REVIEW ARTICLE

Genetic polymorphism in *FOXP3* gene: imbalance in regulatory T-cell role and development of human diseases

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Abstract

The *FOXP3* gene encodes a transcription factor thought to be important for the development and function of regulatory T cells (Treg cells). These cells are involved in the regulation of T cell activation and therefore are essential for normal immune homeostasis. Signals from microenvironment have a profound influence on the maintenance or progression of diseases. Thus, Tregs have an important marker protein, FOXP3, though it does not necessarily confer a Treg phenotype when expressed. *FOXP3* polymorphisms that occur with high frequency in the general populations have been studied in common multifactorial human diseases. Dysfunction of *FOXP3* gene product could result in lack of Treg cells and subsequently chronically activated CD4+ T cells which express increased levels of several activation markers and cytokines, resulting in some autoimmune diseases. In contrast, high Treg levels have been reported in peripheral blood, lymph nodes, and tumour specimens from patients with different types of cancer. The present study discusses the polymorphisms located in intron, exon and promoter regions of *FOXP3* which have already been investigated by many researchers. *FOXP3* has received considerable attention in attempts to understand the molecular aspect of Treg cells. Therefore, in the present study, the relationship between genetic polymorphism of *FOXP3* in Treg-cell role and in disease development are reviewed considering the interactive effect of genetic factors.

[Oda J. M. M., Hirata B. K. B., Guembarovski R. L. and Watanabe M. A. E. 2013 Genetic polymorphism in *FOXP3* gene: imbalance in regulatory T-cell role and development of human diseases. *J. Genet*. **92**, 163–171]

Introduction

The immune system, a highly effective and dynamic cellular network, protects a host from pathogens. Therefore, the immune system should distinguish self from non-self structures, but also between harmful and innocuous foreign antigens (Ags) to prevent nonessential and self-destructive immune responses (Jonuleit and Schmitt 2003).

Regulatory T cells (Tregs) are a unique CD4+ T cell lineage that plays an indispensable role in maintaining immunological unresponsiveness to self-Ags and in suppressing excessive immune responses deleterious to the host. However, they also limit beneficial responses by suppressing sterilizing immunity and limiting antitumour immunity (Sakaguchi *et al.* 2008; Vignali *et al.* 2008; Toker and Huehn 2011).

Most CD4+ cells are commonly characterized by high surface expression of the interleukin 2 (IL-2) receptor α - chain (CD25) and their phenotype is now generally accepted as $CD4^+CD25^{hi}CTLA4^+GITR^+$*FOXP3*$^+$ $CD45RO^+CD45RA^-CD69^-Ki\text{-}67^-$ (Sakaguchi 2005; Betts *et al.* 2006). FOX (forkhead box) is now used as the symbol for all chordate forkhead transcription factors. A phylogenetic analysis has resulted in the definition of 15 classes for all known FOX proteins, so these transcription factors are classified in terms of structure not function. The *FOXP3* (transcription factor forkhead box3) is a member of the forkhead winged-helix transcription-factor family and has three discernible functional domains: a single C2H2 zinc-finger motif (amino acids 200–223), a leucine-zipper-like motif (amino acids 240–261) and a carboxy-terminal forkhead domain (amino acids 338–421). FOXP3 is expressed primarily in a subset of CD4+ T-cells expressing CD25, known as regulatory T cells where it appears to be a key lineage commitment factor for the development of this important subset

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Keywords. disease; exon region; *FOXP3* gene; intron region; mutation; polymorphism; promoter region; Treg cells.

of cells (Coffer and Burgering 2004; Anover *et al.* 2006; Campbell and Ziegler 2007; Eastell *et al.* 2007).

Therefore, FOXP3 has a defining role in regulating the development and function of regulatory T cells. Tone and Greene (2011) and Williams and Rudensky (2007) demonstrated that the continued expression of FOXP3 in mature Treg cells is indispensable for the maintenance of the dominant tolerance that those cells mediate.

Although various signals that induce the expression of *FOXP3* have been identified, the precise mechanisms by which the expression of this protein is controlled in Treg cells are not well understood. So far, it has been established that the synergistic action of signals downstream of the T-cellreceptor (TCR), costimulatory molecules and cytokine receptors is required for the active transcription of *FOXP3* (Huehn *et al.* 2009).

Caution needs to be exercised when interpreting data on FOXP3 expression in tumours. Increased levels of *FOXP3* mRNA expression may be a result of not only an increased influx of immune cells that express *FOXP3*, like Treg CD4+, Treg CD8+ (Liu *et al.* 2011), macrophages (Leavy 2011; Manrique *et al.* 2011) but also increased expression of *FOXP3* directly in tumour cells. This understanding is importance for developing assays on the basis of FOXP3 for prognosis or drug monitoring. The expression of FOXP3 in tumour cells indicates that FOXP3-targeted drugs must be able to penetrate the tumour bed, which is much more challenging than depleting FOXP3 in the periphery (Lu 2009).

Polymorphisms have been described in various regions of the *FOXP3* gene, such as the promoter, intron and exon regions. According to Hoogendoorn *et al.* (2003), promoters are involved in initiating transcription and are therefore among the many important *cis*-acting elements that regulate gene expression that might harbour functionally relevant polymorphisms.

Polymorphisms of *FOXP3* gene may change its role functionally or quantitatively, therefore leading to lack of functional $CD4^{+}CD25^{+}$Tregs, resulting in some autoimmune diseases (Wildin *et al.* 2002), such as immunodysregulation, polyendocrinopathy, enteropathy, X-linked IPEX (immunodysregulation polyendocrinopathy enteropathy) syndrome (van der Vliet and Nieuwenhuis 2007), type 1 diabetes (T1D) (Bassuny *et al.* 2003) and autoimmune thyroid diseases (Ban *et al.* 2007).

In this context, *FOXP3* polymorphisms that occur with high frequency in the general population have been studied in common multifactorial human diseases, and some of these studies are discussed in this review.

Forkhead box 3 (FOXP3)

Most transcriptional factors are modular proteins composed of DNA-binding domains and/or motifs that interact with other transcriptional regulators and modifying enzymes. Many of these interacting proteins do not bind to DNA directly, but modulate DNA binding by conferring transcriptional activating or repressing activity to the DNA binding-partner. This activity is often related to either compaction or relaxation of chromatin, thus restricting or permitting access of other transcriptional regulatory proteins (Li *et al.* 2004).

FOXP3 gene was identified in 2001 as the disease-causative gene in Scurfy mice, which spontaneously develop severe autoimmunity/inflammation as a result of a single-gene mutation on the X chromosome (Brunkow *et al.* 2001). The human full-length *FOXP3* gene is 1296 bp in size and has been reported to consist of 11 different exons. This gene is located at the small arm of the X-chromosome (Xp11.23), that is subject to X-chromosomal inactivation, and encodes a 431 amino-acid protein (Gambineri *et al.* 2003; Fontenot *et al.* 2005; Ban *et al.* 2007; Torgerson and Ochs 2007). Sequence analyses have revealed three highly conserved noncoding regions in *FOXP3* locus, all of which have been found to be subject to epigenetic modifications and involved in regulating the transcription of *FOXP3* (Huehn *et al.* 2009).

The first highly conserved region is the *FOXP3* promoter, which is located 6.5-kb upstream of the first coding exon of *FOXP3*. This promoter is a classic TATA and CAAT-box-containing sequence that is activated in response to TCR signalling through binding of NFAT (nuclear factor of activated T lymphocytes) and AP1 (activator protein 1) (Mantel *et al.* 2006). The second highly conserved noncoding region in the *FOXP3* locus has been identified as a TGFβ-sensitive element that contains binding sites for NFAT and SMADs (Tone *et al.* 2008). The most striking differences regarding the methylation pattern of the *FOXP3* locus have been observed in a third, highly conserved, CpG-rich enhancer and/or stabilizer region. This site was found to be fully demethylated in Treg cells and methylated in conventional T cells (Baron *et al.* 2007; Floess *et al.* 2007; Kim and Leonard 2007; Nagar *et al.* 2008), and normally referred as the Treg-cell-specific demethylated region (TSDR).

The three functional domains described above, single C2H2 zinc-finger motif, a leucine-zipper-like motif and a carboxy-terminal forkhead domain, are involved in DNA binding, nuclear transport (Hancock and Ozkaynak 2009), homomeric and heteromeric complex formation (Li *et al.* 2006), and transcriptional repressor activity (Li *et al.* 2006; Lopes *et al.* 2006). FOXP3 interacts with multiple transcription factors known to be involved in activation, differentiation, and response of CD4+ T cells to TCR stimulation, NFAT), nuclear factor—kappa B (NF-kB), runt-related transcription factor 1 (RUNX1), retinoic acid receptor-related orphan receptors (RORs) (RORα and RORγT), IFN regulatory factor 4 (IRF4), signal transducer and activator of transcription 3 (STAT3), and Jun (Bettelli *et al.* 2005; Wu *et al.* 2006; Ono *et al.* 2007; Du *et al.* 2008; Zhou *et al.* 2008; Chaudhry *et al.* 2009; Rudra *et al.* 2009; Zheng *et al.* 2009).

Genomewide analysis has shown that FOXP3 binds to the promoter region of 700–1100 bp of some genes, many of

those being associated with TCR signalling. A large number of FOXP3-bound genes were upregulated or downregulated in FOXP3+ T cells, indicating that FOXP3 may act as both a transcriptional activator and repressor (Marson *et al.* 2007; Zheng *et al.* 2007). Chromatin immunoprecipitation combined with microarray analyses revealed numerous transcriptional targets of human and murine FOXP3, including genes whose expression is upregulated (*CD25* (cluster of differentiation 25), *CTLA4* (cytotoxic T-lymphocyte antigen 4), *TNFRSF18* (GITR, glucocorticoid-induced TNFR family related gene) or repressed (*IL2* (interleucin-2), *PTPN22* (protein tyrosine phosphatase, nonreceptor type 22)) (Marson *et al.* 2007; Zheng *et al.* 2007).

FOXP3 cooperates with additional transcription factors, such as NFAT1 and RUNX1, to regulate gene expression (Wu *et al.* 2006; Ono *et al.* 2007; Kitoh *et al.* 2009; Rudra *et al.* 2009). Transcriptional repression by FOXP3 may reflect its ability to recruit histone deacetylase (HDAC) family members through an N-terminal repressor domain (Li and Greene 2007; Li *et al.* 2007a). However, the same N-terminal domain is also responsible for transcriptional activation by FOXP3 (Wu *et al.* 2006), and the stimulatory effects of FOXP3 on gene expression cannot be explained by recruitment of HDAC corepressor complexes alone.

The C-terminal forkhead domain, where the largest number of IPEX missense mutations cluster, is required for DNA binding and nuclear import. The leucine zipper of *FOXP3* is necessary and sufficient to mediate both homo-association (Chae *et al.* 2006; Lopes *et al.* 2006; Li *et al.* 2007b) and hetero-association with FOXP1 (Wang *et al.* 2003). Although the forkhead domain alone may bind to DNA *in vitro* (Stroud *et al.* 2006; Bandukwala *et al.* 2011), the disease-associated mutations of the leucine zipper domain disrupting *FOXP3* dimerization can substantially reduce the binding of FOXP3 to promoter regions *in vivo* (Chae *et al.* 2006; Lopes *et al.* 2006; Li *et al.* 2007b). Moreover, the leucine zipper is also important for the interaction between histone H1.5 and FOXP3, which cooperatively repress IL-2 transcription in human T cells (Mackey-Cushman *et al.* 2011). A repression domain in the N-terminal proline-rich region is required for FOXP3 to suppress transcription (Lopes *et al.* 2006).

Genetic polymorphism in *FOXP3* gene: promoter region

Genetic polymorphisms of *FOXP3* in the promoter region have been widely studied in the context of autoimmune diseases (table 1).

Mutations in the open-reading frame of *FOXP3* are associated with IPEX, a rare fatal paediatric condition (Bennett *et al.* 2001), exhibiting aggressive autoimmune features. *FOXP3* mutations in IPEX patients result in heterogeneous biological abnormalities, leading not necessarily to a lack of differentiation of $CD4^+CD25^{high}$Tregs but rather to a dysfunction in these cells and in effector T cells (Bacchetta *et al.* 2006). Thus, polymorphisms of the *FOXP3* gene may change FOXP3 functionally or quantitatively, thus leading to the lack of functional $CD4^+CD25^+$Tregs and subsequently chronically activated $CD4^+$ T cells which express increased levels of several activation markers and cytokines, resulting in autoimmune diseases (Wildin *et al.* 2002; Bjornvold *et al.* 2006).

Polymorphisms in the promoter region may potentially alter gene expression by changing the binding specificity of transcription factors to their binding sites and by modifying the kinetics of transcription initiation (Hanel *et al.* 2011). There are five single-nucleotide polymorphisms (SNP) in the promoter region of *FOXP3*: −924A/G (rs2232365), −1383C/T (rs2232364), −2383C/T (rs3761549), −3279C/A (rs3761548) and −3499A/G (rs3761547) (Bassuny *et al.* 2003).

The *AA* genotype of the −3279C/A polymorphism causes the loss of binding with some transcription factors, such as E47 and C-Myb, leading to defective transcription of *FOXP3*. Moreover, the *A* allele of this polymorphism is associated with a dramatic reduction in luciferase activity compared with the *C* allele (Shen *et al.* 2010). Conversely, there is no report on the functional effects of −2383C/T and −3499A/G polymorphisms on gene expression (Inoue *et al.* 2010).

The −3279 (rs3761548) polymorphism has been largely studied and various associations with diseases have been described. Gao *et al.* (2010) evaluated the susceptibility to psoriasis in a Han Chinese population. These authors found an increased risk for psoriasis associated with the *AC* genotype and also to the combined *AC* + *AA* genotypes compared with the wild *CC* genotype. An association between the −3279 polymorphism and allergic rhinitis (AR) in heterozygous form was also identified in this population, especially in response to house dust mite (Zhang *et al.* 2009). Fodor *et al.* (2011) confirmed the findings of Zhang *et al.* (2009) when they examined whether the association detected in the Chinese population also exists in a European population of Hungarian Caucasian patients with ragweed pollen allergy. According to this study, females homozygous for the rare *FOXP3* rs3761548 allele (*AA*) are protected against AR; otherwise, females who are either wild-type (*CC*) or heterozygote carriers (*CA*) of the rare allele are more susceptible to AR. Bottema *et al.* (2010) also found that in females, the −3279 (rs3761548) was significantly associated with sensitization to egg at age one and two years, and with sensitization to indoor allergens at age two, but not at age four and eight years.

The −3279 (rs3761548) polymorphism in the *FOXP3* gene was also associated with the development and intractability of Graves' disease (GD) (Inoue *et al.* 2010), with lower anti-dsDNA levels in female systemic lupus erythematosus patients (Lin *et al.* 2011), and also significantly associated with unexplained recurrent spontaneous abortion (URSA) in the Chinese Han population (Wu *et al.* 2012).

Table 1. *FOXP3* polymorphisms in promoter region.

Polymorphism	Diseases	Year	Country	Reference
rs2232365	Crohn's disease	2005	USA	Park *et al.* (2005)
	Graves' and Addison's disease	2006	UK	Owen *et al.* (2006)
	Psoriasis	2010	China	Gao *et al.* (2010)
	Unexplained recurrent spontaneous abortion	2012	China	Wu *et al.* (2012)
rs3060515	Allergic rhinitis	2009	China	Zhang *et al.* (2009)
	Systemic lupus erythematosus	2011	Taiwan	Lin *et al.* (2011)
rs3761547	Juvenile idiopathic arthritis	2007	UK	Eastell *et al.* (2007)
	Allergic rhinitis	2009	China	Zhang *et al.* (2009)
	Hashimoto's disease and Graves' disease	2010	Japan	Inoue *et al.* (2010)
rs3761548	Crohn's disease	2005	USA	Park *et al.* (2005)
	Breast cancer	2009	Israel	Raskin *et al.* (2009)
	Allergic rhinitis	2009	China	Zhang *et al.* (2009)
	Atopy	2010	The Netherlands	Bottema *et al.* (2010)
	Psoriasis	2010	China	Gao *et al.* (2010)
	Hashimoto's disease and Graves' disease	2010	Japan	Inoue *et al.* (2010)
	Endometriosis and infertility	2011	Brazil	Andre *et al.* (2011)
	Allergic rhinitis	2011	Hungary	Fodor *et al.* (2011)
	Systemic lupus erythematosus	2011	Taiwan	Lin *et al.* (2011)
	Unexplained recurrent spontaneous abortion	2012	China	Wu *et al.* (2012)
rs3761549	Graves' and Addison's disease	2006	UK	Owen *et al.* (2006)
	Hashimoto's disease and Graves' disease	2010	Japan	Inoue *et al.* (2010)
	Endometriosis and infertility	2011	Brazil	Andre *et al.* (2011)
rs5902434	Psoriasis	2010	China	Gao *et al.* (2010)
	Unexplained recurrent spontaneous abortion	2012	China	Wu *et al.* (2012)
(GT)*n*	Type 1 diabetes	2003	Japan	Bassuny *et al.* (2003)
	Type 1 diabetes	2004	Italy	Zavattari *et al.* (2004)
	Systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, Crohn's disease and celiac disease	2005	Spain	Sanchez *et al.* (2005)
	Type 1 diabetes and coeliac disease	2006	Norway	Bjornvold *et al.* (2006)
	Graves' and Addison's disease	2006	UK	Owen *et al.* (2006)
	Autoimmune thyroid diseases	2007	Japan	Ban *et al.* (2007)
	Type 1 diabetes	2007	Japan	Nakanishi and Shima (2007)

Although several studies have found associations between −3279 (rs3761548) polymorphism and diseases, this is not always the case in all studies. Andre *et al.* (2011) found no association with endometriosis related infertility group or the idiopathic infertility group. There was also no association between this polymorphism with Crohn's disease (Park *et al.* 2005) and breast cancer risk (Raskin *et al.* 2009).

Various studies with $(GT)_n$ microsatellite polymorphisms in the promoter region of *FOXP3* gene have been conducted, particularly examining association with T1D, but with varying results. Bassuny *et al.* (2003) demonstrated that the $(GT)_{15}$ allele showed a significant higher frequency in patients with T1D than in controls. According to these results, the authors concluded that the *FOXP3*/*scurfin* gene appears to confer a significant susceptibility to T1D in the Japanese population. This association, however, was not found by Nakanishi and Shima (2007) who also studied microsatellite polymorphism in the promoter region of *FOXP3* gene in the Japanese population. Further, attempts to confirm this association in independent cohorts of different ethnic origin and other autoimmune diseases have been unsuccessful. Using 418 T1D families and a further 268 male patients and 326 healthy males for a case–control analysis from Sardinia, Zavattari *et al.* (2004) detected no association between the polymorphisms and T1D, nor with seven SNPs and four other microsatellites in the *FOXP3* gene region. This conclusion was also obtained by Bjornvold *et al.* (2006) who analysed the same microsatellite analysed by Bassuny *et al.* (2003) in a Caucasian population. Howson *et al.* (2009) tested six SNPs in *FOXP3* for association with T1D, among these the rs4824747, but found no evidence of association.

The $(GT)_n$ microsatellite polymorphism in *FOXP3* was also investigated in other autoimmune diseases, like the work of Sanchez *et al.* (2005) that investigated this polymorphism in relation with systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, Crohn's disease and celiac disease. They found no evidence of association between this genetic variant with autoimmune diseases in a Spanish population. Ban *et al.* (2007) studied autoimmune thyroid diseases and found that the $(GT)_n$ microsatellite polymorphism is associated with these diseases in a Caucasian cohort but not in a Japanese cohort. Also, no evidence was found that these $(GT)_n$ microsatellite polymorphism contributed to susceptibility to Graves' or Addison's diseases in the northeast England population (Owen *et al.* 2006).

The −924 (rs2232365) SNP is significantly associated with unexplained recurrent spontaneous abortion in the Chinese Han population, highlighting the important role of *FOXP3* in successful pregnancy (Wu *et al.* 2012). But no associations were found between this SNP with susceptibility to psoriasis in a Han Chinese population (Gao *et al.* 2010), Crohn's disease (Park *et al.* 2005) and Graves' disease (Owen *et al.* 2006).

Lin *et al.* (2011) found an evidence for association of the −6054 (rs3060515) SNP with lower risk of lupus nephritis. This SNP was also related to AR, whose disease is also associated with the −3499 (rs3761547) polymorphisms (Zhang *et al.* 2009). However the −3499 (rs761547), was not related to juvenile idiopathic arthritis (JIA) (Eastell *et al.* 2007). This polymorphism also was genotyped by Inoue *et al.* (2010) in Japanese patients to clarify their effects on the development and prognosis of autoimmune thyroid diseases, such as Hashimoto's disease (HD) and Graves' disease. The authors found no difference in genotypes and allele frequencies among the groups. In this same work the authors genotyped −2383 (rs3761549) polymorphism in *FOXP3* gene, which was related to severity of HD. The −3499 (rs3761549) have also significant association with endometriosis, regardless of the stage of the disease (Andre *et al.* 2011). However, this SNP was not associated with the susceptibility to Graves' disease or Addison's disease (Owen *et al.* 2006).

The −6054 (rs5902434) SNP was evaluated in relation to psoriasis in a Han Chinese population by Gao *et al.* (2010) and no association was found, but the evaluation of −6054 (rs5902434) SNP with respect to URSA by Wu *et al.* (2012) showed that this polymorphism of the *FOXP3* gene may confer an important susceptibility in Chinese population.

Genetic polymorphism in *FOXP3* gene: intron region

Some mutations in *FOXP3* gene are localized in intron region, as shown in table 2. Andre *et al.* (2011) analysed three SNPs in intron regions of *FOXP3* gene: −20 (rs2232368) located in the intron 1, +87 (rs2232366) located in intron 5 and +459 (rs2280883) located in intron 9. Their results revealed a possible association of +459 (rs2280883) and −20 (rs2232368) with idiopathic infertility whereas no association was found for +87 (rs2232366) either for the endometriosis-related infertility group or the idiopathic infertility group. When Park *et al.* (2005) investigated the SNP +459 (rs2280883) located in intron 9 of *FOXP3* they observed a significant difference between patients with primary billiary cirrhosis (PBC) and controls. This SNP is also associated with severe psoriasis patients (Gao *et al.* 2010), but not related to susceptibility to Graves' and Addison's

Table 2. *FOXP3* polymorphisms in intron region.

Polymorphism	Disease	Year	Country	Reference
rs2232366	Endometriosis and infertility	2011	Brazil	Andre *et al.* (2011)
rs2232367	Juvenile idiopathic arthritis	2007	UK	Eastell *et al.* (2007)
rs2232368	Endometriosis and infertility	2011	Brazil	Andre *et al.* (2011)
rs2280883	Crohn's disease	2005	USA	Park *et al.* (2005)
	Graves' and Addison's disease	2006	UK	Owen *et al.* (2006)
	Juvenile idiopathic arthritis	2007	UK	Eastell *et al.* (2007)
	Psoriasis	2010	China	Gao *et al.* (2010)
	Endometriosis and infertility	2011	Brazil	Andre *et al.* (2011)
rs2294019	Atopy	2010	The Netherlands	Bottema *et al.* (2010)
rs2294020	Juvenile idiopathic arthritis	2007	UK	Eastell *et al.* (2007)
	Breast cancer	2009	Israel	Raskin *et al.* (2009)
rs2294021	Graves' and Addison's disease	2006	UK	Owen *et al.* (2006)
	Atopy	2010	The Netherlands	Bottema *et al.* (2010)
	Unexplained recurrent spontaneous abortion	2012	China	Wu *et al.* (2012)
rs4824747	Juvenile idiopathic arthritis	2007	UK	Eastell *et al.* (2007)
	Type 1 diabetes	2010	UK	Howson *et al.* (2009)
rs5906761	Breast cancer	2009	Israel	Raskin *et al.* (2009)
	Atopy	2010	The Netherlands	Bottema *et al.* (2010)
rs6609857	Graves' and Addison's disease	2006	UK	Owen *et al.* (2006)
	Juvenile idiopathic arthritis	2007	UK	Eastell *et al.* (2007)
	Atopy	2010	The Netherlands	Bottema *et al.* (2010)
	IPEX syndrome	2001	USA	Bennett *et al.* (2001)
$(TC)_n$	Type 1 diabetes	2003	Japan	Bassuny *et al.* (2003)
	Graves' and Addison's disease	2006	UK	Owen *et al.* (2006)
	Type 1 diabetes	2004	Italy	Zavattari *et al.* (2004)
	Type 1 diabetes and coeliac disease	2006	Norway	Bjornvold *et al.* (2006)
	Autoimmune thyroid diseases	2007	Japan	Ban *et al.* (2007)

Table 3. *FOXP3* polymorphisms in exon region.

Exon	Disease	Year	Country	Reference
1	IPEX syndrome	2006	Italy/France/USA	Anover *et al.* (2006)
12	Autoimmune disease and the risk of FVIII inhibitor development	2010	Italy	Bafunno *et al.* (2010)
2–12	Acute leukemias	2011	Korea	Kim *et al.* (2011)

diseases (Owen *et al.* 2006) and juvenile idiopathic arthritis (Eastell *et al.* 2007).

Located in intron 5 of the *FOXP3* gene, there is also a $(TC)_n$ microsatellite polymorphism. This was analysed by Ban *et al.* (2007), showing an association with autoimmune thyroid disease in a Caucasian cohort, especially in male patients, however this association was not seen in a Japanese cohort. The $(TC)_n$ microsatellite polymorphism also was not related to Graves' and Adisson's diseases (Owen *et al.* 2006) and T1D (Bassuny *et al.* 2003; Zavattari *et al.* 2004; Bjornvold *et al.* 2006).

Bottema *et al.* (2010) studied four intron regions of *FOXP3* gene: rs5906761 (located upstream of exon −1) and rs2294021, rs2294019 and rs6609857 (located in 3'UTR) and observed significant association between these SNPs with sensitization, in girls, to egg at age one and two years, and with sensitization to indoor allergies at age two, but not at four and eight years. They also found that rs5906761 and rs2294021 were associated with remission of sensitization to food allergens in boys. The rs5906761 was also studied by Raskin *et al.* (2009), but in their work no association was found between this polymorphism and breast cancer risk. The rs2294020 SNP was also not associated with juvenile idiopathic arthritis (Eastell *et al.* 2007).

Of the SNPs located in 3'UTR of *FOXP3* gene, two of them were also investigated by Owen *et al.* (2006): the rs2294021 and rs6609857, but the authors did not find any evidence that these contribute to Graves' or Addison's disease susceptibility in northeast England population. The polymorphism rs2294021 was not related with recurrent spontaneous abortion (Wu *et al.* 2012) and rs6609857 was not associated with JIA (Eastell *et al.* 2007). The same authors studied the rs2232367 and rs4824747, such as the other SNPs, in relation to JIA, and also found no associations.

Bennett *et al.* (2001) identified an A→G transition within the first polyadenylation signal (AAUAAA→AAUGAA), which resides 878–883 bp downstream of the stop codon. This mutation was not detected in over 212 normal individuals, suggesting that it is causal of IPEX by a mechanism of nonspecific degradation of the *FOXP3* gene message.

Genetic polymorphism in *FOXP3* gene: exon region

There are few studies involving the expression control and modulation in the exon region of *FOXP3* gene (table 3). Anover *et al.* (2006) showed a novel 1388 bp deletion mutation that included the 5' half exon −1 (noncoding) and a large segment of the first intron on *FOXP3* gene. This aberrantly spliced mRNA species is rapidly degraded leading to overall low mRNA abundance in the patients and subsequent lack of FOXP3+ Treg, resulting in IPEX.

Kim *et al.* (2011) analysed coding region (exons 2–12) of human *FOXP3* gene in acute leukaemia, but no mutations were detected, indicating that *FOXP3* gene mutations may be specific to just for few cancers, such as breast and prostate cancers. Polymorphisms in exon 12 were also studied by Bafunno *et al.* (2010), whose work revealed no positive or negative association between this polymorphism and the susceptibility to haemophilia A treatment.

Conclusion

The *FOXP3* gene is a central molecule in the function of Tregs cells, both in the context of maintenance of immune tolerance and also in regulation of response. Therefore, this transcription factor is very important to play a crucial role in generation of Treg phenotype. In both autoimmune diseases and cancer, FOXP3 may play a role in immunopathology, due to potent suppressive T-cell activation and effector function (Watanabe *et al.* 2010). Polymorphisms in *FOXP3* gene can lead to immune system imbalance and mediate clinically serious human disease development. The study of these polymorphisms may contribute to a better understanding of their pathogenesis and progression.

Acknowledgements

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq, CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES), PROCAD, PNPD, Fundação Araucária do Paraná (FAP), Brasil and Pró-Reitoria de Pós-Graduação - State University of Londrina - PROPPG-UEL. The entire article was revised by a British-born scientific text editor.

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Received 8 May 2012, in revised form 20 September 2012; accepted 31 October 2012
Published on the Web: 2 April 2013