Common variants in FOXP1 are associated with generalized vitiligo

Ying Jin, Stanca A. Birlea, Pamela R. Fain, Christina M. Mailloux, Sheri L. Riccardi, Katherine Gowan, Paulene J. Holland, Dorothy C. Bennett, Margaret R. Wallace, Wayne T. McCormack, E. Helen Kemp, David J. Gawkrodger, Anthony P. Weetman, Mauro Picardo, Giovanni Leone, Alain Taïeb, Thomas Jouary, Khaled Ezzedine, Nanny van Geel, Jo Lambert, Andreas Overbeck & Richard A. Spritz

SUPPLEMENTARY METHODS

SUBJECTS

The initial GWA study included 1514 generalized vitiligo patients of North American and United Kingdom non-Hispanic/Latino white origin. All patients met strict clinical diagnostic criteria for generalized vitiligo\(^1\); subjects with other causes of localized hypopigmentation or uncertain diagnoses were excluded.

The case-control replication study included 647 additional unrelated patients and 1056 non-vitiligo controls of North American and European white origin, principally spouses of the vitiligo patients; all of these controls had no other known relatives with vitiligo. The family-based replication study included 183 unrelated simplex parents-affected offspring trios, and 332 unrelated multiplex families, consisting of two or more affected family members and relevant unaffected relatives (1383 individuals), of North American and European non-Hispanic/Latino white origin. Patients, relatives, and controls in the two replication studies provided clinical history regarding vitiligo and other autoimmune diseases.
Written informed consent was obtained from all study subjects. This study was approved by each institutional review board and was conducted according to Declaration of Helsinki principles.

GENOTYPING AND QUALITY CONTROL

Genomic DNA was prepared from peripheral-blood specimens by standard methods or from saliva specimens with use of a DNA self-collection kit using the manufacturer’s instructions (Oragene, DNA Genotek). DNA concentrations were assayed by ultraviolet A260 spectrophotometry (NanoDrop, Thermo Scientific). Genomewide genotyping and quality control filtering of the genomewide genotyping data has been described elsewhere. Briefly, we initially determined genotypes for approximately 610,000 SNPs in 1514 CEU GV cases using the Illumina 610-Quad BeadChip, and compared SNP allele frequencies to those of 2813 “public” controls genotyped using the Illumina 1M BeadChip, obtained from the Databases of Genotypes and Phenotypes (dbGaP) (n = 2731) and the Illumina iControlDB (n = 82). After stringent quality control filtering of SNP data and removal of genetic outliers and unmatchable individuals after genetic matching using GEM, we performed unadjusted and eigenvector-adjusted Cochran-Armitage trend tests of the remaining 520,460 SNPs in the remaining 1392 cases and 2629 controls using PLINK and EIGENSTRAT, correcting test statistics for the remaining genomic inflation factor of 1.048 using the genomic control method.

Genotypes for additional SNPs across each of the candidate association regions were imputed from the GWA genotype dataset by use of MACH, ver.1.0 (http://www.sph.umich.edu/csg/abecasis/MACH/download/), based on phased haplotype data in the HapMap CEU samples (phase II release 24, and phase III release 2). Only imputed genotypes with r² > 0.3 were used for further analyses.
In the present replication study we successfully determined genotypes for the most significant SNP from each of the seven loci that showed suggestive association in the initial GWA analysis, defined as nominal $P$ values better than $10^{-4}$ for multiple SNPs across a contiguous genomic region, using the Sequenom MassArray iPLEX genotyping system. *FOXPI* SNP rs17008713 could not be genotyped using the MassArray system for technical reasons; accordingly, we imputed genotypes for nearby SNP rs17008723 (imputed genotype $r^2 = 0.995$), which is in almost complete LD with rs17008713 in the GWA dataset ($r^2 = 0.99$), and in the replication study we therefore genotyped rs17008723. Family-based data were subjected to Mendelian error-checking, and incompatibilities were either resolved or the incompatible individual or entire family was excluded. Two SNPs were excluded on the basis of apparent deviation from Hardy-Weinberg equilibrium in controls.

**STATISTICAL ANALYSES**

Statistical analyses of the GWA study have been described in detail elsewhere\(^2\). In the case-control replication study, after quality control filtering, we compared allele frequencies for the remaining SNPs in 647 patients and 1056 controls using the Cochran-Armitage trend test\(^5\). Odds ratios and 95% confidence limits were calculated by logistic regression analysis. In the family-based replication study, we calculated the association of each SNP with vitiligo using the family-based association test (FBAT)\(^9\), version 1.5.5. Odds ratios (ORs) and 95% confidence limits were calculated by conditional logistic regression analysis, using matched case-pseudocontrol data derived from the family data\(^10\). To obtain combined $P$ values and ORs, we performed meta-analysis using a Cochran-Mantel-Haenszel test with cases and controls from the GWA study and the case-control replication study, and cases and pseudocontrols derived from the family-based
dataset, and with just the two replication datasets. The fraction of variance accounted for by each of the confirmed GV association signals, and by all 12 loci combined, was calculated as Pseudo R² by logistic regression analysis of the GWA and replication study case-control dataset using STATA, assuming a multiplicative model for each SNP and a polygenic multiplicative model for all 12 loci combined.

To test whether the P-values of the GV-associated CCR6 SNP rs6902119 and the Crohn’s disease-associated CCR6 SNP rs2301436 were independent of each other in the vitiligo data, we used STATA to compare the fit of the logistic regression model including both loci to a model including only the conditioning locus, assuming a multiplicative genotypic effect for the high-risk allele of each locus.

REFERENCES

1. Taïeb, A. & Picardo, M. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment Cell. Res. 20, 27-35 (2007).

2. Jin, Y., et al. Variant of TYR and autoimmunity susceptibility loci in generalized vitiligo. New Engl. J. Med. Epub. ahead of print 2010 Apr 21; PMID: 20410501 (2010).

3. Luca, D., et al. On the use of general control samples for genome-wide association studies: Genetic matching highlights causal variants. Am. J. Hum. Genet. 82, 453-463 (2008).

4. Purcell, S., et al. PLINK: a toolset for whole-genome association and population-based linkage analysis. Am. J. Hum. Genet. 81, 559-575 (2007).

5. Price, A.L., et al. Principal components analysis corrects for stratification in genome-wide association studies. Nat. Genet. 38, 904-909 (2006).

6. Devlin, B., Roeder, K., & Wasserman, L. Genomic control, a new approach to genetic-based association studies. Theor. Popul. Biol. 60, 155-166 (2001).
7. Li, Y., Ding, J., & Abecasis, G.R. Mach 1.0: Rapid haplotype reconstruction and missing genotype inference. *Am. J. Hum. Genet.* **79**, S2290 (2006).

8. International HapMap Consortium, *et al.* A second generation human haplotype map of over 3.1 million SNPs. *Nature.* **449**, 851-861 (2007).

9. Horvath, S., *et al.* Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. *Genet. Epidemiol.* **26**, 61-69 (2004).

10. Cordell, H.J., Clayton, D.G. A unified stepwise regression procedure for evaluating the relative effects of polymorphisms within a gene using case/control or family data: application to HLA in type 1 diabetes. *Am. J. Hum. Genet.* **70**, 124-141 (2002).

11. Schaid, D.J. General score tests for associations of genetic markers with disease using cases and their parents. *Genet. Epidemiol.* **13**, 423-449 (1996).

12. Clayton, D.G. Prediction and interaction in complex disease genetics: experience in type 1 diabetes. *PloS Genet.* **5**, e1000540 (2009).
### Supplementary Table 1  Suggestive Association of Seven Loci in the GWA Dataset

| Chr | SNP     | nt Position (GRCh37) | Risk | Risk Allele Frequency | EIGENSTRAT P value | PLINK P value | Odds Ratio (95%CI) |
|-----|---------|----------------------|------|-----------------------|--------------------|---------------|-------------------|
| 3p13| rs11720980 | 71505650             | A    | 0.371                 | 5.30E-05           | 4.03E-05      | 1.23(1.12-1.35)   |
|     | rs6549391  | 71529915             | A    | 0.710                 | 1.00E-03           | 1.20E-03      | 1.19 (1.07-1.31)  |
|     | rs6549392  | 71538696             | G    | 0.713                 | 1.05E-03           | 1.10E-03      | 1.19 (1.07-1.31)  |
|     | rs11720523 | 71545170             | C    | 0.619                 | 2.40E-04           | 1.91E-04      | 1.20 (1.09-1.32)  |
|     | rs6779258  | 71549639             | T    | 0.618                 | 9.57E-05           | 8.12E-05      | 1.21 (1.10-1.33)  |
|     | rs2133627  | 71551870             | C    | 0.250                 | 8.86E-05           | 5.54E-05      | 1.26 (1.13-1.40)  |
|     | rs17008713 | 71566417             | T    | 0.215                 | 6.28E-06           | 3.70E-06      | 1.32 (1.18-1.48)  |
|     | rs9866496  | 71568501             | C    | 0.282                 | 1.36E-05           | 1.04E-05      | 1.27 (1.15-1.41)  |
|     | rs1499895  | 71571667             | A    | 0.607                 | 2.34E-04           | 2.04E-04      | 1.20 (1.09-1.32)  |
| 3q13| rs697963  | 107078487            | T    | 0.992                 | 9.58E-04           | 9.08E-04      | 2.18 (1.38-3.46)  |
|     | rs6779094  | 108081277            | A    | 0.257                 | 3.47E-05           | 3.96E-05      | 1.26 (1.13-1.41)  |
|     | rs11707076 | 108098637            | T    | 0.292                 | 5.02E-04           | 4.61E-04      | 1.21 (1.09-1.34)  |
|     | rs10511271 | 108114572            | C    | 0.246                 | 3.30E-04           | 4.79E-04      | 1.22 (1.09-1.36)  |
|     | rs4273371  | 108119071            | T    | 0.549                 | 7.12E-05           | 4.95E-05      | 1.22 (1.11-1.33)  |
|     | rs9810676  | 108132759            | T    | 0.500                 | 3.80E-04           | 3.01E-04      | 1.19 (1.09-1.31)  |
|     | rs3996020  | 108179729            | A    | 0.347                 | 2.02E-06           | 1.15E-06      | 1.28 (1.16-1.42)  |
|     | rs4299484  | 108189627            | T    | 0.344                 | 1.58E-06           | 9.86E-07      | 1.29 (1.17-1.42)  |
|     | rs2603127  | 108243551            | A    | 0.265                 | 4.50E-07           | 2.69E-07      | 1.34 (1.20-1.49)  |
|     | rs6784002  | 108260695            | A    | 0.555                 | 1.39E-03           | 9.30E-04      | 1.17 (1.07-1.29)  |
|     | rs2060986  | 108264948            | T    | 0.394                 | 1.26E-03           | 8.90E-04      | 1.18 (1.07-1.30)  |
|     | rs7633235  | 108454848            | T    | 0.237                 | 2.46E-06           | 1.19E-06      | 1.33 (1.19-1.49)  |
|     | rs9815057  | 108464197            | T    | 0.234                 | 6.59E-06           | 3.83E-06      | 1.31 (1.17-1.47)  |
|     | rs7637614  | 108508966            | G    | 0.557                 | 5.94E-04           | 4.43E-04      | 1.19 (1.08-1.30)  |
|     | rs9879707  | 108580778            | A    | 0.191                 | 2.25E-04           | 1.50E-04      | 1.27 (1.13-1.44)  |
| 6q27| rs1358786  | 166054922            | A    | 0.148                 | 7.83E-04           | 1.02E-03      | 1.25 (1.10-1.43)  |
|     | rs11755875 | 166216338            | C    | 0.808                 | 6.41E-04           | 7.55E-04      | 1.22 (1.09-1.37)  |
|     | rs9366076  | 167373708            | C    | 0.818                 | 7.15E-04           | 1.52E-03      | 1.21 (1.08-1.36)  |
|     | rs9355610  | 167383075            | G    | 0.711                 | 2.99E-04           | 6.33E-04      | 1.19 (1.08-1.32)  |
|     | rs429083   | 167383972            | G    | 0.581                 | 8.38E-04           | 1.34E-03      | 1.17 (1.07-1.28)  |
|     | rs9366078  | 167399512            | A    | 0.709                 | 3.43E-04           | 7.29E-04      | 1.19 (1.08-1.32)  |
rs933243   167403873   G   0.709   0.671   3.26E-04   7.01E-04   1.19 (1.08-1.32)
rs400837   167411008   C   0.581   0.543   9.36E-04   1.47E-03   1.17 (1.06-1.28)
rs2301436  167437988   A   0.524   0.479   2.41E-04   2.27E-04   1.19 (1.09-1.30)
rs12529876 167461501   A   0.464   0.419   1.91E-04   1.68E-04   1.20 (1.09-1.31)
rs10484530 167461562   A   0.446   0.405   6.61E-04   6.16E-04   1.18 (1.08-1.29)
rs12183084 167473685   G   0.446   0.404   5.82E-04   5.47E-04   1.18 (1.08-1.29)
rs6921588  167494397   A   0.462   0.419   4.00E-04   3.65E-04   1.19 (1.08-1.29)
rs204295   167500562   C   0.580   0.540   8.91E-04   9.84E-04   1.17 (1.07-1.28)
rs6902119  167505791   C   0.495   0.446   7.23E-05   5.72E-05   1.21 (1.11-1.33)
rs6456156  167522300   C   0.541   0.502   1.59E-03   1.57E-03   1.16 (1.06-1.27)
rs4708777  167814784   A   0.922   0.904   2.86E-03   1.71E-03   1.32 (1.11-1.56)
7p21.3     
rs2192346  8176301   A   0.778   0.737   5.40E-05   6.59E-05   1.26 (1.13-1.40)
rs887848   8179846   A   0.756   0.722   1.23E-03   1.37E-03   1.20 (1.07-1.33)
rs2110333  8185089   T   0.755   0.719   6.77E-04   8.00E-04   1.20 (1.08-1.34)
9q22.33    
rs7870439  100951838   G   0.778   0.744   9.81E-04   1.02E-03   1.21 (1.08-1.34)
rs10818610 100969348   G   0.654   0.612   5.02E-04   4.12E-04   1.19 (1.08-1.31)
rs7868451  100987622   A   0.695   0.650   6.80E-05   8.37E-05   1.22 (1.11-1.35)
rs1573025  100991430   G   0.838   0.804   2.63E-04   2.40E-04   1.26 (1.12-1.42)
rs879368   100995758   C   0.748   0.705   8.42E-05   9.23E-05   1.24 (1.11-1.37)
rs7853442  101008885   A   0.693   0.649   8.94E-05   1.08E-04   1.22 (1.10-1.34)
rs10115971 101024887   A   0.834   0.801   6.61E-04   6.36E-04   1.24 (1.10-1.39)
rs10818692 101026301   A   0.834   0.801   5.48E-04   5.37E-04   1.24 (1.10-1.39)
rs10760233 101034626   G   0.745   0.708   7.61E-04   7.43E-04   1.20 (1.08-1.32)
rs4743196  101049252   G   0.662   0.623   6.63E-04   8.05E-04   1.18 (1.07-1.29)
12q13.2    
rs11171710 56368078   G   0.582   0.541   5.10E-04   4.94E-04   1.19 (1.08-1.30)
rs773107   56369506   G   0.376   0.319   1.03E-06   6.60E-07   1.28 (1.17-1.41)
rs10876864 56401085   G   0.467   0.411   5.57E-06   2.74E-06   1.25 (1.14-1.37)
rs1701704  56412487   C   0.389   0.329   2.54E-07   1.66E-07   1.30 (1.18-1.42)
rs705708   56488913   A   0.507   0.463   1.53E-04   2.15E-04   1.20 (1.09-1.31)
rs10783779 56491880   G   0.445   0.405   4.34E-04   6.25E-04   1.18 (1.08-1.30)
12q24.12   
rs12311063 110557312   A   0.656   0.619   1.49E-03   1.73E-03   1.17 (1.06-1.29)
rs12313068 110597304   C   0.865   0.836   9.70E-04   6.98E-04   1.26 (1.11-1.44)
rs11065287 110609714   T   0.342   0.296   8.82E-05   3.53E-05   1.24 (1.12-1.36)
rs12318836 110642190   A   0.883   0.855   7.49E-04   4.76E-04   1.29 (1.12-1.48)
| SNP          | Chromosome Position | Allele | Minor Allele Frequency | Total Allele Frequency | Minor Allele Odds Ratio | P Value | Log10 P Value | 95% CI | Log10 95% CI |
|-------------|---------------------|--------|------------------------|------------------------|-------------------------|---------|--------------|--------|-------------|
| rs10774599  | 110697448           | T      | 0.667                  | 0.630                  | 2.67E-03                | 1.53E-03| 1.17          | 1.07-1.29 |
| rs3026445   | 110723203           | T      | 0.669                  | 0.631                  | 4.25E-04                | 1.26E-03| 1.18          | 1.07-1.29 |
| rs7957299   | 111002311           | G      | 0.361                  | 0.319                  | 3.81E-04                | 1.83E-04| 1.21          | 1.10-1.34 |
| rs7975139   | 111221131           | T      | 0.908                  | 0.884                  | 1.64E-03                | 1.24E-03| 1.30          | 1.11-1.52 |
| rs850511    | 111239821           | A      | 0.482                  | 0.441                  | 9.50E-04                | 4.84E-04| 1.19          | 1.08-1.30 |
| rs7970490   | 111756438           | A      | 0.741                  | 0.701                  | 4.75E-04                | 3.60E-04| 1.21          | 1.09-1.34 |
| rs3847953   | 111765464           | G      | 0.747                  | 0.711                  | 1.32E-03                | 1.12E-03| 1.19          | 1.07-1.31 |
| rs3184504   | 111884608           | T      | 0.544                  | 0.490                  | 1.47E-05                | 6.91E-06| 1.24          | 1.13-1.36 |
| rs653178    | 112007756           | G      | 0.545                  | 0.492                  | 2.05E-05                | 9.58E-06| 1.24          | 1.13-1.35 |
| rs11065987  | 112072424           | G      | 0.475                  | 0.432                  | 6.75E-04                | 3.72E-04| 1.19          | 1.08-1.30 |
| rs17696736  | 112486818           | G      | 0.480                  | 0.440                  | 1.41E-03                | 8.22E-04| 1.17          | 1.07-1.28 |
| rs11066188  | 112610714           | A      | 0.462                  | 0.423                  | 2.13E-03                | 1.23E-03| 1.17          | 1.07-1.28 |
| rs11066320  | 112906415           | A      | 0.475                  | 0.436                  | 2.28E-03                | 1.42E-03| 1.17          | 1.06-1.27 |
| rs233716    | 113039943           | G      | 0.437                  | 0.397                  | 1.85E-03                | 9.82E-04| 1.17          | 1.07-1.28 |

Listed SNPs are from the 2000 with highest-ranked PLINK P values in the GWA study.
## Supplementary Table 2  Genotype counts for SNPs in novel candidate GV susceptibility loci

| SNP     | Genotypes | GWA Study | Replication 1 | Replication 2 |
|---------|-----------|-----------|---------------|---------------|
| Chr     | Locus Region | Region ID | Location (nt) | Cases | Controls | Cases | Controls | Cases |
| Replicated loci | | | | | | | | |
| 3p13    | FOXP1 | rs17008713 | 7156417 | GG   | 61 (4.4) | 83 (3.1) | 25 (4.1) | 25 (2.8) | 30 (3.2) |
|         |       |           |           | GT   | 476 (34.2) | 735 (28.0) | 210 (34.8) | 253 (28.3) | 313 (33.6) |
|         |       |           |           | TT   | 855 (61.4) | 1811 (68.9) | 369 (61.1) | 617 (68.9) | 590 (63.2) |
| 6q27    | CCR6   | rs6902119 | 167505791 | CC   | 361 (25.9) | 527 (20.0) | 124 (21.3) | 158 (18.9) | 243 (24.9) |
|         |       |           |           | CT   | 655 (47.1) | 1290 (49.1) | 353 (60.5) | 459 (54.8) | 485 (50.7) |
|         |       |           |           | TT   | 376 (27.0) | 812 (30.9) | 106 (18.2) | 220 (26.3) | 239 (24.4) |
| Unconfirmed loci | | | | | | | | |
| 3q13.13 | MYH15  | rs3603127 | 108243551 | AA   | 92 (6.6) | 118 (4.5) | 20 (3.5) | 30 (3.6) | 67 (7.6) |
|         |       |           |           | AG   | 553 (39.7) | 880 (33.5) | 167 (28.8) | 217 (26.1) | 285 (32.2) |
|         |       |           |           | GG   | 747 953.7) | 1624 (61.9) | 392 (67.7) | 585 (70.3) | 533 (60.2) |
| Excluded loci | | | | | | | | |
| 7p21.3  | ICA1   | rs2192346 | 8176361 | AA   | 840 (60.4) | 1413 (53.6) | 347 (57.0) | 513 (57.6) | 581 (59.8) |
|         |       |           |           | AG   | 485 (34.9) | 1049 (39.9) | 221 (36.3) | 332 (37.3) | 350 (36.1) |
|         |       |           |           | GG   | 66 (4.7) | 166 (6.3) | 40 (6.6) | 45 (5.1) | 40 (4.1) |
| 9p22.33 | TBC102 | rs7866451 | 10097622 | AA   | 686 (49.3) | 1127 (42.9) | 214 (37.3) | 370 (46.8) | 418 (44.0) |
|         |       |           |           | AG   | 563 (40.4) | 1162 (44.2) | 288 (50.3) | 332 (42.0) | 422 (44.5) |
|         |       |           |           | GG   | 143 (10.3) | 340 (12.9) | 71 (12.4) | 88 (11.1) | 109 (11.5) |
### Supplementary Table 3 Replication analysis of novel candidate GV susceptibility loci – Excluded loci

| SNP     | Risk allele | AF<sub>RA</sub> cases | AF<sub>RA</sub> controls | PLINK P | OR | Replication 1 | PLINK P | OR | Replication 2 | PLINK P | OR | Meta-analysis Replication 1 + Replication 2 | PLINK P | OR | Meta-analysis GWA + Replication 1 + Replication 2 | PLINK P | OR |
|---------|-------------|------------------------|--------------------------|---------|----|---------------|---------|----|---------------|---------|----|-----------------------------------------------|---------|----|-------------------------------------------------|---------|----|
| rs2192346 | A           | 0.778                  | 0.734                    | 6.59 x 10<sup>-5</sup> | 1.26 | 0.510          | 0.94   | 0.572        | 0.90   | 0.627 | 0.96                                         | 1.42 x 10<sup>-3</sup> | 1.16 |
| rs7868451 | A           | 0.695                  | 0.650                    | 8.37 x 10<sup>-5</sup> | 1.22 | 3.45 x 10<sup>-3</sup> | 0.79   | 0.346        | 1.01   | 0.016 | 0.82                                         | 0.022   | 1.10 |

SNP rs2192346 and rs7868451 were located at nt 8,176,301 in the *ICA1* region of 7p21.3, and nt 100,987,622 in the *TBC1D2* region of 9q22.33, respectively. SNP nucleotide positions are from GRCh37 and genes in close proximity to the designated SNP are denoted. AF<sub>RA</sub>, allele frequency of the risk allele EIGENSTRAT GWA P-values for SNPs rs2192346 and rs7868451 were 5.40 x 10<sup>-5</sup>, and 6.80 x 10<sup>-5</sup>, respectively. PLINK and EIGENSTRAT GWA test statistics were calculated and adjusted for the genomic inflation factor 1.048 as described in the Supplementary Methods. The Bonferroni adjusted significance threshold for the combined replication stage 1 + 2 meta-analysis was P < 1.00 x 10<sup>-2</sup>, and the significance threshold for the overall combined GWA + replication stage 1 + 2 meta-analysis was P < 5 x 10<sup>-8</sup>.