The allele frequencies of A_1 and A_2 are denoted by p and q, respectively, and are estimated using the following formulas:

$$p=(2a+b+d)/(2n_1+n_2)$$
$$q=(b+2c+e)/(2n_1+n_2)$$

Then, the expected values for the A_1A_1, A_1A_2, and A_2A_2 genotypes in the female samples, calculated using the estimated p and q, are equal to p2, 2pq, and q2, respectively. Finally, the observed and expected values for the genotypes should be compared using the chi-squared test. The degree of freedom is 1.

Because the number of X chromosomes differs between the sexes of the participants, to compare cases with controls (in case-control studies), participants should be stratified by sex. However, when allelic frequency is estimated in the manner described above, it is possible to compare allelic frequencies (and not genotypic frequencies) between all cases and controls, regardless of their sex.

Considering that Yaylacıoğlu Tuncay et al.[1] included both sexes among their participants, the reported results should be interpreted with caution. It is recommended that the authors present their data on the genotypes of each polymorphism according to the sex of the participants and reanalyze their data to address the major issues mentioned above. As emphasized elsewhere,[3,4,5] the researchers should also show that the frequencies of the observed genotypes are not significantly different from their expected frequencies based on HWE. I wish the esteemed authors all the best in their research.

Ethics

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Reply

First of all, we thank the author for evaluating our article.[1] As the author and we pointed out in our article, the Forkhead box P3 (*FOXP3*) gene is on the X chromosome. As far as we know, ten different reports in the literature have investigated the association between *FOXP3* polymorphisms and the development of Graves' disease (GD).[2,3,4,5,6,7,8,9,10,11] Seven of those reports presented the genotype and allele frequencies by pooling both sexes,[2,3,4,5,6,7,8] and the rest stratified the participants by sex and reported the genotype and allele frequencies separately in females and males.[9,10,11] In our article, we aimed to evaluate the frequency of *FOXP3* polymorphisms in GD with or without ophthalmopathy in a Turkish population.[1] Since the number of participants in each group was limited in our study and there were no corrections for the previously published articles,[2,3,4,5,6,7,8] we did not stratify the groups by sex and used the results of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) directly to analyze genotype and allele frequencies.[1] In the PCR-RFLP method, the results were shown by band patterns and those patterns do not indicate whether the sample had one or two alleles of the gene of interest. Additionally, using the same analysis method, we could compare our results with those seven reports that did not stratify the groups by sex.

However, as mentioned by the author, as males have one and females have two X chromosomes, the genotypic patterns differ between the two sexes. There are three genotypes (two homozygotes and one heterozygote) in females but only two hemizygous genotypes in males. Therefore, it will be better to stratify the groups by sex and define the genotype and allele frequencies separately in females and males for X-linked genes. Moreover, the author emphasized the importance of comparing the observed and expected genotypic values based on Hardy-Weinberg equilibrium (HWE) in his letter and explained the formula for an X-linked polymorphic locus. According to the concerns reported by the author, in this reply, we reported our additional analysis by stratifying the groups by sex as shown in Tables 1, 2, 3, and 4.

Firstly, we did the HWE analysis in both the controls and study groups, as suggested by the author. The frequencies of the observed genotypes were not significantly different from their expected frequencies based on HWE for all single nucleotide polymorphisms (SNPs) in female control groups (rs3761547, p=0.926; rs3761548, p=0.881; and rs3761549, p=0.926).

Table 1. Genotypic and allelic distribution of *FOXP3* SNPs in female study and control groups				
Genotype	**Control group n=68**	**Study group (non-GO/GO) n=130**	**p value**	**OR (95% CI)**
rs3761548 -3279 C/A				
CC	39 (57%)	30 (23%)	-	1.0[a]
AC	26 (38%)	74 (57%)	**<0.0001**	3.7 (1.9-7.1)
AA	3 (5%)	26 (20%)	**<0.0001**	11.26 (3.1-40.8)
Allele				
C	104 (76%)	134 (51%)	-	1.0[a]
A	32 (24%)	126 (49%)	**<0.0001**	3.05 (1.9-4.8)
rs3761549 -2383 C/T				
CC	58 (85%)	89 (68%)	-	1.0[a]
CT	10 (15%)	35 (27%)	**0.03**	2.28 (1.04-4.9)
TT	0 (0%)	6 (5%)	-	-
Allele				
C	126 (93%)	210 (81%)	-	1.0[a]
T	10 (7%)	50 (19%)	**<0.0001**	3 (1.4-6.1)
rs3761547 -3499 A/G				
AA	58 (85%)	103 (79%)	-	1.0[a]
AG	10 (15%)	26 (20%)	0.34	1.4 (0.65-3.24)
GG	0	1 (1%)	-	-
Allele				
A	126 (93%)	232 (89 %)	-	1.0[a]
G	10 (7%)	28 (11%)	0.27	1.5 (0.71-3.23)

[a]The first allele or genotype (CC for -3279 C/A, CC for -2383 C/T, AA for -3499 A/G) is considered the reference value. SNP: Single nucleotide polymorphism, GO: Graves' ophthalmopathy, OR: Odds ratio, n: Number, CI: Confidence interval. Frequencies of genotypes and alleles were compared using chi-square test. Bold values indicate statistical significance

Table 2. Genotypic and allelic distribution of *FOXP3* SNPs in non-GO and GO female patients				
Genotype	**Non-GO**	**GO**	**p value**	**(95% CI)**
	n=56	**n=74**		
-3279 C/A				
CC	10 (17.9%)	17 (23.0%)	0.47	1.38 (0.5, 3.2)
AC	34 (60.7%)	40 (54.0%)	0.44	0.8 (0.38, 1.5)
AA	12 (21.4%)	17 (23.0%)	0.84	1.09 (0.4, 2.5)
Allele			0.77	1.07 (0.65, 1.7)
A	54 (48.2%)	74 (50%)		
C	58 (51.8%)	74 (50%)		
-2383 C/T				
CC	42 (75.0%)	47 (63.5%)	0.16	0.58 (0.26, 1.25)
CT	10 (17.9%)	25 (33.8%)	0.06	2.4 (0.98, 5.41)
TT	4 (7.1%)	2 (2.7%)	0.07	0.24 (0.05, 1.24)
Allele			0.46	0.78 (0.42, 1.50)
C	94 (81.0%)	119(80.4%)		
T	18 (19.0 %)	29 (19.6%)		
-3499 A/G				
AA	44 (78.6%)	59 (79.7%)	0.52	1.1 (0.46, 2.52)
AG	12 (21.4%)	14 (18.9%)	0.71	0.86 (0.36, 2.02)
GG	0	1 (1.4%)	-	-
Allele			0.57	0.99 (0.4, 2.19)
A	100 (86.2%)	132 (89.2%)		
G	12 (13.8%)	16 (10.8%)		

SNP: Single nucleotide polymorphism, GO: Graves' ophthalmopathy, OR: odds ratio, n: number, CI: confidence interval. Frequencies of genotypes and alleles were compared using chi-square test

Table 3. Allelic distribution of the male study and control groups				
Allele	**Control group n=32**	**Study group (non-GO/GO) n=44**	**p value**	**OR (95% CI)**
rs3761548				
C	27 (84%)	20 (45%)	-	1.0[a]
A	5 (16%)	24 (55%)	**<0.0001**	6.8 (2.1-19)
rs3761549				
C	30 (94%)	42 (95%)	-	1.0[a]
T	2 (6%)	2 (5%)	0.74	2.3 (1.2-4.2)
rs3761547				
A	30 (94%)	44 (100%)	-	-
G	2 (6%)	0 (0%)	-	-

[a]The first listed allele is considered the reference value. GO: Graves' ophthalmopathy, OR: Odds ratio, n: Number, CI: Confidence interval. Frequencies of alleles were compared using chi-square test. Bold values indicate statistical significance

Table 4. Allelic distribution of the study and control groups regardless of their sex				
Allele	**Control group n=168**	**Study group (non-GO/GO) n=304**	**p value**	**OR (95% CI)**
rs3761548				
C	131 (78%)	154 (51%)	-	1.0[a]
A	37 (22%)	150 (49%)	**<0.0001**	3.4 (2.24-5.2)
rs3761549				
C	156 (93%)	252 (83%)	-	1.0[a]
T	12 (7%)	52 (17%)	0.0025	2.3 (1.2-4.2)
rs3761547				
A	156 (93%)	276 (91%)	-	1.0[a]
G	12 (7%)	28 (9%)	0.43	1.31 (0.65-2.67)

[a]The first listed allele is considered the reference value. GO: Graves' ophthalmopathy, OR: Odds ratio, n: Number, CI: Confidence interval. n=2 x number of females + number of males. Frequencies of alleles were compared using chi-square test. Bold values indicate statistical significance

Secondly, when the groups were stratified by sex, we found that the frequency of the AC and AA genotypes of -3279 (rs3761548) and the CT genotype of -2383 (rs3761549) were significantly increased in our female study group (Table 1). Additionally, the A allele of -3279 had a significantly increased frequency both in the female (Table 1) and male study groups (Table 3) when compared separately or when compared between controls and patients regardless of sex (Table 4). The frequency of the T allele of -2383 was significantly increased both in our female study group when compared separately (Table 1) and in the whole study sample when compared regardless of sex (Table 4). However, the frequency of the T allele of -2383 was not significantly increased in our male study group when compared separately (Table 3). For polymorphism -3449 (rs3761547), allele and genotype frequency distribution were not significantly different in any comparisons between the control and study groups (Table 1, Table 3, and Table 4).

Thirdly, comparing the genotypic and allelic distributions of each of the three *FOXP3* SNPs between female patients with Grave's ophthalmopathy (GO) and GD without ophthalmopathy (non-GO) showed no statistically significant difference (Table 2).

Consequently, the results in our published article and the analysis made by stratifying the participants by sex were similar. However, as the author emphasized in his letter, polymorphic loci on X-chromosome should be analyzed differently than the loci on autosomal chromosomes. Therefore, his letter and our reply will be helpful for researchers who will investigate associations with X-linked polymorphic loci.

Ethics

Authorship Contributions

Surgical and Medical Practices: K.S.C., B.T., O.K., Concept: F.Y.T., K.S.C., S.G.E., O.K., Design: F.Y.T., K.S.C., S.G.E., O.K., Data Collection or Processing: F.Y.T., K.S.C., S.G.E., Analysis or Interpretation: F.Y.T., K.S.C., S.G.E., Literature Search: F.Y.T., K.S.C., Writing: F.Y.T., K.S.C., S.G.E.

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