*STAT*1 gene mutation is not implicated in upper aerodigestive cancers

Polat Dura[1], Rene H. M. te Morsche[1], Martin Lacko[2], Mihai G. Netea[1], Jos W. M. van der Meer[1], Joost P. H. Drenth[1], Wilbert H. M. Peters[1*]

[1]Departments of Gastroenterology and Internal Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
[2]Department of Otorhinolaryngology, Maastricht University Medical Center, Maastricht, The Netherlands
Email: *w.peters@mdl.umcn.nl

ABSTRACT

Autosomal Dominant Chronic Mucocutaneous Candidiasis (AD-CMC) is characterized by defective T cell immunity, leading to fungal infections limited to mucosal surfaces. Recently it was discovered that mutations in the coiled-coil (CC) domain of *STAT*1 are the cause of AD-CMC. STAT1 deficiency has been implicated in experimental models of oesophageal cancer (EC) and head and neck carcinoma (HNC). Both carcinoma types are prevalent among CMC patients. Consequently, we postulated that the same mutation in the *STAT*1 gene triggering AD-CMC, could also be involved in oesophageal or head and neck carcinogenesis. However we failed to identify the c.820C>T mutation in the *STAT*1 *CC* domain in 3 cohorts of Dutch Caucasian origin: being 351 EC patients, 325 HNC patients and 309 controls. Although it seems valuable to investigate the relationship between AD-CMC and upper aerodigestive neoplasms, the c.820C>T mutation in the *STAT*1 gene does not seem implicated in EC and HNC aetiology.

1. INTRODUCTION

Signal transducers and activators of transcription (*STAT*) are dormant cytoplasmic proteins that upon activation regulate a wide variety of cellular processes, such as immune regulation, apoptosis, differentiation and proliferation [1,2]. In humans, seven *STATs* (1-4, 5a, 5b, 6) have been discovered [2]. Phosphorylation of tyrosine residues precedes activation of *STAT* and is achieved through two main signalling routes: the growth factor-and cytokine-signalling pathway [1,2]. Growth factor signalling occurs primarily via the Epidermal Growth Factor Receptor (EGFR). Conversely, cytokine activation of Janus Tyrosine Kinases (JAKs), is an effective alternative *STAT* activation pathway.

Dysregulation of these pathways is frequently observed in primary malignant tumours. Particularly *STAT*1 and *STAT*3 play an important role in carcinogenesis, and both are well studied in a range of malignancies [3]. *STAT*1 target genes include tumour suppressor genes, whereas target genes of *STAT*3 are oncogenes that stimulate cell cycle progression and inhibit apoptosis [3]. However, the exact role of *STAT* in oncogenesis remains complex and is incompletely understood. This is illustrated by the fact that *STAT*1 and *STAT*3 are sometimes expressed simultaneously [3].

*STAT*1 appears also to be involved in inflammatory and immunological diseases as it regulates immune effector genes [4], and plays for example a causative role in chronic mucocutaneous candidiasis (CMC) [5]. CMC can be divided into several diseases: CMC with Auto-immune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED), CMC without APECED but associated with primary hypothyroidism, and sporadic CMC [6]. APECED has an autosomal recessive manner of inheritance due to mutations in the *AIRE* gene, which expresses a protein that acts as a transcriptional activator [7]. The disease is characterized by the presence of autoreactive T lymphocyte responses, and subsequently neutralizing autoantibodies against cytokines of the Th17 family such as IL-17 and IL-22 [7], leading to fungal infections (mainly Candida) limited to mucosal surfaces. The second syndrome is inherited through an autosomal dominant pattern. Recently it was discovered that mutations in the *CC* domain of *STAT*1 are the cause of autosomal dominant CMC (AD-CMC) [5], resulting in defective type 1 and type 17 helper T cell immunity. The c.820C>T mutation in the *STAT*1 gene results in an arginine to tryptophan amino acid substitution at codon 274 [5] and leads to a defective CC domain of the *STAT*1

protein. It has been shown that this defect results in defective IL-12R and IL-23R signalling pathways, with absent production of IL-17, IL-22 and IFNγ [5].

*STAT*1 deficiency has been implicated in experimental models of oesophageal cancer (EC) and head and neck cancer (HNC) [8,9]. Both carcinoma types are prevalent among CMC patients [6,10]. Interestingly, Veerdonk *et al.* reported that 3 out of their 14 AD-CMC patients also suffered from EC or HNC [5].

Consequently we postulated that the same mutation in the *STAT*1 gene, triggering AD-CMC as described by Veerdonk *et al.*, could also be involved in oesophageal or head and neck carcinogenesis (**Figure 1**).

2. MATERIALS AND METHODS

Three cohorts of Dutch Caucasian origin were studied: 351 patients with oesophageal cancer [11], 325 patients with head and neck carcinoma [12], and a group of 309 healthy controls, recruited from the same geographical area as the patients [11]. A 60 bp region of exon 10 of the *STAT*1 gene, including the c.820C>T mutation, was examined by means of PCR followed by High Resolution Melting analysis. After using the forward primer 3'-CTTGTGTCTTCCCAGGTTCA-5', the reverse primer 3'-CCAATTCCTCCAACTTTTTAAGC-5' and EvaGreen (Biotium, Hayward, CA) as the fluorescent dye in the PCR, melting curves from 65°C to 95°C with a ramp rate of 0.1°C/10 seconds were obtained with the CFX96^{TM} Real-Time PCR Detection System (Biorad Laboratories, Hercules, CA). Melting curves were analyzed using the

Precision Melt software (Biorad). Samples with deviant curves were sequenced.

3. RESULTS AND DISCUSSION

We failed to identify the c.820C>T mutation in the *STAT*1 *CC* domain in our two cohorts of 676 patients with upper aerodigestive cancer. Likewise, the mutation was also absent in 309 healthy controls. Demographics of patients and controls are shown in **Table 1**.

It has been suggested that oral or oesophageal cancer in APECED-CMC may be induced by the production of carcinogens by *C. albicans* [13]. Although EC and HNC are reported in AD-CMC [5,6], a clear correlation has not been proven, and the possible mechanism remains unclear. However, as with APECED-CMC, prolonged exposure to *C. albicans* infections in the upper GI tract

Table 1. Characteristics of patients with esophageal cancer, head and neck cancer and controls.

Characteristics	Patients		Controls
	EC	HNC	
Number (% of total)	351 (100%)	325 (100%)	309 (100%)
Age (yrs; mean ± SD)	65.0 ± 10.9	61.8 ± 11.6	42.0 ± 16.3
Gender			
Male	283 (80.6%)	260 (80.0%)	188 (60.9%)
Female	68 (19.4%)	65 (20.0%)	121 (39.1%)

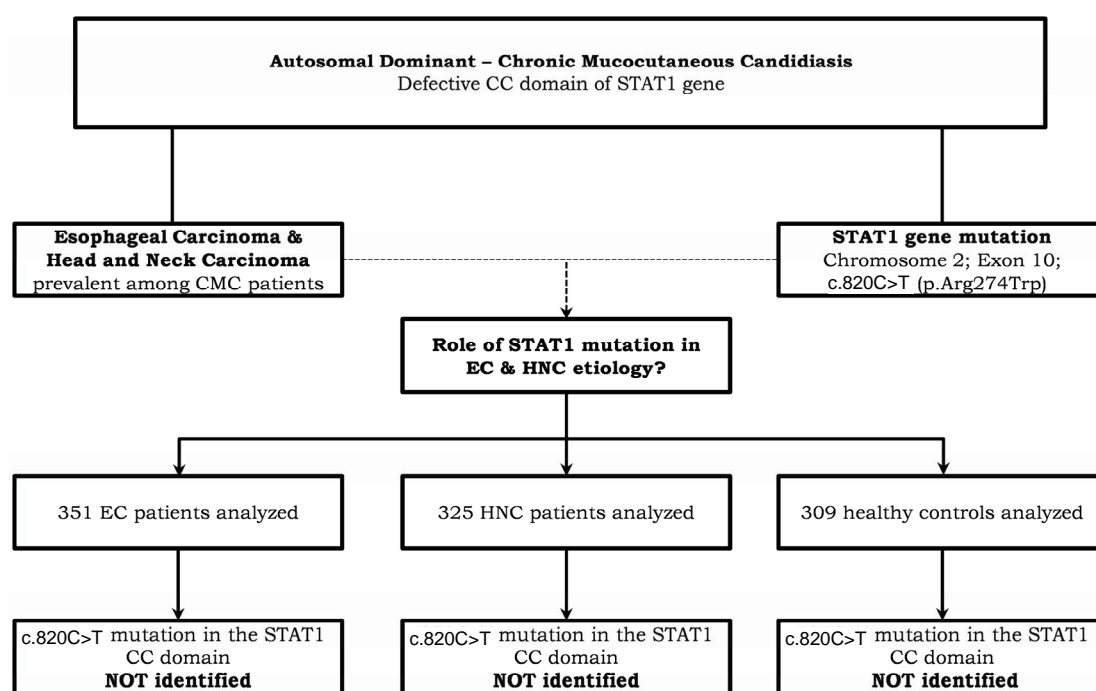EC, esophageal carcinoma; HNC, head and neck carcinoma.

Figure 1. Flowchart.

can be an etiological factor [14]. IgA deficiency might be another immunological clarification for EC and HNC in AD-CMC. IgA deficiency, linked with neoplasm development [14], has also been reported in APECED negative CMC patients [14,15]. Although there is no known correlation with EC and HNC, IgA deficiency is reported to influence oesophageal dysplasia [16].

Our findings do not support a role of the c.820C>T mutation in the *STAT*1 gene with oesophageal or head and neck carcinogenesis. The only mutation that was found twice in both patient groups and twice in the control group, was the c.796G>A mutation (rs41473544). However, its occurrence in both patients and controls suggests no pathophysiological relevance.

Furthermore, there is no other clear evidence that the *STAT*1 mutation is involved in EC risk, although there are reports that *STAT*1 activation induces apoptosis in EC cells in vitro [17,18], and loss of *STAT*1 activity was suggested to result in EC progression [8]. To our knowledge no case-control or GWAS studies examining the role of *STAT*1 mutations in EC and HNC are known. For HNC as well, most of the *STAT*1 related basic research concentrates on biomarker and therapeutic potential [19, 20].

In conclusion, it seems valuable to investigate the possible relationship between oesophageal and head and neck malignancy and APECED-CMC and AD-CMC. However, pursuing the c.820C>T mutation in the *STAT*1 gene as an etiological factor for the development of EC/HNC in the absence of CMC does not seem worthwhile. Moreover, the *STAT*1 overexpression may not be independent, as *STAT*3 activation seems to occur simultaneously in a wide range of neoplasms. Consequently, adding the fact that *STAT*3 is an oncogene, haplotyping *STAT*1 and *STAT*3 genes in both carcinoma groups may be a more promising approach.

4. ACKNOWLEDGEMENTS

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