



Nasal potential difference of carriers of the W493R ENaC variant with non-cystic fibrosis bronchiectasis

To the Editor:

Common underlying conditions leading to bronchiectasis are infection, immune deficiency or cystic fibrosis (CF) [1]. Transgenic mice that overexpress the amiloride-sensitive epithelial sodium channel ENaC mimic the typical features of CF lung disease [2], and correspondingly, it has been proposed that ENaC hyperactivity may predispose to bronchiectasis [3]. The ENaC ion channel consists of three subunits which are encoded by the genes *SCNN1A*, *SCNN1B* and *SCNN1G*. Sequencing of these three genes in 71 subjects with CF-like disease in whom a mutation could not be identified on both *CFTR* (*cystic fibrosis transmembrane conductance regulator*) genes uncovered singular cases of disease caused by mutations in *SCNN1A* [3] and *SCNN1B* [4], and a higher incidence of *ENaC* polymorphisms [3]. Of nine identified amino acid sequence polymorphisms, one variant in the alpha subunit, named p.W493R-*SCNN1A*, encoded a hyperactive ENaC channel when tested in a heterologous expression system [3]. Hence AZAD *et al.* [3] proposed that the p.W493R-*SCNN1A* ENaC variant could be a global risk factor for the development of lung diseases.

We tested this hypothesis by screening 69 adults with non-CF bronchiectasis from the adult bronchiectasis clinic at Hannover Medical School for the carriage of p.W493R-*SCNN1A* (rs5742912) (table 1). The study was conducted with the approval of the Ethics Committee of Hannover Medical School (study number: 5391). CF had been excluded by chloride concentrations below the pathological range in the sweat test and/or the absence of any disease-causing mutation in the *CFTR* coding region in genetic testing. Genomic DNA was amplified with the forward primer 5'-TGCAGGTTAGTGTCCTTC and the reverse mismatch primer 5'-CTGGGATGTCACCGATGG by PCR. The 166-bp long PCR product was digested with BseI (Fermentas; Thermo Fisher Scientific, Vilnius, Lithuania), which cleaves the common W493 allele, and separated by 4% agarose gel electrophoresis. Six of the 69 subjects with non-CF bronchiectasis were identified as heterozygous carriers of p.W493R-*SCNN1A*. Thus the incidence of p.W493R-*SCNN1A* of 8.7% was significantly higher in the investigated disease cohort than that of 3.1% in a previously studied healthy control cohort of 739 Mid-European subjects of similar ethnic descent [3] ($p=0.022$, Fisher's exact test). This data demonstrates an association between the carrier status for p.W493R and the manifestation of bronchiectasis.

TABLE 1 Characteristics of the patient cohort[#]

| | |
|--|----------------------|
| Subjects n | 69 |
| Age years | 51±18 |
| Sex | |
| Male | 24 (35) [carriers=3] |
| Female | 45 (65) [carriers=3] |
| FEV₁ % predicted | 47±27 |
| Chronic airways colonisation with <i>Pseudomonas aeruginosa</i> | 34 (49) [carriers=3] |
| Associated conditions[¶] | |
| Idiopathic | 33 (48) [carriers=2] |
| COPD/emphysema | 8 (12) |
| Asthma/ABPA | 6 (9) [carriers=1] |
| Post infectious | 5 (7) |
| Primary ciliary dyskinesia | 5 (7) |
| Immunodeficiency | 5 (7) [carriers=2] |
| CFTR-related disorder | 3 (4) [carriers=1] |
| Other | 4 (6) |

Data are presented as mean±SD or n (%), unless otherwise stated. FEV₁: forced expiratory volume in 1 s; COPD: chronic obstructive pulmonary disease; ABPA: allergic bronchopulmonary aspergillosis; CFTR: cystic fibrosis transmembrane conductance regulator. [#]: the number of p.W493R-*SCNN1A* carriers is listed in brackets; [¶]: indicates the diagnosis at the beginning of the study.

Next, we wanted to know whether carriage of p.W493R-SCNN1A translates into higher sodium channel activity as indicated by a larger hyperpolarisation response to amiloride in nasal potential difference measurements (NPD) of the upper respiratory epithelium. Five of the six p.W493R carriers were available for an assessment by NPD. NPD measurements were performed according to the Standard Operating Procedure NPD_EU001, version 1.7 (March 2013) of the European Cystic Fibrosis Society Diagnostic Network Working Group, which primarily differs from a publicly available protocol [5] by superfusion of room temperature solutions through a Marquat catheter (model I0202US; Marquat Genie Biomedical, Boissy-Saint-Leger, France). Two subjects presented normal NPD tracings, and two subjects showed a normal response to amiloride, but only a minute response upon exposure to chloride-free solution consistent with the diagnosis of a CFTR-related disorder [6, 7]. However, one subject, a 58-year old female with non-CF bronchiectasis and underlying asthma complicated by allergic bronchopulmonary aspergillosis, produced a tracing suggestive of a hyperactive ENaC. The basic potential was normal (right nostril -21 mV, left nostril -27 mV), but inhibition of the sodium channel caused a large hyperpolarisation to a final value close to 0 mV ($+19$ mV, $+25$ mV), which was followed by a similarly large depolarisation upon the exposure to chloride-free gluconate solution (-21 mV, -21 mV). The high sodium conductance caused by the ENaC channel was balanced by an equivalent chloride conductance mediated mainly by open CFTR channels. Virtually identical NPD profiles have been reported previously for two subjects with CF-like lung disease who were heterozygous carriers for p.W493R-SCNN1A and a mutation in the CFTR gene [3]. Thus there are now three documented cases with bronchiectasis who presented with a large hyperpolarisation upon exposure to amiloride in the NPD.

NPD tracings with normal basic and large hyperpolarisation potentials have been observed in subjects with genetically proven Liddle syndrome which is caused by heterozygous mutation in either the beta or gamma subunit of ENaC [8]. Liddle syndrome is an autosomal dominant disorder characterised by early-onset salt-sensitive hypertension, hypokalaemia and metabolic alkalosis [9]. These clinical features were absent in our index case with non-CF bronchiectasis. The known non-synonymous mutations in the ENaC subunits cause a broad spectrum of loss-of-function to gain-of-function renal phenotypes, which range from pseudoaldosteronism to Liddle syndrome. According to AZAD *et al.* [3] and our data a mutant ENaC may also be occasionally involved in disorders of the lung, including bronchiectasis.

In conclusion, carriage of the p.W493R-SCNN1A polymorphism in the alpha subunit of ENaC has been identified as a risk factor for bronchiectasis, but it is not invariably associated with the phenotype of a hyperactive ENaC channel in the lung, which promotes the hyper-absorption of sodium ions from the periciliary fluid of the respiratory epithelium and thereby may cause CF-like lung disease. This finding is consistent with the observations that transgenic mice overexpressing SCNN1A do not present with CF-like lung disease [2] and that, of 11 sequence variants identified in the SCNN1A gene of individuals with CF-like lung disease, only the most frequent p.W493R-SCNN1A polymorphism and the singular missense mutation p.F61L-SCNN1A substantially modulate the sodium ion transport capability of the ENaC ion channel in a heterologous test system by more than two-fold [3]. However, factors not covered by the sequencing of the coding region and the exon/intron junctions [3] could also influence ENaC function, *i.e.* sequence variants located in deep intronic regions or genetic factors acting in trans that are located elsewhere in the genome. Hence, based on our experience that the most frequent sequence variant in the coding region is not predictive of whether the ENaC channel will be involved in causing lung disease, in particular bronchiectasis, we would like to recommend that one should start the diagnostic work-up of ENaC in the context of bronchiectasis of unknown aetiology with NPD measurements followed by genetic testing, if applicable. Based on current knowledge, if there are no other clinical symptoms or implications for ENaC disease and no indication for ENaC disease in the NPD measurements, testing of the SCNN1ABG genes is not indicated in a clinical setting because of the low clinical consequences for the patient and high costs attached to the extra genetic testing.



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ENaC polymorphism p.W493R is associated with bronchiectasis but does not necessarily lead to aberrant ion conductance <http://ow.ly/S5Mwj>

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