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*J Immunol* published online 15 February 2012
[http://www.jimmunol.org/content/early/2012/02/15/jimmunol.1102775](http://www.jimmunol.org/content/early/2012/02/15/jimmunol.1102775)

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[http://www.jimmunol.org/content/suppl/2012/02/15/jimmunol.1102775.5.DC1](http://www.jimmunol.org/content/suppl/2012/02/15/jimmunol.1102775.5.DC1)

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A 17q12 Allele Is Associated with Altered NK Cell Subsets and Function

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NK cells play an important role in innate immunity. A previous genome-wide association study demonstrated an association between a 17q12 allele (rs9916629C) and lower frequency of CD3+CD56+ NK cells in peripheral blood. We performed an analysis that not only replicates the original result of the genome-wide association study (p = 0.036) but also defines the specific cell subpopulations and functions that are modulated by the rs9916629 polymorphism in a cohort of 96 healthy adult subjects using targeted multiparameter flow cytometric profiling of NK cell phenotypes and functions. We found that rs9916629C is associated with alterations in specific NK cell subsets, including lower frequency of predominantly cytotoxic CD56dim NK cells (p = 0.011), higher frequency of predominantly regulatory CD56bright NK cells (p = 0.019), and a higher proportion of NK cells expressing the inhibitory NKG2A receptor (p = 0.0002). Functionally, rs9916629C is associated with decreased secretion of macrophage inflammatory protein-1β by NK cells in the context of Ab-dependent cell-mediated cytotoxicity (p = 0.039) and increased degranulation in response to MHC class I-deficient B cells (p = 0.017). Transcriptional profiling of NK cells suggests that rs9916629 influences the expression of transcription factors such as TBX21, which has a role in NK cell differentiation, offering a possible mechanism for the phenotypic and functional differences between the different alleles. The rs9916629C allele therefore has a validated effect on the proportion of NK cells in peripheral blood and skews NK cells toward a specific phenotypic and functional profile, potentially influencing the impact that these innate immune cells have on infection and autoimmunity. *The Journal of Immunology, 2012, 188: 000–000.

Natural killer cells represent a key component of the innate immune system and are able to rapidly eliminate tumor cells or virally infected cells without prior Ag sensitization (1–4). NK cells are broadly defined as a lymphocyte subset expressing CD56 and/or CD16 on their surface in the absence of T cell activation of regulatory CD3+CD56+ NK cells (14, 15). Thus, CD56+ NK cells in peripheral blood. We performed an analysis that not only replicates the original result of the genome-wide association study (p = 0.036) but also defines the specific cell subpopulations and functions that are modulated by the rs9916629 polymorphism in a cohort of 96 healthy adult subjects using targeted multiparameter flow cytometric profiling of NK cell phenotypes and functions. We found that rs9916629C is associated with alterations in specific NK cell subsets, including lower frequency of predominantly cytotoxic CD56dim NK cells (p = 0.011), higher frequency of predominantly regulatory CD56bright NK cells (p = 0.019), and a higher proportion of NK cells expressing the inhibitory NKG2A receptor (p = 0.0002). Functionally, rs9916629C is associated with decreased secretion of macrophage inflammatory protein-1β by NK cells in the context of Ab-dependent cell-mediated cytotoxicity (p = 0.039) and increased degranulation in response to MHC class I-deficient B cells (p = 0.017). Transcriptional profiling of NK cells suggests that rs9916629 influences the expression of transcription factors such as TBX21, which has a role in NK cell differentiation, offering a possible mechanism for the phenotypic and functional differences between the different alleles. The rs9916629C allele therefore has a validated effect on the proportion of NK cells in peripheral blood and skews NK cells toward a specific phenotypic and functional profile, potentially influencing the impact that these innate immune cells have on infection and autoimmunity.

The online version of this article contains supplemental material.

Abbreviations used in this article: ADCC, Ab-dependent cell-mediated cytotoxicity; KIR, killer Ig-related receptor; MIP, macrophage inflammatory protein; SNP, single nucleotide polymorphism.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1102775
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a vast repertoire of subsets based on their cell receptor profiles, each with a unique capacity to recognize infected or malignant target cells (16). The major classes of receptors include the killer Ig-related receptors (KIRs) that bind to MHC molecules, the c-type lectins (NKG2) that bind to stress inducible molecules such as MICA/B and ULBP, the natural cytotoxicity receptors that bind to viral hemagglutinins, as well as the FcγRIIIa receptor (CD16) that binds to the Fc region of IgG Abs (16). NK cell clones, either constitutively or upon activation, express a wide range of additional receptors that can further modulate target cell recognition. A recent genome-wide association study in healthy subjects identified two single nucleotide polymorphisms (SNPs) on chromosome 17q12 (rs18381349 and rs9916629; $r^2 = 1.0$ in HapMap 2 CEU subjects, Utah residents with ancestry from northern and western Europe) that are associated with the frequency of CD3−CD56+ NK cells (7). However, this study only addressed how these polymorphisms affected the overall frequency of NK cells without investigating whether specific NK cell subsets or functions were modulated preferentially. Given the complexity of NK cell population structure and function, we were thus interested in not only replicating this result but also defining whether this locus is associated with alterations in specific NK cell subpopulations or functions that could predict differential disease outcomes. In this study, multiparameter flow cytometric phenotyping was coupled with functional profiling of NK cells and gene expression analysis of purified NK cell populations of individuals carrying the different variants at this locus. We report a replication of the original association and a novel observation that the candidate SNP (rs9916629) is associated with an altered NK phenotypic and functional profile.

Materials and Methods

Study subjects and genotyping of the candidate gene

The Institutional Review Board of Partners Healthcare approved the study. Subjects gave written informed consent for their DNA analysis and immune cell profiling. To study the association between the candidate SNP and NK cell phenotype and function, we drew subjects from a living biobank of >1600 healthy adult blood donors between 18 and 50 y age who were recruited from the Boston area as part of the Brigham and Women’s Hospital PhenoGenetic Project, which is an ongoing effort to understand how genetic variations affect the immune system and influence the risk of disease. Participants were recruited from the Boston area as part of the Brigham and Women’s Hospital PhenoGenetic Project, which is an ongoing effort to understand how genetic variations affect the immune system and influence the risk of disease. In this study, we recruited a total of 20 subjects with genotype data at rs9916629. RNA expression profiles in purified NK cell populations were generated using the Illumina Bead-Array platform (Illumina, San Diego, CA). Briefly, NK cells were purified using the RosetteSep separation kit (Stemcell Technologies, Vancouver, BC, Canada) on whole blood following cell lysis. RNA was extracted using an RNA isolation kit (Qiagen, Valencia, CA). The Illumina platform reported average signal, bead SE, total number of beads detected per probe, and detection p value. Our samples contained expression data from 48,760 probes that met the Illumina threshold for significant detection (detection p value cutoff of 0.01). The CG sites cover a total of 37,780 transcripts. After removing probes without expression in any sample, expression data for 14,431 probes remained. With C as the reference allele, we performed linear regression using an additive model for each probe against the genotype at rs9916629.

Statistical analysis

We used linear regression models to explore the correlation between the candidate SNP (rs9916629) and the quantitative traits of NK profile obtained in the flow cytometric assessment. To meet the assumptions of our linear regression models, and to limit the impact of outliers, we assessed quantitative NK cell frequencies for normality and performed a square root or transformation of the respective expression. To limit the impact of outliers, we assessed quantitative NK cell functions for normality and performed a square root or log transformation of the respective expression. For frequencies of NK cells that express cytokines or degranulate was examined following stimulation of PBMCs with different NK target cell lines, each at a 10:1 E:T ratio: 1) Ab-dependent cell-mediated cytotoxicity (p815 cells; a mouse leukemic cell line [American Type Culture Collection], pre-incubated for 1 h with 1 mg/ml p815-specific Ab [Abcam, Cambridge, MA]), 2) MHC-devoid target cell line (K562 cells; American Type Culture Collection), and 3) MHC class I-deficient B cell line (721.221 cells; American Type Culture Collection). Treatment with PMA (2.5 μg/ml) and ionomycin (1 μg/ml) was used as positive control (Sigma-Aldrich), and incubation of PBMCs with media alone (RPMI 1640 medium with 10% FCS) or PBMCs with p815 cells without the coating Ab was used as the negative control for the respective experiments. Brefeldin A (0.5 μg/ml; Sigma-Aldrich), 0.3 μg/ml monensin (GolgiStop; BD Biosciences), and anti-CD107a-PE-Cy5 Ab (a surrogate marker of cell degranulation and cytotoxicity; BD Biosciences) were added directly to the stimulation conditions and cells were incubated at 37°C in 5% CO2 for 6 h. Following this period of coculture, cells were washed and stained with the immunophenotype marker (CD3, CD16, CD56) as described above. Cells were then washed, fixed, and permeabilized using Fix/Perm solutions (BD Biosciences) according to the manufacturer’s instructions. Intracellular cytokine staining for IFN-γ and macrophage inflammatory protein (MIP)-1β (also known as CCL4) was performed using anti–IFN-γ-FITC and anti-MIP-1β Abs (both BD Biosciences). Data were acquired on a BD LSRII flow cytometer and analyzed using FlowJo software v9.3.

RNA expression and expression quantitative trait locus analysis

From 20 subjects with genotype data at rs9916629, RNA expression profiles in purified NK cell populations were generated using the Illumina Bead-Array platform (Illumina, San Diego, CA). Briefly, NK cells were purified using the RosetteSep separation kit (Stemcell Technologies, Vancouver, BC, Canada) on whole blood following cell lysis. RNA was extracted using an RNA isolation kit (Qiagen, Valencia, CA). The Illumina platform reported average signal, bead SE, total number of beads detected per probe, and detection p value. Our samples contained expression data from 48,760 probes that met the Illumina threshold for significant detection (detection p value cutoff of 0.01). The CG sites cover a total of 37,780 transcripts. After removing probes without expression in any sample, expression data for 14,431 probes remained. With C as the reference allele, we performed linear regression using an additive model for each probe against the genotype at rs9916629.

Results

A previous study found that a polymorphism at the 17q12 locus was associated with the frequency of the CD3−CD56+ NK cells in
circulation (7), but whether this SNP affects all NK cells or specific NK cell populations was not examined. In this study, we sought to dissect the impact of the 17q12 locus on specific NK cell subpopulations and their functions. To address these questions, we leveraged a living biobank of >1600 healthy adult blood donors between 18 and 50 years of age (the Brigham and Women’s Hospital PhenoGenetic Project) and captured a detailed profiling of NK cell immunophenotypes and functional capacity from 96 randomly selected subjects (see Supplemental Table I for demographic details). Outcome measures included NK cell subpopulation frequency and functional response to different activation pathways. In our data processing pipeline, we comprehensively assessed the frequency of nine specific cell surface markers on NK cells using multicolor flow cytometry. Additionally, NK cell function was assessed in response to three different stimuli using intracellular cytokine staining for IFN-γ and MIP-1β, and NK cell degranulation, which serves as a surrogate marker for NK cell-mediated cytotoxicity (18). The frequency of NK cell subsets was expressed as a percentage of CD3⁺ CD56⁺ bulk NK cells, CD56bright, or CD56dim cells. We performed association analyses between the rs9916629 variant and the quantitative traits derived from NK cell cytometric profiles to define the impact of this SNP on NK cells.

In our primary analysis, we set out to replicate the previously published association between the rs9916629C allele and reduced NK cell frequency in the peripheral circulation. We replicated the finding by Ferreira et al. (7) in our cohort of 96 healthy adult subjects: rs9916629C is associated with a lower frequency of CD3⁺ CD56⁺ NK cells (p = 0.015 unadjusted; p = 0.036 after adjusting for age and sex, which independently influence the frequency of NK cells; median NK cell frequency [quartile 1–quartile 3] for each genotype as below: CC = 5.86% [4.23–9.63%], CT = 5.71% [4.39–9.02%], TT = 8.15% [5.54–13.4%]). There is no statistically significant association between the rs9916629 variant and the quantitative traits derived after adjusting for age and sex as covariates. Direction of effect is relative to the C allele and is determined from the beta value. Direction of effect was adjusted for age and sex as covariates.

Thus, we cannot distinguish whether the effect of rs9916629C is additive or dominant at this time.

We next assessed whether rs9916629 C influences the overall NKG2A expression or expression of this inhibitory NK cell receptor on one particular NK cell receptor. Interestingly, we found an association between rs9916629C and the frequency of CD56bright NK cells, it is expressed with a higher frequency and at higher levels to analyze separately. Overall, the rs9916629C allele is thus associated with NK cell subset redistribution that includes a higher frequency of the regulatory CD56bright NK cells in addition to a general decrease in the total frequency of circulating NK cells.

**Multiparameter flow cytometric phenotypic profiling of NK cells**

To further define whether the rs9916629C allele modulated the frequency of specific NK cell subpopulations, we next used targeted immunophenotypic profiling to extend our understanding of the impact of the SNP on the frequency of NK cells expressing particular NK cell receptors. Interestingly, we found an association between rs9916629C and the frequency of NK cells expressing the inhibitory NK cell receptor NKG2A (or CD159A) (Table II). Using our default additive model, the frequency of NK cells expressing the inhibitory NKG2A receptor increases for each additional rs9916629C allele (p = 0.00046 unadjusted; p = 0.0002 after adjusting for age and sex) (Fig. 2A), meeting a Bonferroni-corrected p value threshold for multiple comparisons of 0.0056 (given that one SNP was tested with nine markers of NK cell subpopulations). Because rs9916629CT and rs9916629CC subjects had similar median NKG2A frequencies (67.8 and 67.3%, respectively), we also tested the association between rs9916629C and NKG2A using a dominant model (rs9916629CT/CC versus rs9916629TT) and observed a similar result (Fig. 2B, Supplemental Table II) as that seen in the additive model for this SNP. Thus, we cannot distinguish whether the effect of rs9916629C is additive or dominant at this time.

Whereas NKG2A is expressed on both CD56bright and CD56dim NK cells, it is expressed with a higher frequency and at higher intensity on CD56bright NK cells than on CD56dim NK cells. We next assessed whether rs9916629C influences the overall NKG2A expression or expression of this inhibitory NK cell receptor on one

**FIGURE 1.** Representative flow cytometry data for the primary outcome from two subjects. (A) Homozygote for the reference allele C; (B) homozygote for the T allele at the rs9916629 SNP.

| Candidate SNP (rs9916629) and primary outcome of NK cytometric profile in the healthy cohort | Unadjusted | Multivariate* |
|----------------------------------|------------|--------------|
| **NK Cells** | **Direction of Effect** | **p** | **Direction of Effect** | **p** |
| CD56bright | ↑ | 0.019 | ↑ | 0.019 |
| CD56dim | ↓ | 0.0069 | ↓ | 0.011 |

Genotype and quantitative trait association was analyzed using PLINK with the C allele of rs9916629 as the reference allele. Direction of effect is relative to the C allele and is determined from the beta value.

*In the multivariate analysis, the association between rs9916629 and the phenotype was adjusted for age and sex as covariates.
of the two major NK cell subsets. We found that rs9916629C was associated with a higher frequency of CD56dim NK cells expressing NKG2A (p = 0.0024 unadjusted; p = 0.0017 after adjusting for age and sex) but did not influence the frequency of NKG2A-expressing CD56bright NK cells (Supplemental Table II). Of note, we also found a suggestive association between rs9916629C and a higher frequency of bulk NK cells expressing CD161 (p = 0.025 unadjusted; p = 0.038 after adjusting for age and sex) and a higher frequency of bulk NK cells expressing CD94 (p = 0.064 unadjusted; p = 0.030 after adjusting for age and sex). Thus, rs9916629C is specifically associated with an enrichment of NKG2A2CD56dim NK cells that also express higher levels of the NKG2A-hetero- }

| Variable                              | Median Percentage of Expression (Quartile 1–Quartile 3) | Unadjusted | Multivariatea |
|---------------------------------------|----------------------------------------------------------|------------|--------------|
|                                       | CC (n = 83) | CT (n = 43) | TT (n = 45) | Direction of Effect | Direction of Effect |
| CD161/NK                              | 71.8 (49.9–76.6) | 72.7 (61.8–80.4) | 58.75 (47.85–72.85) | ↑ | 0.026 | ↑ | 0.038 |
| CD94/NK                               | 68.4 (58.6–74.4) | 64.4 (48.8–71.6) | 57.4 (45.25–67.6) | ↑ | 0.064 | ↑ | 0.030 |
| KIR/NK                                | 30.8 (25.7–36.4) | 28.8 (23.5–41) | 33.9 (24.7–40.5) | ↑ | 0.81 | ↑ | 0.97 |
| NKG2A2/NK                             | 67.3 (62.5–76.2) | 67.8 (53.3–77.2) | 54.75 (44.3–65.5) | ↑ | 0.00046 | ↑ | 0.00020 |
| NKG2D2/NK                             | 88.1 (80.7–96.6) | 85.8 (81.8–92.1) | 87.55 (80.55–91.45) | ↑ | 0.50 | ↑ | 0.66 |
| NKp30/NK                              | 70.7 (70.7–70.7) | 87.45 (87.2–92.2) | 87.05 (84.2–92.4) | ↑ | 0.11 | ↑ | 0.299 |
| NKp30/NK                              | 4.09 (2.59–6.19) | 3.7 (3.19–5.46) | 3.56 (2.89–5.04) | ↑ | 0.41 | ↑ | 0.64 |
| NKp46/NK                              | 94.6 (91.4–95.6) | 91.6 (86.9–95.2) | 90.8 (83.7–94.4) | ↑ | 0.068 | ↑ | 0.076 |
| Perforin/NK                           | 71.25 (64.85–75.75) | 77.9 (70.4–83.1) | 81.3 (74.5–86.7) | ↑ | 0.11 | ↑ | 0.11 |
| CD161/CD56bright                      | 47.3 (39.2–72.1) | 62.65 (44.8–69.9) | 50.65 (39.63–65.9) | ↑ | 0.086 | ↑ | 0.10 |
| CD94/CD56bright                       | 97.3 (93.1–99.4) | 96.15 (91.5–98.7) | 97.7 (95.3–98.75) | ↑ | 0.094 | ↑ | 0.14 |
| KIR/CD56bright                        | 5.94 (2.33–9.56) | 5.33 (2.68–8.65) | 4.42 (3.05–6.65) | ↑ | 0.61 | ↑ | 0.60 |
| NKG2A2/CD56bright                     | 93.5 (91.2–97.7) | 92.7 (86.5–95.6) | 93.05 (89.4–95.4) | ↑ | 0.62 | ↑ | 0.74 |
| NKG2D2/CD56bright                     | 86.9 (80.9–95.5) | 83.7 (76.1–91.1) | 86.5 (78.3–92.5) | ↑ | 0.80 | ↑ | 0.74 |
| NKp30/CD56bright                      | 97.5 (97.5–97.5) | 96.35 (93–99.7) | 95.9 (95.8–96.6) | ↑ | 0.55 | ↑ | 0.66 |
| NKp44/CD56bright                      | 15.9 (8.47–19.4) | 16.3 (10.8–20.7) | 18.1 (12.2–22.2) | ↑ | 0.32 | ↑ | 0.26 |
| NKp6/CD56bright                       | 98.7 (97.3–98.9) | 98.6 (96.99–99) | 99 (98.2–99.5) | ↑ | 0.079 | ↑ | 0.095 |
| Perforin/CD56dim                       | 6.61 (3.71–11.3) | 7.38 (4.21–10.9) | 6.53 (4.18–13.5) | ↑ | 0.51 | ↑ | 0.60 |
| CD161/CD56dim                          | 77.2 (50.5–87.9) | 76.15 (62.8–83.3) | 61.05 (49.1–76.6) | ↑ | 0.024 | ↑ | 0.043 |
| CD161/CD56dim                          | 66.2 (47.6–73.3) | 61.2 (46.6–70.9) | 56.4 (41.5–71.2) | ↑ | 0.22 | ↑ | 0.16 |
| KIR/CD56dim                           | 37 (31.7–42.75) | 35.3 (26.4–45.3) | 36.5 (26.8–45.1) | ↑ | 0.89 | ↑ | 0.73 |
| NKG2A2/CD56dim                         | 65.5 (61.8–75.6) | 66.05 (51.2–79.2) | 53.85 (38.3–66.15) | ↑ | 0.0024 | ↑ | 0.0017 |
| NKG2D2/CD56dim                         | 88.9 (82.1–97.1) | 88.25 (83.7–93.1) | 87.65 (80.35–92.3) | ↑ | 0.39 | ↑ | 0.51 |
| NKp30/CD56dim                          | 63.9 (63.9–63.9) | 84.45 (76.9–88.2) | 84.8 (80.8–93.2) | ↑ | 0.12 | ↑ | 0.35 |
| NKp44/CD56dim                          | 2.46 (2.3–3.97) | 2.31 (1.92–3.09) | 2.36 (2.03–3.19) | ↑ | 0.66 | ↑ | 0.48 |
| NKp6/CD56dim                           | 95.3 (91.3–95.9) | 93.3 (87.1–96.3) | 91.2 (83.4–94.7) | ↑ | 0.068 | ↑ | 0.085 |
| Perforin/CD56dim                       | 82.4 (77.7–84.45) | 87.8 (82.6–90.6) | 89.5 (85.6–92.6) | ↑ | 0.25 | ↑ | 0.21 |

Genotype and quantitative trait association were analyzed using PLINK with the C allele of rs9916629 as the reference allele. Direction of effect is relative to the C allele and is determined from the beta value. Boldface indicates a p value meeting a Bonferroni-corrected threshold for multiple comparisons. Alternative marker names: CD94 (KLRD1), CD159A (NKG2A), CD161 (KLRB1, NKR-P1A), CD314 (NKG2D), CD335 (Nkp46), CD336 (NKP44).

a In the multivariate association, association between rs9916629 and the phenotype was adjusted for age and sex as covariates.

Table II. Candidate SNP (rs9916629) and secondary outcome of NK cell subpopulations in the healthy cohort.
its effect on the level of expression of all Schlafen genes that are all found in a cluster at 17q12, we assessed rs9916629 is located near SLFN13 directly influenced by genetic variation in their vicinity. Because expression quantitative trait locus analysis, we first assessed genes on which rs9916629C was associated with increased expression of TBX21 (p = 0.0037), which encodes the T-box transcription factor 21 (also known as T-BET) (Supplemental Fig. 1) previously implicated in the development and differentiation of NK cells (19). When applying the Ingenuity pathway analysis tools (Ingenuity Systems, http://www.ingenuity.com) to identify the networks of genes whose expressions are coordinately influenced by rs9916629C, we observed, in this unsupervised analysis, that the best scoring model (score of 20) contained TBX21 as well as 12 other genes, suggesting that increased TBX21 expression may be part of a transcriptional program influenced by rs9916629C.

**Discussion**

NK cells are an important effector component of the innate immune system that play a central role in eliminating infected or malignantly transformed cells as well as in qualitatively modulating adaptive immunity. Thus, identifying the genetic factors influencing NK cell frequency, phenotype, or function may lead to improved prediction models for disease susceptibility or clinical outcomes and discovery of potential therapies that specifically target the function of NK cells. Following the recent report of an SNP (rs9916629) linked to reduced NK cell frequency (7), we sought to replicate this finding and to examine the previously unaddressed questions of whether genetic variation at this locus affects particular NK cell subpopulations and functional profiles. Informed by the functionally distinct NK cell subsets distinguished based on the intensity of CD56 staining, CD56bright and CD56dim, our analytic approach uncovers a more complex effect of the 17q12 locus on NK cells and refines the original association within a much smaller subject sample size (n = 96) than the original study (n = 2538), which used a coarser CD3+CD56+ definition for NK cells (7). Given their distinct functions, the failure to distinguish the CD56+ NK cell subsets may, as in this case, result in averaging opposing effects in different subpopulations and dilution of an association with the NK cell phenotype. Because age affects NK cells, it is also important that our study extends previous findings to an adult population since the subjects in the cohort studied by Ferreira et al. (7) were predominantly adolescents.

The detailed profiling of NK cell immunophenotypes and functional responses that we have generated provides additional insights into how the rs9916629C variant influences NK cell frequency and response. Overall, several observations support the conclusion that individuals bearing one or two copies of the rs9916629C allele exhibit an altered peripheral NK cell profile when compared with individuals lacking the rs9916629C allele. First, rs9916629C is associated with an intriguing redistribution of NK cell subsets with a reduction in the CD56dim NK cells and an expansion of the CD56bright NK cells. These immunoregulatory CD56bright NK cells secrete copious amounts of cytokines and chemokines that are essential in initiating the early inflammatory response. Second, this allele is associated with the preferential accumulation of NK cells expressing the inhibitory c-type lectin NKG2A and its heterodimeric partner, CD94. Third, rs9916629C data were available: the level of expression of probe sets interrogating SLFN1, 5, 11, 12, 12L, and 13 were not associated with rs9916629C. On an exploratory basis, we assessed our RNA data for an effect of rs9916629C on all other interrogated transcripts, and we observed changes in the expression levels of 126 genes within the purified NK cell population that were suggestively associated with rs9916629C (p < 0.01; Supplemental Table III). Given our small sample size, no single transcript had significant evidence of association after correcting for the testing of multiple hypotheses. Among the suggested associations, interestingly, we found that rs9916629C was associated with increased expression of T-BET (also known as T-BET) (Supplemental Fig. 1) previously implicated in the development and differentiation of NK cells (19).
show a robust correlation with enhanced degranulation (CD107a) by bulk NK cells as well as by CD56^{bright} and CD56^{dim} cells following maximum stimulation in response to PMA/ionomycin, with a median percentage of expression of 11.8 (9.02–14.9) in CD107a/NK cells compared to 7.09 (5.65–10.54) in IFN-γ/NK cells. This indicates that the rs9916629 variant may be associated with increased activity in NK cells, possibly due to infection or malignancy, which has been shown to alter the expression of this NKG2A ligand and this interaction between NK cells and target cells.

Note that because this variant emerged from a genome-wide association scan, it is probably not the causal variant within this locus. Fine mapping with interrogation of all genetic variants in the region will be necessary to identify the causal variant. In the interim, rs9916629 is a good surrogate marker that captures the effect of the 17q12 locus, which may be dominant or additive, as our current data fit either model equally well. Although the impact of rs9916629 on the distribution of NK cell surface phenotypes is more clearly appreciated, the functional impact of the SNP is not as clear in part due to the heterogeneity in NK cell activity from subject to subject. Functional heterogeneity is linked to differences in KIR/HLA backgrounds that alter the functional licensing of NK cells, resulting in different levels of activity against generic target cell lines. Only extremely large cohort studies could normalize for the impact of the extreme genetic variability within the KIR and HLA loci. In our relatively small cohort, the rs9916629 C was more readily than MHC-devoid target cell lines, strongly suggesting as a marker of terminal differentiation in NK cells, although it is also expressed on the CD56 dim subset of NK cells, it is also expressed on the CD56 dim subset of NK cells, it is also expressed on the CD56 dim subset of NK cells.
NKG2A and HLA-E likely plays an important role in providing inhibitory signals to peripheral NK cells. Furthermore, there is evidence that the NKG2A/HLA-E interaction may also play a critical role during NK cell development, where NKG2A is expressed early in the developmental pathway of this lymphocytic cell subset (20, 25). Given that our study involved detailed characterization of NK cells but not their targets, we did not examine the expression of HLA-E to determine whether increased ligand expression may increase NKG2A expression or NK cell expansion. We did not observe any obvious change in HLA-E expression in the transcriptional analysis of purified NK cell populations in individuals that possess the rs9916629\(^c\) allele (data not shown). It is plausible that changes in ligand expression on other more relevant cells (e.g., stromal cells, APCs) may more profoundly impact NK cell development and warrant further investigation to define the specific mechanism by which the SNP may alter NK cell development. Taken together, the observations that rs9916629 is associated with not only a shift toward the CD56\(^{bright}\) NK subset, which typically expresses more NKG2A, but also with an overall increased expression of this marker on CD56\(^{dim}\) NK cells as well as an accompanying enhancement of NK cell degranulation (a hallmark effector function of CD56\(^{dim}\) NK cells) potentially reveal a previously unappreciated role of this inhibitory receptor in NK cell licensing (16) or education (4, 26). The functional activity of NK cells is determined early in NK cell development through the interaction between self-ligands and inhibitory NK cell receptors such as KIRs and NKG2A. It is plausible that rs9916629\(^c\), by affecting NKG2A expression, may also influence NK cell licensing, as we observed enhanced NK cell degranulation and elevated NKG2A expression that are associated with this SNP.

To gain further insights into the role of this SNP in modulating NK cell development, we performed an analysis of RNA expression in a subset of subjects. Among these individuals, we did not find an effect on nearby genes of the SLFN family but noticed suggestive associations between the rs9916629\(^c\) allele and many different transcripts, which include increased TBX21 RNA expression with rs9916629\(^c\). TBX21 has been implicated in autoimmune diseases, including experimental autoimmune encephalomyelitis (27, 28). Intriguingly, TBX21 is also a master regulator of commitment to the Th1 lineage, is expressed at the T/NK cell lymphocyte progenitor phase, and regulates the expression of IFN-\(\gamma\) in Th1-derived cells and NK cells (19, 29–31). NK cell differentiation is dramatically reduced in mice lacking the murine TBX21 homolog (19), demonstrating the essential role of this transcription factor in NK cell development. Furthermore, the observation that TBX21 regulates the expression of sphingosine 1-phosphate receptor 5 on NK cells, which is essential for NK cell egress from the lymph nodes, suggests that TBX21 may also influence the distribution of NK cells in blood (32). We therefore speculate that elevated TBX21 expression, in the presence of rs9916629\(^c\), may affect NK cell differentiation. This is supported by our observation that the rs9916629\(^c\) allele was associated with higher TBX21 RNA expression in human NK cells as well as a lower frequency of CD56\(^{dim}\) NK cells and higher frequency of CD56\(^{bright}\) NK cells in the peripheral circulation. Thus, rs9916629\(^c\) may alter NK cell frequency and phenotype by influencing the expression of early transcription factors required for NK cell development. This interesting hypothesis will require further investigation to be rigorously tested and elaborated.

Although we have provided possible explanations for the association between the candidate SNP and the observed NK phenotype, the exact mechanism by which the 17q12 locus affects TBX21 expression and NK cell biology remains unclear. Given the large distance between TBX21 and rs9916629 (12 Mb), it is unlikely that the rs9916629 variant has a direct effect on TBX21 expression. The influence on TBX21 expression is likely mediated by an indirect effect of the chromosomal region containing rs9916629. Interestingly, the rs9916629 SNP is found between two members of the Schlafen family of genes (SLFN12L and SLFN13) (University of California at Santa Cruz Genome Browser, http://genome.ucsc.edu) (33). Although little is known about these genes, other members of this gene family, which are located nearby, have been implicated as negative regulators of lymphocyte and thymocyte proliferation (34). Furthermore, SLFN2 and SLFN5 are involved in responses to type 1 IFN stimulation (35, 36), which plays an important role in NK cell activation. Thus, it is plausible that rs9916629 influences the function of a specific Schlafen family gene that may regulate the expression of TBX21, which may in turn regulate NK cell differentiation and maturation. Fine mapping of the 17q12 locus will provide insights into the identity of the gene(s) that may mediate the association between rs9916629 and NK cell phenotypes and functions.

In conclusion, we validate and refine the association of the 17q12 locus with NK cell frequency by demonstrating that an SNP in this locus is associated with the frequency of two major subsets of NK cells: the rs9916629\(^c\) allele is associated with a larger proportion of regulatory CD56\(^{bright}\) and a smaller proportion of cytotoxic CD56\(^{dim}\) cells. The shift toward the CD56\(^{bright}\) NK cell profile occurs in conjunction with immunophenotypic and functional alterations that suggest a possible mechanism by which the 17q12 locus influences the differentiation and function of NK cells: it may alter the function of members of the Schlafen gene family with repercussions on transcriptional programs that include the NK cell fate-determining gene TBX21.

Acknowledgments
We thank participants of the Brigham and Women’s Hospital PhenoGenetic Project. We also acknowledge Drs. David Goldstein and Kevin Shianna’s laboratory for generating RNA expression data from NK cells.

Disclosures
The authors have no financial conflicts of interest.

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