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Evidence from autoimmune thyroiditis of skewed X-chromosome inactivation in female predisposition to autoimmunity

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The etiologic factors in the development of autoimmune thyroid diseases (AITDs) are not fully understood. We investigated the role of skewed X-chromosome inactivation (XCI) mosaicism in female predisposition to AITDs. One hundred and ten female AITDs patients (81 Hashimoto's thyroiditis (HT), 29 Graves' disease (GD)), and 160 female controls were analyzed for the androgen receptor locus by the *Hpa*II/polymerase chain reaction assay to assess XCI patterns in DNA extracted from peripheral blood cells. In addition, thyroid biopsy, buccal mucosa, and hair follicle specimens were obtained from five patients whose blood revealed an extremely skewed pattern of XCI, and the analysis was repeated. Skewed XCI was observed in DNA from peripheral blood cells in 28 of 83 informative patients (34%) as compared with 10 of 124 informative controls (8%, *P* < 0.0001). Extreme skewing was present in 16 patients (19%), but only in three controls (2.4%, *P* < 0.0001). The buccal mucosa, and although less marked, the thyroid specimens also showed skewing. Analysis of two familial cases showed that only the affected individuals demonstrate skewed XCI patterns. Based on these results, skewed XCI mosaicism may play a significant role in the pathogenesis of AITDs.

Introduction

Hashimoto's thyroiditis (HD) and Graves' disease (GD) are autoimmune thyroid diseases associated with multiple genetic factors. Although the pathogenesis is poorly understood, a widely accepted model suggests an inherited

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background, which predisposes the subjects to autoimmunity. Additional intrinsic and extrinsic factors such as hormones and the environment may ultimately trigger or contribute to the development of the disease phenotype.[1] Extensive linkage genome screens during the past decade have resulted in the identification of several thyroid-specific susceptibility genes and/or loci, but confirmation through multiple population studies is still awaited for the majority of these loci.[1,2] A common feature of auto-immune diseases, including autoimmune thyroid diseases (AITDs), is an increased prevalence in women when compared with men. The most striking sex differences are

observed in AITDs, scleroderma, Sjögren's syndrome, and systemic lupus erythematosus, which are diseases where over 80% of the patients are females.[3]

It has been demonstrated that risk of autoimmunity could be increased by a lack of exposure to self-antigens in the thymus and the presence of autoreactive T cells.[4–6] Disturbances in the X-chromosome inactivation (XCI) process provide a potential mechanism whereby the lack of exposure to self-antigens could occur,[7,8] including AITDs.[9,10] X-chromosome inactivation is a physiologic process that takes place in early female development and results in the transcriptional silencing of one of the pair of X chromosomes.[11] As a result of this epigenetic regulation, a random inactivation of the X chromosome inherited from either parent occurs and normal female subjects are thus a mosaic of two cell populations. It is therefore an attractive hypothesis that skewed XCI could lead to the escape of X-linked self-antigens from presentation in the thymus or in other peripheral sites that are involved in tolerance induction, inadequate thymic deletion, and finally loss of T-cell tolerance. Indeed, we recently observed skewed XCI in blood cells of women with scleroderma.[12]

Based on our observation that an association exists between skewed XCI and female predisposition to auto-immunity, we hypothesized that skewed XCI may be involved in the pathogenesis of AITDs, particularly in the hematopoietic compartment. We observed extremely skewed XCI in the blood samples of a significant proportion of female patients with AITDs.

Methods
Patients and pedigree analysis
Caucasian women diagnosed with AITDs ($n = 110$), and healthy female controls with no history of autoimmune disease and cancer ($n = 160$) were included in the study. Among the patients, 81 were diagnosed with HT and 29 with GD. The mean ages were 44.8 ± 14.1 (mean $\pm$ SD) years for AITDs (46 ± 14.2 years in the Hashimoto patients, and 40.6 ± 13.2 years in the Graves' patients), and 46 ± 10 for controls. The duration of the symptoms was 5.7 ± 7.4 years among the AITDs patients (5.7 ± 7 years in the Hashimoto patients and 6 ± 8.5 years in the Graves' patients). The mean age of diagnosis was 39 ± 12 years. All of the patients had attended the outpatient clinics of the Endocrinology and Metabolic Diseases Department of Ankara University School of Medicine for at least 1 year since the onset of disease. Patients were randomly chosen for the study.

All clinical investigations described in this manuscript were conducted in accordance with the guidelines in the Declaration of Helsinki (http://www.wma.net). The ethics review board of the participating institutions approved the study protocol. Informed consent was obtained from all subjects.

The diagnosis of HD was made by the existence of a firm goitre in combination with elevated thyroid auto-antibodies (thyroglobulin and/or thyroid peroxidase), a low ultrasonographic echogenity of the gland, and demonstration of lymphocytic infiltration by fine-needle aspiration biopsy and/or biochemical hypothyroidism. The diagnosis of GD was based on biochemical hyperthyroidism, and a diffuse symmetrical goitre in combination with positive thyroid antibodies (thyroglobulin, thyroid peroxidase or TSH receptor). In addition, thyroid ophthalmopathy and/or diffuse hyperplasia on an isotope scan or ultrasonography demonstrating homogenous echo texture may accompany the clinical picture.

Following the XCI studies, a complete pedigree analysis was carried out for 64 individuals informative for the AR polymorphism with medical follow-up of reported AITDs among family members when possible. Owing to emigration or unwillingness to contribute family information, data could not be obtained from the remaining 19 participants. Family history of AITDs was determined by reviewing the probands' pedigree to determine the number of relatives affected by these autoimmune diseases. Only first- and second-degree relatives were counted. A positive family history was noted if one additional AITD was documented by medical review.

X-chromosome inactivation analysis
Genotyping of a highly polymorphic CAG repeat in the androgen-receptor (*AR*) gene was performed to assess the XCI patterns as described elsewhere.[12,13] Densitometric analysis of the alleles was performed at least twice for each sample using the MultiAnalyst version 1.1 software. A corrected ratio (CrR) was calculated by dividing the ratio of the predigested sample (upper/lower allele) by the ratio of the nonpredigested sample for normalization of the ratios that were obtained from the densitometric analyses. The use of CrR compensates for preferential amplification of the shorter allele when the number of PCR cycles increases.[14] A skewed population is defined as a cell population with greater than 80% expression of one of the *AR* alleles. This corresponds to CrR values of <0.33 or >3.

Haplotype analysis
Human MapPairs Version 10 purchased from Research Genetics (currently available by Invitrogen, CA, USA) was used to screen the X chromosome. Site-specific PCR, 6% polyacrylamide gel electrophoresis, and silver staining techniques were used for genotyping the individuals. Gels were manually pictured and genotyped. Cyrillic program (version 2) was used to generate the haplotypes. A total of 27 X-chromosome-specific DNA markers from the Map-Pairs Panel were genotyped. Map order and physical positions (Mb) of the additional polymorphic DNA markers were obtained from USCS genome browser (The University of California Santa Cruz, CA, USA http://genome.ucsc.edu/).

Statistical methods

The results from control and test groups in XCI studies were compared by χ^2 test with Yate's correction.

Results

PCR-based X-inactivation study of peripheral blood

XCI status was informative in 83 of the 110 AITDs patients and in 124 of the 160 controls. Some heterozygous individuals were considered uninformative since only those whose alleles resolve adequately for densitometric analyses were included in the study. Skewed XCI ($>80\%$ skewing) was observed in 28 of the 83 patients (34%), and 10 of the 124 controls (8%) ($P<0.0001$). When the data for the two groups of AITDs patients was analyzed independently, 23/67 (34.33%, $P<0.0001$) of the Hashimoto's patients and 5/16 (31.25%, $P=0.0167$) of the Graves' patients were found to display the skewed XCI in blood. More importantly, extremely skewed XCI, defined as $>90\%$ inactivation of one allele, was present in 16 patients (19%), and in only three controls (2.4%, $P<0.0001$) (see Table 1). Extremely skewed XCI is a rare event in the general population. It has been reported in only 1–2% of women aged 20–40 years, and in 2–4% of women aged 55–72 years.[15,16] The distribution of XCI skewing in the general population is thought to be mainly due to chance deviations from 50:50 as a result of the limited number of embryonic cells present (4–20) at the time of XCI.[17] Age alone is unlikely to influence the strikingly bimodal data in our AITDs patients (Figure 1). We did not observe a shift towards the skewed range in older patients and controls.

PCR-based X-inactivation study of thyroid biopsy, buccal mucosa, and hair follicle specimens

Thyroid biopsy, buccal mucosa, and hair follicle specimens were obtained from five patients (04-121, 04-198, 04-214, 04-221, and 04-225). Their blood XCI profile displayed almost exclusive representation of only one allele of the *AR* polymorphism in their methylation-sensitive PCR assay, which indicates extremely skewed XCI. Five randomly selected patients showed skewing in the same direction for all tissues, except hair follicle, that in the thyroid being less marked than blood and buccal cells (Figure 2). Hair follicle specimens had a random XCI pattern. The allele ratios are given in Table 2.

Pregnancy history and pedigree analysis

Characteristics of the AITDs patients with skewed and random XCI are shown in Table 3. Only those patients with a complete pregnancy and family history are included in this table. The pedigrees of many AITDs probands with skewed XCI *versus* those with random XCI were interesting in two aspects. First, recurrent spontaneous abortions (defined as three or more pregnancy losses), which have been shown to be associated with skewed XCI,[16,18] occurred in four of 25 (16%) of our AITDs probands with skewed XCI. Conversely, a history of recurrent spontaneous abortions was negative both in the patients with random XCI and in the control group subjects ($P<0.0199$). Although the etiology of recurrent abortions in thyroid autoimmunity remains unknown, women who present with thyroid antibodies in the first trimester of pregnancy have a two-to four-fold increase in their miscarriage rates.[19]

Second, a positive family history, particularly in the skewed group, was apparent (12/25, 48% in the skewed; and 10/39, 25.6% in the random groups). We therefore contacted all of the 12 probands in an attempt to extend the X-chromosome inactivation studies to other family members. Initially, a positive response was received from three families, but blood samples could be obtained from

Table 1 Proportion of patients and controls with skewed X-chromosome inactivation

Degree of skewing (%)	No. (%) observed with skewing	
	Autoimmune thyroiditis (n = 83)	Control females (n = 124)
90+	16 (19.27)	3 (2.41)
80–89	12 (14.45)	7 (5.64)
70–79	6 (7.22)	22 (17.74)
60–69	16 (19.27)	29 (23.38)
50–59	33 (39.75)	63 (50.80)

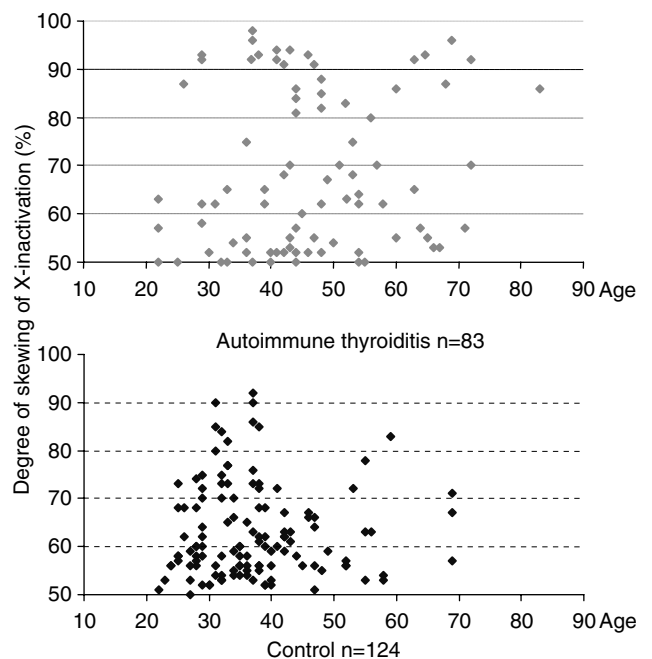For comparison by χ^2, $P<0.0001$ ($>80\%$ skewing); $P<0.0001$ (90+% skewing).

Figure 1 Distribution of X-inactivation patterns according to age in AITDs patients and control subjects.
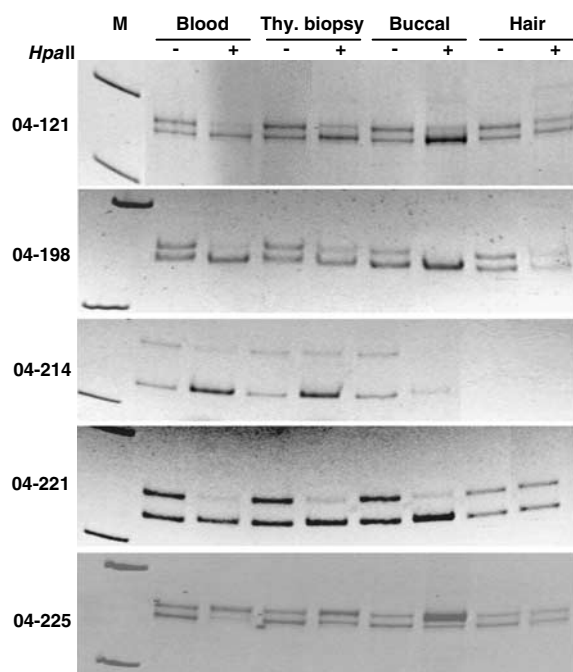
Figure 2 X-inactivation analysis of androgen receptor locus. PCR products of undigested (−) and *Hpa*II-digested (+) DNA from peripheral blood, thyroid biopsy, buccal, and hair follicle samples of AITDs patients 04-121, 04-198, 04-214, 04-221, and 04-225 are shown. Two alleles are seen in undigested samples, whereas a single allele resulting from extremely skewed XCI is clearly visible in all peripheral blood samples. Allele ratios are given in the text and in Table 2. M: marker (pUC mix 8), 331 and 242 bp fragments are visible.

the family members of only two probands (04-445, Family 1; and 04-298, Family 2). An important observation emerges from a study of these families: only the affected individuals demonstrate skewed XCI patterns. For example, XCI is extremely skewed in the affected sister and mother of 04-445 (Family 1), but random in the two unaffected sisters. The inactive X chromosome here is of maternal origin. In patient 04-298 (Family 2), skewing in the 80–89% range is noted for her affected sister, but unfortunately her mother was not informative for the AR polymorphism. Interestingly, the inactive X chromosome appears to be of paternal origin in Family 2 (Supplementary Figures 1 and 2).

Haplotype analysis

Because XCI segregates as a heritable trait associated with the disease in two generations of Family 1, we performed haplotype analysis by using polymorphic X-chromosomal markers to determine possible segregation between the

Table 2 X-chromosome inactivation patterns in blood, thyroid, buccal mucosa, and hair follicle specimens

Sample	04-121	04-198	04-214	04-221	04-225
Blood	94:6	91:9	84:16	92:8	91:9
Thyroid	72:28	79:21	76:24	74:26	64:36
Buccal	86:14	97:3	87:13	89:11	82:18
Hair	60:40	50:50	(−)	59:41	52:48

Table 3 Characteristics of the patients who are informative for X-chromosome inactivation status

Patient	Birth date	Disease onset	Pregnancy history	Sex and birth date of children	Family history of first-degree relatives
90+% skewing					
1 04-136[a]	1975	2004	G0,P0,A0	(−)	(−)
2 04-127[b]	1975	2003	G0,P0,A0	(−)	(−)
3 04-138[b]	1962	1980	G7,P0,A7	(−)	Two sisters
4 04-298[b]	1979	2003	G1,P1,A0	F03	Mother, one sister
5 04-445[b]	1961	2000	G1,P1,A0	F88	Mother, one sister
6 04-198[b,c]	1935	2004	G2,P2,A0	M68,M72	(−)
7 04-221[b,c]	1958	2000	G4,P2,A2	M91,F94	Two sisters
8 04-250[b]	1967	1996	G7,P2,A5	M89,M93	One son
9 04-226[b,c]	1963	2004	G3,P3,A0	M83,F88,M98	One sister
10 04-233[a]	1967	1990	G4,P3,A1	M88,F94,M01	(−)
11 04-121[b,c]	1957	1988	G7,P4,A3	F78,M83,F91,F94	(−)
12 04-205[b]	1927	2003	G6,P5,A1	M47,M50,F52,F53,M55	(−)
13 04-225[b,c]	1936	1975	G6,P6,A0	F56,F58,F60,F62,M64,M66	One daughter
80–89% skewing					
14 04-132[b]	1960	2002	G0,P0,A0	(−)	Mother, one sister
15 04-223[b]	1956	1988	G0,P0,A0	(−)	Mother, one sister
16 04-105[a]	1978	1999	G5,P1,A4	M98	(−)
17 04-131[b]	1944	2002	G3,P2,A0	M69,M71	(−)
18 04-120[b]	1956	1994	G3,P3,A0	F75,M77,F78	(−)
19 04-107[b]	1948	1998	G4,P3,A1	F70,F72,F76	(−)
20 04-98[b]	1956	2000	G8,P3,A1	F79,F81,F87	Two daughters
21 04-218[b]	1941	1991	G4,P3,A1	M61,F63,F67	(−)
22 04-108[b]	1952	1999	G5,P3,A2	F77,F78,M83	(−)
23 04-208[b]	1960	1999	G5,P3,A0	F83,F85,M88	Mother

Table 3 (*Continued*)

Patient	Birth date	Disease onset	Pregnancy history	Sex and birth date of children	Family history of first-degree relatives
24 04-110[a]	1960	1998	G4,P4,A0	M80,M83,M85,F96	(−)
25 04-214[b,c]	1921	1999	G9,P8,A1	F44,M45,F47,F48,M54,F56,F58,M60	One daughter
70–79% skewing					
26 04-203[b]	1961	2004	G3,P1,A0	M82	(−)
27 04-230[b]	1951	1999	G2,P2,A0	M77,M86	One son
28 04-213[b]	1947	1998	G7,P3,A0	F67,M68,M71	(−)
29 04-228[b]	1953	2001	G3,P3,A0	M71,M73,M82	(−)
30 04-137[b]	1932	1981	G5,P4,A0	F50,F53,M55,F59	(−)
60–69% skewing					
31 04-206[a]	1946	1964	G1,P0,A1	(−)	(−)
32 04-92[b]	1971	1998	G3,P1,A0	F96	Mother
33 04-240[b]	1975	2003	G2,P1,A0	M00	(−)
34 04-139[b]	1959	2002	G1,P1,A0	M97	(−)
35 04-257[b]	1973	2004	G3,P2,A1	M95,M01	(−)
36 04-112[b]	1952	1999	G2,P2,A0	F72,F77	Mother
37 04-220[a]	1955	1998	G3,P2,A0	M74,M78	(−)
38 04-103[b]	1962	1986	G6,P2,A1	F82,M92	(−)
39 04-251[b]	1941	1984	G5,P3,A1	M60,M63,M65	Two sisters
40 04-99[b]	1961	1997	G6,P3,A2	F81,F85,F87	Mother, one sister
41 04-224[a]	1950	2001	G6,P5,A1	M69,M72,F73,M75,F78	(−)
50–59% skewing					
42 04-96[b]	1939	1996	G0,P0,A0	(−)	(−)
43 04-242[b]	1960	1999	G0,P0,A0	(−)	(−)
44 04-129[b]	1982	1998	G0,P0,A0	(−)	Mother
45 04-196[b]	1956	1999	G6,P1,A0	F93	(−)
46 04-231[b]	1964	2003	G1,P1,A0	M93	(−)
47 04-201[a]	1971	2001	G2,P1,A1	F93	Mother
48 04-95[b]	1975	2004	G3,P2,A0	F98,M00	(−)
49 04-239[b]	1951	2003	G3,P2,A0	M68,M75	(−)
50 04-246[b]	1961	1996	G2,P2,A0	M78,F81	(−)
51 04-200[b]	1954	1992	G5,P2,A0	M73,M75	Three sisters
52 04-237[b]	1970	2004	G2,P2,A0	M93,F97	(−)
53 04-102[b]	1964	2003	G3,P2,A1	F95,F87	(−)
54 04-204[b]	1949	2002	G4,P2,A0	F71,M73	(−)
55 04-93[a]	1960	2003	G2,P2,A0	M91,F94	(−)
56 04-116[a]	1960	2003	G4,P2,A0	F86,M89	One brother
57 04-197[b]	1961	1976	G6,P3,A2	M82,M84,M98	(−)
58 04-229[b]	1938	1980	G3,P3,A0	M61,M63,F65	One sister
59 04-255[b]	1974	1993	G3,P3,A0	F92,M96,F01	(−)
60 04-212[a]	1958	2002	G4,P3,A0	M76,F80,M84	(−)
61 04-238[b]	1939	2002	G6,P4,A0	M61,M65,F67,M72	(−)
62 04-117[b]	1944	2004	G6,P4,A0	M60,M64,F66,F67	(−)
63 04-211[a]	1950	2002	G6,P6,A0	F66,F67,F72,M84,M85,M86	(−)
64 04-243[a]	1937	1994	G12,P7,A1	M57,M59,F60,F62,M65,M67,F68	(−)

G, number of pregnancies; P, para (pregnancies carried to term and delivered); A, spontaneous abortions.
[a]Graves' disease.
[b]Hashimoto's thyroiditis.
[c]Patients from whom thyroid biopsy samples were obtained.

disease and marker alleles. Although the size of this family is not large enough to prove linkage, it still provides valuable information about the exclusion area on the X chromosome. This helps to define a minimal critical region on the X chromosome, which might be associated with AITDs. Xp11-q13 (GATA144DO4, DXS7132, and AR) and Xp22 DNA markers (DXS8022, DXS987, and DX9902) showed concordance among the affected individuals indicating positive segregation between the disease and marker alleles. The haplotype structure is shown in

Figure 3. However, lod score[20] analysis did not allow formal acceptance of linkage to any loci mainly due to the small size of the family.

Discussion
The autoimmune diseases include more than 70 chronic disorders that affect approximately 5% of the population. A reduction in sex ratio (male:female) is characteristic of most such diseases, including AITDs.[3] Even though the

female prevalence of autoimmune diseases has been recognized for over a hundred years, candidate mechanisms that could be important in pathogenesis have been uncovered only during the past two decades. These include

genetic traits associated with autoimmunity,[21] pregnancy-related microchimerism,[22] and disturbances in XCI mosaicism in female subjects.[12] In this study, we demonstrate skewed XCI patterns in peripheral blood mononuclear cells of a significant proportion (34%) of female subjects with AITDs. Approximately 8% of female control subjects demonstrate skewed X-inactivation patterns $\geqslant$80:20, which is consistent with previous estimates.[16,18,23] The effect is more pronounced at patterns of X-inactivation $\geqslant$90:10; nearly 20% of AITDs patients show such skewing (Supplementary Figure 3), compared with only a few percent of female control subjects. Our results show that factors associated with extremely skewed XCI could account for a significant proportion of female patients with AITDs.

Skewed XCI is a result of primary or secondary causes. The former is bias in the initial choice of which X chromosome is inactivated due to germline *XIST* (X-inactive-specific transcript) mutations.[24] The secondary causes are deleterious X-linked mutations, X chromosome rearrangements, aging, twinning, or monoclonal expansion of cells (for a review, see Brown[25]). We believe that deleterious X-linked mutations or X chromosome rearrangements and their differential expression patterns could provide a disadvantage to blood and buccal cells, and possibly to thyroid cells in AITDs patients, and lead to skewed XCI. This has been supported by our observation that maternally inherited skewed XCI profile accompanies the disease phenotype for our AITDs Family 1. We observed segregation between the disease and marker alleles with the DNA markers residing on the distal short arm and pericentromeric regions of the X chromosome in this family. Although examples of skewed X-inactivation segregating with a trait have been reported previously,[18,26] this is the first example in AITDs to the best of our knowledge. In a recently published study on a three-generation kindred, extreme skewing of X inactivation was documented in three female subjects who have hemophilia A.[26] Since the inactive X was always of paternal origin in affected female subjects, the authors concluded that skewing in the family resulted from an abnormality in the initial choice process. This prevented the X chromosome, which carried the mutant *FVIII* allele, from being an inactive X. In our Family 2 with two affected sisters, the inactive X chromosome was of paternal origin like the
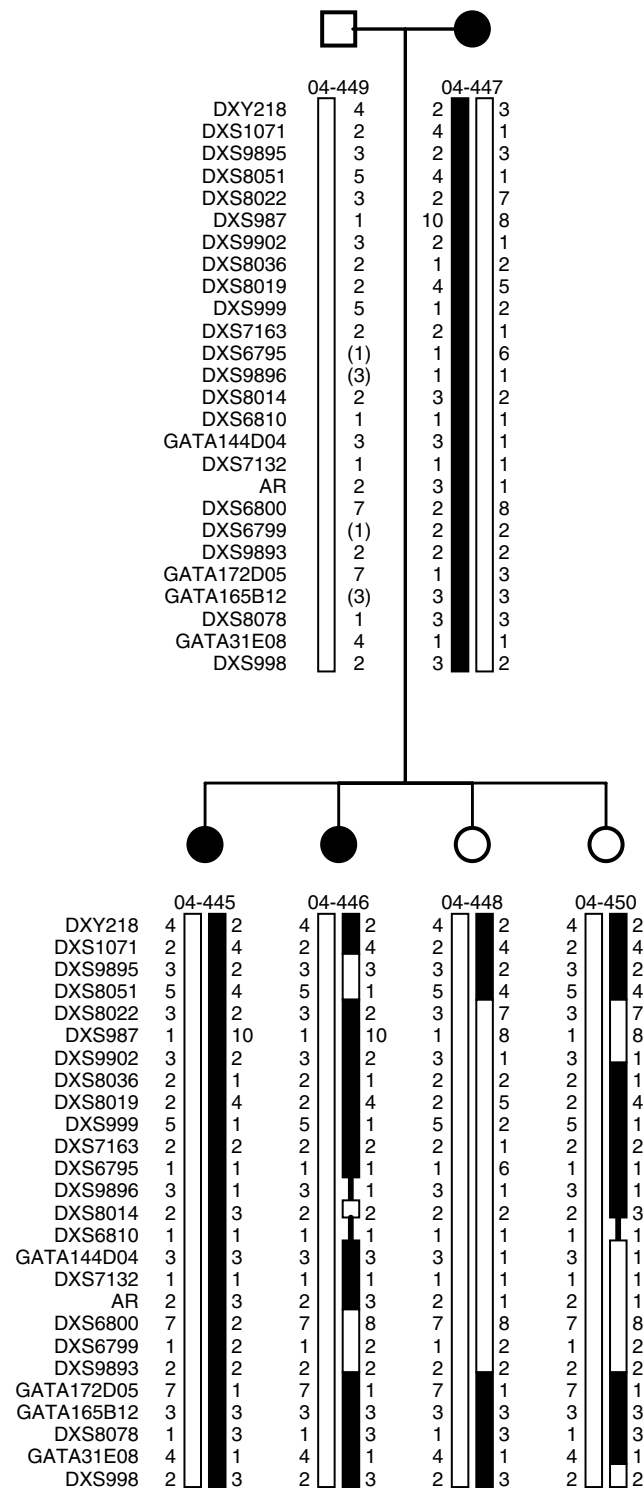
Parents: 04-449 and 04-447

Marker	04-449	04-447 (a)	04-447 (b)
DXY218	4	2	3
DXS1071	2	4	1
DXS9895	3	2	3
DXS8051	5	4	1
DXS8022	3	2	7
DXS987	1	10	8
DXS9902	3	2	1
DXS8036	2	1	2
DXS8019	2	4	5
DXS999	5	1	2
DXS7163	2	2	1
DXS6795	(1)	1	6
DXS9896	(3)	1	1
DXS8014	2	3	2
DXS6810	1	1	1
GATA144D04	3	3	1
DXS7132	1	1	1
AR	2	3	1
DXS6800	7	2	8
DXS6799	(1)	2	2
DXS9893	2	2	2
GATA172D05	7	1	3
GATA165B12	(3)	3	3
DXS8078	1	3	3
GATA31E08	4	1	1
DXS998	2	3	2

Offspring: 04-445, 04-446, 04-448, 04-450

Marker	04-445		04-446		04-448		04-450	
DXY218	4	2	4	2	4	2	4	2
DXS1071	2	4	2	4	2	4	2	4
DXS9895	3	2	3	3	3	2	3	2
DXS8051	5	4	5	1	5	4	5	4
DXS8022	3	2	3	2	3	7	3	7
DXS987	1	10	1	10	1	8	1	8
DXS9902	3	2	3	2	3	1	3	1
DXS8036	2	1	2	1	2	2	2	1
DXS8019	2	4	2	4	2	5	2	4
DXS999	5	1	5	1	5	2	5	1
DXS7163	2	2	2	2	2	1	2	2
DXS6795	1	1	1	1	1	6	1	1
DXS9896	3	1	3	1	3	1	3	1
DXS8014	2	3	2	2	2	2	2	3
DXS6810	1	1	1	1	1	1	1	1
GATA144D04	3	3	3	3	3	1	3	1
DXS7132	1	1	1	1	1	1	1	1
AR	2	3	2	3	2	1	2	1
DXS6800	7	2	7	8	7	8	7	8
DXS6799	1	2	1	2	1	2	1	2
DXS9893	2	2	2	2	2	2	2	2
GATA172D05	7	1	7	1	7	1	7	1
GATA165B12	3	3	3	3	3	3	3	3
DXS8078	1	3	1	3	1	3	1	3
GATA31E08	4	1	4	1	4	1	4	1
DXS998	2	3	2	3	2	3	2	2

Figure 3 Haplotype structure of Family 1. Patient 04-445 was arbitrarily selected to construct the haplotype. Maternally inherited haplotype was highlighted with solid black bar. Haplotypes of the remaining sibs were compared with the reference individual (04-445), and shared portions were also marked with solid bars. Noninformativeness in the crossover regions were demonstrated with thin bars. The regions between the DNA markers DXS8051 and DXS8036 as well as DXS8014 and AR regions on Xp22 and Xp11-q13 regions, respectively, were not excluded since positive segregation between the disease and marker alleles was observed.

hemophilia A family. Extension of both the XCI and linkage studies to large cohorts with familial AITDs cases could prove to be very rewarding in understanding the relation between skewed XCI and autoimmune thyroidites.

Studies that aim to delineate the medical consequences of skewed X-inactivation have shown that clinical manifestation of X-linked disorders in female subjects could be influenced by disturbances in the XCI process.[27] In addition, it has been hypothesized that skewed XCI could be a factor that influences female predisposition to autoimmunity.[7,8] Now that we have demonstrated skewed patterns of XCI in a significant proportion of female AITD patients, deviation from the physiological range of XCI mosaicism could be considered as a potential mechanism contributing to disease pathogenesis. This is further supported by the recently reported observation that female twins with AITDs have a high frequency of skewed XCI.[28]

Although extremely skewed XCI is rare, it does not always lead to the development of AITDs. A subsequent event, such as environmental exposure to viral, chemical, or other agents may trigger a cascade that results in AITDs. In addition, the co-inheritance of genetic susceptibility factors, such as functional variants in vital negative regulatory molecules of the immune system,[29,30] may exacerbate the effects of skewed XCI and contribute to the development of autoimmune diseases including AITDs.

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