapy [17]. Undoubtedly, house dust mite allergy is also a major risk factor in the development of AR [18].

This study showed a 2-fold higher risk of developing AR in those 13–14-year-olds who had started attending kindergarten at the age of 2, whereas the risk of developing AR in the adult group was 2 times lower in those who had begun attending kindergarten at the age of 2. Attending kindergarten is associated with massive exposure to infectious agents (viruses and bacteria). The authors findings regarding the effect of infectious agents on allergy development were inconclusive. Exposure to infections may either protect against or induce allergy. To date, there is no consensus as to the ultimate effect of these factors on allergy development [19]. A study in Japanese children showed positive tuberculin responses to be inversely proportional to atopy. This indicates that subclinical exposure to *Mycobacterium tuberculosis* may have a beneficial effect on the balance of Th cell types and, thus, reduce the incidence of allergies [17].

A study by Tantilipikorn [20] showed that viral infections may either promote AR or protect against it, which is most likely dependent on the condition of the individual's adaptive and innate immunity and on the virulence of the pathogen. The significance of viral infections in allergy development has been largely documented with respect to asthma. Follow-up data collected over many years show frequent recurrences of wheezing and the development of allergy [21]. Of particular importance are respiratory syncytial virus (RSV) infections, whose harmful effect is due to destruction of respiratory epithelium and stimulation of Th_2 cells. Currently, asthma development is being also attributed to rhinovirus infections. Such long-term effects of infections may be due to the release of tissue-remodeling factors, such as tumor growth factor β (TGF-β), epidermal growth factor, and vascular endothelial growth factor (VEGF) [22].

This study showed a 2 times higher risk of asthma in those who had been exposed to livestock in childhood. This observation undermines the hygiene hypothesis, which is based on the assumption that environmental

exposures in early childhood sensitize the immune system towards Th_1 cell production, whereas a sterile environment shifts the immune cell balance towards Th_2 cells and induces allergy development. Therefore, the results of this study show the need to reinvestigate the accuracy of the hygiene hypothesis in light of the considerable effect of interindividual genetic and epigenetic differences in allergy development [23].

This study demonstrated that living together with numerous people within 1 households, particularly before the age of 5 years, reduces the risk of AR. Having a large number of siblings was also shown to have a protective effect against AR. Moreover, having many siblings and attending kindergarten at an early age reduced the risk of asthma. Conversely, the evaluated adult subgroup showed a higher risk of asthma if they came from families with many children. A number of studies demonstrated that the incidence of atopy, AR, and asthma is inversely proportional to the number and order of siblings. Families with many children have lower rates of AR and asthma [24]. The results of this study are partly consistent with the hygiene hypothesis and partly cast doubt on its tenets. This is probably due to epigenetic variations.

This study demonstrated a protective effect of exposure to farm animals and non-pasteurized milk consumption on AR, whereas no such effect was observed in the case of asthma. There have been a number of studies attempting to assess the incidence of allergy in children residing in rural areas in comparison with that in children from urban areas. The evaluated factors included exposure to farm animals (spending time in stables, cattle sheds, and barns) and regular consumption of dairy products (milk, butter, cheeses). The results indicated a reduced risk of allergies [25]. Multicenter cohort studies conducted in Europe evaluated the effect of prenatal exposure to environmental factors (the PASTURE study) and the mechanisms of early exposure to allergens (the EFRAIM study). These studies showed a beneficial, allergy-protective effect of maternal exposure to livestock during pregnancy on the immune system in neonates [25].

The 30-year-old hygiene hypothesis has not been replaced by other concepts, but its validity is continually being challenged. The key arguments derive from the increasing incidence of allergies despite an unchanged incidence of infectious diseases, diagnosing allergies in adults, and the cases of allergy in children with Th_1-mediated disorders. Recent years saw the hygiene hypothesis evolving towards the microbial diversity hypothesis. The current, modified, version of the hygiene hypothesis assumes that the Western lifestyle limits not only the number of infections but also exposure to microorganisms. This disrupts the development of the immune system, affects the development and maintenance of immune tolerance, and alters gut colonization, thus predisposing to allergy development [26]. Another factor of great importance in allergy development is epigenetics, which involves the associations between the environment and genes [23]. This study has found that asthma was twice as frequent in patients who were exposed to livestock and drank unpasteurized milk in their childhood years. This contradicts the principles of the hygiene hypothesis that the early childhood environment makes the immune system more sensitive in terms of the production of Th_1 lymphocytes (non-allergic phenotype) whereas a sterile environment shifts the balance in favour of Th_2 lymphocytes and the development of allergy. The results of this research show that it is necessary to verify the hygiene hypothesis and to consider the significant effect of interpersonal genetic variation on the development of allergies. Interactions between genes and the environment are clearly paramount to the development of asthma and allergies. The best-researched genetic associations with an asthma phenotype include variants of the HLA-DQ and DB genes, variants of enzymes and receptors of the routes of lipid mediators derived from arachidonic acid: *ALOX5*, *ALOX12*, *LTC4S*, *CYSLTR1*, *CYSLTR2* and a variant of the *CH13L1* gene

promoter, which encodes the chitinase-3-like protein. It seems that epigenetic mechanisms also play a major role in the development of asthma. Oxidative stress in cells may be caused by many environmental factors. The DNA so damaged causes DNA methyltransferases to malfunction. DNA methylation is affected by factors such as air pollution, stress or smoking. This makes epigenetics a mechanism that acts an intermediary between genetics, the environment and the disease [23].

CONCLUSIONS

The authors would like to conclude by emphasizing that our study confirms the role of hygiene theory in the development of allergic diseases: the age at which children attend the nursery school is critical to the development of allergic diseases; in allergic rhinitis, the risk of an IgE-dependent reaction is 2 times higher in the second than in the first year of life while in asthma, having a large number of siblings increases the risk of developing obstructive disease by almost 6 times. On the other hand, the results of this research (a 6-fold increase in the risk of asthma developing in adults living in large families and twice as many cases of asthma in patients who were exposed to livestock in their childhood years) also show that it is necessary to verify the hygiene hypothesis and to pay special attention to the effect of epigenetic mechanisms on the development of allergic diseases.

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