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Pilot screening study of targeted genetic polymorphisms for association with seasonal influenza hospital admission

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Abstract

Host response to influenza is highly variable, suggesting a potential role of host genetic variation. To investigate the host genetics of severe influenza in a targeted fashion, 32 single nucleotide polymorphisms (SNPs) within viral immune response genes were evaluated for association with seasonal influenza hospitalization in an adult study population with European ancestry. SNP allele and genotype frequencies were compared between hospitalized influenza patients (cases) and population controls in a case-control study that included a discovery group (26 cases and 993 controls) and two independent, validation groups (one with 84 cases and 4,076 controls; the other with 128 cases and 9,187 controls). Cases and controls had similar allele frequencies for variant rs12252 in interferon-inducible transmembrane protein 3 (*IFITM3*) ($P > 0.05$), and the study did not replicate the previously reported association of rs12252 with hospitalized influenza. In the discovery group, the preliminary finding of an association with a nonsense polymorphism (rs8072510) within the schlafen family member 13 (*SFLN13*) gene ($P = 0.0099$) was not confirmed in either validation group. Neither rs12252 nor rs8072510 showed an association according to the presence of clinical risk factors for influenza complications ($P > 0.05$), suggesting

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CONFLICTS OF INTEREST

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that these factors did not modify associations between the SNPs and hospitalized influenza. No other SNPs showed a statistically significant association with hospitalized influenza. Further research is needed to identify genetic factors involved in host response to seasonal influenza infection and to assess whether rs12252, a low-frequency variant in Europeans, contributes to influenza severity in populations with European ancestry.

Keywords

influenza; host susceptibility; *IFITM3*; polymorphisms; virus

INTRODUCTION

Human influenza infections are common, and outcomes range from mild or asymptomatic infection to life-threatening illness. Both animal and human studies suggest that host genetic variation is a contributor to influenza infection severity,[1–3] and several human studies have investigated a potentially functional single nucleotide polymorphism (SNP), rs12252, located at a predicted splice acceptor site in interferon-inducible transmembrane protein 3 (*IFITM3*), an anti-viral effector that mediates the host innate immune response to influenza infection.[4–7] The minor CC genotype of rs12252 was associated with hospitalized influenza in patients of European ancestry and with severe influenza in a Han Chinese study population.[3, 8] Further, the CC genotype was associated with severe clinical infection by avian-origin H7N9 influenza virus.[9] However, three other studies were unable to confirm the association between the CC genotype and severe influenza.[10–12]

Knowledge of host genetic variants that are associated with influenza severity could be useful for identifying subpopulations at higher risk for severe influenza infection and might also yield insight into molecular mechanisms that underlie aberrant response to influenza infection. Because the role of host genetics as a modulator of influenza illness severity is understudied and evidence from animal studies suggests that host susceptibility to influenza is not controlled by a single genetic locus,[13] this study sought to evaluate a set of candidate SNPs in genes involved in immune response to viral infection, including *IFITM3* rs12252, for association with severe (hospitalized) seasonal influenza. It also attempted to validate the results of hospitalized influenza analyses using independent study samples.

MATERIALS AND METHODS

Study subjects

The study included three groups of subjects: a discovery group and two validation groups. For the discovery group, samples and data were obtained from subjects who had participated in two unrelated studies at Marshfield Clinic, Wisconsin, USA. Subject selection began with a cohort of 5,368 adults with acute respiratory illness who had been tested for influenza in seasonal studies of influenza vaccine effectiveness from 2004–05 through 2011–12, including the 2009 pandemic.[14–18] Of this cohort, 2,294 (42.7%) individuals had independently provided DNA for future research as part of the Personalized Medicine Research Project (PMRP), a population-based bio-bank of about 20,000 adults

(approximately 98% of European ancestry) from central Wisconsin, USA.[19] This provided an opportunity to investigate the association of influenza-related hospital admission with specific SNPs in an ethnically homogeneous, community population.

Of 2,294 subjects, 500 (21.8%) had a nasopharyngeal swab that tested positive for influenza using real-time reverse transcription polymerase chain reaction (rRT-PCR) to detect the genes encoding for the matrix protein (M1) of influenza A virus and the non-structural protein 1 (NS1) of influenza B virus. Of the 500 patients with laboratory-confirmed influenza, 26 (5.2 %) were hospitalized within 14 days after illness onset, 24 (4.8%) were seen at the Emergency Department within 14 days of influenza infection but were not hospitalized, and the remaining 450 (90.0%) were outpatients. Hospitalization was used as an indicator of severe influenza infection in this study, and the discovery group sample consisted of the 26 patients hospitalized for seasonal influenza (cases) and a random sample of 993 PMRP participants (controls) (Fig. 1).

To confirm associations observed in the discovery group, two validation groups were used (Fig. 1). The first was an independent group of 84 cases and 4,076 controls from the PMRP. The second consisted of 128 cases and 9,187 controls (all non-Hispanic white adults) from the Vanderbilt University Medical Center's BioVU bio-bank resource.[20] Validation group cases were subjects whose medical record data indicated a positive laboratory test for influenza A or B (mostly between the years 2001 and 2015) and hospitalization within 14 days after the laboratory test. The approach to case identification was different for the discovery and validation groups. In the discovery group, cases were patients with acute respiratory infection who had been actively recruited in an outpatient setting into studies of influenza vaccine effectiveness based on self-report of the presence and duration of respiratory symptoms. By contrast, in the validation groups, cases were identified as a result of having undergone diagnostic testing for influenza at or before hospital admission, based on clinician orders. Therefore, cases in the validation groups may have had more chronic illnesses compared with cases in the discovery group. Validation group controls were subjects who did not have a positive laboratory test for influenza documented in their medical records and who had genome-wide SNP genotype data available due to participation of the PMRP and BioVU bio-banks in the Electronic Medical Records and Genomics (eMERGE) network,[21] a consortium that uses genomic data linked to electronic medical records to study personalized medicine.

All PMRP subjects gave informed consent at enrollment in PMRP for future use of their medical record data and DNA samples in research studies. The Vanderbilt BioVU resource operates as non-human subjects research according to the provisions of the Code of Federal Regulations 45, part 46.[20] This study was approved by the Marshfield Clinic Institutional Review Board.

Data collection

For cases in the discovery group, hospital discharge summaries were reviewed for all hospital admissions to confirm that acute influenza infection was a contributing factor. A review of hospital discharge summaries was not performed for cases in the two validation groups. However, among the 84 cases in the PMRP validation group, 71 (84.5%) were

hospitalized within 24–48 hours, and 83 (98.8%) were hospitalized within eight days, after testing positive for influenza. Data on time to hospital admission were available for 122 of the 128 Vanderbilt cases, and the corresponding numbers of subjects were 117 (95.9%) and 118 (96.7%). Therefore, it was considered likely that the influenza infection contributed to the hospitalization of cases in the validation groups. For all cases in the discovery and validation groups, comorbid conditions (listed in Table S1) that were present within one year before the influenza diagnosis, mean body mass index (BMI), and use of immunosuppressive medications within six months before the influenza diagnosis were obtained from the electronic medical record. For comorbidities, a timeframe of one year before the influenza diagnosis was chosen to identify new or existing, unresolved, chronic conditions that could influence the risk of influenza complications. Mean BMI was calculated from weight and height measurements obtained during the one-year period before influenza diagnosis. For cases from the PMRP, receipt of an influenza vaccination within six months before the influenza diagnosis was determined using a validated immunization registry.[22]

SNP selection

Three main criteria were used to select 51 SNPs located in or near genes that are thought to be involved in host response to viral infection (references listed in Table S2): (1) SNPs that are putative expression quantitative trait loci (eQTL) based on a statistically significant ($P < 0.01$) Spearman correlation coefficient between SNP genotype and mRNA expression in lymphoblastoid cell lines from the HapMap population with Northern European ancestry (HapMap CEU);[23] (2) putative functional nonsense and missense SNPs; and (3) tagging SNPs for human leukocyte antigen alleles. While designing the multiplexed genotyping assay, 19 SNPs were removed from the assay design to improve the predicted performance of the assay, leaving 32 SNPs for genotyping.

Genotyping

In the discovery group, 30 SNPs were genotyped using a multiplexed assay on the MassARRAY iPLEX SNP genotyping system (Agena Bioscience, San Diego, California, USA) and two SNPs (rs12252 and rs2476601) using TaqMan assays (ThermoFisher Scientific, Grand Island, New York, USA). The sequences of primers used for genotyping are listed in Table S3. Primers for the rs12252 and rs2476601 TaqMan assays were custom-designed and pre-designed, respectively. The rs12252 TaqMan assay was tested by Sanger sequencing with the use of previously published primers.[8] Five HapMap samples (NA07357 T/T, NA11830 T/T, NA11831 C/T, NA12763 C/T, and NA12873 C/T) and a subset of 12 samples (1 C/C, 6 C/T, 5 T/T) from subjects in the discovery group were sequenced, and the TaqMan and Sanger sequencing results were 100% concordant. Genotyping of the 32 SNPs was attempted, and successfully performed, for all subjects in the discovery group. The genotype call rate in this group was 99.99%: only one genotype for one study subject was not called.

Two SNPs were genotyped in the PMRP validation group: *IFITM3* rs12252 and *SFLN13* rs8072510 (Fig. 1). The aforementioned TaqMan assay with custom-designed primers was used to genotype rs12252 in all 84 cases and in 301 controls in the PMRP validation group.

All 84 cases were also genotyped for rs8072510 using a TaqMan assay with pre-designed primers (Table S3). For the two assays, genotype concordance was 100% when replicate samples for 10% of subjects were genotyped, and the genotype call rate was 100%. In the PMRP validation group, genotype data for rs8072510 were available for 3,775 controls that had been genotyped previously using the Illumina Human660 W-Quadv1_A genotyping platform.[24]

In the Vanderbilt validation group, subjects had been genotyped previously for rs12252 and rs8072510 on one of four Illumina platforms: Human660W-Quadv1_A, HumanOmni1-Quad, HumanOmni5-Quad, and Human1M-Duo.[24, 25] SNP rs8072510 was genotyped in all 128 cases and 9,187 controls; SNP rs12252 was genotyped in 70 cases and 8,719 controls (Fig. 1).

Statistical analysis

To examine differences between hospitalized cases and other patients with influenza in the PMRP, selected characteristics of hospitalized cases in the discovery and validation groups were compared to characteristics of the 450 outpatients with laboratory-confirmed influenza in the discovery group cohort. Statistical tests employed were the Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables. The comparisons were made using SAS software (version 9.3; SAS Institute, Cary, North Carolina, USA).

Genotype association analyses were performed using PLINK.[26] For each SNP, Weir's exact test was used to assess Hardy-Weinberg equilibrium in cases and controls, separately, in the discovery and validation groups. To test for SNP associations with hospitalized influenza, allele and genotype frequency association tests were performed using Fisher's exact test. For *IFITM3* rs12252, previously reported to be associated with hospitalized influenza, and for any other SNPs showing associations in the discovery group, tests for associations with hospitalized influenza were also performed in the PMRP validation group, the combined PMRP discovery and validation groups, and the Vanderbilt validation group. To minimize confounding, analyses of the combined group and the Vanderbilt validation group were repeated after excluding hospitalized cases with any risk factors that increase the risk of influenza complications:[27] ≥ 65 years of age, morbid obesity (BMI ≥ 40 kg/m^2), diagnosis of at least one comorbid condition (listed in Table S1) within one year before influenza diagnosis, and use of immunosuppressive medications within six months before influenza diagnosis. SNP association analyses were corrected for testing of 32 SNPs using the Bonferroni method, and a *P*-value < 0.0015 (0.05/32) was considered statistically significant.

RESULTS

Hospitalized influenza cases in the PMRP discovery and validation groups were older, had a higher prevalence of comorbid conditions and immunosuppressive medication use, and were more likely to have received an influenza vaccine during the prior six months compared with outpatients who had influenza (Table I). More than one-third of hospitalized cases in the discovery group and of outpatient influenza infections occurred during the 2007–08 season dominated by influenza A (H3N2), compared with only 12% of hospitalized cases in the validation group. Influenza subtype was not collected consistently during the study period

and many subjects had missing data for subtype. One hospitalized case in the discovery group, five in the validation group, and 70 outpatients were infected with pandemic H1N1pdm09. A comparison of the proportions of the non-missing subtypes among the three groups showed no statistically significant differences. The characteristics of cases in the Vanderbilt validation group are described in Table S4. Compared with hospitalized cases in the PMRP discovery and validation groups, a smaller percentage of Vanderbilt cases were ≥ 65 years and a larger percentage were males or had used immunosuppressive medications. Mean BMI and the percentage of subjects who were morbidly obese or had comorbid conditions were similar between Vanderbilt and PMRP cases. Also, similar to PMRP cases, most Vanderbilt cases were infected with influenza A virus. No data were available on influenza subtype for hospitalized cases in the Vanderbilt validation group. Although case ascertainment was different in the discovery and validation groups, the proportion of cases with chronic diseases was not larger in the two validation groups than in the discovery group: approximately 50% of cases in all three groups had comorbid conditions (Table I and Table S4).

Of the 32 SNPs genotyped in the discovery group, one (interferon regulatory factor 2 (*IRF2*) rs59219184) was found to be monomorphic as no subject carried the minor allele. After excluding this SNP, 31 SNPs remained for analysis (Table II). All 31 SNPs were consistent with Hardy-Weinberg equilibrium ($P > 0.01$) in cases and controls in the discovery group. *IFITM3* rs12252 and *SFLN13* rs8072510 were also in Hardy-Weinberg equilibrium ($P > 0.01$) in the cases and controls genotyped for these SNPs in the PMRP and Vanderbilt validation groups.

In tests of allele frequency, two SNPs were associated with hospitalized influenza at the nominal *P*-value threshold of 0.05: schlafen family member 13 (*SLFN13*) rs8072510 and interferon alpha and beta receptor subunit 2 (*IFNAR2*) rs1131668 (Table II and Table S5). The *SLFN13* rs8072510 minor T allele had the strongest association with increased risk of hospitalized influenza in the discovery group (allelic *P*-value = 0.0099 for comparison with PMRP controls; Table S5) but the *P*-value was not below the threshold value of 0.0015 for statistical significance based on Bonferroni adjustment. Because rs8072510 had the lowest allelic *P*-value of all the SNPs tested, associations with genotype frequency and with dominant and recessive genetic models for rs8072510 were also examined in the discovery group. Statistically significant associations were observed with genotype frequency and the recessive model (when PMRP controls were used as the comparison group in Table S5), suggesting that the rs8072510 minor TT genotype was associated with hospitalized influenza. However, no association between rs8072510 and hospitalized influenza was observed in the PMRP validation group, the combined PMRP discovery and validation groups, or the Vanderbilt validation group (Table S6). In addition, none of the other SNPs evaluated in this study showed associations that remained statistically significant after Bonferroni adjustment (Table II).

Because no associations were observed, a post-hoc analysis of the PMRP discovery group was performed to calculate the minor allele frequencies in hospitalized influenza cases that would show a statistically significant difference when compared with the observed minor allele frequencies in controls (Table S7). The ratio of the calculated minor allele frequencies

in cases to the observed minor allele frequencies in controls was considered as the magnitude of the fold-change in minor allele frequency that would produce a statistically significant association, assuming alpha = 0.0015 and 80% power. For the 31 SNPs, the ratio ranged from 1.9 to 60.0 with a median value of 2.8. This range indicated that a modest to large fold-change in minor allele frequency was required, depending on the SNP, for a statistically significant difference to be detected. The ratio of the observed minor allele frequencies in cases to that in controls ranged from 0.7 to 2.2 with a median value of 1.0 (Table S7), suggesting that no associations were detected in Table II because the minor allele frequencies of most of the 31 SNPs were similar in cases and controls.

For *IFITM3* rs12252, the frequency of the minor C allele was zero in hospitalized cases in the discovery group, 0.036 in controls in the discovery group, and 0.041 in the 1000 Genomes[28] European ancestry population (Table II). There was no statistically significant difference in the C allele frequency when hospitalized cases were compared with either controls ($P = 0.26$) or the 1000 Genomes European ancestry population ($P = 0.26$), using Fisher's exact test (Table S8). One hospitalized case in the PMRP validation group and four controls but no cases in the Vanderbilt validation group had the minor CC genotype (Table III). No statistically significant associations between rs12252 and hospitalization for seasonal influenza were observed in analyses involving the two validation groups, including when subjects at risk for influenza complications were excluded (Table III).

To determine whether risk factors for influenza complications modified the associations between the SNPs and hospitalized influenza, gene-environment interactions were investigated by testing for associations within risk factor categories (Table IV). Age is an important risk factor for influenza hospitalization;[29] therefore, associations were examined separately for cases < 65 years and ≥ 65 years at diagnosis. Associations were also tested according to the presence or absence of other clinical risk factors for influenza complications. These exploratory analyses, performed for rs12252 (Table IV) and rs8072510 (Table S9), did not detect differences in SNP associations with hospitalized influenza by risk factor categories, based on the *P*-value threshold of 0.0015.

DISCUSSION

This pilot screening study to evaluate the association between hospitalized influenza and 32 SNPs in genes postulated to play a role in host response to seasonal influenza infection did not show statistically significant results for any of the interrogated SNPs, including *IFITM3* rs12252 previously reported[3, 8, 9] to be associated with hospitalized influenza. One reason could be low power to detect associations due to the small number of hospitalized cases in the discovery group; the sample size of this group was limited by the number of individuals who both contributed DNA to the PMRP and were enrolled and tested for influenza during an acute respiratory illness. The PMRP and Vanderbilt validation groups each had a larger sample size (≥ 70 cases and ≥ 1,200 controls) than the discovery group but no association between hospitalized influenza and *IFITM3* rs12252 was observed in analyses of these groups. In the combined PMRP discovery and validation groups, the frequency of the rs12252 minor C allele in the 110 cases would need to be 0.131 to achieve at least 80% power to observe a difference in C allele frequency between cases and controls at alpha =

0.0015, given that the C allele frequency in the 1,294 controls was 0.035. However, the C allele frequency was similar in the cases and controls. Further, the rs12252 homozygous risk genotype was found in only one hospitalized case in the PMRP validation group and was absent among hospitalized cases in the discovery group and the Vanderbilt validation group. Therefore, similar to previous reports[10–12] that observed no association between rs12252 and hospitalized influenza, the rs12252 minor C allele and homozygous risk genotype were present at low frequency in study populations with European ancestry.

IFITM3 restricts infection by viruses that exploit endocytosis pathways to enter host cells (such as the influenza virus) by becoming localized to endocytic vesicles and preventing fusion of the virus with endosomal cell membranes, thereby inhibiting release of virions into the host cell cytosol.[5, 7] Obstructing the endocytosis of IFITM3 protein reduces its ability to inhibit influenza virus infection.[30] The rs12252 minor C allele creates a predicted splice site that would generate a putative, truncated *IFITM3* transcript lacking the first 21 N-terminal amino acids (Δ1-21 variant),[3] including the Y20 amino acid, located in a motif that is recognized and bound by the AP-2 complex leading to trafficking of IFITM3 protein to endosomes and lysosomes.[30] The Δ1-21 variant protein was shown to accumulate at the plasma membrane rather than at endosomal membranes and to have reduced activity to restrict influenza virus infection *in vitro*.[31, 32] Mutation or deletion of Y20 resulted in similar effects.[30, 31, 33, 34] Lymphoblastoid cells and peripheral blood mononuclear cells from individuals with the rs12252 minor CC genotype did not express the Δ1-21 variant protein;[3, 35] therefore, the relevance of the Δ1-21 variant protein to the level of severity of influenza infection in humans is unclear. At present, it is unknown whether the predicted splice site is used for splicing *in vivo*, and experimental studies of splice site usage are needed to determine whether the rs12252 minor C allele has a functional effect that impedes the ability of *IFITM3* to restrict influenza virus infection.

A conservative approach was applied to correct for multiple testing in this study. The Bonferroni method was used when performing allele and genotype association tests for 32 SNPs to control for the probability of rejecting the null hypothesis at alpha = 0.05, given that the null hypothesis is true. Because this was a screening study, concern about generating false positive results prompted use of stringent methods to reduce the chances of false positive associations.

Hospital admission for influenza was used as a proxy for severe influenza infection in this study; however, multiple non-genetic factors increase the risk for hospital admission, including chronic disease.[36] The study accounted for some of these factors in statistical analyses, but it was not possible to adjust for all potential confounders due to the small sample size. In addition, the study was unable to account for protection from pre-existing neutralizing antibodies[37] and the virulence of different influenza viral strains.[38] Influenza-related hospitalizations and deaths are greater during seasons in which H3N2 is the dominant subtype[38] but this study could not evaluate the effect of influenza subtype on the associations between the SNPs and hospitalized influenza because it was limited by missing data on subtype for many subjects. Evidence that influenza subtype may not modify associations between host genetic variants and hospitalized influenza includes the observation that the same subtype infects both hospitalized cases and outpatients in any

given season[39] and the findings of a study in a Chinese population that associations between influenza-related mortality and two SNPs (rs12252 in *IFITM3* and rs5743313 in the toll-like receptor 3 gene) were consistent for the two subtypes (H1N1pdm09 and H7N9) examined.[40] An important limitation is that genetics may make only a small contribution to influenza severity in older adults. In the PMRP discovery and validation groups, age at influenza diagnosis was ≥ 65 years for > 60% of cases. Chronic disease, smoking, frailty, immunosenescence, and circulating levels of antibodies against influenza may be far more important in this age group. The analyses performed for low-risk cases in this study accounted for some of these factors, but the low-risk groups were small in size and no data were available on influenza antibody titers. If host genetics have a significant impact on risk of influenza-related hospitalization, it would most likely be observed in children who have a more limited lifetime exposure to influenza and fewer comorbid conditions. Notably, a recent study in a pediatric population observed no association between *IFITM3* rs12252 and severe influenza infection,[35] similar to this study's findings for adults. Another limitation was the inability to distinguish whether hospital admission within 14 days after illness onset was a direct consequence of the primary viral infection or was due to a secondary bacterial infection. Secondary bacterial pneumonia was considered to be a complication of the primary influenza infection and to be associated with the severity of the primary infection. Therefore, regardless of whether hospital admission was because of the primary influenza infection or a secondary infection, hospitalization was considered as an indicator of severe influenza.

In conclusion, this study examined a wider selection of genetic variants for association with severe influenza in humans than previous studies, but no association was observed between the 32 SNPs in genes involved in immune defense against viral infection, including *IFITM3* rs12252, and hospitalization for seasonal influenza. More research is necessary to investigate the independent contributions of non-genetic and genetic factors to influenza severity, and one possible approach is to focus on pediatric populations because children often lack the clinical (non-genetic) risk factors that increase the risk of influenza-related complications and hospitalization. Identifying host genetic factors involved in the pathogenesis of influenza severity is important because these factors are potential molecular targets for therapies aimed at mitigating influenza severity, and by offering the therapies to patients most likely to benefit from them (patients who carry the genetic factors), clinical care for severe influenza may be improved through personalized medicine in future. The immune response to influenza is complex and affected by multiple factors such as immunosenescence, host fragility, prior vaccination and infection, virus subtype and virulence, and host genetics. A systems biology approach may be helpful for understanding how these factors interact to determine influenza severity and outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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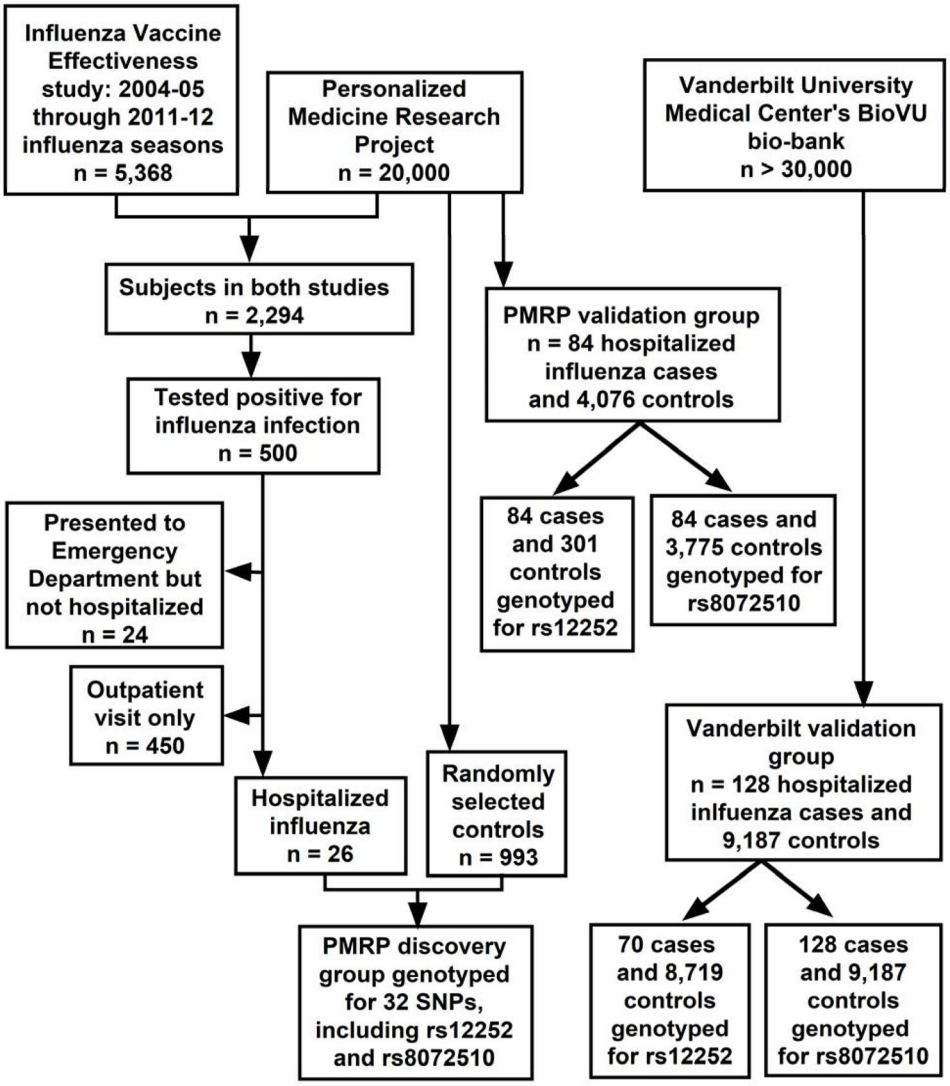

FIGURE 1.

Numbers of subjects in the discovery and validation groups and the genotype data available for each group

TABLE I

Characteristics of hospitalized and outpatient cases with influenza in the Personalized Medicine Research Project[a]

Characteristic	Hospitalized cases from discovery group (n = 26)	Hospitalized cases from validation group (n = 84)	Outpatient influenza from discovery cohort (n = 450)
Age at influenza diagnosis (years), mean ± SD	65 ± 16[b]	71 ± 17[b]	49 ± 16
Age ≥ 65 years at influenza diagnosis, n (%)	16 (61.5)[b]	57 (67.9)[b]	89 (19.8)
Males, n (%)	11 (42.3)	31 (36.9)	170 (37.8)
Body mass index (kg/m^2), mean ± SD[c]	28.9 ± 7.2	31.5 ± 9.1	31.4 ± 7.3
Obesity (body mass index ≥ 30 kg/m^2), n (%)[c]	6 (37.5)	41 (51.9)	156 (50.5)
Morbid obesity (body mass index ≥ 40 kg/m^2), n (%)[c]	1 (6.3)	7 (8.9)	39 (12.6)
Co-morbid conditions, n (%)[d]	14 (53.9)[b]	40 (47.6)[b]	59 (13.1)
Immunosuppressive drug use, n (%)[e]	3 (11.5)[f]	8 (9.5)[f]	9 (2.0)
Influenza vaccine, n (%)[g]	18 (69.2)[f]	52 (61.9)[f]	214 (47.6)
Influenza type, n (%)			
A	19 (73.1)	72 (85.7)	347 (77.1)
B	7 (26.9)	12 (14.3)	100 (22.2)
A and B	0 (0.0)	0 (0.0)	3 (0.7)
Influenza subtype, n (%)			
A(H3N2)	13 (50.0)	6 (7.1)	205 (45.5)
A(H1N1pdm09)[h]	1 (3.8)	5 (6.0)	70 (15.6)
A(H1N1)	2 (7.7)	0 (0.0)	29 (6.4)
B(Yamagata)	1 (3.8)	2 (2.4)	30 (6.7)
B(Victoria)	1 (3.8)	0 (0.0)	9 (2.0)
Unknown	8 (30.8)	71 (84.5)	107 (23.8)
Influenza season, n (%)			
2001 – 2002	0 (0.0)	4 (4.8)	0 (0.0)
2002 – 2003	0 (0.0)	1 (1.2)	0 (0.0)
2003 – 2004	0 (0.0)	7 (8.3)	0 (0.0)
2004 – 2005	6 (23.1)	7 (8.3)	53 (11.8)
2005 – 2006	1 (3.8)	5 (6.0)	18 (4.0)
2006 – 2007	2 (7.7)	1 (1.2)	16 (3.6)
2007 – 2008	10 (38.5)	10 (11.9)	194 (43.1)
2008 – 2009	6 (23.1)	1 (1.2)	95 (21.1)
2009 – 2010	0 (0.0)	4 (4.8)	3 (0.7)
2010 – 2011	1 (3.8)	2 (2.4)	38 (8.4)
2011 – 2012	0 (0.0)	3 (3.6)	33 (7.3)
2012 – 2013	0 (0.0)	24 (28.6)	0 (0.0)
2013 – 2014	0 (0.0)	11 (13.1)	0 (0.0)
2014 – 2015	0 (0.0)	4 (4.8)	0 (0.0)

[a] Comparison of each group of hospitalized cases with the outpatient group using Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables.

[b] $P < 0.0001$.

[c] Body mass index averaged from multiple weight and height measurements obtained during the one year period before influenza diagnosis (10 hospitalized cases in the discovery group, 5 hospitalized cases in the validation group, and 141 outpatients with influenza had missing data for body mass index).

[d] Diagnoses of co-morbid conditions within one year before influenza diagnosis (conditions are listed in Table S1).

[e] Medication use within the six month period before influenza diagnosis.

[f] $P < 0.05$.

[g] Influenza vaccination in six-month period before influenza diagnosis.

[h] Pandemic H1N1 influenza subtype.

TABLE II

Tests for association between 32 single nucleotide polymorphisms and hospitalized influenza in the discovery group

dbSNP identification number	Chr	Position[a]	Gene	Location of SNP in/near gene	Major/minor alleles	Minor allele frequencies		
						Hospitalized influenza group (n = 26)[b]	Control group (n = 993)	1000 Genomes European sample (n = 503)[c]
rs2476601	1	113834946	*PTPN22*	exon 14 / exon 12	G/A	0.096	0.094	0.094
rs2564978	1	207321071	*CD55*	5′ near gene	C/T	0.308	0.310	0.255
rs7517810	1	172884320	*FASLG*	3′ near gene	C/T	0.173	0.240	0.233
rs1801274	1	161509955	*FCGR2A*	exon 4	T/C	0.481	0.461	0.489
rs3024505	1	206766559	*IL10*	3′ near gene	C/T	0.173	0.158	0.168
rs1919366	2	200145055	*CLK1*	3′ near gene	C/T	0.404	0.446	0.459
rs4535048	2	111893828	*IL1A*	3′ near gene	C/T	0.135	0.124	0.113
rs59219184	4	184388899	*IRF2*	exon 9	G/A	0.000	0.000	0.002
rs1887415	6	137198101	*IFNGR1*	exon 7	T/C	0.000	0.003	0.008
rs11246059	11	305961	*IFITM1*	5′ near gene	A/C	0.135	0.151	0.151
rs10398	11	308180	*IFITM2*	5′ UTR	A/G	0.135	0.194	0.193
rs1059091	11	309127	*IFITM2*	exon 2	A/G	0.327	0.316	0.332
rs12252	11	320772	*IFITM3*	exon 1	T/C	0.000	0.036	0.041
rs7948108	11	323507	*IFITM3*	5′ near gene	T/C	0.442	0.458	0.502
rs7944917	11	325800	*IFITM3*	5′ near gene	G/A	0.000	0.031	0.030
rs57285449	11	299411	*IFITM5*	exon 1	C/G	0.288	0.310	0.348
rs7481685	11	384140	*IFITM5*	5′ near gene	C/T	0.250	0.320	0.327
rs4693	11	62796537	*NXF1*	exon 15	G/A	0.346	0.379	0.377
rs4075090	11	766791	*PDDC1*	3′ near gene	T/C	0.154	0.194	0.218
rs3782578	12	12897485	*GPRC5A*	intron	G/A	0.250	0.314	0.304
rs1849645	17	6357260	*C1QBP*	5′ near gene	T/C	0.212	0.182	0.158
rs12761	17	5422825	*RPAIN*	exon 3	G/C	0.327	0.271	0.305
rs72483216	17	35444977	*SLFN13*	exon 3	G/A	0.038	0.034	0.035
rs8072510	17	35445639	*SLFN13*	exon 3	G/T	0.231[d,e]	0.105	0.102
rs17875834	21	33349476	*IFNAR1*	exon 8	C/T	0.000	0.002	0.001

dbSNP identification number	Chr	Position[a]	Gene	Location of SNP in/near gene	Major/minor alleles	Minor allele frequencies		
						Hospitalized influenza group (n = 26)[b]	Control group (n = 993)	1000 Genomes European sample (n = 503)[c]
rs2229207	21	33241945	*IFNAR2*	exon 2	T/C	0.077	0.094	0.074
rs1131668	21	33262573	*IFNAR2*	exon 9	G/A	0.481[d,e]	0.331	0.323
rs4986958	21	33414987	*IFNGR2*	exon 2	C/G	0.000	0.005	0.006
rs9808753	21	33415005	*IFNGR2*	exon 2	A/G	0.096	0.143	0.143
rs2834215	21	33424579	*IFNGR2*	intron	G/A	0.442	0.457	0.435
rs13047599	21	33553954	*SON*	exon 3	T/C	0.327	0.337	0.328
rs2834317	21	33984405	*SON*	3′ near gene	G/A	0.154	0.153	0.149

Chr, chromosome number; dbSNP, National Center for Biotechnology Information database of single nucleotide polymorphisms; HapMap CEU, sample of persons with Northern European ancestry in the HapMap Project; ID, identification number; SNP, single nucleotide polymorphism; UTR, untranslated region.

[a]Based on GRCh38 Genome Reference Consortium Human Reference 38 (GRCh38/hg38).

[b]Minor allele frequencies of hospitalized influenza in the discovery group compared with minor allele frequencies of controls in the discovery group and the 1000 Genomes European sample using Fisher's exact test.

[c]1000 Genomes European sample consisted of 503 individuals from five populations: British in England and Scotland (GBR), Finnish in Finland (FIN), Iberians in Spain (IBS), Toscans in Italy (TSI), and Utah residents with Northern and Western European ancestry (CEU).

[d]$P < 0.05$ comparing minor allele frequencies between hospitalized influenza and controls in the discovery group.

[e]$P < 0.05$ comparing minor allele frequencies between hospitalized influenza in the discovery group and the 1000 Genomes European sample.

TABLE III

IFITM3 rs12252 polymorphism and hospitalized influenza

	PMRP validation group		Combined PMRP discovery and validation groups			Vanderbilt validation group		
rs12252	**Cases (n = 84)[a]**	**Controls (n = 301)**	**Cases – all (n = 110)[b]**	**Cases – low-risk (n = 22)[b,c]**	**Controls (n = 1,294)**	**Cases – all (n = 70)[d]**	**Cases – low-risk (n = 20)[c,d]**	**Controls (n = 8,719)**
Genotype, n (%)								
TT	78 (92.8)	282 (93.7)	104 (94.6)	21 (95.5)	1203 (93.0)	66 (94.3)	19 (95.0)	8209 (94.2)
TC	5 (6.0)	19 (6.3)	5 (4.5)	1 (4.5)	91 (7.0)	4 (5.7)	1 (5.0)	506 (5.8)
CC	1 (1.2)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (0.0)
C allele frequency	0.042	0.032	0.032	0.023	0.035	0.029	0.025	0.029
Genotype *P*-value	0.32		0.05	1.00		1.00	1.00	
Allelic *P*-value	0.48		1.00	1.00		1.00	1.00	
Allele trend test *P*-value	0.53		0.79	0.65		0.95	0.87	
Dominant model *P*-value	0.80		0.70	1.00		1.00	1.00	
Recessive model *P*-value	0.22		0.08	1.00		1.00	1.00	

PMRP, Personalized Medicine Research Project.

[a] Fisher's exact test used to compare cases and controls in the PMRP validation group.

[b] Fisher's exact test used to compare cases and controls in the combined PMRP discovery and validation groups.

[c] Excluded cases who met any of the following criteria: ≥ 65 years at influenza diagnosis, morbidly obese (body mass index ≥ 40 kg/m^2), diagnosis of at least one co-morbid condition within one year before influenza diagnosis, or use of immunosuppressive medications within six months before influenza diagnosis.

[d] Fisher's exact test used to compare cases and controls in the Vanderbilt validation group.

TABLE IV

Association between *IFITM3* rs12252 and hospitalized influenza according to the presence of risk factors for influenza complications

	Combined PMRP discovery and validation groups[a]				Vanderbilt validation group[b]			
	Age at influenza diagnosis in cases		Presence of clinical risk factors in cases[c]		Age at influenza diagnosis in cases		Presence of clinical risk factors in cases[c]	
rs12252	**< 65 years (n = 37)**	**≥ 65 years (n = 73)**	**Yes (n = 59)**	**No (n = 51)**	**< 65 years (n = 51)**	**≥ 65 years (n = 19)**	**Yes (n = 55)**	**No (n = 15)**
Genotype, n (%)								
TT	33 (89.2)	71 (97.3)	55 (93.2)	49 (96.1)	47 (92.2)	19 (100.0)	52 (94.5)	14 (93.3)
TC	3 (8.1)	2 (2.7)	3 (5.1)	2 (3.9)	4 (7.8)	0 (0.0)	3 (5.5)	1 (6.7)
CC	1 (2.7)	0 (0.0)	1 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C allele frequency	0.068	0.014	0.042	0.020	0.039	0.000	0.027	0.033
Genotype *P*-value	0.024	0.23	0.040	0.57	0.55	0.63	1.00	0.60
Allelic *P*-value	0.19	0.24	0.61	0.58	0.55	0.63	1.00	0.59
Allele trend test *P*-value	0.14	0.16	0.68	0.39	0.56	0.28	0.89	0.90
Dominant model *P*-value	0.33	0.23	1.00	0.57	0.54	0.62	1.00	0.60
Recessive model *P*-value	0.028	1.00	0.044	1.00	1.00	1.00	1.00	1.00

PMRP, Personalized Medicine Research Project.

[a] Fisher's exact test used to compare each case stratum with all 1,294 controls in the combined PMRP discovery and validation groups. Genotype and allele frequencies for the controls are given in Table III.

[b] Fisher's exact test used to compare each case stratum with all 8,719 controls in the Vanderbilt validation group. Genotype and allele frequencies for the controls are given in Table III.

[c] Clinical risk factors included any of the following: morbid obesity (body mass index ≥ 40 kg/m^2), diagnosis of at least one co-morbid condition within one year before influenza diagnosis, and use of immunosuppressive medications within six months before influenza diagnosis.