TMEM173 variants and potential importance to human biology and disease

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Abstract

TMEM173 gene encodes the protein STING (stimulator of interferon genes), a key player in host defense against pathogens. Mutations in the human TMEM173 gene cause a life-threatening auto-inflammatory disease called SAVI (STING-associated vasculopathy with onset in infancy). Human STING is also a promising therapeutic target for cancers and infectious diseases. Recently, Aduro Biotech and Novartis announced a $250M-plus initiative to develop STING-targeting cancer immunotherapies. Thus, understanding the genetics of the human TMEM173 gene is important for both basic and translational research. The human TMEM173 gene has great heterogeneity and population stratification. R232 of STING is the most common human TMEM173 allele. However, >50% of Americans are not R232/R232. HAQ (R71H-G230A-R293Q) is the second most common human TMEM173 allele. While R232/R232 is the dominant TMEM173 genotype in Europeans, R232/HAQ is the most common TMEM173 genotype in East Asians. Importantly, recent studies suggested that HAQ and H232 are likely loss-of-function TMEM173 alleles. In all, ~30% of East Asians and ~10% of Europeans are HAQ/HAQ, HAQ/H232, or H232/H232. Here, we reviewed human TMEM173 alleles, mutations and their potential impact on human health and medicine.

Introduction

DNA, including pathogen DNA from infection and mammalian DNA from damaged cells, stimulates STING (stimulator of interferon genes)-dependent type I interferon (IFN) production and promotes inflammation [1, 2]. STING is a four-transmembrane endoplasmic reticulum (ER) resident protein (Fig. 1) [1, 3]. It exists as a homodimer and undergoes a conformational change when binding to its ligands cyclic dinucleotides (CDNs) [3–5]. Activated STING homodimer then traffics through Golgi to the perinuclear region where it activates TANK Binding Kinase 1 (TBK1) leading to type I IFN production [1]. Human transmembrane protein 173 (TMEM173) gene, which encodes the STING protein, is a ~7kb-long gene at 5q31.2 (Fig. 1). STING is a critical player in host defense against pathogens, including HIV [6, 7], Plasmodium [8–10], and Mycobacterium tuberculosis [11–13]. STING also influences the development of autoimmune diseases [14, 15]. Last, pharmaceutical companies are developing STING-targeting immunotherapies [16–18]. The DNA-STING pathway has been reviewed extensively elsewhere [19, 20]. Here, we review the genetics of human TMEM173 alleles, mutations and their potential impact on human health and medicine.

Human population is highly heterogeneous for the TMEM173 gene

The initial identified human STING has a Histidine at amino acid 232 (H232) [1, 2, 21]. We later found that H232 was a minor allele [22]. The most common TMEM173 allele in the human population has an Arginine at amino acid 232 (R232) [22]. In two cohorts of ~1000 Americans, ~45% are R232/R232, and only ~2% are H232/H232 [22]. Notably, the H232 allele is defective in response to CDNs in vitro [23, 24].

We also found that >50% of Americans contain at least one copy of non-R232 TMEM173 allele, which suggested
that the human population is highly heterogeneous for the TMEM173 gene [22]. Common TMEM173 alleles (>1% population frequency) are R232, HAQ (R71H-G230A-R293Q), AQ (G230A-R293Q), Q293 and H232 (Fig. 1) [22, 25]. HAQ is the second most common human TMEM173 allele that contains triple non-synonymous single nucleotide polymorphisms (SNPs) (R71H-G230A-R293Q) [22]. Using B-cell lines derived from homozygous HAQ/HAQ individuals, we recently showed that HAQ/HAQ cells had extremely low STING protein expression and decreased TMEM173 transcript compared to R232/R232 cells [26]. Data from Genotype-Tissue Expression (GTEx) Portal (https://www.gtexportal.org/home/) showed that HAQ individuals had decreased TMEM173 transcript in organs such as artery, skin, lung, and esophagus that are not B-cell dominant [26]. It is likely that HAQ is a null allele [26]. This notion was strengthened in vivo by studies in a knock-in HAQ mouse, which did not respond to CDNs [26]. Last, HAQ and H232 alleles are in linkage disequilibrium with rs13181561 that is associated with >10-fold decrease of
IFNα production to vaccinia virus stimulation in Europeans [27, 28]. It is worth noting that Sivick et al. [29] found no functional difference in PBMC among HAQ and R232 individuals although their study had a small sample size with unknown ethnic origins.

The HAQ allele has significant population stratification. In Europeans, R232/R232 is the most common genotype, while in East Asian, the dominant genotype is HAQ/R232 [26]. Furthermore, ~16% of East Asians are HAQ/HAQ compared to ~3% in Europeans [26]. Interestingly, Africans have no HAQ/HAQ [26]. Instead, ~4% of Africans are AQ/AQ, which is absent in other ethnic populations [26]. Africans also have the Q293 allele [26]. It is likely that Q293 is the founder allele, where AQ, then HAQ, derived during the human migration out of Africa continent.

In summary, the human TMEM173 gene has (i) great heterogeneity; (ii) significant population stratification; (iii) two possible loss-of-function alleles: HAQ and H232. In all, the HAQ/HAQ, H232/HAQ, and H232/H232 genotypes account for ~30% of East Asians and ~10% of Europeans [26].

**TMEM173 alleles in human health**

STING is essential for host defense against DNA virus and some retroviruses [30]. Considering the vital role of viral infection in human evolution [31], it is surprising that such high percentages of the human population have possible loss-of-function TMEM173 alleles. For example, Herpes simplex virus-1 (HSV-1), a DNA virus, has a high seroprevalence in the human population [32]. When infecting the central nervous system, HSV-1 causes herpes simplex encephalitis, the leading cause of viral encephalitis [33]. The initial study found that STING deficient mice were extremely susceptible to intravenous infection of HSV-1 [34]. So how HAQ/HAQ, H232/H232, and HAQ/H232 individuals survived with HSV-1? Two recent studies may shed some light on it. They found that although STING−/− mice were much more susceptible to intravenous infection of HSV-1 than the WT mice, there were no difference in survival between the STING−/− and WT mice following a mucosal HSV-1 infection route, which is a natural route of infection in humans [35, 36]. Furthermore, STING was not required for viral clearance and had a minimal effect on type I IFN production during the mucosal HSV-1 infection [35, 36]. Thus, the physiological role of STING in pathogen infection, especially in humans, need to be carefully evaluated.

Genome-wide association study (GWAS) identified two SNPs rs13181561 [28] and rs7447927 [37] within or near the human TMEM173 gene (Fig. 1). In a search for genes associated with cytokine responses to vaccinia virus stimulation, Kennedy et al. [28], linked rs13181561 to decreased IFNα production in European (492 individuals), but not Africans (196 individuals) . rs13181561 is in linkage disequilibrium with HAQ, H232 in Europeans [27], which indicates that these loss-of-function TMEM173 alleles are associated with decreased IFNα production in response to vaccinia virus stimulation in Europeans.

In a joint analysis of three GWAS of esophageal squamous cell carcinoma (ESCC) in Chinese populations (5337 ESCC cases and 5787 controls), Wu et al. [37] found that rs7447927 was associated with ESCC in Chinese populations. rs7447927 is in linkage disequilibrium with rs13181561 [37], which is linked to HAQ, H232 alleles. Thus HAQ and H232 alleles are likely associated with susceptibility to the development of ESCC. It remains to be determined whether the loss of STING function in humans leads to the predisposition to ESCC.

**TMEM173 mutations in SAVI (STING-associated vasculopathy with onset in infancy)**

Activating mutations in the TMEM173 gene lead to a newly classified rare auto-inflammatory disease call SAVI [38] (Table 1). It is an autosomal-dominant disease characterized by systemic inflammation, interstitial lung disease, cutaneous vasculitis, and recurrent bacterial infection [38, 39]. Both inherited, and de novo TMEM173 mutations were found in SAVI patients (Table 1). SAVI with the de novo TMEM173 mutations tended to have an early-onset (<8 weeks) and severe phenotype [38, 40], whereas familial TMEM173 mutations had late-onset (teenager or adulthood) and milder clinical manifestations [39, 41]. For instance, SAVI patients with the inherited V155M mutation.
had a less severe disease penetration than patients with the de novo V155M mutation [38, 39, 42]. Jeremiah et al. [39], first found that the V155M mutation, at the steady state, localized mainly in the Golgi and in perinuclear vesicles of patient fibroblasts, which is a hallmark of the STING activation.

SAVI as a unique interferonopathy with lung manifestation

SAVI is considered as a type I Interferonopathy that includes chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature, Aicardi-Goutieres syndrome, and TREX1-SAMHD1-mediated familial chilblain lupus [40, 43, 44]. For example, familial SAVI mutations caused familial chilblain lupus [39, 41]. However, SAVI is unique because it is the only known type I Interferonopathy with pulmonary involvement [40, 43, 44]. In fact, all three reported fatalities from SAVI patients were due to the pulmonary complications [38, 40]. We showed that activating STING in the mouse lung by intranasal administration of CDNs, induced lung production of IFNγ and IFNλ but not IFNβ [45]. Interestingly, IFNγ+CD4+ T cells and serum IFNγ were markedly increased in a recent SAVI patient [46]. Notably, serum IL-18, a known IFNγ inducer, was also elevated in several SAVI patients [47]. Whether the increased IFNγ production contributes to the lung symptoms in SAVI patients is worth further investigation.

Treating SAVI with JAK inhibitors

Current anti-inflammatory treatments corticosteroid, DMARDs, anti-TNF, steroids, anti-CD20, IVIG, were...
ineffective in SAVI patients [42, 47]. SAVI patients died of lung complication, and lung damage was irreversible [40, 47]. In fact, one SAVI patient died after double lung transplantation due to acute complications [40]. Thus, any SAVI treatment should result in improved lung function and prevent the irreversible lung damage.

Encouragingly, in a 2-year study with three SAVI children, ruxolitinib dramatically improved pulmonary function, resolved the cutaneous lesions and led to a better overall well-being of the patients [42, 48]. In a separate study, after a 3-month tofacitinib treatment, Seo et al. [46], saw an improved skin lesion in a SAVI teenager but the pulmonary defect remained. Eli Lilly is currently conducting a clinical trial (ClinicalTrials.gov number, NCT01724580) to examine the efficacy of baricitinib in SAVI patients.

Ruxolitinib and baricitinib are JAK1 and JAK2 inhibitors while tofacitinib is a JAK3 and to a lesser degree, JAK2 inhibitor. IFNα/β signals via JAK1/Tyk2 while IFNy activates JAK1/JAK2. Thus, ruxolitinib and baricitinib are more suitable for treating SAVI than tofacitinib. Notably, baricitinib, at a high dose, also inhibits Tyk2, which mediates IL-10, IL-12/23, IL-6, and IL-4/13 signaling. Proper dosing may be important when treating SAVI patients with baricitinib.

Loss-of-function human TMEM173 allele as a natural inhibitor of SAVI

SAVI is caused by gain-of-function human TMEM173 mutations [38] (Table 1). An intriguing question is whether the loss-of-function TMEM173 alleles could serve as natural genetic inhibitors [49]. Cerboni et al. [49] found that in vitro, introducing HAQ into the V155M SAVI mutation (HAQ-V155M) relocated STING back to ER, restored T cell proliferation, and corrected NF-κB activation. Recently, a de novo SAVI patient was identified in a HAQ family [46]. In this case, the activating TMEM173 mutation acts in trans with the HAQ allele [46]. The patient exhibited SAVI symptoms but with a late-onset (3 years) [46]. Thus, the presence of the HAQ allele could be advantageous to SAVI patients.

TMEM173 mutations in human cancers

TMEM173 can promote [50, 51] or suppress [52, 53] tumorigenesis in mice. Xia et al., sequenced the TMEM173 gene in 11 human colon cancer cell lines and 11 human melanoma-derived cell lines [54, 55]. No somatic TMEM173 mutations were found, although 2 out of the 11 colon cancer lines and 7 out of the 11 melanoma lines carried the HAQ allele [54, 55]. Data from the COSMIC database, however, out of 9110 primary human cancer tissues, 313 samples (3.44%) have overexpressed TMEM173 gene expression (Z-score > 2) compared to the 17 samples (0.19%) that have underexpressed TMEM173 gene (Z-score < −2) [56, 57]. Samples from lung cancers, brain cancers, and kidney cancers have the highest rate of TMEM173 gene overexpression [56, 57].

Why do cancer samples have overexpressed TMEM173 gene? The vast majority of those cancer samples did not have somatic TMEM173 mutations. Wang et al. [61], recently reported that c-Myc binds to the −124 to 1 bp of the human TMEM173 gene and promotes its transcription. c-Myc is activated in many cancers. It is tempting to suggest that TMEM173 overexpression in human cancer samples is driven by the activated c-Myc. Whether the overexpressed TMEM173 gene in human cancer samples contributes to tumorigenesis, remains to be determined.

TMEM173 alleles in human medicine

STING is a promising therapeutic target for cancer immuno-therapies [16–18, 62]. Pneumovax23® vaccine efficacy depends on STING in mice [26, 63]. STING may also contribute to the efficacy of the radiotherapy [16] and chemotherapy [55, 64] likely due to DNA released during these treatments. Using a mouse model of the human HAQ allele, we found that Pneumovax23® was ineffective in the HAQ mouse [26]. Furthermore, CDNs lost its adjuvant
activity in the HAQ mouse [26]. The low expression of STING in the HAQ/HAQ individuals likely will affect the efficacy of STING-targeting cancer immunotherapies.

On the other hand, increased STING expression in primary human cancer samples provides a rationale for targeting STING for cancer treatments. STING/MPYS was initially identified as an apoptotic molecule mediating anti-MHC II mAb induced cell death in mouse B-cell lymphomas [21]. Recently, several studies showed that direct activation of STING by CDNs could kill tumors [65, 66]. Thus, tumors with increased STING expression may be killed directly by the activation of STING/MPYS-mediated cell death pathway. On the other hand, activation of the STING pathway in DCs promotes DCs maturation [67, 68]. These mature DCs can initiate CD8+ T cells-mediated cytotoxic response and generate memory response to prevent cancer relapse [17, 18, 69–73].

**Future directions**

Since its discovery in 2008, most of our knowledge on STING has been from mouse studies. These studies have established a critical role of STING in infectious diseases and autoimmune diseases. The identification of SAVI mutations in 2014 established an unambiguous role of the TMEM173 gene in the human auto-inflammatory disease. However, the role of TMEM173 in human infectious diseases remains to be determined. A recent study done in two independent European cohorts (150 patients and 188 controls) found that the population frequency of HAQ increased in human Legionnaires’ disease patients as compared to healthy controls, which suggested that HAQ carriers may be more susceptible to Legionnaires’ disease than the R232 carriers [74]. Questions remain whether HAQ affects individual’s susceptibility/resistance to other human pathogens such as HIV, *Plasmodium*, or *Mycobacterial tuberculosis*. Meanwhile, a GWAS study revealed an association between the TMEM173 gene and ESCC [37]. It remains to be determined whether the HAQ and H232 alleles associated with ESCC are causative, which will greatly enhance the mechanistic understanding of this disease.

The animal and in vitro studies indicated that STING might influence the efficacy of some human medicines [16, 26, 64]. It will be beneficial to determine whether the HAQ and H232 alleles cause decreased efficacy for Pneumovax23®, chemotherapy, and radiotherapy so that right patients can be benefited from these medications.

Lastly, the HAQ allele is common in East Asians and rare in Africans [26]. The H232 allele, however, does not show such population stratification [26]. Thus, the environmental pressure selecting for the HAQ allele seems to be different from the H232 allele. Determining the environmental factor (s) selecting for these loss-of-function TMEM173 alleles will help us understand the physiological function of the human TMEM173 gene.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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