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Increased loss of the Y chromosome in peripheral blood cells in male patients with autoimmune thyroiditis

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ABSTRACT

Multiple mechanisms have been proposed to explain the peculiar distribution of autoimmune thyroiditis (AIT) among women and men. Most attention has been focused on the detection of the role of estrogens and the X chromosome. Specifically, a potential role for X haploinsufficiency has been proposed in the female patient population and an association with the disease has been confirmed. Our knowledge of the etiopathogenesis of autoimmunity in male patients remains, however, limited. Next to the possible role of androgens and their imbalances, the Y chromosome appears as a potential candidate for influence of the immune function in men. Herein we analyzed a population of male patients with AIT ($n = 31$) and healthy controls ($n = 88$) to define a potential association of disease and the loss of the Y chromosome. Y chromosome loss increases in AIT compared to unaffected subjects; these phenomenon increases with aging as expected, however, the degree of loss is significantly increased in the patient population compared to the healthy controls. We were, thus, able to confirm the existence of an analogous mechanism in the male population to previously identified X haploinsufficiency in female patients with AIT. We propose that this commonality might represent a relevant feature in the etiopathogenesis of AIT that should be further investigated.

1. Introduction

The striking gender differences characterizing autoimmune diseases, both systemic and organ-specific, have been a target of investigation for decades. Pathogenetic hypotheses include the possible role of sexual hormones, environmental triggers and a role of genetic and/or epigenetic influences [1–6]. The latter hypothesis initially focused on the relevance of autosome genes, a theory supported by the frequent clustering of autoimmune diseases in twin pairs [7] but has been increasingly revived in recent years by a focus on the sex chromosomes, X and Y [2].

Most attention has been put into the analysis of the role of the X chromosome, leading to the emergence of three fundamental hypotheses: i) loss of mosaicism, ii) reactivation and iii) haploinsufficiency of X-linked genes [8–10]. Our group has mostly focused on the latter, identifying peculiar patterns of X chromosome loss in peripheral cells of individuals affected by several autoimmune diseases [11–13], including autoimmune thyroiditis (AIT). Nonetheless, some other conditions, such as systemic lupus

Abbreviations: AIT, autoimmune thyroiditis; SLE, systemic lupus erythematosus; DYZ3, chromosome Y α-Satellite; DAPI, 4,6-diamidine 2-phenylindole; SRY, sex-determining region of the Y chromosome; Yaa, Y-linked autoimmune acceleration; PARs, pseudoautosomal regions; FOXP3, forkhead box P3; HT, Hashimoto's thyroiditis; GD, Grave's diseases.

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erythematosus (SLE) did not display haploinsufficiency at increased rates compared to the general population [14] but, on the contrary, an increased dose of X chromosomes [15].

AIT displays a marked female prevalence, justifying the attention toward the role of female steroid hormones and the role of the X chromosome, however, the disease does not spare male patients. Frequently, male patients suffering from rheumatologic conditions present different clinical manifestations and might suffer from more rapid disease progression [16].

The role of androgens on the immune system is being analyzed [17], however, the role of the male sex chromosome is not known. The Y chromosome has been widely investigated in its link to male fertility and infertility [18], but only recently the role of other genes within this chromosome has been approached [19]. The Y chromosome contains a large number of genes related to fertility, however it also harbors several X homologs, which might exert a relevant role in the immune function, as e.g. the interleukin receptors IL3RA or IL9R.

Loss of the Y chromosome has been reported in several cancers [20–24] and might act as a potential marker of therapeutic response or disease progression [25]. Whether the loss of the entire chromosome in these cells is due to a more rapid cellular turnover, to cellular aging or to a distinct mechanism remains to be elucidated [26].

In this work, we investigated Y chromosome numbering in males affected by AIT, a disease that has demonstrated an increased incidence of X haploinsufficiency in the female patient population [12]. We verified the incidence of Y chromosome loss in the patient population and a control population identifying a marked increase in the diseased population. This recapitulates our previous findings in the female population with AIT and should thus be considered a common feature to both male and female patients affected by this condition, which warrants further investigation.

2. Materials and methods

2.1. Study population

After obtaining written consent, blood was obtained from 31 male AIT patients (Table 1) and 88 age-matched healthy controls. Twenty-three of the 31 AITD patients were affected with Graves' disease and the 8 remaining had Hashimoto's thyroiditis. AITD were diagnosed according to the presence of abnormal serum TSH levels (<0.15 mU/L in GD or >6 mU/L in HT) accompanied by positive autoantibodies directed against the thyroperoxidase (Ab-TPO > 10 kU/L) and/or the TSH receptor (TRAb > 1 U/L) as previously done in women [12]. Presence of serum markers and autoantibodies was assessed by routine laboratory techniques. The Ethics committee of the University of Milan approved the following study protocol.

2.2. Chromosome preparations and FISH analysis

Chromosome spreads were obtained as previously described [11]. Briefly, blood samples were cultured for 72 h using chromosome medium P containing mitogens (Euroclone, Wetherby, UK). All samples were blindly analyzed. Dual-color FISH analysis was carried out co-hybridizing samples using a rhodamine-labeled chromosome Y α-Satellite (DYZ3) probe (Qbiogene, Illirch Cedex, France) and a fluorescein-labeled DXZ1 probe (Appligene, Gaithersburg, MD, USA), following standard procedures. The slides were then counterstained with DAPI (4,6-diamidine 2-phenylindole) and visualized on a Leitz Diaplan microscope equipped with DAPI and FITC-TRITC epifluorescence optics and a digital camera. For each sample, at least 500 nuclei were scored. Y loss rate is expressed as percentage of the entire number counted.

Table 1
Clinical features of men with Hashimoto's thyroiditis (HT) and Grave's diseases (GD).

		HT ($n = 8$)	GD ($n = 23$)
Serum antibodies	TPOAb	8 (100%)	23 (100%)
	TRAb	–	23 (100%)
Thyroid dysfunction	Mild (Normal FT4/FT3)[a]	6 (75%)	6 (26%)
	Severe	2 (25%)	17 (74%)
Associated autoimmune manifestations[b]		3 (37.5%)	18 (78%)

[a] Normal ranges: FT4, 9–20 pM; FT3, 4–8 pM.
[b] Thyroid associated ophthalmopathy in 18 GD patients, atrophic gastritis in 1 HT, vitiligo in 1HT, polyglandular autoimmune disease type 3 in 1 HT.

2.3. Statistical analysis

Differences in median values between two groups were assessed using Student's t test. All analysis was two-sided and p values minor 0.05 were considered statistically significant. The statistical comparisons were performed with STATA Statistical Software (STATA Corporation, College Station, TX, USA) and GraphPad Prism (GraphPad, San Diego, CA, USA).

3. Results

3.1. Patients with AIT display an increased frequency of Y loss in their peripheral blood cells

Frequency of Y chromosome loss was determined in patients with AIT and healthy controls. Mean Y loss rates was significantly higher in patients with AIT (1.95%, range: 0.56–7.2%) compared to healthy controls (1.31% range: 0.20–5.60%) $p = 0.037$ (Fig. 1). Both GD and HT showed an increased Y loss compared to controls, even though only HT demonstrated a significant difference (Fig. 1b).

3.2. Y loss increases more significantly with age in AIT patients than in healthy controls

We subsequently analyzed the rate of Y chromosome loss in association with increasing age, as this appears to be a confounding factor for haploinsufficiency [27]. In AIT population an increase of Y chromosome loss with aging can be detected, and strikingly increased levels of Y loss can be detected at all ages in the patient population in comparison to the healthy controls (Fig. 2). This trend recapitulates our previous findings on X chromosome haploinsufficiency in the female patient population with autoimmune diseases.

4. Discussion

In general, females are healthier, live longer, and have a better outcome from several illnesses than males [28,29]. The immunological advantage of women has been long known and the X chromosome is partially responsible for it [1,30]; however very little is known regarding the role of the Y chromosome in male immune response. Males less frequently than women suffer from autoimmune diseases. The present work demonstrated for the first time how Y chromosome loss associates with AIT in male patient population. While several studies from our group have identified an increased rate of X monosomy in female patients [12], a possible analogous mechanism in male patients was never investigated. Y loss increases with age [27] and our findings recapitulate this, however, the degree of Y chromosome loss in the patient population exceeds the one in healthy control subjects, mirroring X loss observed in female patient populations.

Phenotypical differences between females and males result from direct genetic differences [8,31]. These genetic differences can