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The effect of the *ATG16L1 Thr300Ala* polymorphism on susceptibility and outcome of patients with epithelial cell-derived thyroid carcinoma

Dear Editor

Epithelial cell-derived thyroid carcinoma (TC) is the most common endocrine malignancy. Increasing evidence suggests that autophagy, a complex process of autodigestion in conditions of cellular stress, plays an important role in the pathophysiology of the TC malignant process. One of the main mammalian autophagy proteins is autophagy related 16-like 1 (ATG16L1), which is essential for autophagosome formation, induction of autophagy, and modulation of inflammation (Saitoh *et al*. 2008). Subsequently, defective autophagy in *ATG16L1* knockout mice results in an increased production of the proinflammatory cytokine interleukin 1β (IL1β; Saitoh *et al*. 2008) that is also known to affect the growth and differentiation of different malignant cell types (Apte & Voronov 2002).

Considering the potential role of both autophagy and IL1β in the pathology of TC, we hypothesized that genetic variation in *ATG16L1* influences the susceptibility for and the outcome of TC. One single nucleotide polymorphism of the *ATG16L1* gene (c.898A>G, *Thr300Ala*, rs2241880) has been shown to affect the autophagy process (Cooney *et al*. 2010) and also to modulate production of IL1β in human cells (Plantinga *et al*. 2011). We investigated whether this *ATG16L1* polymorphism is associated with the susceptibility or clinical outcome of TC.

One hundred and thirty nine patients (75% women, mean age 39±13 (s.d.) years) with histologically confirmed TC (papillary (70%), follicular TC (24%), or both (6%)), who visited the outpatient clinic at the Department of Endocrinology of our centre, were included. Primary treatment consisted of (near-) total thyroidectomy in all patients and modified radical neck dissections in patients with confirmed nodal metastases, followed by ablation with radioactive iodine (I^{131}, RAI) of residual thyroid tissue. Initial cure is defined as undetectable thyroglobulin (Tg) in the absence of anti-Tg antibodies, and no evidence of locoregional or distant metastasis on the whole-body iodine scans and/or neck ultrasonography at 6 months after RAI ablation. Current remission was defined as undetectable Tg in the absence of anti-Tg antibodies and no evidence of loco-regional or distant metastases at the last follow-up visit. Persistent disease was defined as either detectable Tg or evidence of loco-regional or distant metastases. The population-based control group consisted of 1964 Dutch healthy controls (48% women, mean age 61+10 (s.d.) years) from the Nijmegen Biomedical Study, a population-based survey conducted by the Department of Epidemiology and Biostatistics and the Department of Clinical Chemistry of the Radboud University Nijmegen Medical Centre.

The results indicate that the *ATG16L1* 300Ala (G) allele showed a clear trend towards a protective effect on TC susceptibility in a gene dose-dependent model, $P=0.054$ (Table 1). The gene dose-dependent model suggests a dominant model of action, with odds ratio for *Thr/Ala* heterozygotes and *Ala/Ala* homozygotes of 0.67 and 0.57 respectively. Indeed, the dominant model showed an odds ratio of 0.63 ($P=0.021$), whereas no significant association was observed in a recessive model ($P=0.171$). For these analyses, gender was excluded as a possible confounder (data not shown). The analysis of the association between the *ATG16L1* genotype and the clinical parameters revealed no differences with respect to tumor size ($P=0.432$) and the current disease status ($P=0.308$; Table 2). In contrast, patients heterozygous or homozygous for the 300Ala (G) allele had a higher chance of successful ablation therapy ($P=0.017$), were treated with a lower cumulative RAI dose to achieve remission ($P=0.014$) and received a lower number of treatments than patients with the *Thr300Thr* genotype ($P=0.038$). This observation has important

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Table 1 Difference in genotype frequencies between the patient and control group, and the effect of the genotypes on epithelial cell derived thyroid carcinoma susceptibility

	Patients	**Controls**	**OR** (95% CI)	***P* value**[a]
Gene dose-dependent model			Fisher's exact test	0.054
Thr/Thr	38 (27%)	378 (19%)	1.00 (reference)	0.055[b]
Thr/Ala	69 (50%)	1029 (52%)	0.667 (0.441–1.008)	
Ala/Ala	32 (23%)	557 (28%)	0.571 (0.351–0.931)	
Total	139	1964		
Dominant model				
Thr/Thr	38 (27%)	378 (19%)	1.00 (reference)	0.021[b]
Thr/Ala+*Ala/Ala*	101 (73%)	1586 (81%)	0.633 (0.429–0.935)	
Total	139	1964		
Recessive model				
Thr/Thr+*Thr/Ala*	107 (77%)	1407 (72%)	1.00 (reference)	0.171[b]
Ala/Ala	32 (23%)	557 (28%)	0.755 (0.503–1.135)	
Total	139	1964		

[a]*P* values are nominal without correction.
[b]Calculated by χ^2 analysis.

implications considering the fact that, besides the surgery, the treatment with RAI represents the only effective treatment for patients with TC, including those with metastatic disease. The disease becomes incurable when the tumor loses its ability to accumulate I^{131}. These data imply that patients carrying a 300Ala (G) allele may have a better outcome than subjects homozygous for the 300Thr (A) allele.

Table 2 Summary of genotype–phenotype association parameters within the Dutch patient group ($n=139$)

Variable	***Thr/Thr*** (%)	***Thr/Ala*** (%)	***Ala/Ala*** (%)	**Total**	***P* value**[a]
T stage					0.432
T1	7 (18%)	20 (29%)	14 (44%)	41	
T2	14 (37%)	22 (32%)	9 (28%)	45	
T3	7 (18%)	12 (17%)	4 (13%)	23	
T4	4 (11%)	6 (9%)	1 (3%)	11	
Tx	6 (16%)	9 (13%)	4 (13%)	19	
N stage					0.489
N0	18 (47%)	36 (52%)	18 (56%)	72	
N1	10 (26%)	22 (32%)	14 (44%)	46	
Nx	10 (26%)	11 (16%)	0 (0%)	21	
M stage					0.357
M0	22 (58%)	47 (68%)	27 (85%)	96	
M1	0	1 (2%)	2 (6%)	3	
Mx	16 (42%)	21 (30%)	3 (9%)	40	
RAI treatments (*n*)[b]					0.038
0–1	19 (50%)	38 (55%)	25 (78%)	82	
≥ 2	19 (50%)	31 (45%)	7 (22%)	57	
Cumulative RAI dose (GBq)					0.014
≤ 3.7	4 (11%)	21 (30%)	10 (31%)	35	
3.8–7.4	15 (39%)	19 (28%)	16 (50%)	50	
>7.4	19 (50%)	29 (42%)	6 (19%)	54	
Disease status after ablation					0.017
Remission	17 (46%)	36 (52%)	25 (78%)	78	
Persistent	20 (54%)	33 (48%)	7 (22%)	60	
Missing data	1	0	0	1	
Current disease status					0.308
Remission	28 (74%)	52 (75%)	28 (88%)	108	
Persistent	10 (26%)	14 (21%)	3 (9%)	27	
Recurrent	0	3 (4%)	1 (3%)	4	

[a]Calculated by χ^2 analysis, *P* values are nominal without correction.
[b]Including radio-ablation.

In a previously published genome-wide association study, no effect of the ATG16L1 *Thr300Ala* polymorphism on susceptibility to TC was observed in patients from Iceland (Gudmundsson *et al*. 2009). A plausible explanation may be represented by the differences between the two populations studied. While in The Netherlands, the incidence of TC is low (Netea-Maier *et al*. 2008), this is far higher in Iceland (Duntas & Doumas 2009). The volcanic nature of Iceland has been invoked as a potential culprit, as the incidence of TC in Iceland is much higher than that in the founder populations from Denmark and Sweden (Kilfoy *et al*. 2009). It is therefore rational to hypothesize that the effect of these local geographical factors that lead to the higher TC incidence in Iceland would likely mask the moderate effect of the *ATG16L1* polymorphism acting in low TC incidence countries such as The Netherlands.

One possible explanation for the observed association between the *ATG16L1* genotype and TC is that this effect is mediated through modulation of the proinflammatory cytokine IL1β. Besides its functional role in immune responses, IL1β also affects cell growth and differentiation of different cell types. IL1β has anti-proliferative effects on several malignant cell lines, including some of the human epithelial cell-derived TC cell lines (Kimura *et al*. 1992, Ohta *et al*. 1996). We therefore propose that genetic variants of *ATG16L1* that are shown to influence IL1β production may affect TC risk through modulation of the IL1β response. However, other effects of the *ATG16L1* polymorphism on the ability of TC cells to prevent cell death by induction of autophagy cannot be excluded.

In conclusion, we report that the *300Ala (G)* allele of *ATG16L1* is associated with decreased risk for epithelial cell-derived TC in a Dutch population. Furthermore, we report that the 300Ala allele is associated with a higher sensitivity to RAI, with less chance of persistent disease after thyroidectomy and RAI ablation. Although by itself the *ATG16L1* genotype is not suitable as a sole prognostic marker, this polymorphism may represent a new marker that could be added to a complex multifactorial model to predict the response of TC patients to RAI treatment. Further investigations are needed to confirm our observations. Furthermore, additional studies are required to unravel the underlying mechanism.

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Declaration of interest

Co-author J Gudmundsson is employed by deCODE, Reykjavik, Iceland. The remaining authors declare that there are no conflicts of interest.

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Author contribution statement

A Huijbers, T S Plantinga, L A B Joosten, M G Netea, A R M M Hermus and R T Netea-Maier conceived the experiments; A Huijbers and T S Plantinga performed the experiments; A Huijbers, T S Plantinga, J Gudmundsson, L A L M Kiemeney and R T Netea-Maier performed the statistical analysis; K K H Aben, J Gudmundsson, M den Heijer, L A L M Kiemeney and R T Netea-Maier collected and managed the patient samples; and all authors contributed to the writing of the manuscript.

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