
Article:
Jin, Y., Birlea, S.A., Fain, P.R. et al. (19 more authors) (2010) Common variants in FOXP1 are associated with generalized vitiligo. Nature Genetics, 42 (7). 576 - 578. ISSN 1061-4036

https://doi.org/10.1038/ng.602

Published in final edited form as:
Nat Genet. 2010 July ; 42(7): 576–578. doi:10.1038/ng.602.

Common variants in *FOXP1* are associated with generalized vitiligo

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Abstract

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METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

AUTHOR CONTRIBUTIONS

Y.J. performed statistical analyses. K.G. managed computer databases and genotype data. S.L.R. and C.M.M. managed DNA samples and contributed to experimental procedures. P.J.H. managed subject coordination. S.A.B., D.C.B., M.R.W., W.T.M., E.H.K., D.J.G., A.P.W., M.P., G.L., A.T., T.J., K.E., N.vG., J.L., and A.O. provided subject samples and phenotype information. P.R.F. and R.A.S. oversaw and managed all aspects of the study. R.A.S. wrote the first draft of the manuscript. All authors contributed to the final paper.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

In a recent genome-wide association study of generalized vitiligo (GV) we identified 10 confirmed susceptibility loci. By testing additional loci that showed suggestive association in the genome-wide study, using two replication cohorts of European descent, we observed replicated association of GV with variants at 3p13 encompassing *FOXP1* (rs17008723, combined $P = 1.04 \times 10^{-8}$) and with variants at 6q27 encompassing *CCR6* (rs6902119, combined $P = 3.94 \times 10^{-7}$).

Generalized vitiligo (GV) is a common, complex autoimmune disease in which patchy depigmentation of skin and hair results from loss of melanocytes from involved areas[1], and which is epidemiologically associated with several other autoimmune diseases[2]. A number of potentially contributory genes have been suggested for GV on the basis of candidate gene association and genetic linkage studies, though few of these have received consistent support[3].

We recently carried out a genome-wide association (GWA) study of GV patients and families of European descent, identifying 10 different loci that contribute to GV risk[4]. In addition, seven other loci showed suggestive association with GV in the initial GWA analysis (Supplementary Table 1), defined as nominal *P* values < 10^{-4} for multiple SNPs clustered across a contiguous genomic region. At 3p13, we observed suggestive association of 9 SNPs spanning nt 71,505,650-71,571,667; most significant was rs17008713 ($P = 3.70 \times 10^{-6}$, OR = 1.32), located within *FOXP1* (Figure 1a). At 3q13.13, we observed suggestive association of 15 SNPs spanning nt 107,078,487-108,580,778; most significant was rs2603127 ($P = 2.69 \times 10^{-7}$, OR = 1.34), located within *MYH15*. At 6q27 we observed suggestive association of 17 SNPs spanning nt 166,054,922-167814784; most significant was rs6902119 ($P = 5.72 \times 10^{-5}$, OR = 1.21), upstream of *CCR6* (Figure 1b). At 7p21.3 we observed suggestive association of three SNPs spanning nt 8,176,301-8,185,089; most significant was rs2192346 ($P = 6.59 \times 10^{-5}$, OR 1.26), located within *ICA1*. At 9q22.33 we observed suggestive association of 10 SNPs spanning nt100,951,838-101,049,252; most significant was rs7868451 ($P = 8.37 \times 10^{-5}$, OR = 1.22), located within *TBC1D2*. At 12q13.2 we observed suggestive association of 6 SNPs spanning nt 56,368,078-56,491,880; most significant was rs1701704 ($P = 1.66 \times 10^{-7}$, OR = 1.30), located upstream of *IKZF4*. At 12q24.12 we observed suggestive association of 18 SNPs spanning nt 110,557,312-113,039,943; most significant was rs3184504 ($P = 6.91 \times 10^{-6}$, OR = 1.24), located within *SH2B3*.

To test replication of association of these seven candidate signals, we genotyped the most significant SNP in each locus (Supplementary Table 2) in two independent replication cohorts of European descent: Replication 1 consisted of 647 unrelated GV cases and 1056 non-GV controls (principally spouses of GV cases) and Replication 2 consisted of 183 simplex GV trios and 332 GV multiplex families. SNP rs17008713 could not be genotyped 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 SNP rs17008723. *SH2B3* region SNP rs3184504 and *IKZF4* region SNP rs1701704 deviated significantly from Hardy-Weinberg equilibrium in the Replication 1 controls; therefore, these SNPs were excluded from further analysis. Case-control association statistics were calculated using the Cochran-Armitage trend test implemented in PLINK[5] and family-based association statistics were calculated using FBAT[6]. We carried out a combined meta-analysis of the two replication studies using a Cochran-Mantel-Haenszel test with cases and controls from Replication 1, and cases and pseudocontrols derived from Replication 2, and an overall combined meta-analysis of the two replication studies plus the GWA study. We considered as conservative joint criteria for confirmed association: 1) identification of the same high-risk allele in the GWA study and both of the replication studies; 2) nominally

significant ($P < 0.05$) association in both replication cohorts or significant association in one and marginal association in the other replication cohort; 3) significant (Bonferroni uncorrected $P < 1.0 \times 10^{-2}$; 0.05 / 5) combined replication stage 1 + 2 P value; and 4) a genome-wide significant ($P < 5 \times 10^{-8}$) ([ref. 7]) overall combined P value.

As shown in Table 1, we replicated association for *FOXP1* region SNP rs17008723 (risk allele G; combined replication stage 1 + 2 $P = 1.36 \times 10^{-3}$; overall combined $P = 1.04 \times 10^{-8}$, OR = 1.33; Figure 1a) and for *CCR6* upstream SNP rs6902119 (risk allele C; combined replication $P = 3.79 \times 10^{-3}$; overall combined $P = 3.94 \times 10^{-7}$, OR = 1.23; Figure 1b), although the latter did not achieve the genome-wide significance threshold in the combined analysis. *MYH15* SNP rs2603127 showed a consistent high-risk allele across the three study cohorts, and near genome-wide significant association in the combined analysis ($P = 5.36 \times 10^{-8}$), but showed marginal or no association in the replication cohorts and marginal combined replication P values and so was considered unconfirmed. SNPs at two loci, *ICA1* and *TBC1D2*, failed to even show consistent high-risk alleles across the three study cohorts and thus are considered most likely excluded.

FOXP3 and *CCR6* both encode proteins that play important roles in immune regulation. *FOXP1* encodes a widely expressed transcription factor that is essential for the development of B-cells[8], quiescent naïve T-cells[9], and monocytes[10], and is paralogous to *FOXP3*, which encodes a transcriptional regulator of regulatory T-cell development and function and is the defective gene in the IPEX multiple autoimmune disease syndrome[11]. *Foxp1* conditional knock-out mice have premature cell-autonomous hyper-activation of early thymocytes to CD4+ and CD8+ mature T-cells with effector functions[9].

CCR6 encodes a receptor for macrophage inflammatory protein-3a (CCL20) expressed on unactivated memory B- and T-cells, T-helper 17 cells, and some dendritic cells, and plays a key role in B-cell differentiation and tissue specific migration of dendritic and T cells during inflammatory and immunological responses[12]. Another *CCR6* SNP, rs2301436, has been associated with inflammatory bowel disease[13], with the same high-risk allele observed in the GV GWA dataset ($P = 2.27 \times 10^{-4}$). SNPs rs6902119 and rs2301436 are in moderate LD ($r^2 = 0.64$), and logistic regression analysis indicated that association of GV with rs2301436 might be secondary to LD with rs6902119 ($P = 0.0712$). Recently, we described a small GWA study of GV in an isolated Romanian founder population[14], identifying association in that group with another SNP in 6q27, rs13208776, located 1.44 Mb distal to rs6902119, within *SMOC2*. We find no association between GV and SNPs in the vicinity of rs13208776 in the present study, and no apparent long-range LD between rs6902119 and rs13208776 ($r^2 = 0$); nevertheless, we cannot exclude the possibility that in the Romanian founder population a variant near rs13208776 might influence expression of *CCR6* at a distance. Interestingly, these vitiligo-associated 6q27 SNPs are in close proximity to *IDDM8* (http://www.t1dbase.org), a locus with linkage and association with type I diabetes[15–18] and rheumatoid arthritis[19], autoimmune diseases that are epidemiologically associated with GV[2].

Our findings provide additional evidence that variation in genes encoding proteins with roles in the immune system contribute to susceptibility towards GV. Moreover, many of these genes also contribute to other autoimmune diseases, particularly those with which GV is epidemiologically associated. While each of the GV susceptibility loci thus far identified accounts for only a small increase in relative risk, the biological pathways they highlight provide insights into the pathogenesis of GV and other autoimmune diseases, and may afford relatively tractable targets for therapeutic intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the membership of Vitiligo Support International, the Vitiligo Society, the National Vitiligo Foundation, the American Vitiligo Research Foundation, and Associazione Ricerca Informazione per la Vitiligine for their enthusiastic participation. Supported by grants AR45584 and AR056292 from the National Institutes of Health.

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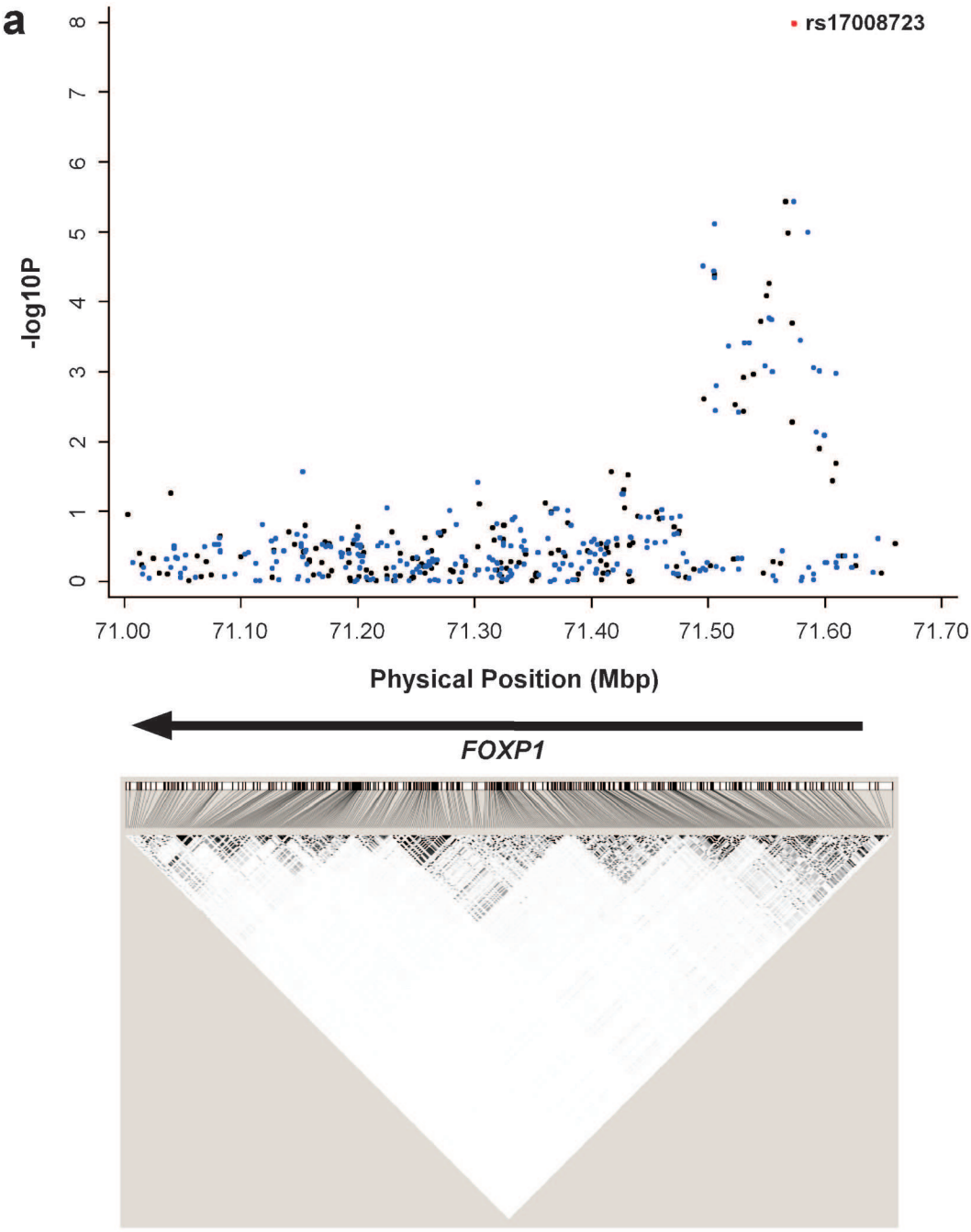

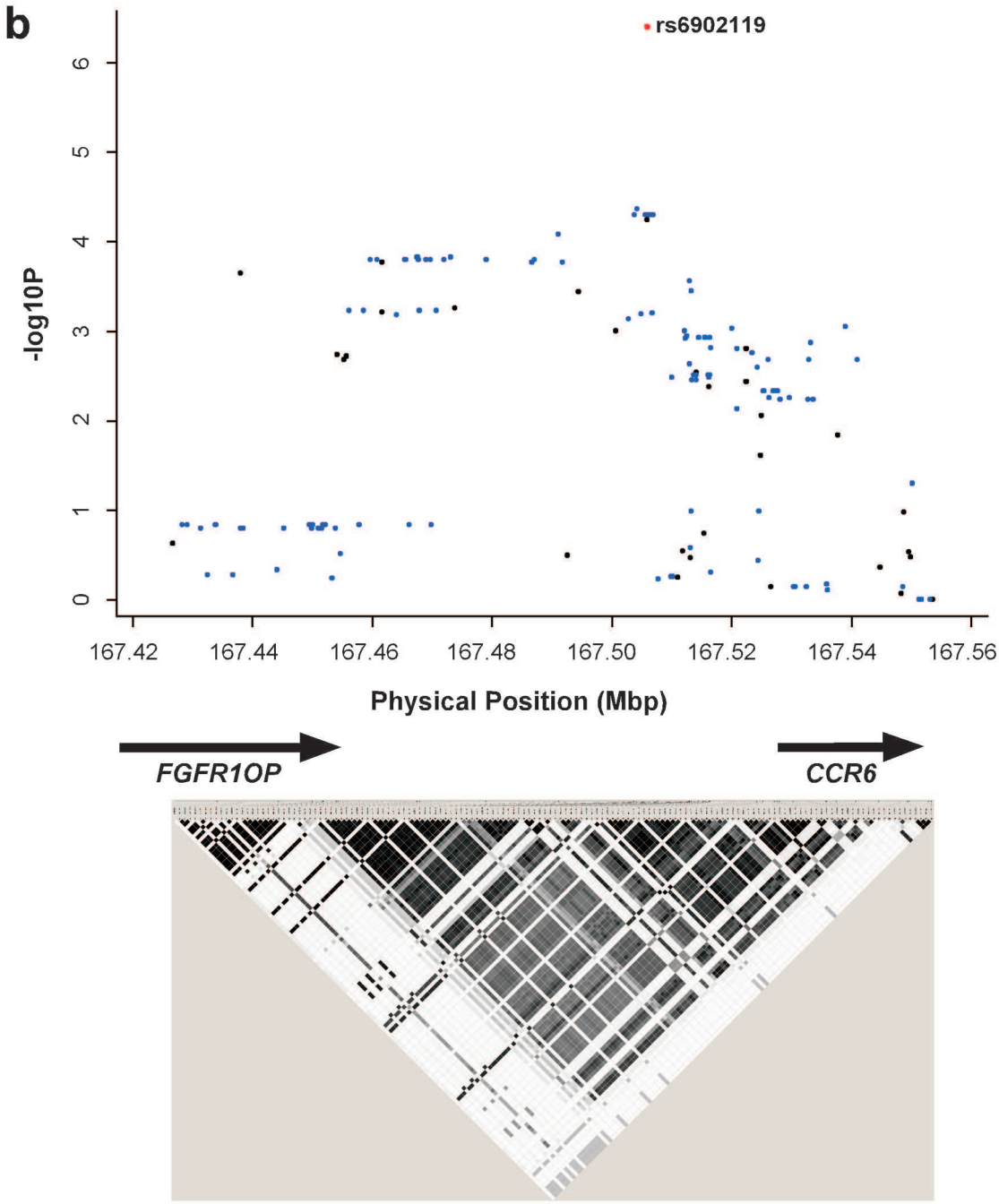

Figure 1.

Newly replicated associations in GV. Upper panel shows genomic control-corrected PLINK association results from the GWA scan for genotyped (black) and imputed (blue) SNPs on the y axis versus chromosomal nucleotide position (GRCh37) on the x axis surrounding (**a**) *FOXP1* and (**b**) *CCR6*. Red squares indicate Cochran-Mantel-Haenszel combined *P*-values for the most strongly associated SNP in each locus. LD patterns for SNPs across the regions are shown below. Arrows indicate gene positions and transcriptional orientation. Lower panel shows pairwise r^2 values for LD; darker boxes indicate stronger disequilibrium for SNPs in the upper panel.

Table 1

Replication analysis of novel candidate GV susceptibility loci

		GWA Study				Replication 1		Replication 2		Meta-analysis Replication 1 + Replication 2		Meta-analysis GWA + Replication 1 + Replication 2	
SNP	**Risk allele**	**AF_{RA}, cases**	**AF_{RA}, controls**	**PLINK *P***	**OR**	***P***	**OR**	***P***	**OR**	***P***	**OR**	***P***	**OR**
Replicated													
rs17008723	G	0.214	0.171	3.65×10^{-6}	1.32	1.47×10^{-3}	1.35	0.017	1.26	1.36×10^{-3}	1.36	1.04×10^{-8}	1.33
rs6902119	C	0.495	0.446	5.72×10^{-5}	1.21	2.92×10^{-3}	1.28	0.058	1.09	3.79×10^{-3}	1.25	3.94×10^{-7}	1.23
Uncertain													
rs2603127	A	0.247	0.213	2.69×10^{-7}	1.34	0.404	1.09	0.078	1.00	0.085	1.19	5.36×10^{-8}	1.30

SNPs rs17008723, rs2603127, and rs6902119 were located at nt 71,573,135 in the *FOXP1* region of 3p13; nt 167,505,791 in the *CCR6* region of 6q27; and nt 108,243,551 in the *MYH15* region of 3q13.13, respectively. SNP nucleotide positions are from GRCh37 and genes in close proximity to the designated SNP are denoted. AF_{RA}, allele frequency of the risk allele. EIGENSTRAT GWA *P*-values for SNPs rs17008723, rs6902119, and rs2603127 were 6.25×10^{-6}, 7.23×10^{-5}, and 4.50×10^{-7}, 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 $P < 1.00 \times 10^{-2}$, and the significance threshold for the overall combined GWA + replication stage 1 + 2 meta-analysis was $P < 5 \times 10^{-8}$. *FOXP1* SNP rs17008713 from the GWA study could not be genotyped in the replication study for technical reasons; therefore, genotypes for nearby SNP rs17008723 ($r^2 = 0.99$) were imputed, and rs17008723 was genotyped in the replication study. Data for SNPs rs2192346 and rs7868451, for which association was excluded, are shown in Supplementary Table 3.