Familial aggregation of nasal conditioning capacity

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Sahin-Yilmaz A, Pinto JM, deTineo M, Elwany S, Naclerio RM. Familial aggregation of nasal conditioning capacity. J Appl Physiol 103: 1078–1081, 2007. First published July 19, 2007; doi:10.1152/japplphysiol.00299.2007.—In our previous studies on nasal conditioning, we observed a large variability among individuals to condition inspired air. Although we previously investigated various physiological parameters (age, sex, nasal mucosal temperature, heart rate, blood pressure, and nasal volume) that might underlie these differences, we have been unable to explain this variability. Many proteins and molecules, which are under genetic control and could affect nasal conditioning, are involved in water transport. In this study, we hypothesized that familial factors may contribute to the differences in nasal conditioning capacity (NCC). We performed a prospective study of 47 sibling pairs. Cold dry air was delivered to the nose and the total water gradient (TWG) was calculated to determine the NCC. We found a highly significant intraclass correlation of 0.53 (P < 0.0001) between sibling pairs for the TWG. These results suggest that there is a familial basis for nasal conditioning and a large enough genetic component to search for genes explaining the observed correlation.

nasal conditioning; genetics; siblings; nose; temperature

The fundamental function of the nose is to warm and humidify inspired air (13). In this way, the nose protects the lower airway from ambient conditions that range from temperatures from −42 to 48°C and from 0 to 100% relative humidity (RH) (16). It has been shown that nasal breathing reduces the drying and cooling effects of increased ventilation caused by exercise and reverses the tendency to bronchoconstriction in lower airway (22).

Our laboratory previously developed a technique to measure the ability of the human nose to warm and humidify air by using inhalation of cold dry air (CDA) (20). In our laboratory’s previous studies (3–5, 18, 20), our group observed a large variability among individuals to condition inspired air but were unable to explain this variability.

We investigated different parameters that might influence this ability, including age, sex (18), nasal mucosal temperature (NMT), pulse, blood pressure, and nasal volume (4, 5). Another possibility for the difference among individuals is variability of water transport across the epithelium. There are multiple channels, transporters, and proteins involved in water transport. Variations in the genes that code for these proteins could explain the variability.

A remaining possibility is that the physiological function of the nose has a heritable component, like a number of other measures of airway physiology. In a recent study of subjects with sleep apnea, the volume of the upper airway soft tissue structures was shown to be heritable (21). A familial correlation has been found in the decline of forced expiratory volume in 1 s (15). Similarly, genetic factors were shown to play an important role in other physiological parameters such as blood pressure, serum lipid levels, and body mass index (12). Therefore, we hypothesized that familial factors contribute to the differences in nasal conditioning capacity (NCC) in humans.

In the absence of data on NCC in families and without access to sufficient numbers of mono- and dizygotic twins, we chose to study siblings and hypothesized that NCC would correlate more closely between siblings than unrelated individuals. Others have used this approach successfully to determine heritability of respiratory-related phenotypes (15).

METHODS

Subjects. We recruited 47 consecutive sibling pairs between the ages of 18 and 35 yr who met our inclusion criteria and who had no medical conditions other than allergic rhinitis and/or mild asthma (Table 1). The atopic status was confirmed by skin prick testing with a panel of common allergens present in the Chicago area (Dermatophagoides farinae, cat, tree, Timothy grass, Giant ragweed, and mold; Multitest II, Lincoln Diagnostics). Subjects with seasonal allergies were studied outside of their allergy season. We excluded subjects with an upper respiratory infection within 14 days before study visit; those taking intranasal medication during the previous 72 h; and those taking oral decongestants, antihistamines, or leukotriene inhibitors during the previous 7 days. Subjects with significant nasal septal deviation, nasal polyps, or history of smoking in the previous 6 mo, which might affect NCC, were also excluded. The only medications allowed within 24 h of the assessment of nasal conditioning were acetaminophen and oral contraceptives, or inhaled medications for the control of mild asthma. The Institutional Review Board of the University of Chicago approved the study, and written consent was obtained from all participants.

Experimental protocol. We performed a cross-sectional study consisting of a single visit. On arrival, subjects waited for 15 min to allow equilibration of the nasal mucosa with the environmental conditions of the laboratory, as in prior studies. Baseline nasal symptoms of rhinorrhea, congestion, and itching of the nose or throat of each subject were recorded. The baseline volume of the nasal cavity was measured by acoustic rhinometry. The more patent side was chosen for probe insertion and remaining measurements were taken on the opposite side [nasal conditioning, nasal mucosal temperature (NMT), secretion weights, and nasal volume]. The side of probe insertion was sprayed with two puffs (0.2 ml) of 0.05% oxymetazoline hydrochloride (Nasotrila, Ciba Self-Medication, Woodbridge, NJ), followed by two puffs (0.2 ml) of 4% topical lidocaine hydrochloride (Roxane Laboratories, Columbus, OH). A probe containing a temperature sensor was inserted through the nose along the floor of the nasal cavity so that the tip was suspended in the nasopharynx, thus positioned to measure the temperature of the air exiting the nonprobe, nondecongested, nonanesthetized side. Flexible nasopharyngoscopy (Flex View Nasopharyngoscope, Smith and Nephew ENT, Barlett, TN) was used to verify position. The nostril

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Table 1. Demographic data of all subjects

<table>
<thead>
<tr>
<th>Number of subjects</th>
<th>94 (47 pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range)</td>
<td>23 (18–35)</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>1.47</td>
</tr>
<tr>
<td>FE</td>
<td>18 pairs</td>
</tr>
<tr>
<td>MM</td>
<td>9 pairs</td>
</tr>
<tr>
<td>FM</td>
<td>20 pairs</td>
</tr>
<tr>
<td>Race (%)</td>
<td>38.3</td>
</tr>
<tr>
<td>C</td>
<td>51.1</td>
</tr>
<tr>
<td>AA</td>
<td>4.3</td>
</tr>
<tr>
<td>AS</td>
<td>4.3</td>
</tr>
<tr>
<td>H</td>
<td>2.1</td>
</tr>
<tr>
<td>I</td>
<td>73.4</td>
</tr>
<tr>
<td>Atopy (%)</td>
<td>26.6</td>
</tr>
<tr>
<td>Nonatopic</td>
<td>73.4</td>
</tr>
</tbody>
</table>

F = Female, M = Male, C = Caucasian, AA = African American, AS = Asian, H = Hispanic, I = Indian.

containing the probe was then occluded using a silicone wax plug (Mack’s Ear-plug, McKeon Products, Pleasant Ridge, MI); thus none of the air to be conditioned was passed through the nostril containing the probe or the nostril that had received the decongestant and the local anesthetic. After 5 min, NMT, symptom scores, secretion weight, and nasal volume measurement were recorded.

CDA exposure of the nonprobe, nonmedicated side was then performed as in prior studies (see below) (4, 5, 18, 20). At the end of CDA challenge, before removal of the mask, NMT and the symptom scores were recorded. After the mask was removed, post-CDA secretion weight and nasal volume were measured. The nasal probe was then removed.

Nasal conditioning measurement. The measurement of nasal conditioning was done as described previously (20). Briefly, a nasal continuous positive mask (Respirronics, Murrysville, PA) was applied to the face with head straps. A probe was inserted into one nasal cavity and used to continuously measure air temperature in the nasopharynx and a second temperature probe continuously measured the temperature before entry into the nose in the mask, thus standardizing the experimental conditions in all subjects. All other measurements (NMT, volume) were taken on the opposite side. Cold air at 0% RH was delivered to the patient’s nose via the mask at flow rates of 5, 10, and 20 l/min at air temperatures 17, 10, and −1°C, respectively for 12 min each. In prior studies, the RH under the experimental conditions described was always 100% in the nasopharynx and 0% in the mask; thus they were not measured in this study. The difference between water content of air before entry into the nose and that in the nasopharynx is the water gradient (WG) across the nose; this represents the amount of water evaporated by the nose to condition air under the conditions of the experiment (20). The total WG (TWG) represents the sum of WGs obtained at each of the three flow rates and represents NCC.

All the studies were done between 9 AM and 5 PM, and siblings were studied at the same time of day; thus diurnal variation would not be expected to be a problem. The coefficient of variation is 8.8% for an individual studied on 3 different days under the conditions of this experiment (20).

Symptom scores. Symptoms of rhinorrhea, nasal congestion, and itching in the nose or throat were graded on a scale as follows: 0 = no symptoms, 1 = very mild, 2 = mild, 3 = moderate, 4 = severe, 5 = very severe.

Nasal volume measurement. Nasal volume measurement was performed with an Eccovision Acoustic Rhinometry System (Hood Laboratories, Pembroke, MA). The volume was measured between 0 and 6 cm from the tip of the rhinometry probe. Each measurement was performed in triplicate, and the average values are reported.

NMT measurement. The NMT was measured as described previously (4). In brief, a thermistor attached to a Q-tip was inserted through a hole in the continuous positive airway pressure mask and contacted the septum distal to the transitional epithelium. The mean of the data collected during a 30-s period was recorded.

Secretion weight measurements. Secretions were collected from the anterior nasal septum by use of filter paper disks as previously described (6). The disks were placed for 30 s and removed. The change in weight of the disks before and after CDA exposure is reported as the amount of secretion.

Statistical analysis. The number of subjects required for the experiment was determined according to standard power calculations. We determined the number of subjects needed to provide 80% power for detecting a correlation between siblings by the use of a two-sided P value of <0.05 as 47 sibling pairs. The intraclass correlation coefficient was used to detect a correlation among siblings and was calculated as follows: ρ = σ²λ / (σ²λ + σ²), where σ²λ = variability between sibling pairs and σ² = variability within sibling pairs. A 95% confidence interval (CI) for the correlation was then obtained. For comparison of the TWG, statistical analysis was performed by use of parametric statistics. For other parameters, unpaired t-test was used for analysis. Remaining correlations were performed by the Spearman rank method.

RESULTS

As in prior studies, for all subjects (n = 94), increasing the CDA flow rate progressively increased the WG (P < 0.001) (data not shown). Similarly, symptom scores (rhinorrhea and congestion) increased significantly after CDA exposure in all subjects as in prior studies (P = 0.006) (Table 2). Thirty-three sibling pairs were studied on the same day, 6 pairs 1–2 days apart, 3 pairs 1 wk apart, 3 pairs ~2 wk apart, and 2 pairs ~1 mo apart, but all were studied in same season. Eighty percent of sibling pairs were currently living in the Chicagoland area.

To determine whether there was a correlation between sibling pairs in TWG, we calculated the sibling intraclass correlation coefficient for this parameter. The intraclass correlation coefficient (ρ) for TWG was highly significant (ρ = 0.53, P < 0.0001; 95% CI: 0.33–0.74).

We then performed post hoc subgroup analyses. We investigated whether atopic status and volume of the nasal cavity might affect the ability of the nose to condition the air. The subgroups evaluated include sibling pairs with both individuals atopic, both nonatopic, and mixed atopy status. The ρ between atopic sibling pairs (n = 26 pairs) was statistically significant (ρ = 0.54, P = 0.0015; 95% CI: 0.26–0.81). The intraclass correlation coefficient between nonatopic pairs (n = 4 pairs) was 0.59 (P = 0.10). The intraclass correlation coefficient between discordant pairs (1 atopic, 1 nonatopic) sibling pairs (n = 16 pairs) was 0.25 (P = 0.15).

The nasal volume measurements obtained using acoustic rhinometry were available in 76 subjects. Nasal volume after

Table 2. Comparison of symptom scores and secretion weights following cold dry air exposure

<table>
<thead>
<tr>
<th>ProCDA Median (Range)</th>
<th>Post CDA Median (Range)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinorrhea Score (0–5)</td>
<td>2 (0–3)</td>
<td>3 (1–4)</td>
</tr>
<tr>
<td>Congestion Score (0–5)</td>
<td>0 (0–0)</td>
<td>3 (2–4)</td>
</tr>
<tr>
<td>Secretion Weight (mg)</td>
<td>21 (4–31)</td>
<td>36 (16.6–48)</td>
</tr>
</tbody>
</table>

CDA = Cold dry air.
CDA exposure in these subjects decreased significantly compared \((P < 0.001, \text{each comparison})\), consistent with prior studies. There was a statistically significant negative correlation between the change in nasal volume after CDA exposure and TWG \((r = -0.35, P = 0.0018; \text{Fig. 1})\).

We calculated the sibling intraclass correlation coefficient for nasal volumes. The sibling intraclass correlation was statistically significant for the baseline nasal volumes among sibling pairs \((\rho = 0.64, P = 0.000; \text{95% CI}: 0.45–0.82)\). There was also a statistically significant sibling intraclass correlation for the change in nasal volume after CDA exposure \((\rho = 0.68, P = 0.000; \text{95% CI}: 0.50–0.85)\).

The nasal secretion weights were available in 90 subjects. The secretion weights in these subjects increased significantly after CDA exposure \((P < 0.001)\), consistent with prior studies (Table 2). There was no correlation between the change in secretion weight and TWG of all subjects \((r = -0.11, P = 0.2)\). Similarly, there was no correlation between the change in nasal volume and the change in secretion weight after CDA exposure \((r = -0.1, P = 0.35)\). However, the sibling \(\rho\) was significant for the change in nasal secretion weights after CDA exposure \((\rho = 0.68, P = 0.000)\) (95% CI: 0.52–0.84).

There was no significant correlation between the TWG value and baseline nasal volume, and between baseline nasal symptoms and age of all subjects \((P > 0.05 \text{for both})\). There was a significant correlation between baseline secretion weights and TWG of all subjects \((\rho = 0.38, P = 0.0004)\). The TWG between male \((n = 38)\) and female \((n = 55)\) subjects was not significantly different \((P = 0.9)\).

**DISCUSSION**

Our data show a significant intraclass correlation among siblings in their ability to condition inspired air, measured as the TWG. The reduced variability of TWG among sibling pairs compared with nonsiblings suggests that genetic factors contribute to the differences in NCC of individuals.

Because siblings share a higher proportion of genetic information than unrelated individuals, their phenotypes are correlated as noted by a classic study by Fisher (10). Because data on familial clustering are not available and twin studies were not possible, we took advantage of this phenomena to determine a genetic basis of the non-Mendelian trait of NCC using a sibling-pair study design. Heritability is linked to the \(\rho\), based on assumptions about the genes involved. Although correlation can be used to infer heritability, the genetic component of the trait variance may be overestimated because of unknown, shared environmental influences that could affect NCC. Familial resemblance arises when related individuals who share genes and/or environmental exposures exhibit greater similarity than do pairs of unrelated individuals (19). It is possible that some aspect of a shared childhood home environment could significantly influence nasal conditioning in siblings. However, our subjects were adults at the time of measurement of NCC and were living independently of their sibling. We cannot rule out early or developmental effects, although genetic-environment interactions remain an important question for all genetic studies.

Our approach of studying sibling pairs only predicts heritability and does not distinguish whether NCC is monogenic or polygenic, although given the many possible molecular mechanisms affecting water transport, multiple genes are likely to be involved. It should be noted that taking advantage of the shared genetic components between siblings is a standard design of genetic trait mapping studies (11).

Demonstration of the existence of heritable variability in water transport in the nose could potentially explain the mechanism by which the upper airway physiology affects the lower airway. If air is not fully conditioned by the nose, then more conditioning must occur in the lower airway. We posit that, when the conditioning of inspired air is decreased in the nose, it must occur in the lower airway, with resultant derangements of lower airway physiology leading to disease such as asthma and other forms of pulmonary dysfunction.

Theoretically, a heritable defect in water transport could also be responsible for atopic diseases. If the water transport problem is in the tight junctions between cells, a disruption of this barrier may permit greater movement of antigens across the epithelium, leading to an enhanced immune response. Such a mechanism has been proposed for the development of celiac disease (8, 9).

To determine whether the heritable defects in water transport may be related to atopy, we grouped the sibling pairs according to their atopy status. There was a significant intraclass correlation among atopic siblings. Unfortunately, there were too few discordant or nonallergic sibling pairs to comment on their responses. If the defect occurs in both the atopic and nonallergic population, then the responsible gene(s) probably explains the variability among all subjects. It may also be associated with the gene(s) responsible for atopy. If the defect is seen only in the atopic sibling pairs, the water defect may contribute to the pathophysiology of atopic disorders including allergic rhinitis and asthma.

In our patient population, we had a large number of atopic individuals (73%), those with a positive skin test. This number is high, until you consider the data from the National Health and Nutrition Examination Survey (NHANES) III study conducted in 1994 in which 53% of the US population was atopic. This represented a 5.5-fold increase over the NHANES II study conducted in 1980 (1). The fact that our laboratory is known for studies of lower airway physiology leading to disease such as asthma and other forms of pulmonary dysfunction.

After CDA exposure, there were significant decreases in nasal volume and significant increases in nasal secretion weights and nasal symptoms in all subjects, findings that are consistent with previous studies (3, 5, 18). Additionally, we showed that the amount of change in nasal volume as a result of exposure to CDA paralleled the increase in nasal humidification.
cation in response to CDA. This supports the theory that exposure to CDA results in hyperosmolarity of nasal secretions which triggers the parasympathetic system to cause vasodilation of the vascular bed with a subsequent decrease in nasal volume and an increase in nasal conditioning.

We grouped all individuals to see whether other parameters predicted the TWG. We found that baseline nasal volume values were not correlated to the TWG consistent with our previous studies (3, 4). Recently, in a transient simulation in three-dimensional models of the nasal cavity, Naftali et al. (17) demonstrated that the air conditioning efficacy of the nasal cavity is unaffected by local variations in nasal geometry, supporting our previous and present results. There was no significant correlation between the baseline symptoms scores or the ages of the subjects. Baseline secretion weights were correlated with TWG.

In summary, our results suggest that there is a genetic basis for nasal conditioning.

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