Loss of Function of Transient Receptor Potential Vanilloid 1 (TRPV1) Genetic Variant Is Associated with Lower Risk of Active Childhood Asthma*

Received for publication, June 30, 2010

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Transient receptor potential cation channels of the vanilloid subfamily (TRPV) participate in the generation of Ca2+ signals at different locations of the respiratory system, thereby controlling its correct functioning. TRPV1 expression and activity appear to be altered under pathophysiological conditions such as chronic cough and airway hypersensitivity, whereas TRPV4 single nucleotide polymorphisms (SNP) are associated with chronic obstructive pulmonary disease. However, to date, there is no information about the genetic impact of either TRPV1 or TRPV4 on asthma pathophysiology. We now report on the association of two functional SNPs, TRPV1-I585V and TRPV4-P19S, with childhood asthma. Both SNPs were genotyped in a population of 470 controls without respiratory symptoms and 301 asthmatics. Although none of the SNPs modiﬁed the risk of suffering from asthma, carriers of the TRPV1-I585V genetic variant showed a lower risk of current wheezing (odds ratio = 0.51; p = 0.01), a characteristic of active asthma, or cough (odds ratio = 0.57; p = 0.02). Functional analysis of TRPV1-I585V, using the Ca2+-sensitive dye fura-2 to measure intracellular [Ca2+] concentrations, revealed a decreased channel activity in response to two typical TRPV1 stimuli, heat and capsaicin. On the other hand, TRPV4-P19S, despite its loss-of-channel function, showed no signiﬁcant association with asthma or the presence of wheezing. Our data suggest that genetically determined level of TRPV1 activity is relevant for asthma pathophysiology.

TRPV12 and TRPV4 are nonselective cation channels, members of the vanilloid subfamily of transient receptor potential cation channels (1). TRPV1 is expressed primarily on nociceptive neurons and can be activated by capsaicin, noxious heat, and protons (2). The widely distributed TRPV4 cationic channel participates in the transduction of mechanical and/or osmotic stimuli in different tissues (3, 4). TRPV4 channels also respond to heat, acidic pH, and endogenous arachidonic acid metabolites (5, 6).

Both channels are expressed in airway sensory nerves (TRPV1) (7) and epithelial (8, 9) and smooth muscle cells (TRPV4) (10). As integrators of different physical and chemical stimuli, they participate in the generation of Ca2+ signals (11, 12), that contribute to airway defense mechanisms such as cough (13, 14) and mucociliary clearance (9), but TRPV1 and TRPV4 also show a pathophysiological downside. TRPV1 activity has been related to different aspects of chronic respiratory disease such as neurogenic inﬂammation (15, 16), irritant-induced chronic cough (13, 14), and airway hypersensitivity (17). TRPV4 activation disrupts alveolar barrier in animal models (18) and has been associated with chronic obstructive pulmonary disease in humans (19). Together, these evidences make TRPV1 and TRPV4 interesting candidate genes for asthma. To understand their implication in asthma pathophysiology, i.e. whether the etiology or the pathological symptoms are associated with a gain or loss of channel function, we have only considered two non-synonymous variants that may alter channel activity.

EXPERIMENTAL PROCEDURES

Population Characteristics—Data for this analysis were obtained from the Childhood Respiratory Health Study (CRHS). The study was conducted in two phases (I and II). Phase I had a population-based cross-sectional design and was carried out in the cities of Barcelona and Sabadell (Spain). Parents of 12,382 children aged 7–8 and registered in all existing primary schools (public and private) were invited to participate. For a total of 10,821 children, a parent self-administered questionnaire was completed (response rate 87.4%). The questionnaire was based on the International Study on Asthma and Allergies in Childhood (ISAAC) (20).

Phase II had a two-stage population-based case-control study design. From Phase I completed questionnaires, children were classified in different groups according to their answers to respiratory symptom questions. Parents of all children with asthma diagnosed by a doctor (cases) and a random sample of children free of symptoms (controls) were invited to complete a self-administered questionnaire including questions on respiratory symptoms, drugs prescribed for asthma/wheeze, rhinitis...
A. **TRPV1-1585V**

- **II** (n = 104)
- **V** (n = 153)
- **VV** (n = 43)
- **V carrier** (n = 196)

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<th>OR (95% CI)</th>
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Risk of wheezing and cough by **TRPV1** and **TRPV4** genotype in asthmatics. A–D, crude odds ratio (95% CI) of wheezing (A and C) and cough (B and D) as a function of **TRPV1-1585V** (A and B) and **TRPV4-19P** (C and D) by multivariate logistic regression analysis. Adjusted ORs did not vary significantly from the crude ones. For **TRPV1**, values are: II, n = 104 (76 of whom presented wheezing and 69 cough); VI, n = 153 (94 of whom presented wheezing and 84 cough); VV, n = 43 (23 of whom presented wheezing and 20 cough); V carrier, n = 196 (117 of whom presented wheezing and 79 cough). For **TRPV4**, values are: PP, n = 282 (184 of whom presented wheezing and 163 cough); S carrier, n = 19 (10 of whom presented wheezing and 10 cough); II, Ile-Ile; VI, Val-Ile; VV, Val-Val; V carrier, Val carrier; PP, Pro-Pro; S carrier, Ser carrier.

B. **COUGH**

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C. **TRPV4-19P**

- **PP** (n = 282)
- **S carrier** (n = 19)

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D. **COUGH**

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**RESULTS AND DISCUSSION**

**Population-based Genetic Association Studies**—An SNP corresponding to an isoleucine-to-valine mutation at position 585
of the TRPV1 protein (rs8065080; TRPV1-I585V) (24) and an SNP generating a proline-to-serine mutation at amino acid 19 of the TRPV4 protein (rs3742030; TRPV4-P19S) (25) were evaluated for their association with asthma. The most frequent alleles, TRPV1-Ile-585 (61% of the population) and TRPV4-Pro-19 (97% of the population), were used as the reference alleles. We first tested for association between diagnosed asthma and the presence of TRPV1-I585V and TRPV4-P19S SNPs by multivariate logistic regression analysis. Asthma was not associated with TRPV1-I585V allele neither in homozygous (OR, 0.96, 0.62–1.49 95% CI, n = 122) nor in heterozygous children (OR, 1.17, 0.86–1.60 95% CI, n = 392) when compared with noncarriers (n = 297; p = 0.4)). Similarly, the OR of TRPV4-P19S carriers (1.39, 0.75–2.58; n = 43; homozygous and heterozygous were pulled together due to the low number of the former) was not different (p = 0.29) from noncarriers (n = 770). Next, within asthmatics, we evaluated the association of these SNPs with the presence of wheezing or cough within the last year. The magnitude and direction of the association (OR < 1 implies a decrease risk) were consistent with a progressive protective effect of the genetic variants, we ex-

The 20–30% loss of channel function shown by TRPV1-Val-585 responded to heat (50 °C; Fig. 2A) and capsaicin (10 nM; Fig. 2B) with significantly higher increases in intracellular [Ca2+] than those transfected with TRPV1-Val-585. A quantitative analysis of the Ca2+ signal, calculating the mean area under the curve as an indicator of the magnitude of the Ca2+ signal, is shown in Fig. 2, C and D.

The impact of TRPV4-P19S mutation on TRPV4 channel activity has already been evaluated. TRPV4-P19S induces a loss of channel function in response to mild hypotonic stimuli and has been recently associated with hyponatremia (25).

Our combined functional and population-based genetic epidemiological studies provide evidence for the involvement of TRPV1, but not TRPV4, in symptoms typically associated to asthma: wheezing and cough. We also acknowledge that given the low frequency of TRPV4-P19S, the statistical power to detect its real impact on asthma pathophysiology is also low. TRPV1 is generally viewed as a molecular integrator of nociceptive stimuli and inflammatory reactions. In the lung, Ca2+ plays a crucial role in the activation of almost all cells. In fact, TRPV1-mediated Ca2+ entry in response to irritants and endogenous activators triggers pulmonary chemoreflexes leading to bronchoconstriction, mucus hypersecretion, airway irritation, and cough (17). It also appears to be responsible for the release of neuropeptides that favor the inflammatory response (17). Besides, sensitivity to TRPV1 activators is elevated in both animal models of asthma (15) and human patients (26). Consistent with all these previous reports, we observed that the TRPV1-Val-585 variant that decreases the channel response to different agonists is associated with lower risk of asthmatics to present wheezing and cough. Although we could not show any evidence that TRPV1-I585V was associated with asthma or with other asthma outcomes, our data suggest that this genetic variant can be implicated in the clinical heterogeneity of childhood asthma and eventually in the remission of asthma.
In summary, our data provide the first genetic evidence for the involvement of the TRPV1 nonselective cation channel in human disease and provide support to recent reports pointing toward the important role of intracellular Ca\(^{2+}\) dysregulation in asthma pathophysiology (28).

Acknowledgment—We thank B. Nilius (Katholieke Universiteit Leuven, Belgium) for the TRPV1 plasmid. The CRHS study was funded by Fondo de Investigaciones Sanitarias (FIS/1198) and by an unrestricted grant from GlaxoSmithKline and Pharmacia Diagnostics, AB.

REFERENCES