

ORIGINAL ARTICLE

Variation in the *TEK* gene is not associated with asthma but with allergic conjunctivitis

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Summary

The Tie2 receptor is an important player in angiogenesis. The Tie2 mRNA and protein are abundantly expressed in the lungs and the associated pathway also has an important role in the development and function of the eye. Tie2 is encoded by the *TEK* gene in humans. Recently, variations in the *TEK* gene have been found associated with asthma. The objective of the present study was to investigate whether variations in the *TEK* gene influenced the susceptibility to pediatric asthma and/or associated phenotypes like GINA status, viral- or exercise-induced asthma, allergic asthma, indoor, outdoor, inhalative allergies, IgE and eosinophil levels, allergic rhinitis and allergic conjunctivitis. Three single nucleotide polymorphisms (SNPs, rs3780315, rs581724 and rs7876024) in the *TEK* gene were genotyped in 1189 unrelated individuals, out of which 435 were asthmatic children and 754 healthy controls. Different types of asthma, allergies and co-morbidities were defined in 320 patients. Among the fully phenotyped 320 asthmatic patients 178 (55.6%) also had allergic rhinitis and 100 (31.3%) had conjunctivitis. Among the rhinitis patients 98 (55.1%) also had conjunctivitis. Two patients had conjunctivitis without rhinitis. The genotyped SNPs showed no association with asthma. However, SNP rs581724 was significantly associated with allergic conjunctivitis in a recessive way ($p=0.007$; $OR=2.3$ (1.3-4.4)) within the asthmatic population. The risk remained significant when the whole population (asthmatics and healthy controls) was included in the calculation ($p=0.003$; $OR=2.1$ (1.3-3.6)). The minor allele of the rs581724 SNP which is associated with the increased risk to conjunctivitis is also associated with reduced Tie2 expression. There was a significant association between SNP rs581724 and the occurrence of allergic conjunctivitis in asthmatic children. If additional studies can confirm the role of the Tie2 pathway in allergic conjunctivitis, it can be a potential novel therapeutic target in the disease.

KEYWORDS

allergic conjunctivitis, allergy, angiogenesis, asthma, genetics, Tie2

1 | INTRODUCTION

Asthma is a chronic respiratory disease influenced by a wide range of environmental and genetic factors. It is characterized by coughing,

wheezing, bronchoconstriction, airway hyperresponsiveness or airway remodelling. Processes such as cytokine production, inflammatory cell infiltration to the lungs and injury to epithelial cells play a role in the development and severity of asthma. Structural changes

in the airway walls due to both neovascularization and angiogenesis are also an important feature of asthma (Bischof et al., 2009). Increased vascularity and angiogenesis in asthmatic patients contribute to airway occlusion due to an increase in airway wall thickness (Makinde & Agrawal, 2011). Further, Salvato has found that the number of vessels as well as vascularity has a positive correlation with asthma severity (Salvato, 2001).

Angiogenesis is regulated by angiopoietins, such as Ang1 (angiopoietin 1) and Ang2 (angiopoietin 2) in humans. Ang1 and Ang2 are glycoproteins and both have been characterized by acting as ligands for tyrosine protein kinase receptor, Tie2. It has been found that Tie2 receptor mRNA and protein are abundantly expressed in the lungs (Kanazawa, 2007). Ang1 and Ang2 have a similar affinity for binding to Tie2. Ang1 stimulates kinase activity by binding to Tie2; on the other hand, Ang2 may act as an agonist or an antagonist for Tie2 in a tissue- and cell-dependent manner (Augustin, Koh, Thurston, & Alitalo, 2009). Ang1 provides an anti-inflammatory effect on blood vessels during angiogenesis, whereas Ang2 and vascular endothelial growth factor (VEGF) contribute to a pro-inflammatory effect (Gamble et al., 2000; Makinde & Agrawal, 2011).

Tie2 is encoded by the *TEK* (TEK receptor tyrosine kinase) gene in humans. In a recent genomewide association study in American populations of European origin, the strongest signals were identified at the 9p21.2 locus consisting of four single nucleotide polymorphisms (SNPs) in weak linkage disequilibrium (LD) with each other and spanning three genes (*EQTN*, *MOB3B* and *TEK*) (Almoguera et al., 2017). Based on its function, it was hypothesized that variations in the *TEK* gene were responsible for the association. This hypothesis, however, was not proved with additional data. In another study, three expression quantitative trait locus (eQTL) SNPs, rs3780315, rs581724 and rs7876024 in the introns of the *TEK* gene, were identified, which were not in LD, but all of them were associated with lower *TEK* gene expression in HapMap3 cell lines and with increased risk for developing acute respiratory distress syndrome (Ghosh et al., 2016).

It is well known that asthma is not a single disease but rather a series of overlapping individual diseases or phenotypes, each defined by its unique interaction between genetic and environmental factors (Borish & Culp, 2008; Lötvall et al., 2011). In children, the most frequent endotype is the IgE-mediated allergic asthma which can also have heterogeneous symptoms. The most frequent asthma-associated allergic diseases are allergic rhinitis and conjunctivitis which often occur together (Lee et al., 2013; Rosario & Bielory, 2011; Shaker & Salcone, 2016). In different studies, between 50% and 65% of patients with rhinitis also had conjunctivitis, although conjunctivitis could also exist without rhinitis (Rosario & Bielory, 2011). Conjunctivitis is an inflammatory disease of the eye characterized by flushing, swelling, itching and watering of the eyes (Rosario & Bielory, 2011). The Tie2 pathway also has an important role in the development and function of the eye, and the *TEK* gene is highly expressed in the Schlemm's canal endothelium in the eye (Souma et al., 2016). Mutations in the *TEK* gene are associated with primary congenital glaucoma, and Tie2 is a highly investigated target

in different eye diseases such as subretinal and choroidal neovascularization, macular oedema or diabetic retinopathy (Campochiaro, 2015; Campochiaro & Peters, 2016; Tan, Cheung, Simó, Cheung, & Wong, 2016).

In this study, we investigated whether the above-mentioned SNPs in the *TEK* gene associated with lower *TEK* gene expression influenced the susceptibility to paediatric asthma and/or associated phenotypes.

2 | MATERIALS AND METHODS

2.1 | Patients

The genotyping analysis included 1,189 unrelated individuals, out of which 435 were asthmatic children and 754 healthy controls. The recruitment of the study participants started in 2005. The patients and controls were all Hungarian (Caucasian), from which about 5%, based on state population data, were of Gypsy origin. Asthma diagnosis was based on GINA guidelines, as before (Ungvari et al., 2012). Atopy was identified by a positive skin prick test and/or positive total or allergen-specific serum IgE levels. The IgE levels were tested with the 3gAllergy blood tests in the IMMULITE 2000 Immunoassay system (Tarrytown, NY, USA). The specific IgE was considered positive in those having detectable allergen-specific IgE (>0.35 kU/L). The total IgE levels were considered high based on internationally established age-specific ranges (<https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8159>), adjusted to our laboratory.

Different types of asthma, allergies and comorbidities were defined in 320 patients. Other asthmatic children could be not characterized in terms of asthma phenotypes due to missing information. Asthma was grouped into allergic and nonallergic asthma types. Within allergic asthmatics, subgroups of indoor, outdoor and inhalative allergies have been identified in patients depending on what type of allergy triggered their asthma. Moreover, comorbidities of allergic rhinitis and allergic conjunctivitis have also been included in the analysis. The nonallergic asthma group includes the viral-induced asthma, when the onset of asthma or the asthma exacerbations have been associated with an infection-related acute respiratory illness. If asthma exacerbation was provoked by exercise in the medical history of the patients, the asthma was categorized as exercise-induced asthma.

Eosinophil cell counts from blood were measured with the Coulter AXM analyser, where the normal relative range was between 1% and 6%, and the normal range of absolute eosinophil count was between 0.050 and 0.200 G/L. The patients had no change in therapy before the blood sample was taken. Neither had they exacerbations nor respiratory infections for at least four weeks prior to the blood test.

The control children were from the Orthopaedic Department in the Budai Children's Hospital or from the Urological Department from the Heim Pál Hospital, both in Budapest. The controls had no symptoms of asthma, nor any need for medication. The adult controls were healthy blood donors. Out of the 754 healthy controls, 453 were males and 297 females with a mean age of 13.8 ± 11.8 years.

TABLE 1 Detailed characteristics of asthmatic patients participating in SNP analysis

Clinical and biological characteristics	Asthmatic patients, n = 435
Age $\pm$ SD	10.3 $\pm$ 4.4
Sex (Male/Female)	268/165
Asthma phenotypes:	n = 320
Exercise-induced, yes (%) / no ^a	111 (35) / 209
Viral-induced, yes (%) / no ^a	134 (42) / 186
Allergic asthma, yes (%) / no ^a	47 (44) / 60
Inhalative allergy, yes (%) / no ^a	244 (76) / 75
Outdoor allergy, yes (%) / no ^a	202 (63) / 117
Indoor allergy, yes (%) / no ^a	190 (60) / 129
Comorbidity rhinitis, yes (%) / no ^a	178 (56) / 142
Comorbidity conjunctivitis, yes (%) / no ^a	100 (31) / 220
GINA status ^a	
Number of patients in GINA 1	84 (26)
Number of patients in GINA 2 (%)	206 (65)
Number of patients in GINA 3 (%)	28 (9)
Number of patients in GINA 4 (%)	0 (0)
Absolute eosinophil count $\pm$ SD (G/L)	0.3 $\pm$ 0.3
Number of patients with normal or high absolute eosinophil count (normal (%) / high)	72 (36) / 127
Total IgE $\pm$ SD (kU/L)	487 $\pm$ 2,066
Number of patients with normal or high IgE level (normal (%) / high)	66 (32) / 143

^aData are available on a limited data set only.

Written informed consent was signed by all patients or by their parent/guardian. Details of the subjects can be found in Table 1. The study was carried out according to the Declaration of Helsinki and was approved by the Ethics Committee of the Hungarian Medical Research Council.

2.2 | Genotyping

Genomic DNA was isolated from whole blood samples of 1,189 individuals using the QIAamp Blood DNA Midi Kit (Qiagen, MD, USA) or the iPrep PureLink gDNA Blood Kit on iPrep Purification Instrument (Invitrogen, Carlsbad, CA, USA). The KBiosciences Competitive Allele-Specific PCR (KASP) version 4.0 genotyping system was used (LGC Genomics, Berlin, Germany) along with the TaqMan ABI 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) to genotype three *TEK* SNPs, rs3780315, rs581724 and rs7876024.

2.3 | Statistics

Hardy–Weinberg equilibrium (HWE) was tested using the chi-square goodness-of-fit test implemented in the online DeFinetti HWE application (Helmholtz Zentrum München, Institut für Humangenetik

<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>). The significance level was set to .01. SNP data were analysed using SPSS version 19 (SPSS Inc., Chicago, IL, USA) software. Logistic regression analyses adjusted for age and gender were used to evaluate the association between *TEK* genotypes and asthma, its intermediate phenotypes, the discretized (normal/high) serum IgE and eosinophil levels (see at Subjects) and the different asthma phenotypes. Multiple comparisons were corrected for using the Benjamini–Hochberg correction, and a new significance level of $p = .008$ with the false discovery rate (FDR) $< 4.5\%$ was estimated. The figure was made using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA).

3 | RESULTS

3.1 | Prevalence of comorbidities among the asthmatic patients

The clinical characteristics of the asthmatic patients are presented in Table 1. Altogether, 435 asthmatic children and 754 healthy controls participated in the genotyping study. Among the fully phenotyped 320 asthmatic patients, 178 (55.6%) also had allergic rhinitis and 100 (31.3%) had conjunctivitis. Among the rhinitis patients, 98 (55.1%) also had conjunctivitis. Two patients had conjunctivitis without rhinitis.

3.2 | SNP association study

The distribution of the genotypes has been checked for deviation from the Hardy–Weinberg equilibrium (HWE). One of the three SNPs, rs3780315, showed a significant deviation from the HWE in the control population ($p = .000013$); therefore, it was excluded from our analysis.

Neither of the two SNPs, rs581724 and rs7876024, showed an association with asthma (Table 2). However, SNP rs581724 was significantly associated with allergic conjunctivitis in a recessive way ($p = .007$; OR = 2.4 (1.3–4.4) Table 2) which means that the homozygote carriers of the minor allele (AA genotype) had a significantly increased risk of developing allergic conjunctivitis within the asthmatic population (27% vs. 14% with vs. without conjunctivitis, respectively). The risk remained significant when the whole population (asthmatics and healthy controls) was included in the calculation ($p = .003$; OR = 2.1 (1.3–3.6)).

No other phenotypes (GINA status, viral- or exercise-induced asthma, allergic asthma, indoor, outdoor, inhalative allergies, IgE and absolute eosinophil levels and allergic rhinitis) were associated with either of the two SNPs in the statistical analysis (data not shown).

4 | DISCUSSION

In the present study, we investigated whether eQTL SNPs in the *TEK* gene influenced the risk for asthma or associated phenotypes. In our population, we did not find any association between these SNPs and

TABLE 2 Results of association of TEK SNPs rs581724 and rs7876024 with asthma and allergic conjunctivitis

Phenotype	SNP	Phenotype	MAF	Genotype ^a 11 n (%)	Genotype ^a 12 n (%)	Genotype ^a 22 n (%)	Difference between allelic frequencies		Recessive model (11 + 12 vs. 22)	
							p	OR (95% CI)	p	OR (95% CI)
Asthma	rs581724	Present	0.4	138 (35)	188 (48)	66 (17)	.3	1.1 (0.9-1.3)	.4	1 (0.8-1.3)
		Absent	0.39	246 (38)	311 (47)	98 (15)				
	rs7876024	Present	0.24	208 (58)	126 (35)	26 (7)	.7	1 (0.8-1.3)	.9	1 (0.6-1.7)
		Absent	0.25	386 (59)	216 (33)	48 (8)				
Allergic conjunctivitis	rs581724	Present	0.4	31 (35)	34 (38)	24 (27)	.2	1.3 (0.9-1.8)	.007	2.4 (1.3-4.4)
		Absent	0.46	68 (33)	107 (53)	28 (14)				
	rs7876024	Present	0.25	51 (62)	21 (26)	10 (12)	1	1 (0.7-1.5)	.045	2.6 (1-6.5)
		Absent	0.25	104 (55)	74 (39)	10 (6)				

MAF, minor allele frequency. Significant associations are presented by bold values.

^aIn case of rs581724, genotypes 11, 12 and 22 are CC, AC and AA, respectively. In case of rs7876024, genotypes 11, 12 and 22 are AA, AG and GG, respectively.

asthma; however, one of the variations showed a rather strong association with allergic conjunctivitis. To the best of our knowledge, this is the first publication which shows that a genetic variation associates only with allergic conjunctivitis and not with other atopic diseases such as allergic rhinitis or asthma. Figure 1 shows the possible role of Tie2 signalling in allergic conjunctivitis.

Several lines of evidences indicate, however, that the Tie2 pathway can have a role in asthma.

Earlier, our research group has carried out measurements of gene expression levels by Agilent Whole Mouse Genome Oligo Microarray 4x44K chips in the lungs of mice with allergic airway inflammation and control mice (GSE11911 record number in GEO database) (Tolgyesi et al., 2009). Comparing the expression level of the genes in the lungs of mice with OVA-induced allergic airway inflammation and control mice, among the genes in the Tie2 pathway, the expression of *Ang1* (*Angpt1*) was significantly lower in the lung of mice with allergic airway inflammation (in average with 2.3-fold; corrected $p = .001$). This result was also confirmed by Simoes et al. in a mouse model of allergic airway disease (Simoes et al., 2008). As *Ang1* has an anti-inflammatory role in the lung by inhibiting leucocyte transendothelial migration, cytokine production and vascular permeability, its reduced expression can contribute to the development of the asthmatic airway inflammation (Simoes et al., 2008).

Furthermore, in a recent large genomewide association study, four low-frequency SNPs on chromosome 9p21.2 were found to be significantly associated with asthma. Although the detected SNPs were closest to the *EQTN* gene and quite far from the *TEK* gene, they were in LD with a missense variant in the *TEK* gene. Because of this linkage and the known function of the Tie2, the *TEK* gene was suggested as a candidate gene. However, fine mapping of the region showed no eQTL effects in any of the tissues relevant to asthma (Almoguera et al., 2017). In our study, we tested three eQTL SNPs within the *TEK* gene with known respiratory disease association, whether they influenced the risk for asthma or any associated phenotypes. We found no association with asthma, but a quite strong association with allergic conjunctivitis.

Perhaps allergic conjunctivitis is the least well studied among the atopic diseases. Because in most cases it occurs together with rhinitis, they are often studied together as rhinoconjunctivitis and most studies report only on either the proportion of rhinitis patients suffering from ocular symptoms or the associated burden (Klossek, Annesi-Maesano, Pribil, & Didier, 2012). However, not all rhinitis patients develop conjunctivitis and conjunctivitis can exist without rhinitis indicating a partially different genetic and pathological background. In contrast, there is hardly any published genetic study in allergic conjunctivitis and there are no genetic variants which associate only or mainly with allergic conjunctivitis. Although several studies indicated that ocular symptoms could have greater negative impact on the quality of life of the patients than nasal problems, they are underappreciated and often undertreated (Pitt et al., 2004; Smith et al., 2005). The presence of ocular symptoms has been shown to be correlated with sleep impairment, limitations in daily activities and emotional distress (Stull, Schaefer, Crespi, & Sandor, 2009).

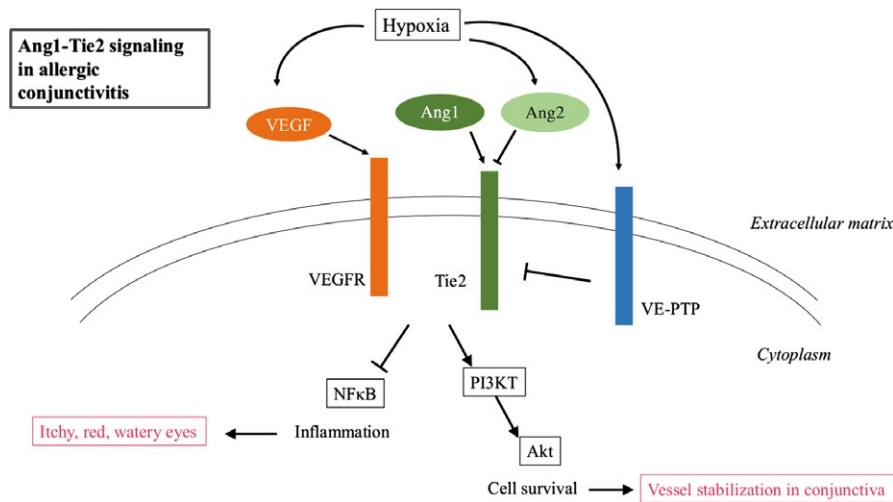


FIGURE 1 Possible role of Ang1-Tie2 signalling pathway in allergic conjunctivitis. Ang1 and Ang2 bind to Tie2 with a similar affinity. Ang1 stimulates kinase activity by binding to Tie2 providing an anti-inflammatory effect and vessel stabilization in the conjunctiva. On the other hand, Ang2 may act as an antagonist for Tie2. Hypoxia can induce Ang2, VEGF (vascular endothelial growth factor) and VE-PTP (vascular endothelial protein tyrosine phosphatase) expression which can drive the expressions of inflammatory molecules resulting in the characteristic symptoms of itchy, red, watery eyes of allergic conjunctivitis

In our study group, the prevalence of allergic conjunctivitis among the patients with rhinitis (55.1%) was within the range of the results of other studies (50%–65%) (Rosario & Bielory, 2011). The gene for the Tie2 receptor is a plausible candidate gene in allergic conjunctivitis. It is highly expressed in the eye, its mutations can cause congenital glaucoma, and it is a potential drug target in different eye diseases (Souma et al., 2016). The associated SNP is located in an intron near the transcription start site which is annotated as an endothelial cell-specific enhancer region and associated with heavily acetylated histones and/or endothelial cell-specific euchromatin. The minor allele of the rs581724 SNP which is associated with the increased risk to conjunctivitis is also associated with reduced Tie2 expression in HapMap3 cell lines.

Presently, it cannot be explained how exactly this SNP increases the risk to conjunctivitis, but it has been shown that Tie2 participates in the regulation of the barrier function of the endothelial cells (David, Kümpers, Van Slyke, & Parikh, 2013; Rübzig et al., 2016). It can be speculated that the rs581724 SNP, which is associated with lower Tie2 expression, can weaken this barrier function of the endothelial cells. Furthermore, it is well known that the inflammatory response to allergens causes nearby blood vessels to dilate and become more permeable. It can be hypothesized that this second hit to the microvascular barriers already weakened by reduced Tie2 expression may increase the risk that the activated inflammatory cells leak out of the blood vessels into the surrounding tissues in the eye causing the characteristic symptoms of the allergic conjunctivitis.

Although there are different types of ophthalmic antiallergic medications available for the treatment of the patients with allergic conjunctivitis, it is generally accepted that the patients are undertreated. For example, in a large French study, it was shown that, despite having received treatment for ocular symptoms, in more than 20% of the patients, the symptoms persisted indicating that the treatments were not

effective (Klossek et al., 2012). Based on our results, the Tie2 pathway can play a role in the pathomechanism of the disease and it is a potential novel therapeutic target in allergic conjunctivitis. In the last decade, several different agents which can activate the Tie2 pathway have been investigated in diseases where leaky blood vessels and/or downregulated Tie2 receptor contributed to their development. These diseases included sepsis, acute kidney injury, influenza, stroke and eye diseases, and it was shown that these drugs could provide additional benefit to the prevailing therapy (Bourdeau et al., 2016; Cui et al., 2013; David et al., 2013; Rübzig et al., 2016; Sugiyama et al., 2015). It was also shown that compensatory changes in Ang1 expression might help preserve basal Tie2 signalling when Tie2 gene expression is low. Our results suggest that these or similar drugs might also be potential candidates in allergic conjunctivitis.

It must be noted, however, that the study has some limitations. First, because of the retrospective nature of the study, about one-third of the asthmatic patients have not been fully phenotyped which could cause bias in the results. Second, the number of patients with conjunctivitis is rather low which can reduce the power of the study. Third, the allergic disorders, particularly in childhood, are not static. Because of this, with time, the symptoms of the patients and controls can change and this could affect the classification of the patient. This is true, however, for all such studies. This can be corrected by prospective studies or by following up the patients for years. We are already involved in a prospective study to try to confirm these findings in a larger patient group.

5 | CONCLUSION

Although several lines of evidences indicate that the Tie2 pathway might have a role in asthma, the investigated variations in

the *TEK* gene, which are associated with lower Tie2 expression, did not influence the susceptibility to the disease. However, we found that the homozygote carriers of the rs581724 SNP had significantly increased risk to allergic conjunctivitis. If additional studies can confirm the role of the Tie2 pathway in allergic conjunctivitis, this can be a potential novel therapeutic target in the disease.

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CONFLICT OF INTEREST

The authors report no conflict of interests. The authors alone are responsible for the content and writing of the manuscript.

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