CASE REPORT

Concurrent FOXP3- and CTLA4-associated genetic predisposition and skewed X chromosome inactivation in an autoimmune disease-prone family

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Abstract

CLTA4 is relevant for FOXP3+ Treg cells, and the link between skewed X chromosome inactivation (XCI) and autoimmunity is recognized. The observation of immune dysregulation polyendocrinopathy enteropathy X-linked syndrome and multiorgan endocrine autoimmune phenomena in various members of one family, associated with a CTLA4 polymorphism and skewed XCI, provides an in vivo model of how mechanisms of immune dysregulation may cooperate.

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Introduction

Approximately 80% of patients with autoimmune diseases, which often occur in a familial setting and in genetically predisposed individuals, are women. The most prominent genetic susceptibility factors for autoimmune thyroid disease (AITD) in particular are located in the HLA region and conferred by a specific CTLA4 single nucleotide polymorphism (SNP) (1, 2, 3). One of the rare X-linked syndromes that specifically predispose affected boys to the development of severe multiorgan autoimmune diseases with unclear genotype–phenotype correlation is the ‘immune dysregulation polyendocrinopathy enteropathy X-linked’ (IPEX) syndrome (4). It is caused by frameshift and missense mutations in the fork-head DNA-binding box protein P3 (FOXP3) gene on Xp11.23 that impairs the function of the encoded protein (4, 5). FOXP3 is primarily but not exclusively expressed in CD4+CD25+ regulatory T (Treg) cells that are essential for maintaining the balance between immune tolerance and suppression (5, 6, 7). Partial FOXP3 deficiency is associated with overstimulation of T cells, which in turn is an essential component of many immune disorders and autoimmune phenomena, whereas a complete lack of the protein is the root for severe forms of the IPEX syndrome (4, 5, 6, 8). Although the majority of affected males die within the first 2 years of life, some of them may survive into adulthood (4, 9). Despite positivity of autoantibodies in many asymptomatic patients and increasing incidence of abnormal thyroid-stimulating hormone levels and thyroid disease with age in the normal population (10, 11), the accumulation of autoimmune endocrinopathies within the presented pedigree prompted detailed work-up of potential genetic predispositions, especially in the context of skewed X chromosome inactivation (XCI) in female relatives of an IPEX patient.

Patient family presentation

The clinical and genetic data of relevant family members are shown in Fig. 1 and laboratory features in Table 1. All material from patients was obtained upon informed consent in accordance with the Declaration of Helsinki; and the study was approved by the Institutional Review Board. We observed a FOXP3 mutation in a now 25-year-old patient with a long history of typical but mild IPEX-associated symptoms. He was born as the third child after an uneventful pregnancy. His brother, who was born 9 years earlier, had severe IPEX typical symptoms and died. The patient became symptomatic at 7 months of age with diarrhea and failure to thrive. At 4 years of age, he developed an autoimmune hepatitis that required ongoing treatment with cortisone and azathioprine. Hashimoto type of AITD was diagnosed in the boy’s mother, who also developed type 1 diabetes mellitus (DMT1) at 45 years of age. Furthermore, one of her three sisters and the maternal grandmother suffered from thyroid disease (Graves’ disease and Hashimoto respectively) and
needed corresponding treatment, as reported by family members and the family’s primary care physician. The patient’s three years older sister was healthy but had positive antinucleic acid antibody titers and elevated IgE levels without symptoms or history of atopy. The index patient’s further medical course remained uneventful until he was 18, when he also developed DMT1 as well as a marked eczema on both his upper extremities and trunk. At present, his disease symptoms are under control and his clinical condition remains stable.

Results

The FOXP3 mutation p.R347H (c.1040G>A) in this family is located in the gene’s forkhead domain and had already been reported in other IPEX patients (4). Subsequent screening also revealed its presence in the patient’s sister, mother, and maternal grandmother (Fig. 1). Because the mutation was found in two of the three females with AITD, we further explored the females’ XCI (see legend) (12, 13, 14, 15, 16). Skewing was found in the peripheral blood of the mother and the sister of the patient (both FOXP3+/-), but not in the aunt with AITD without a FOXP3 mutation. Equally, XCI was skewed in sorted CD4+CD25hi T-cells of the patient’s mother and sister (not shown). Based on the repeat length of the male patient, we were able to infer that in both women, the X with the mutated FOXP3 was preferentially inactivated. Global XCI skewing is a rather unusual feature of female FOXP3 mutation carriers (12, 14), whereas it is quite common in women with AITD (12, 13, 15). Therefore, we continued to screen all family members for a potentially relevant CTLA4 SNP (rs231775, A>G) with allele-specific oligonucleotide PCR that enables the simultaneous discrimination and quantification of the unequal length and allele-specific methylation status of polymorphic triplet repeats in the FMR1 and androgen receptor (AR) genes. Numbers beside symbols are provided for patient identification in Table 1. *, year of birth; †, year of death.

Conclusions

We assume that the clinical aspects and skewed XCI in this family are associated with the concurrence of a FOXP3 and a CTLA4-predisposing trait. The products of these two genes interact and cooperate in the same immune regulatory signal transduction pathway (17).
Taking into consideration the normal physiological function and interaction of the respective FOXP3 and CTLA4 products in the maintenance of a balanced T-cell immune regulation (3, 5), one is able to deduce the consequences of the concurrence of their respective mutated and variant alleles in the individual family members as follows. FOXP3 is an X-linked gene whose mutated copy will be selectively silenced in the relevant female carriers (14, 16). Thus, no adverse effects ensue from this specific genetic defect, and any risk of autoimmunity in these individuals is therefore most likely due to the respective CTLA4 variant. As, on the other hand, skewed X inactivation is quite a common phenomenon in females with various forms of autoimmune diseases, the pronounced skewing in one cell compartment other than the Treg subset supports the notion that X-linked factors may also be important in this context as was, for instance, demonstrated in crossbreeding experiments of female mice that were double heterozygote knockouts for FOXP3 and the common gamma chain of the interleukin 2, 7, and 15 receptor genes (8). These experiments confirmed that two X-linked recessive genes can interact to cause disease whereas either alone would not, but also that additional factors are operating in modifying disease severity (8). Based on these experiments and compatible with the presented pedigree, we speculate that particular CTLA4 variants may in turn represent one of the autosomal modifiers of FOXP3 (and/or other similar X-linked immune regulatory gene variants). The notion that specific CTLA4 variants may act as such potentially disease-attenuating genetic modifying factors in IPEX is perhaps further corroborated by the fact that the male FOXP3-mutated patient with the mild clinical course

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**Table 1** Laboratory features in affected family members with immune dysregulation.

<table>
<thead>
<tr>
<th>Family member</th>
<th>1a,b</th>
<th>2</th>
<th>3a</th>
<th>4c</th>
<th>5</th>
<th>6</th>
<th>7c</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH (μU/ml)</td>
<td>1.15</td>
<td>1.63</td>
<td>0.61</td>
<td>NA</td>
<td>1.34</td>
<td>1.92</td>
<td>NA</td>
</tr>
<tr>
<td>FT4 (ng/dl)</td>
<td>1.09</td>
<td>1.48</td>
<td>1.38</td>
<td>NA</td>
<td>1.38</td>
<td>1.17</td>
<td>NA</td>
</tr>
<tr>
<td>Thyroid Ab's (TG)</td>
<td>Neg.</td>
<td>Neg.</td>
<td>Neg.</td>
<td>NA</td>
<td>Neg.</td>
<td>Neg.</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetes-associated</td>
<td>Ab's (U/ml)</td>
<td>1750</td>
<td>1630</td>
<td>610</td>
<td>NA</td>
<td>1340</td>
<td>1920</td>
</tr>
<tr>
<td>ANA</td>
<td>Neg.</td>
<td>Neg.</td>
<td>1:160</td>
<td>Neg.</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>dsDNA-Ab's (IgG, -M, -A)</td>
<td>Neg.</td>
<td>Neg.</td>
<td>Borderline</td>
<td>(IgG and IgM)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cardiolipin Ab's</td>
<td>Neg.</td>
<td>Neg.</td>
<td>Neg.</td>
<td>Borderline</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Liver IgG (mg/dl)</td>
<td>768</td>
<td>1110</td>
<td>958</td>
<td>NA</td>
<td>530</td>
<td>1080</td>
<td>NA</td>
</tr>
<tr>
<td>IgA</td>
<td>213</td>
<td>285</td>
<td>287</td>
<td>112</td>
<td>223</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>IgM</td>
<td>90</td>
<td>118</td>
<td>110</td>
<td>121</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>IgG subclasses</td>
<td>Normal, except IgG4 (0.34)</td>
<td>Normal, except IgG3 (180)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>IgE (IU/ml)</td>
<td>74.7</td>
<td>174</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
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<tr>
<td>CD3⁺ T cells (μl)</td>
<td>600</td>
<td>1750</td>
<td>1435</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>CD4⁺/B ratio</td>
<td>1.9</td>
<td>0.95</td>
<td>3.7</td>
<td>4</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CD4⁺ CD25⁺ (% CD4)</td>
<td>32</td>
<td>30</td>
<td>41</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CD4⁺ CD25⁺ (% CD4)</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Treg cells (μl)</td>
<td>15</td>
<td>100</td>
<td>30</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CD19⁺ B cells (μl)</td>
<td>20</td>
<td>320</td>
<td>110</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CD5⁺ CD3⁻ NK cells</td>
<td>85</td>
<td>170</td>
<td>170</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Differential blood</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>count, eosinophils</td>
<td>Hb, Alc</td>
<td>Hb, Alc</td>
<td>Hb, Alc</td>
<td>Hb, Alc</td>
<td>Hb, Alc</td>
<td>Hb, Alc</td>
<td>Hb, Alc</td>
</tr>
<tr>
<td>Other lab chemistry</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Ab’s: antibodies: thyroid Ab’s: TG, thyroglobulin; TPO, thyroperoxidase; TSHR, thyroid-stimulating hormone receptor autoantibodies; GAD, anti-glutamate-decarboxylase; IA2, anti-insulin antibodies; IA2, anti-tyrosine phosphatase IA2; liver autoantibodies include antibodies against smooth muscle, actin, LKM1 (liver kidney microsomal type 1), soluble liver antigen; LFP, liver function parameters; ND, not done; NA, specimen not available.

aUnder immunosuppressive treatment (azathioprine and low-dose prednisolone).

bUnder thyroid hormone (3) and insulin (1, 3) substitution therapy.

cPatient not present for clinical and laboratory examinations, but oral report from primary care physician and family members documented Graves’ disease in patient 4 and Hashimoto’s thyroiditis in patient 7 with a need for corresponding treatment. All family members were invited for thorough endocrinology and immunological work-up; most were seen at least once at our clinics and were examined physically and by blood investigations. From one aunt (patient 4) and the grandmother (patient 7), only one blood sample was available, which was needed for DNA analysis, and only patients 1, 2, and 3 were available for follow-up investigations.
even carried the CTLA4 risk allele in homozygous form. These observations imply that the CTLA4 variant has either a neutral or protective but certainly not an aggravating effect on the FOXP3 mutation in this particular case. Naturally, the available clinical and laboratory features of this pedigree are derived from a limited time segment of clinical observation, and a longitudinal lifelong follow-up with regular monitoring of many endocrinological and immunological parameters would be needed to allow firm conclusions regarding the full impact of genes on disease development in this pedigree.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
B Rami, E Schober, W D Huber, ND, A Heitger, and O A Bodamer were responsible for the clinical diagnosis, took care of the patient and his family, and provided the relevant clinical and laboratory data; C Item, A Heitger, P Zeithofer, and O A Haas performed genetic and immunological analyses and interpreted the ensuing results; B Rami and M G Seidel designed the tables/figures; M G Seidel and O A Haas wrote the manuscript.

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References


