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Olfactory Receptor Gene Polymorphisms and Nonallergic Vasomotor Rhinitis

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Abstract

We sought a genotype-phenotype association: between single-nucleotide polymorphisms (SNPs) in olfactory receptor (*OR*) genes from the two largest *OR* gene clusters and odor-triggered nonallergic vasomotor rhinitis (nVMR). In the initial pedigree screen, using transmission disequilibrium test (TDT) analysis, six SNPs showed "significant" *p*-values between 0.0449 and 0.0043. In a second case-control population, the previously identified six SNPs did not re-emerge, whereas four new SNPs showed *p*-values between 0.0490 and 0.0001. Combining both studies, none of the SNPs in the TDT analysis survived the Bonferroni correction. In the population study, one SNP showed an empirical *p*-value of 0.0066 by shuffling cases and controls with 10^5 replicates; however, the *p*-value for this SNP was 0.83 in the pedigree study. This study emphasizes that underpowered studies having *p*-values between <0.05 and 0.0001 should be regarded as inconclusive and require further replication before concluding the study is "informative." However, we believe that our hypothesis that an association between *OR* genotypes and the nVMR phenotype remains feasible. Future studies using either a genomewide association study of all *OR* gene-pseudogene regions throughout the genome —at the current recommended density of 2.5 to 5 kb per tag SNP—or studies incorporating microarray analyses of the entire "*OR* genome" in well-characterized nVMR patients are required.

Keywords

vasomotor rhinitis; olfactory receptor genes; genotype-phenotype association study; transmission disequilibrium test; case-control study; multiple-testing; Bonferroni correction; idiopathic environmental intolerance

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Introduction

A number of complex, poorly characterized clinical syndromes fall under the umbrella of "idiopathic environmental intolerance" (IEI) (a.k.a. "multiple chemical sensitivity"). Whether the etiology is psychogenic or environmental continues to be debated (1–4). Several medical conditions appear to be related to overlap with IEI—such as sick-building syndrome and nonallergic vasomotor rhinitis (nVMR). In both of these, an odor or taste often precipitates one or more organ-system responses. Examples of initiating culprits include exposure to chemicals, such as formaldehyde being released from new furniture or carpeting, pesticides in ventilation systems, sterilizing agents (quaternary amines) in indoor cleaning solutions, mildew odors found in damp environments, freon circulating in closed-ventilation systems, perfumes/potpourris, and fresh paint and volatile organic compounds released from various products used in the home and workplace.

The current consensus for establishing a diagnosis of IEI requires that the patient's syndrome satisfies six essential criteria: (1) reproducible symptoms upon repeated exposures to chemicals/irritants; (2) chronicity; (3) later manifestation of symptoms to levels of exposure lower than those previously tolerated; (4) improvement or complete resolution of symptoms upon avoidance; (5) similar symptoms when exposed to chemically unrelated substances; and (6) multiple-organ symptoms—including, but not limited to, runny nose, itchy eyes, headache, scalp pain, scratchy throat, ear ache, mental confusion or sleepiness, heart palpitations, upset stomach, nausea, diarrhea, abdominal cramping, and aching joints (5).

To study IEI as quantitatively as possible, we searched for an experimental paradigm and decided upon nVMR, a clinical phenotype that should be quantifiable and unequivocal. nVMR satisfies all of the six essential IEI criteria except neurocognitive symptoms.

nVMR is a common condition that affects between 20% and 40% of the American population suffering from chronic rhinitis symptoms. The costs of treating chronic rhinitis (both allergic and nonallergic subtypes) and its associated comorbidities (e.g., sinusitis, asthma, otitis media) exceed $10 billion annually in the United States—ranking this condition among the most expensive outpatient health care problems that physicians encounter (6–8). nVMR patients differ from allergic rhinitis patients in that they commonly experience nasal congestion, postnasal drip, sinus pressure, and ear-plugging. Triggers for nVMR include temperature and/or barometric pressure changes, postural changes, and nasal irritants, such as those listed above (9). The present study focused only on olfactory triggers. By definition, nVMR patients do not exhibit nasal eosinophilia, and allergen skin testing to seasonal and perennial allergens is negative (9). Thus, a successful diagnosis of nVMR is dependent on the exclusion of allergic rhinitis.

The mechanism of nVMR is poorly understood (10–13). It has previously been hypothesized (14) that nVMR patients, because of their intolerance to odors and irritants, might be distinguished from patients having other forms of chronic rhinitis, due to an abnormality in their olfactory transduction pathway. Paradoxically, the limited number of studies investigating odorant discrimination found a greater magnitude of olfactory loss in nVMR patients in response to common odorants, such as formalin, camphor, asafetida (India saffron spice), and oil of peppermint—as compared with allergic rhinitis patients or control subjects without rhinitis (15). The one exception was the response to musk odor; in this study (15), males manifested a greater olfactory loss than females for all odorants except asafetida. These observations are in contrast to findings by our group (16), which recently reported that nVMR patients have an olfactory threshold response similar to allergic and mixed rhinitis patients. However, both studies (16) indicate that a hyperacute sense of smell does not account for the clinical symptoms induced by odorants in nVMR patients and support our hypothesis that these

patients may have olfactory receptor (*OR*) gene polymorphisms predisposing them to this response.

Because we chose to focus only on olfactory triggers, we searched for SNPs in *OR* genes. The *OR* gene superfamily is perhaps the largest in the mammalian genome (17,18). The pseudogene/gene ratio is highest in human, lower in chimpanzee, and lowest in mouse—most likely reflecting the rodent's greater dependence on olfaction for survival than humans. Presently in human, there are 390 putatively functional *OR* genes and 465 *OR* pseudogenes located in multiple clusters of varying sizes scattered throughout all autosomes except chromosome (Chr) 18 and Chr 20, and on the X but not the Y chromosome (http://www.gene.ucl.ac.uk/nomenclature/). This total of 855 genes is grouped into 18 families and 238 subfamilies. By far, the two largest *OR* gene clusters are located on Chr 11, with at least 81 functional genes (20.7%) on 11p (Cluster II) and 89 functional genes (22.7% of total) on 11q (Cluster I). The total of 372 genes-plus-pseudogenes on Chr 11 represents 43.0% of the total *OR* genes in the human genome. In the hunt for candidate *OR* genes, we, therefore, chose Chr 11 markers within Clusters II and I as the most efficient means to screen inexpensively for the maximal number of single-nucleotide polymorphisms (SNPs) that might be associated with the nVMR phenotype. Our cohorts included first, a pedigree study, followed by a case-control study.

Materials and Methods

Subjects and DNA Isolation

Patients diagnosed with nVMR were recruited from a large community allergy practice, having 7500 active patients (72% Caucasian, 17% African-American, 10% Hispanic, and 1% Asian); about 4000 have rhinitis with an approximate breakdown of 25% allergic, 30% nonallergic, and 45% mixed rhinitis. Rhinitis was well characterized with respect to their atopic status, nasal eosinophilia, and clinical history. The nVMR phenotype is defined as having symptoms of nasal congestion and postnasal drainage triggered by one or more of 20 common irritant exposures (e.g., ammonia, antiperspirants, bleach, cold air, cooking/frying, cosmetics, crude oils, fresh newsprint, hairspray, smog, cleaning products, mildew, paint, perfume, pine, soap powder, solvent, varnish, and tobacco and wood smoke). Patients rated the severity of their symptoms in response to the 20 irritant triggers using a 10-point Likert scale (19,20), and the total irritant index value was obtained by adding each trigger score. Concomitantly, these patients are negative to prick skin testing of common seasonal/perennial allergens and show no evidence of nasal eosinophilia. Patients were selected as having a diagnosis of nVMR if they had a negative skin-prick test (defined by allergen wheal diameter in comparison to negative saline and positive histamine controls) and an irritant index of ≥24 (20). The irritant index scale was, therefore, a quantitative gradient rather than a binary trait.

For TDT analysis (21a), the prerequisite for enrollment was having two living biologic parents who were willing to participate in the study. Bloods were obtained from 30 nVMR patients and their parents (Table 1, *left*). For TDT analysis, one searches for the unbalanced transfer of a specific allele to affected children, no matter what the affected status of their parents. For the case-control analysis, 103 unrelated nVMR patients and 110 unrelated allergic rhinitis patients consented to participation in this study (Table 1 *right*). Whole blood (3 cc) was collected in a tube containing ethylenediaminetetraacetic acid (EDTA). DNA extraction was performed using the GenomicPrep™ (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA) blood DNA isolation kit. All participants signed an informed consent for genetic testing, approved by the University of Cincinnati Institutional Review Board.

SNP Marker Selection and Genotyping

Clusters II and I on human Chr 11 contain a total of 372 *OR* genes and pseudogenes; these were selected from the Olfactory Receptor Gene Source database (ORDB) (http://senselab.med.yale.edu/senselab/ORDB/files/humanor seqanal.html), and their sequences were obtained from the Human Olfactory Receptor Data Exploratorium (HORDE) (http://bioinformatics.weizmann.ac.il/HORDE/). Of these 372 *OR* genes and pseudogenes, 339 were specifically mapped (Figure 1) to Human Genome Reference Sequence by Human Genome Blast (http://www.ncbi.nlm.nih.gov/genome/seq/page.cgi?F=HsBlast.html). Two regions (corresponding to Clusters II and I), having the highest density of *OR* genes, were selected. *OR* Cluster II spans 1.7 Mb (from Chr 11 4.4 to 6.1 Mb); *OR* Cluster I spans 4.2 Mb (from 55.0 to 59.2 Mb). A total of 224 (91 + 133) *OR* genes and pseudogenes from the ORDB database were mapped precisely to these two regions. Forty-four SNP markers were selected from TaqMan® Validated SNP Genotyping Assays (Applied Biosystems, Foster City, California, USA) to cover these two regions (110 kb/SNP for Cluster II; 150 kb/SNP for Cluster I). The overall genotype-calling rate was 99.1%. For the purpose of quality control, we used 16 blind duplicate samples; no genotype inconsistencies were found with any of the markers. Detailed information about the 44 SNP markers is given in Appendix 1. SNPs were genotyped by TaqMan (22).

Statistical Analysis

Single-marker analyses of the 30 nVMR trios and the population-based association were conducted using Haploview 3.2 (22). We used the chi-squared test upon 2 × 2 allele counts (embedded within the Haploview package) in analyzing allelic associations. In order to correct for multiple-testing, the Bonferroni correction procedure was applied for the TDT analysis; empirical p-values were evaluated by the shuffling permutation test with 10^5 replicates for the case-control analysis (23).

Results

Demographics of the Populations Studied

For TDT analysis, the nVMR subjects were predominantly Caucasian, with two-thirds being female (Table 1, *left*). The patient population reported more problems than the parents with odors and irritants—based on the irritant index score. For the case-control study, the population again was predominantly Caucasian, having 81.5% nVMR female cases compared with 61.8% allergic rhinitis female controls (Table 1, *right*). Age and ethnicity were normally distributed between the case and control groups.

Genomic Analysis

As a preliminary screen, the pedigree study identified two SNPs (rs649358 and rs399208), 176.5 kb apart at the 3′ telomeric end of the Cluster I region; these SNPs were statistically significantly associated (p = 0.0043 and 0.0196) with the nVMR phenotype. Additional "significant" SNPs included the adjacent SNPs rs2515366 and rs1938596, both with p = 0.0143, another SNP (rs501828) having a *P*-value of 0.0499 in the Cluster I region, and one SNP (rs17480) in the Cluster II region (p = 0.0164).

Based on these encouraging results, we proceeded to the larger case-control population; we found no significant deviation from Hardy-Weinberg equilibrium across the 44 markers (data not shown). The SNPs that had been significant in the pedigree study were no longer significant in the case-control study; rather, four other statistically significant SNPs emerged: rs1430397 (p = 0.0094) in the Cluster II region and rs2848634 (p = 0.0490), rs1783826 (p = 0.0114), and rs2245676 (p = 0.0001) in the Cluster I region.

Table 2 summarizes the combined results for both the TDT analysis and the population-based association test. Although there were ten associations having p-values of <0.05, no marker showed significance in both studies. Moreover, no significant p-values found in the TDT analysis survived the Bonferroni correction for 44 multiple tests; this is largely due to the limited power provided by only 30 trios.

For the population-based association test, the correlation of rs2245676 with the phenotype was still significant after correcting for multiple testing, the empirical p-value being 0.0066 by shuffling cases and controls with 10^5 replicates; however, this SNP showed a p-value of 0.83 in the pedigree study. This marker is located within the Cluster I pseudogene, *OR9I2P*.

Discussion

The present study was carried out prior to the release of any HapMap consortium data. We reasoned that genetic differences in olfaction might be associated with the nVMR phenotype triggered by various potent odors. Moreover, we posited that the nVMR trait might be associated with SNPs in one or more of the *OR* genes. Just recently, in fact, olfactory detection threshold phenotypes of four odorants were screened against 43 *OR* genes, genomewide, and a strong association was found between the odorant isovaleric acid and SNPs in *OR11H7P*; this predicted receptor-ligand functional relationship was then validated using the *Xenopus* oocyte expression system, in which the intact allele of *OR11H7P* exhibited a positive response to isovaleric acid (24).

Because *OR* genes are dispersed over 20 of 22 autosomes plus the X chromosome, it was too expensive for an initial screening study to select SNPs for all of the known 390 functional *OR* genes and 465 *OR* pseudogenes. We, therefore, chose to focus on the two most densely populated *OR* gene-pseudogene clusters, examining SNP frequencies at a density of 110-150 kb per SNP, in hopes of finding some region that might be highly statistically significant in a genotype-phenotype association study.

We hypothesized that TDT analysis of 30 VMR patients and their unaffected biologic parents would be sufficient as a first-level screen: Could a very highly significant *OR* gene be identified within either of these clusters? Encouragingly, six statistically significant markers were identified by TDT analysis; in fact, two adjacent SNPs (rs649358 and rs399208), 176.5 kb apart at the 3′ telomeric end of the Cluster I region, showed an association with the nVMR phenotype, having p-values of 0.0043 and 0.0196, respectively. At this point, some laboratories would publish these data. In fact, hundreds of such publications have appeared—especially in journals having “pharmacology,” “toxicology,” “drug,” “environmental,” “pharmacogenetics,” or “pharmacogenomics” in their titles. Such studies should be regarded as inconclusive, underpowered, and in need of further replication before the findings can be regarded as “informative” (25).

None of the six sites in the pedigree study survived the Bonferroni multiple-tests correction analysis. Most likely, this is due to the low SNP marker density in this pilot study. From the data obtained by the HapMap consortium, the general rule for genomewide association studies now is to screen the genome at a density of one tag SNP per 5 kb, and for Africans the density should be one tag SNP per 2.5 kb (25).

In the present study, we then compared 103 nVMR cases with 110 allergic rhinitis controls and found that the original SNPs uncovered via TDT analysis had disappeared, whereas four new $p < 0.05$ SNPs emerged. Combination of the two sets of data (Table 2) gave no consistently statistically significant variant site—with the exception of one pseudogene; although the p-value for rs2245676 was 0.0001 in the case-control study, the p-value was 0.83 in the pedigree study. The allelic frequency of this SNP is significantly different between Caucasians and

Africans; we, therefore, cannot rule out the possibility of a false-positive finding due to population stratification. Thus, no definitive conclusions can be drawn due to the limited power of both the pedigree study and the case-control study. A list of power calculations is presented in Table 3, indicating that both the pedigree and population studies were underpowered.

However, we believe that our hypothesis of a relationship between OR genetic polymorphisms and nVMR remains feasible. Either a future genomewide association study of all *OR* gene-pseudogene regions throughout the genome—at the current recommended density (25,26) of 2.5–5 kb per tag SNP—or a microarray analyses of the entire "*OR* genome" in well-characterized nVMR patients is required to uncover an important OR polymorphism associated with the nVMR trait. Selection of these subjects might be based on their olfactory threshold responses and diagnosed using standardized psychophysical tests currently available for the assessment of olfactory function. Very recently, automated self-administered instruments, using the staircase approach for measuring olfactory responses (based on the validated 40-odor University of Pennsylvania Smell Identification Test (UPSIT), the Cross-Cultural Smell Identification Test™, and the Smell Threshold Test™), have facilitated our ability to investigate olfaction in humans (16). The current understanding of olfactory function in humans has controlled for factors such as age, gender, ethnicity, exposure to toxic agents, and various other disease states, such as Parkinson disease and Alzheimer disease (27–30).

Conclusion

In summary, further investigation of the role of *OR* gene polymorphisms in the pathogenesis of nVMR may provide valuable insight into a better understanding of this disorder. Moreover, any highly statistically significant genotype-phenotype association with an *OR* gene and nVMR may also provide important inroads into the more complex, yet poorly characterized, clinical syndromes that fall under the umbrella of IEI or multiple chemical sensitivity.

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Appendix

Appendix I.—Detailed information of the 44 SNP markers

No.	dbSNP rs_id	Celera ID	Chr	NCBI Pos.	Genetic Pos.	NCBI Gene	Celera Pos.	Celera Gene
1	rs1376681	C__3005257_10	11p	4395403	8.64		4555293	
2	rs1430397	C__8798299_10	11p	4534144	8.335		4694035	
3	rs1505204	C__3187363_10	11p	4578975	7.965	LOC390037	4738872	RNF137
4	rs1389464	C__2021191_10	11p	4693821	7.1	LOC390040	4853710	
5	rs1433897	C__8461299_10	11p	4906213	9.115		5065714	
6	rs1378736	C__8797974_10	11p	5030396	9.845	LOC119679	5181285	
7	rs2499949	C__16029966_10	11p	5115825	10.345		5266666	
8	rs916111	C__9599131_1_	11p	5233652	11.04	HBG1	5388010	HBG1
9	rs951748	C__1451737_10	11p	5381476	11.525		5535635	
10	rs934460	C__1451536_10	11p	5499491	11.105	MGC20470	5653762	MGC20470
11	rs1498553	C__1452175_10	11p	5673337	11.735		5828125	TRIM22
12	rs1453424	C__7695347_10	11p	5781842	12.215		5936563	

No.	dbSNP rs_id	Celera ID	Chr	NCBI Pos.	Genetic Pos.	NCBI Gene	Celera Pos.	Celera Gene
13	rs1377512	C__9604514_10	11p	5783926	12.215	LOC390076	5938647	
14	rs1463289	C__9604619_10	11p	5876789	12.625		6032055	
15	rs17480	C__12033057_10	11p	5990420	12.775	LOC120793	6145694	
16	rs1462983	C__9604662_10	11p	6094146	12.435	LOC196335	6249340	
17	rs649358	C__8904618_10	11q	55024839	59.955	LOC219425	52592871	
18	rs399208	C__3109726_10	11q	55202882	59.665		52769375	
19	rs984371	C__3109488_10	11q	55353058	59.415	LOC219437	52917849	
20	rs1384101	C___268897_10	11q	55569236	59.055		53134041	
21	rs2460211	C__8132897_10	11q	55670193	58.885		53234988	
22	rs1481928	C__1870209_10	11q	55797294	58.675		53362064	
23	rs1945245	C__11666818_10	11q	55924487	58.465	LOC390159	53496810	
24	rs637404	C___746124_10	11q	56246728	58.4		53830121	
25	rs502943	C___732012_10	11q	56354976	58.4		53938382	
26	rs1788998	C__8905573_10	11q	56363335	58.4	OR5G3P	53946741	
27	rs1793426	C__8905725_10	11q	56459110	58.4	LOC390180	54042474	
28	rs501828	C__3043337_10	11q	56572812	58.4		54156411	
29	rs1943482	C__7986115_10	11q	56770969	58.4		54354555	AGTRL1
30	rs3781902	C__8131596_10	11q	56895407	58.4	P2RX3	54479076	P2RX3
31	rs2848634	C__2950363_10	11q	57027688	58.4	SLC43A1	54608883	RTN4RL2
32	rs1783826	C__2864618_10	11q	57178324	58.4		54759451	FLJ30213
33	rs530094	C___924939_1_	11q	57306397	58.4	CTNND1	54888301	CTNND1
34	rs1704781	C__1983937_10	11q	57422668	58.4		55004335	
35	rs921134	C__2161631_10	11q	57570756	58.4		55152459	
36	rs2245676	C__1334145_10	11q	57688053	58.4	OR9I2P	55269667	
37	rs1938663	C__11388708_10	11q	57860843	59.495	LOC219964	55443912	
38	rs2515366	C__10094781_10	11q	58049730	58.705	LOC219968	55633478	
39	rs1938596	C__3185821_10	11q	58157046	58.4	ZFP91-CNTF	55740786	
40	rs1938709	C__8137912_10	11q	58311191	58.4		55894916	
41	rs1892866	C__11330707_10	11q	58457509	58.4		56041242	MGC15937
42	rs1938781	C__3160372_10	11q	58690470	58.4	FLJ22794	56274211	FLJ22794
43	rs1545527	C__8141059_10	11q	59029755	58.4	LOC401696	56615677	—
44	rs3758872	C__11668147_10	11q	59196535	58.4	FLJ36874	56783923	FLJ36874

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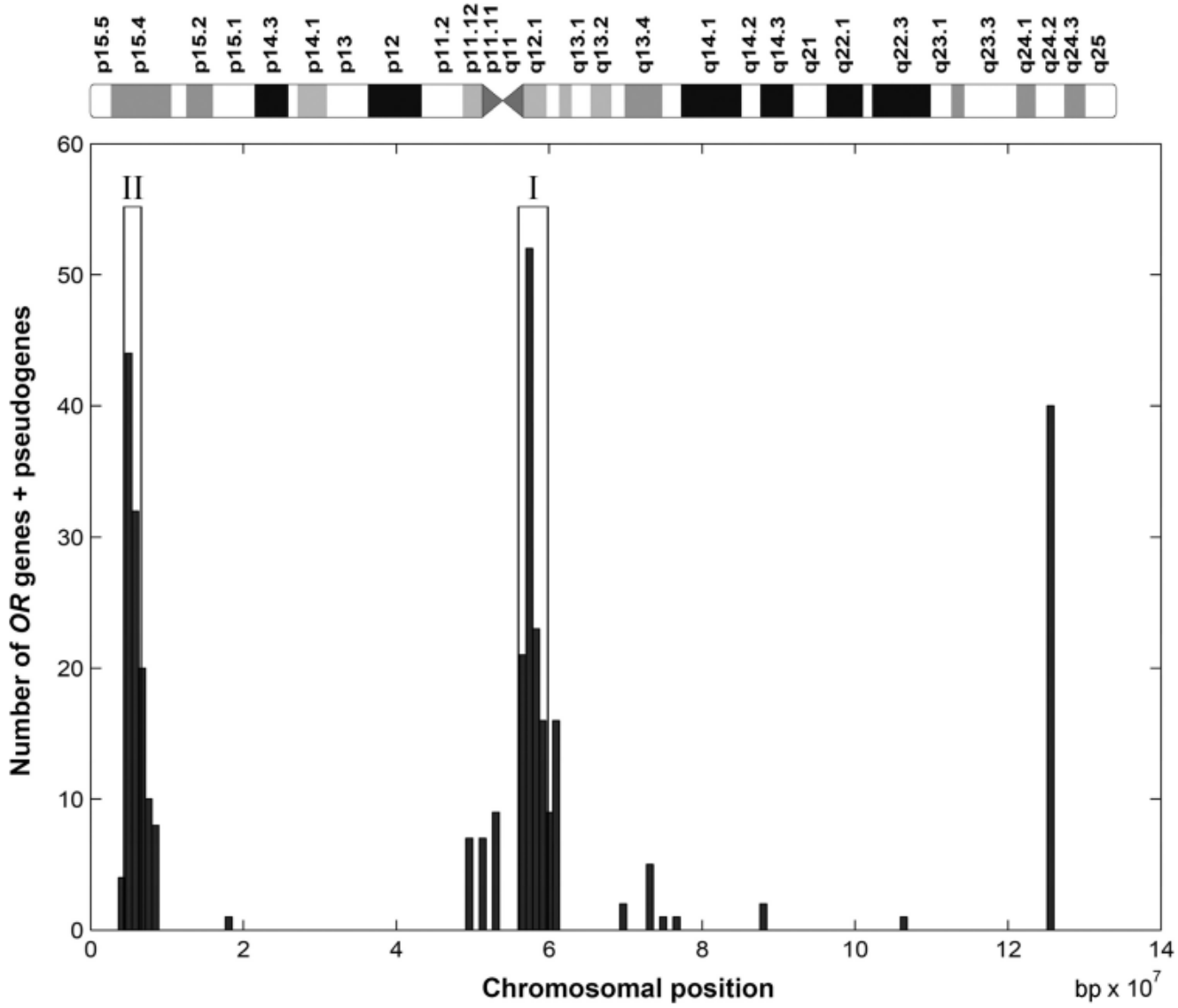

Figure 1.

Distribution of olfactory receptor (*OR*) genes + pseudogenes on chromosome (Chr) 11. Total length of Chr 11 is 134 Mb. The two regions with dense *OR* genes correspond to *OR* gene Cluster II (11p, *left*) and Cluster I (11q, *middle*). The candidate regions in this study were selected as those with the highest density of *OR* genes, using the sliding window technique, and do not cover the entire Cluster II and I regions. Embedded within either cluster are possible candidate genes for nonallergic vasomotor rhinitis other than *OR* genes—such as the gene for C1 esterase inhibitor, an angiotensin II antagonist-like gene, and a purinergic receptor gene.

Table 1

Demographics of the pedigree study and the case-control study.

	Pedigree study		Case-control study	
	Patients (N = 30)	**Parents (N = 58)[a]**	**nVMR cases (N = 103)**	**Allergic rhinitis controls (N = 110)**
Average age (years)	22.8	43	48	44
Gender (F:M)	20:10	29:29	84:19	68:42
Caucasian:African-American	28:2	54:4	99:4	101:9
Irritant index score[b]	29	20.5	—	—

nVMR, nonallergic vasomotor rhinitis.

[a] One subject was also the parent of a subject.

[b] The average irritant index score is based on a scale of 1 to 100; 20 different stimuli were rated from 0 (least) to 5 (most) bothersome, as described in the text.

Table 2

Combined results from TDT and population-based association tests for the SNPs in *OR* Cluster II (markers 1–16) and Cluster I (markers 17–44).

Markers			Allele frequencies			Association tests	
No.	**rs_id**	**Celera ID**	**Minor allele**	**nVMR (103)**	**AR[a](110)**	**TDT p-value**	**nVMR vs. AR[a] p-value**
1	rs1376681	C___3005257_10	C	0.475	0.458	0.5127	0.7182
2	rs1430397	C___8798299_10	T	0.328	0.453	0.5637	0.0094
3	rs1505204	C___3187363_10	C	0.475	0.388	0.5775	0.0720
4	rs1389464	C___2021191_10	C	0.471	0.432	0.1573	0.4329
5	rs1433897	C___8461299_10	A	0.387	0.402	0.2888	0.7600
6	rs1378736	C___8797974_10	G	0.460	0.486	0.6547	0.5947
7	rs2499949	C__16029966_10	A	0.221	0.201	0.7630	0.6222
8	rs916111	C___9599131_1_	A	0.431	0.458	0.6949	0.5848
9	rs951748	C___1451737_10	C	0.475	0.481	0.8527	0.9053
10	rs934460	C___1451536_10	C	0.475	0.500	0.8415	0.6111
11	rs1498553	C___1452175_10	C	0.461	0.453	0.0679	0.8707
12	rs1453424	C___7695347_10	C	0.279	0.338	0.2207	0.1965
13	rs1377512	C___9604514_10	G	0.279	0.349	0.2207	0.1263
14	rs1463289	C___9604619_10	C	0.421	0.386	0.7237	0.4680
15	rs17480	C__12033057_10	G	0.446	0.411	0.0164	0.4715
16	rs1462983	C___9604662_10	T	0.396	0.401	0.2393	0.9189
17	rs649358	C___8904618_10	A	0.453	0.424	0.0043	0.5617
18	rs399208	C___3109726_10	A	0.485	0.495	0.0196	0.8363
19	rs984371	C___3109488_10	C	0.201	0.217	0.2008	0.6883
20	rs1384101	C____268897_10	C	0.119	0.179	0.5271	0.0850
21	rs2460211	C___8132897_10	A	0.325	0.386	0.8575	0.1994
22	rs1481928	C___1870209_10	C	0.328	0.387	0.8575	0.2146
23	rs1945245	C__11666818_10	T	0.267	0.307	0.4328	0.3774
24	rs637404	C____746124_10	C	0.307	0.316	0.3532	0.8415
25	rs502943	C____732012_10	T	0.381	0.429	1.0000	0.3196
26	rs1788998	C___8905573_10	C	0.387	0.420	0.8474	0.4987
27	rs1793426	C___8905725_10	A	0.307	0.362	0.8415	0.2372

Markers			Allele frequencies			Association tests	
No.	**rs_id**	**Celera ID**	**Minor allele**	**nVMR (103)**	**AR[a](110)**	**TDT p-value**	**nVMR vs. AR[a] p-value**
28	rs501828	C___3043337_10	G	0.431	0.395	0.0499	0.4553
29	rs1943482	C___7986115_10	G	0.299	0.302	0.1936	0.9492
30	rs3781902	C___8131596_10	A	0.500	0.533	0.3692	0.5005
31	rs2848634	C___2950363_10	G	0.196	0.278	0.5127	0.0490
32	rs1783826	C___2864618_10	T	0.371	0.495	1.0000	**0.0114**
33	rs530094	C____924939_1_	A	0.275	0.215	0.2253	0.1565
34	rs1704781	C___1983937_10	C	0.289	0.238	0.3173	0.2375
35	rs921134	C___2161631_10	G	0.319	0.330	0.2008	0.8012
36	rs2245676	C___1334145_10	G	0.480	0.297	0.8273	**0.0001**
37	rs1938663	C__11388708_10	G	0.392	0.373	0.0719	0.6822
38	rs2515366	C__10094781_10	T	0.436	0.429	**0.0143**	0.8850
39	rs1938596	C___3185821_10	G	0.436	0.435	**0.0143**	0.9721
40	rs1938709	C___8137912_10	A	0.230	0.250	0.8185	0.6399
41	rs1892866	C__11330707_10	T	0.500	0.552	0.7055	0.2893
42	rs1938781	C___3160372_10	G	0.255	0.298	0.4913	0.3274
43	rs1545527	C___8141059_10	C	0.319	0.294	0.8273	0.5910
44	rs3758872	C__11668147_10	C	0.000	0.000	0.0578	n.a.

TDT, transmission disequilibrium test; SNPs, single-nucleotide polymorphisms; nVMR, nonallergic vasomotor rhinitis.

[a]AR, allergic rhinitis; these patients do not have nVMR.

Table 3

Power calculations for this study, if we assume the test marker is in perfect linkage disequilibrium with the true causative locus.[a]

A: where $\alpha = 0.05$ (without correction for multiple-testing)		
H^2	**TDT**	**Case-control**
0.02	0.121	0.464
0.05	0.231	0.849
0.10	0.403	0.989
0.20	0.669	1.000
B: where $\alpha = 0.001$ (with correction for multiple-testing)		
H^2	TDT	Case-control
0.02	0.006	0.078
0.05	0.019	0.383
0.10	0.058	0.836
0.20	0.186	0.997

TDT, transmission disequilibrium test.

[a]The optimal situation is calculated with a K = 0.20 (incidence of nonallergic vasomotor rhinitis in the United States is estimated to be between 20% and 25%) and the minor allele frequency = 0.1. H^2 denotes heritability (the proportion of phenotypic variation ascribed to the genetic variability at the putative locus under investigation). The reason for including an H^2 of 0.10 and 0.20 is to attain “the highest achievable” statistical power for our small-sample TDT analysis—although such high heritability levels seldom occur in common diseases. Additivity is assumed in these power calculations. These calculations underscore the fact that the power of the TDT analysis is far lower than that of the case-control study.