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Report

Microchimerism in alopecia areata

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Abstract

Background Autoimmunity is the main etiopathogenetic factor in alopecia areata. Microchimerism is the existence of allogeneic DNA in a living creature. There are variable studies investigating the role of microchimerism on the etiopathogenesis of autoimmune diseases. To our knowledge, no report has investigated the relationship between microchimerism and alopecia areata.

Objective We aimed to investigate the possible role of microchimerism on alopecia areata.

Methods We analyzed SRY gene levels as indicators of fetal microchimerism in our patient group. The patients were 29 women with alopecia areata, over 18 years old, who had visited our clinic between 2010 and 2013. Patients were divided into two groups; group 1 consisted of 14 patients having a son and group 2, 15 patients either nulliparous or having a daughter.

Results Seventeen of 29 patients (58.6%) and four of 103 controls (3.9%) showed presence of an SRY gene. The difference between the patient and control groups was statistically significant ($P < 0.001$).

Conclusion As a result of our study, microchimerism may be associated with the etiopathogenesis of alopecia areata. However, we think there is a need for a larger series of studies to support this hypothesis.

Introduction

Alopecia areata (AA) is a common hair disorder, which may have devastating psychological and social consequences and is characterized by the presence of nonscarring alopecia.[1] Various hypotheses have been proposed to explain the pathomechanisms involved in this disease, and studies have shown the participation of autoimmune processes in the pathogenesis of AA.[2] It affects 1.7% of the population at some point in their lives.[3] The term microchimerism is used to indicate the presence of cells from one individual in another. Recently, it has been suggested that microchimerism plays a role in the pathogenesis of autoimmune diseases, including lupus erythematosus, scleroderma, Behçet disease, and Sjögren syndrome.[4–8] These observations have led us to the hypothesis that microchimerism may be involved in the pathogenesis of AA. We recently observed an association between microchimerism and vitiligo.[9] This study was undertaken to evaluate microchimerism among patients with AA.

Material and methods

Patients

The diagnosis of AA was based on a thorough physical examination and evaluation of the patient's medical history. The patient's gender, age, and clinical type of disease (AA, alopecia totalis, or alopecia universalis) were documented. The patients were 29 women with AA over 18 years old who had been seen in our clinic between 2010 and 2013. Patients were divided into two groups: group 1 consisted of 14 patients having a son and group 2, 15 patients either nulliparous or having a daughter. Controls were also divided into two groups according to whether women had a son or not, group 3 ($n = 51$) and group 4 ($n = 52$), respectively. All women gave informed consent before participation in the study. The study was approved by the local ethics committee (no. 2009/112-06). A detailed pregnancy history was taken from patients and healthy control females, including number of pregnancies, age at first pregnancy, outcome of pregnancies whether sons, daughters, or abortions, and age of oldest sons. All women were married and had a history of at least one pregnancy before disease onset (regarding those who had been pregnant). None of the patients or healthy controls gave a history of organ transplantation, blood transfusion, autoimmune diseases, and skin diseases, or was pregnant at the time of the study.

Methods

We analyzed the sex-determining region (SRY) gene as an indicator of fetal microchimerism in our patient and healthy control groups.

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DNA extraction and analysis

Venous blood samples were drawn from each individual. Genomic DNA was extracted from fresh-frozen blood using the High Pure PCR Template Preparation Kit according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany).

Real-time quantitative polymerase chain reaction analysis of the SRY gene using a standard curve

The real-time standard curve quantitative polymerase chain reaction (PCR) analysis was performed using the ABI PRISM 7500 Real-Time PCR System and SDS 2.0.3 software for 7500 Real Time PCR Product (Applied Biosystems, Foster City, CA, USA). Sequence data were obtained from the GenBank Sequence database, NM_001101.3 Homo sapiens Actin Beta (ACTB) and NG_011751.1 Homo sapiens sex determining region Y (SRY) RefSeqGene on chromosome Y. Primer Express 3.0 (Applied Biosystems) was used to design both the PCR primers and the TaqMan probes. The SRY TaqMan system consisted of forward primer 5′-TCCTCAAAAGAAACCGTGCAT-3′, reverse primer 5′-AGATTAATGGTTGCTAAGGACTGGAT-3′, and dual-labeled fluorescent TaqMan probe 5′-FAM-TAA(pdC) *TCC(pdC)CA(pdC)AA(pdC)CT(pdC)TTT-BHQ-1-3′ (*pdCs are a modified nucleotide used in place of cytosine for increasing annealing temperature). The ACTB TaqMan system consisted of forward primer 5′-GGCACCCAGCACAATGAAG-3′, reverse primer 5′-GCCGATCCACACGGAGTACT-3′, and a dual-labeled fluorescent TaqMan probe 5′-Yakima Yellow-TCAAGATCATTGCTCCTCCTGAGCGCBHQ-1-3′. (Yakima Yellow was used as an alternative probe to VIC.) The ACTB gene was used as an endogenous control gene. The real-time standard curve quantitative PCR analysis was performed in 25 μl of reaction solution containing 30 ng of DNA, 12.5 μl of 2 $\times$ TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nmol of each primer (SRY and ACTB), and 200 nmol of each probe (SRY and ACTB). Reaction conditions are preincubation at 60 °C for 1 min, 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Each sample was analyzed in triplicate. The standard curves of SRY and ACTB were run in parallel in each analysis. The concentration of the SRY and ACTB was calculated using standard curves in the same run for all samples in both controls and patients.

Statistical analysis

All statistical analyses were performed using the SPSS version 11.5 for Windows computer software (SPSS, Chicago, IL, USA). The Pearson chi-squared and likelihood ratio chi-squared tests were used to compare SRY genes in all groups. The comparisons between patients and controls were done using Mann–Whitney *U* and Kruskal–Wallis tests. All values are presented as mean $\pm$ SD. $P < 0.05$ was considered statistically significant.

Results

The subjects were 14–50 years old (mean age 32.54 $\pm$ 10.69 years). The mean age of the control subjects was 30 $\pm$ 10.09 years, and the mean age of the patients was 33 $\pm$ 12 years. Of the 29 patients, 25 (86.2%) had AA and four (13.7%) had alopecia totalis. The presence of SRY genes among all groups was statistically significant ($P < 0.001$). Descriptive statistics and *P* values were seen in Tables 1 and 2. According to the tables, the presence of SRY genes was higher in groups 1 and 2, whereas it was lower in groups 3 and 4. There was a statistically significant difference between group 1 (patients with AA and having a son) and group 3 (healthy and having a son) subjects considering the presence of the SRY gene ($P < 0.001$). In addition, there was a statistically significant difference between group 2 (patients with AA and not having a son) and group 4 (healthy and not having a son) subjects considering the presence of the SRY gene ($P < 0.001$). Consequently, 17 of 29 patients (58.6%) and four of 103 control women (3.9%) showed presence of the SRY gene. The difference between the patient and control groups was statistically significant ($P < 0.001$). The distribution of the SRY gene did not differ significantly in patients with AA according to clinical types (Table 3).

Discussion

AA is defined as nonscarring loss of hair characterized by sudden onset independent of age and sex. The cause is unknown. Genetic, environmental, and individual etiologic factors are discussed. Its association with other autoimmune disorders renders an autoimmune pathogenesis very likely, the targeted antigen being specific to the hair follicle. Several observations, including the presence of lesional inflammatory cells, hair follicle-specific autoantibody in the blood of patients with AA, response to treatment with immunosuppressive medications, and association of AA with other autoimmune diseases, support this hypothesis. Histology is characterized by peribulbar lymphocyte or eosinophilic infiltration. Cellular and humoral immunities have been implicated in the development of AA, and their role continues to be investigated.

Table 1 Distribution of the SRY gene according to all groups

	Group 1 (%)	Group 2 (%)	Group 3 (%)	Group 4 (%)	*P*[a]
SRY gene					
Presence	12 (66.6)	5 (45.5)	2 (3.9)	2 (3.8)	<0.001
Absence	6 (33.4)	6 (54.5)	49 (96.1)	50 (96.2)	

[a]Pearson chi-squared test $P < 0.05$.

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Table 2 The distributions of the SRY gene in patients and controls

	Patients	Control	P^a
SRY gene			
Presence	17 (58.6)	4 (3.9)	<0.001
Absence	12 (42.4)	99 (96.1)	

[a]Pearson chi-squared test $P < 0.05$.

Table 3 Distribution of the SRY gene according to clinical type in patients with vitiligo

	Clinical type			
SRY gene	Alopecia areata (%)	Alopecia totalis (%)	Alopecia universalis (%)	P^a
Presence	20 (80)	4 (100)	0	0.201
Absence	5 (20)	0	0	
Total	25	4	0	

[a]Likelihood ratio chi-squared test $P < 0.05$.

Peripheral blood and peribulbar skin biopsies of patients with AA show that T cells, mononuclear cells, various proinflammatory cytokines, and autoantibodies can damage hair follicles.[3] Autoimmune thyroid disease is the most common abnormality associated with AA.[2] The presence of thyroid autoantibody has no clinical correlation with AA severity.[1] Vitiligo occurs in 3–8% of patients with AA compared with 1% in the general US population.[3] Atopy is twice as common in patients with AA as it is in the general population.[2] Other diseases and genetic disorders reported to be associated with AA include Down syndrome, Addison disease, autosomal recessive autoimmune polyglandular syndrome, pernicious anemia, psoriasis, lupus, Sjögren syndrome, intermediate uveitis, rheumatoid arthritis, celiac disease, ulcerative colitis, myasthenia gravis, and multiple sclerosis.[1–3] We recently observed an association between microchimerism and vitiligo.[9]

Individuals can become chimeric in several ways. Pregnancy-related causes of chimerism can lead to chimerism of either the pregnant woman or the fetus. The former comprise completed pregnancy, miscarriage, and induced abortion.[10–12] The latter comprise maternal–fetal cell transfer, cell transfusion from a twin, either a surviving twin or a vanished twin, and transfer of cells, via the maternal circulation, from an older sibling.[13] Causes of chimerism not related to pregnancy comprise blood transfusions and bone marrow or solid organ transplantations.[11] Fetal cell chimerism is considered a natural consequence of normal pregnancy, resulting from local permeability of the placenta. It occurs in most, if not all, women during pregnancy, and therefore fetal cell chimerism has become a primary interest in view of prenatal screening and testing. Chimeric fetal cells may present as hematopoietic progenitor cells (CD34+ and CD34+ CD38+ cells), trophoblast cells, nucleated erythrocytes, T lymphocytes, and other leukocytes.[11]

To determine what types of experiments will most efficiently elucidate the role of chimeric cells in AA and other autoimmune diseases, it is necessary to determine the various pathogenic mechanisms that theoretically could be involved. We can discuss three hypotheses on the role of chimeric cells in immune-mediated diseases, with emphasis on the role of chimerism in AA.

The first hypothesis is that the chimeric cell is a T cell that induces a graft-versus-host reaction.[12] Antibodies to hair follicles are present in the circulation of a significant proportion of patients with AA.[2,3] A genetic predisposition to immune dysregulation can lead to aberrant T-cell reactivity that destroys hair follicles.[3] At least three conditions are required for a chimeric cell to induce a graft-versus-host reaction. First, the host must accept the presence of chimeric cells. Secondly, the chimeric cells must be immunologically competent T cells. Thirdly, the chimeric cells must recognize the cells of the host as foreign.[14] Microchimerism may be associated with the autoreactive T-cell response in AA.

The second hypothesis is that the chimeric cell is the target of a host-versus-graft-like reaction. Antigens from the chimeric cells induce an immune response leading to an autoimmune-like reaction, either by a direct response to chimeric cells or by cross-reactivity due to molecular mimicry.[15,16] Although the exact trigger for the onset of AA is unknown, higher chimeric immune cells can recruit to the site of inflammation once sensitization to several specific AA autoantigens has developed. During and after pregnancy, antipaternal human leukocyte antigen (HLA) antibodies have been found in up to 30% of the mothers.[16,17] A number of studies investigated whether the HLA class alleles of the fetus and mother were related to the occurrence of autoimmune disease.[18] The significant association of alleles HLA-DQB1*03, HLA-B-18, and HLA-A2 and AA was demonstrated in several different populations.[19,20] Microchimerism might clarify this condition.

The third hypothesis is that the chimeric cell is not directly involved in the pathogenesis of autoimmune disease but that its presence in host tissues represents the result of a repair mechanism.[21] Histological studies of skin biopsies from patients with AA have shown that lymphatic inflammatory cells are most prominent at the hair follicles of AA lesions.[1] In AA, chimeric cells might develop from progenitor cells into parenchymal cells and replace damaged host cells after inflammation.

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A new paradigm – the presence of cells other than leukocytes in microchimerism as a possible factor in skin disease – is explored in the paper by Khosrotehrani *et al.*[22] The authors identified maternally derived cytokeratin-positive cells in archival paraffin-embedded sections of the skin of 11 of 12 male children with pityriasis lichenoides by fluorescent *in situ* hybridization with X and Y chromosome-specific probes. These maternally derived cells were also present in biopsies of skin from male control subjects without skin disease but with a lower frequency and density.[22] Increased production of cytokines, including interferon-γ, interleukin-2, and tumor necrosis factor-α from the mononuclear cells of patients with AA, has also been reported. These cytokines can attract neutrophils to AA lesions amplifying destructive inflammatory reactions.[2]

It has been hypothesized that the autoantigen(s) of AA is melanocyte associated. Furthermore, there is an association of AA with vitiligo, and hair bulb melanocytes in AA show both histological and ultrastructural abnormalities.[2] Recently, we showed that microchimerism could be associated with the etiopathogenesis of vitiligo.[9] Our results can clarify these connections.

Recently, Steifert *et al.* observed the complete remission of alopecia universalis after allogeneic hematopoietic stem cell transplantation. The authors concluded that AA was probably a chronic autoimmune disorder, curable by replacement of the immune system with allogeneic hematopoietic stem cell transplantation.[23] These observations as well as this case report all suggest the possibility of treating autoimmune diseases by eradication of autoreactive cells. This possibly could be achieved via high-dose immunoablation or a graft-versus-host immunity effect.

If microchimerism is involved in the pathogenesis of some autoimmune diseases, further understanding potentially may lead to new therapeutic strategies.[23] To the best of our knowledge this study presents for the first time the presence of microchimerism in AA. It remains unclear, however, whether SRY-containing cells trigger an immune response causative of AA. In AA, we might see depigmented skin lesions as a mild graft-versus-host disease-like reaction to maternal cells. One of the factors held responsible for the etiopathogenesis of AA is increased autoreactive T-cell clones. Further analysis of microchimerism and extended samples may clarify the current findings, eventually resulting in a better understanding of the etiopathogenesis of AA.

References

1 Ito T. Recent advances in the pathogenesis of autoimmune hair loss disease alopecia areata. *Clin Dev Immunol* 2013; **2013**: 348546.

2 Giordano CN, Sinha AA. Cytokine pathways and interactions in alopecia areata. *Eur J Dermatol* 2013; **23**: 308–318.

3 Alkhalifah A. Alopecia areata update. *Dermatol Clin* 2013; **31**: 93–108.

4 Hovinga ICLK, Koopmans M, Baelde HJ, *et al.* Tissue chimerism in systemic lupus erythematosus is related to injury. *Ann Rheum Dis* 2007; **66**: 1568–1573.

5 Mosca M, Giuliano T, Curcio M, *et al.* Comparison of real-time PCR and nested PCR for the detection of Y chromosome sequences in the peripheral blood mononuclear cells of patients with systemic sclerosis. *Ann Rheum Dis* 2009; **68**: 155–156.

6 Endo Y, Negishi I, Ishikawa O. Possible contribution of microchimerism to the pathogenesis of Sjögrens syndrome. *Rheumatology* 2002; **41**: 490–495.

7 Regnier S, Aractingi S. Microchimerism and autoimmune diseases. *Eur J Intern Med* 2002; **13**: 365–368.

8 Alp R, Guney AU, Tursen U, *et al.* Microchimerism in Behçets disease. *Int J Dermatol* 2014; **53**: 832–837.

9 Kurtulus PI, Tursen U, Erdal ME. Microchimerism in vitiligo. *J Eur Acad Dermatol Venereol* 2013; **27**: 795–796.

10 Nelson JL. Maternal-fetal immunology and autoimmune disease: is some autoimmune disease auto-alloimmune or allo-autoimmune? *Arthritis Rheum* 1996; **39**: 191–194.

11 Bianchi DW, Zickwolf G, Weil G, *et al.* Male fetal progenitor cells persist in maternal blood for as long as 27 years post partum. *Proc Natl Acad Sci USA* 1996; **93**: 705–707.

12 Lambert NC, Evans PC, Hashizumi TL, *et al.* Cutting edge: persistent fetal microchimerism in T lymphocytes is associated with HLA-DQA*0501: implications in autoimmunity. *J Immunol* 2000; **164**: 5545–5548.

13 Rubbia-Brandt L, Philippeaux MM, Chavez S, *et al.* FISH for Y chromosome in women with primary biliary cirrhosis: lack of evidence of leucocyte microchimerism. *Hepatology* 1999; **30**: 821–822.

14 Aractingi S, Uzan S, Dausset J, *et al.* Microchimerism in human diseases. *Trends Immunol* 2000; **116**: 3–5.

15 Nelson J, Lambert NC. Microchimerism in rheumatic diseases. *Joint Bone Spine* 2001; **68**: 290–296.

16 Leduc M, Aractingi S, Khosrotehrani K. Fetal cell microchimerism, lymphopoiesis, and autoimmunity. *Arch Immunol Ther Exp (Warsz)* 2009; **57**: 325–329.

17 Regan L, Braude PR, Hill DP. A prospective study of the incidence, time of appearance and significance of anti-paternal lymphocytotoxic antibodies in human pregnancy. *Hum Reprod* 1991; **6**: 294–298.

18 Stevens AM, Tsao BP, Hahn BH, *et al.* Maternal HLA class II compatibility in men with systemic lupus erythematosus. *Arthritis Rheum* 2005; **52**: 2768–2773.

19 Haida Y, Ikeda S, Takagi A, *et al.* Association analysis of the HLA-C gene in Japanese alopecia areata. *Immunogenetics* 2013; **65**: 553–557.

20 Megiorni F, Pizzuti A, Mora B, *et al.* Genetic association of HLA-DQB1 and HLA-DRB1 polymorphisms with

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alopecia areata in the Italian population. *Br J Dermatol* 2011; **165**: 823–827.
21 Wang Y, Iwatani H, Ito T, *et al*. Fetal cells in mother rats contribute to the remodeling of liver and kidney after injury. *Biochem Biophys Res Commun* 2004; **325**: 961–967.
22 Khosrotehrani K, Guegan S, Fraitag S, *et al*. Presence of chimeric maternally-derived keratinocytes in cutaneous inflammatory diseases of children: the example of pityriasis lichenoides. *J Invest Dermatol* 2006; **126**: 345–348.
23 Seifert B, Passweg JR, Heim D, *et al*. Complete remission of alopecia universalis after allogeneic hematopoietic stem cell transplantation. *Blood* 2005; **105**: 426–427.

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