Andrzej T. Slominski[1], Wei Li[2], Syamal K. Bhattacharya[3], Richard A. Smith[4], Patti L. Johnson[3], Jianjun Chen[2], Kathleen E. Nelson[5], Robert C. Tuckey[6], Duane Miller[2], Yan Jiao[4], Weikuan Gu[4] and Arnold E. Postlethwaite[3,7,8]

[1]*Department of Pathology, University of Tennessee Health Science Center, Memphis, Tennessee, USA;* [2]*Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, Tennessee, USA;* [3]*Department of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee, USA;* [4]*Orthopedic Surgery, College of Pharmacy, University of Tennessee Health Science Center, Memphis, Tennessee, USA;* [5]*Department of Biology, Christian Brothers University, Memphis, Tennessee, USA;* [6]*School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, Crawley, Western Australia, Australia;* [7]*Division of Connective Tissue Diseases, University of Tennessee Health Science Center, Memphis, Tennessee, USA and* [8]*Department of Veterans Affairs Medical Center, Christian Brothers University, Memphis, Tennessee, USA*

E-mail: aslominski@uthsc.edu

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Bhattacharya SK (1977) Simultaneous determination of calcium and magnesium in human blood serum by atomic absorption spectrophotometer. *Anal Lett* 10:817–30

Bikle DD (2010) Vitamin D: newly discovered actions require reconsideration of physiologic requirements. *Trends Endocrinol Metab* 21:375–84

Holick MF, Garabedian M, Schnoes HK *et al.* (1975) Relationship of 25-hydroxyvitamin D3 side chain structure to biological activity. *J Biol Chem* 250:226–30

Liu S, Tang W, Zhou J *et al.* (2006) Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D. *J Am Soc Nephrol* 17:1305–15

Moro JR, Iwata M, von Andriano UH (2008) Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat Rev Immunol* 8:685–98

Postlethwaite AE, Smith GN, Lachman LB *et al.* (1989) Stimulation of glycosaminoglycan synthesis in cultured human dermal fibroblasts by interleukin 1. *J Clin Invest* 83:629–36

Raghow R, Postlethwaite AE, Keski-Oja J *et al.* (1987) Transforming growth factor-beta increases steady state levels of type I procollagen and fibronectin messenger RNAs posttranscriptionally in cultured human dermal fibroblasts. *J Clin Invest* 79:1285–8

Saito H, Maeda A, Ohtomo S *et al.* (2005) Circulating FGF-23 is regulated by 1alpha, 25-dihydroxyvitamin D3 and phosphorus *in vivo*. *J Biol Chem* 280:2543–9

Shackleton C, Roitman E, Guo LW *et al.* (2002) Identification of 7(8) and 8(9) unsaturated adrenal steroid metabolites produced by patients with 7-dehydrosterol-delta7-reductase deficiency (Smith-Lemli-Opitz syndrome). *J Steroid Biochem Mol Biol* 82:225–32

Slominski A, Zjawiony J, Wortsman J *et al.* (2004) A novel pathway for sequential transformation of 7-dehydrocholesterol and expression of the P450scc system in mammalian skin. *Eur J Biochem/FEBS* 271:4178–88

Slominski AT, Janjetovic Z, Fuller BE *et al.* (2010) Products of vitamin D3 or 7-dehydrocholesterol metabolism by cytochrome P450scc show anti-leukemia effects, having low or absent calcemic activity. *PLoS One* 5:e9907

Slominski AT, Zmijewski MA, Semak I *et al.* (2009) Sequential metabolism of 7-dehydrocholesterol to steroidal 5,7-dienes in adrenal glands and its biological implication in the skin. *PLoS One* 4:e4309

Szodoray P, Nakken B, Gaal J *et al.* (2008) The complex role of vitamin D in autoimmune diseases. *Scand J Immunol* 68:261–9

Zmijewski MA, Li W, Zjawiony JK *et al.* (2009) Photo-conversion of two epimers (20R and 20S) of pregna-5,7-diene-3beta, 17alpha, 20-triol and their bioactivity in melanoma cells. *Steroids* 74:218–28

Genetic Variants in *CTLA4* Are Strongly Associated with Alopecia Areata

Journal of Investigative Dermatology (2011) **131,** 1169–1172; doi:10.1038/jid.2010.427; published online 24 February 2011

TO THE EDITOR

Alopecia areata (AA) is a common hair-loss disorder that affects approximately 1–2% of the general population (Safavi *et al.*, 1995). The occurrence of familial AA is well established (Blaumeiser *et al.*, 2006), and the pattern of familiality strongly suggests that its genetic basis is multifactorial. Our current understanding of the etiopathogenesis of AA is incomplete, but the condition is thought to be a tissue-specific autoimmune disease directed against the hair follicle (Tobin, 2003).

Numerous studies in the past decade have reported an association between variants of the gene coding for the cytotoxic T lymphocyte antigen-4 (*CTLA4*) and some of the autoimmune diseases, including Graves' disease, antineutrophil cytoplasmic antibody–associated vasculitis, type 1 diabetes, and rheumatoid arthritis (Kristiansen *et al.*, 2000; Ueda *et al.*, 2003). CTLA4 is a costimulatory molecule that is expressed on activated T cells and is involved in the negative regulation of T-cell activation (Brunet *et al.*, 1987). Given the autoimmune component shared by the various autoimmune diseases, we aimed to investigate the role of *CTLA4* in the development of AA. We performed a high-resolution association analysis of the *CTLA4* gene locus using 22 tagging single-nucleotide polymorphisms (SNPs) in a sample of 1,196 unrelated AA patients and 1,280 controls of Central European origin. During the final preparation of this report, a genome-wide association study was published by Petukhova *et al.* (2010) that implicates several new gene loci for AA, including *CTLA4*.

In our study, eight variants showed nominal significance in the combined sample (Table 1). The strongest association was found for rs3087243, which is located 236 bp downstream of *CTLA4* (Figure 1). This had a nominal *P*-value (P_{nom}) of 4.66×10^{-7} and an odds ratio

Abbreviations: AA, alopecia areata; CTLA4, cytotoxic T lymphocyte antigen-4; OR, odds ratio; SNP, single-nucleotide polymorphism

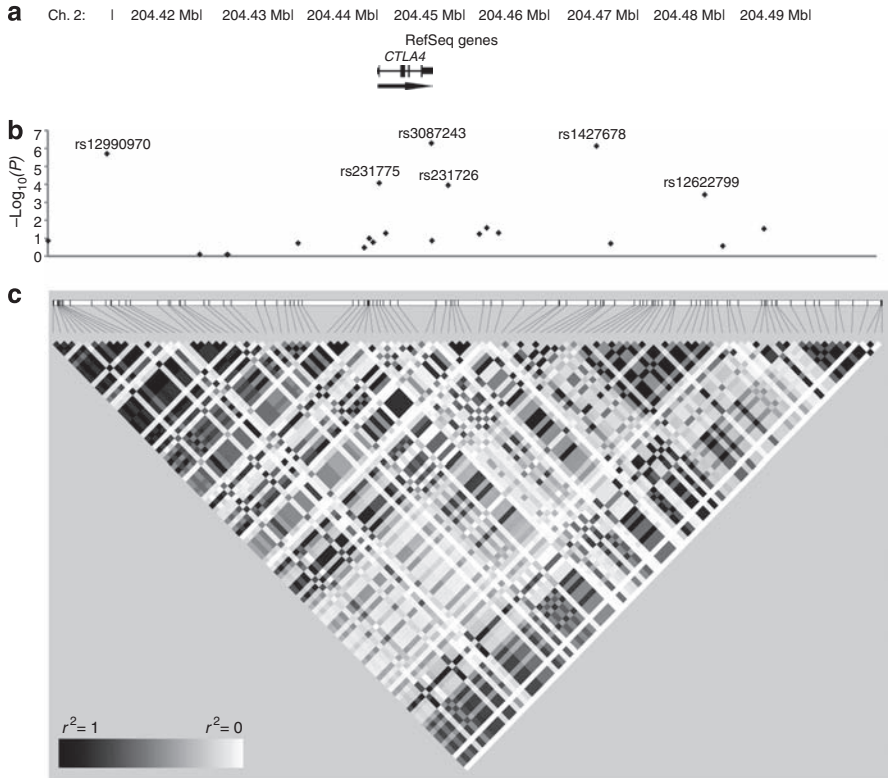

Figure 1. Details of the investigated genomic region (204 402 596–204 498 096 bp; NCBI reference sequence build 36) on chromosome 2. (**a**) Transcript information for the investigated cytotoxic T lymphocyte antigen-4 (*CTLA4*) locus (UCSC Genome Browser, build 36), with arrows indicating the direction of transcription. (**b**) Negative $\log_{10}$ association *P*-values of markers analyzed in the case–control study. (**c**) Linkage disequilibrium (LD) at the *CTLA4* locus is displayed by r^2. LD and haplotype blocks were analyzed using Haploview software (version 4.1).

(OR) of 1.34 (95% confidence interval: 1.20–1.50) (Table 1). In total, six of the eight nominally significant SNPs withstood Bonferroni correction for multiple testing (Table 1). Genotype distributions are shown in Supplementary Table S1 online.

In the subgroup analyses, the highest ORs were observed among the following groups of cases: (i) severe, (ii) early age at onset, and (iii) positive family history (Table 1). The highest OR was observed in the severe group for rs1427678, which is located approximately 20 kb downstream of *CTLA4* ($P_{nom} = 6.38 \times 10^{-10}$; OR = 1.55 (1.35–1.78)). In the analysis of only mild cases, one marker (rs3087243) showed a significant ($P_{nom} = 0.03$) association, although this result did not withstand correction for multiple testing (data not shown).

We then performed a conditional association analysis of the combined sample to test whether the most strongly associated marker (rs1427678) alone was able to explain the association signal observed at this locus. In this analysis, one additional SNP (rs11571290) showed nominal significance ($P_{nom} = 0.017$) after accounting for rs1427678. However, when the conditional analysis was restricted to the severely affected cases, rs1427678 explained the whole association signal, with no additional effect from other SNPs.

We also investigated which of the clinical covariates contributed independently to the association. Severity, in combination with rs1427678, significantly improved the fit of a logistic model ($P = 5.98 \times 10^{-7}$). The other covariates did not improve the model fit (e.g., $P = 0.15$ for early age at onset). A haplotype analysis did not significantly improve the association findings (data not shown).

Our findings and the findings by Petukhova *et al.* (2010) provide strong evidence for the association of *CTLA4* with AA, and indicate that the *CTLA4* locus might be a genetic factor that is shared between AA and other autoimmune diseases. We observed the strongest effect in patients with severe disease, as observed previously for other AA susceptibility genes (Betz *et al.*, 2007, 2008; Redler *et al.*, 2010). The usefulness of the severity criterion in defining the group of patients that drives the association is demonstrated by the results of the logistic regression analysis. In this analysis, inclusion of other covariates, such as age at onset and familiality, yielded no significant improvement in the association finding (Table 1).

Our results revealed that rs3087243 was the best of the 21 analyzed SNPs in the combined sample, with a corrected *P*-value of 4.89×10^{-5} (OR = 1.34 (1.20–1.50)). This is the most consistently implicated SNP in other autoimmune diseases. The size of the genetic effect observed in our sample is comparable to that observed for other autoimmune diseases (Ueda *et al.*, 2003; Plenge *et al.*, 2005). However, the functional impact of this variant, which is located in the 3′ untranslated region of *CTLA4*, remains unclear. It has been suggested that this variant may affect the expression of *CTLA4*, given that decreased levels of soluble CTLA4 have been observed in carriers of the susceptibility allele (Ueda *et al.*, 2003; Maier *et al.*, 2007). However, the present findings cannot exclude the possibility that a variant that is in linkage disequilibrium with rs3087243 is the true causative variant. Petukhova *et al.* (2010) found the strongest association for rs1024161, a SNP that was not examined in our study. The variant rs3087243 was not genotyped in their study, but, based on imputation, it too showed a highly significant association.

The SNP rs231775 is the only validated nonsynonymous SNP in the coding region of *CTLA4*. The results of *in vitro* studies have shown that the amino-acid substitution p.Thr17Ala in the signal peptide of CTLA4 causes defective endoplasmic reticulum processing of a significant portion of the susceptibility allele molecules

Table 1. Case–control association analysis between selected SNPs at the *CTLA4* locus and alopecia areata, with subgroup analyses for severe cases, early-age-at-onset cases, and cases with a positive family history

			MAF[1]					Severe cases			Early age of onset			Positive family history		
SNP	Position[2]	Allele (A/B)	Ca	Co	*P* Armitage	*P* corr.[3]	Allelic OR[4] (95% CI)	MAF[1] Ca	*P* Armitage	OR (95% CI)[4]	MAF[1] Ca	*P* Armitage	OR (95% CI)[4]	MAF[1] Ca	*P* Armitage	OR (95% CI)[4]
rs11571308	204 402 596	C/T	0.134 (T)	0.120 (T)	0.145	1	1.13 (0.96–1.34)	0.143 (T)	0.052	1.22 (1.00–1.49)	0.134 (T)	0.257	1.13 (0.91–1.41)	0.126 (T)	0.671	1.06 (0.82–1.36)
rs12990970	204 408 934	C/T	0.373 (T)	0.439 (T)	1.92×10^{-6}	2.02×10^{-4}	1.32 (1.18–1.48)	0.341 (T)	1.39×10^{-8}	1.51 (1.31–1.74)	0.358 (T)	6.57×10^{-6}	1.41 (1.21–1.64)	0.368 (T)	7.10×10^{-4}	1.34 (1.13–1.59)
rs11903660	204 419 833	C/T	0.057 (T)	0.055 (T)	0.769	1	1.04 (0.81–1.32)	0.051 (T)	0.635	1.08 (0.80–1.46)	0.048 (T)	0.428	1.15 (0.82–1.59)	0.058 (T)	0.726	1.07 (0.75–1.52)
rs6741283	204 423 055	C/T	0.057 (T)	0.055 (T)	0.784	1	1.04 (0.81–1.32)	0.051 (T)	0.643	1.08 (0.79–1.46)	0.049 (T)	0.443	1.14 (0.82–1.59)	0.059 (T)	0.696	1.07 (0.75–1.53)
rs11571290	204 431 386	A/G	0.039 (A)	0.046 (A)	0.190	1	1.20 (0.91–1.59)	0.042 (A)	0.547	1.11 (0.79–1.55)	0.041 (A)	0.430	1.15 (0.81–1.65)	0.032 (A)	0.093	1.47 (0.94–2.32)
rs733618	204 439 189	C/T	0.083 (C)	0.075 (C)	0.353	1	1.10 (0.90–1.36)	0.097 (C)	0.025	1.32 (1.04–1.68)	0.089 (C)	0.162	1.20 (0.93–1.56)	0.085 (C)	0.396	1.14 (0.84–1.54)
rs16840252	204 439 764	C/T	0.192 (T)	0.173 (T)	0.096	1	1.13 (0.98–1.31)	0.196 (T)	0.093	1.16 (0.98–1.39)	0.185 (T)	0.412	1.08 (0.90–1.31)	0.188 (T)	0.379	1.10 (0.89–1.37)
rs11571317	204 440 253	C/T	0.070 (T)	0.081 (T)	0.165	1	1.16 (0.94–1.44)	0.060 (T)	0.025	1.37 (1.04–1.80)	0.058 (T)	0.015	1.44 (1.07–1.94)	0.066 (T)	0.187	1.24 (0.90–1.73)
rs231775	204 440 959	A/G	0.415 (G)	0.361 (G)	9.00×10^{-5}	0.009	1.26 (1.12–1.41)	0.446 (G)	5.84×10^{-7}	1.43 (1.24–1.64)	0.439 (G)	1.38×10^{-5}	1.39 (1.20–1.61)	0.433 (G)	4.78×10^{-4}	1.35 (1.14–1.60)
rs231777	204 441 833	C/T	0.172 (T)	0.152 (T)	0.052	1	1.16 (1.00–1.35)	0.176 (T)	0.055	1.20 (1.00–1.44)	0.163 (T)	0.406	1.09 (0.89–1.33)	0.164 (T)	0.424	1.10 (0.88–1.37)
rs3087243	204 447 164	A/G	0.395 (A)	0.466 (A)	4.66×10^{-7}	4.89×10^{-5}	1.34 (1.20–1.50)	0.362 (A)	2.49×10^{-9}	1.54 (1.33–1.77)	0.378 (A)	1.83×10^{-6}	1.43 (1.24–1.66)	0.381 (A)	5.69×10^{-5}	1.42 (1.20–1.68)
rs11571319	204 447 183	A/G	0.190 (A)	0.174 (A)	0.136	1	1.12 (0.97–1.29)	0.193 (A)	0.144	1.14 (0.96–1.36)	0.183 (A)	0.503	1.07 (0.89–1.29)	0.187 (A)	0.422	1.09 (0.88–1.35)
rs231726	204 449 111	C/T	0.357 (T)	0.306 (T)	1.11×10^{-4}	0.012	1.26 (1.12–1.42)	0.379 (T)	7.58×10^{-6}	1.38 (1.20–1.60)	0.376 (T)	5.13×10^{-5}	1.36 (1.17–1.59)	0.374 (T)	5.24×10^{-4}	1.36 (1.14–1.61)
rs231731	204 452 775	C/T	0.222 (C)	0.200 (C)	0.060	1	1.14 (1.00–1.31)	0.233 (C)	0.021	1.21 (1.03–1.43)	0.219 (C)	0.221	1.12 (0.94–1.33)	0.217 (C)	0.330	1.11 (0.90–1.35)
rs13030054	204 453 672	C/T	0.244 (T)	0.217 (T)	0.027	1	1.16 (1.02–1.33)	0.255 (T)	0.009	1.23 (1.05–1.45)	0.240 (T)	0.137	1.14 (0.96–1.35)	0.244 (T)	0.130	1.16 (0.96–1.41)
rs11571300	204 455 012	A/G	0.123 (G)	0.141 (G)	0.056	1	1.17 (0.99–1.38)	0.113 (G)	0.015	1.29 (1.05–1.60)	0.120 (G)	0.090	1.21 (0.97–1.50)	0.134 (G)	0.631	1.06 (0.83–1.35)
rs1427678	204 466 603	A/G	0.443 (A)	0.486 (G)	7.12×10^{-7}	7.48×10^{-5}	1.33 (1.19–1.49)	0.405 (A)	6.38×10^{-10}	1.55 (1.35–1.78)	0.421 (A)	5.74×10^{-7}	1.45 (1.25–1.68)	0.437 (A)	2.83×10^{-4}	1.36 (1.15–1.61)
rs2882974	204 468 309	C/T	0.477 (T)	0.494 (T)	0.216	1	1.07 (0.96–1.20)	0.463 (T)	0.065	1.13 (0.99–1.30)	0.467 (T)	0.135	1.12 (0.96–1.29))	0.462 (T)	0.125	1.14 (0.96–1.34)
rs12622799	204 479 323	C/T	0.258 (T)	0.303 (T)	4.04×10^{-4}	0.042	1.25 (1.11–1.42)	0.229 (T)	2.96×10^{-6}	1.46 (1.25–1.71)	0.245 (T)	4.98×10^{-4}	1.34 (1.14–1.58)	0.246 (T)	3.59×10^{-3}	1.33 (1.10–1.61)
rs2217202	204 481 598	A/G	0.035 (G)	0.029 (G)	0.274	1	1.19 (0.87–1.64)	0.035 (G)	0.360	1.19 (0.81–1.75)	0.034 (G)	0.472	1.16 (0.77–1.75v	0.025 (G)	0.534	1.18 (0.70–1.98)
rs7597297	204 486 339	G/T	0.219 (G)	0.194 (G)	0.032	1	1.16 (1.01–1.33)	0.233 (G)	0.006	1.26 (1.07–1.48)	0.220 (G)	0.078	1.17 (0.98–1.40)	0.213 (G)	0.266	1.12 (0.91–1.38)
rs1465538	204 498 096	C/T	Marker not biallelic	—	—	—	—	—	—	—	—	—				

Abbreviations: Ca, cases; CI, confidence interval; Co, controls; *CTLA4*, cytotoxic T lymphocyte antigen-4; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.
[1]Minor alleles in parentheses.
[2]In bp, NCBI reference sequence build 36.
[3]*P*-values corrected for multiple testing.
[4]Odds ratio calculation based on the risk allele.

(CTLAAla[17]) and that this results in inefficient glycosylation and decreased cell-surface expression (Anjos *et al.*, 2002). Our association results show that rs231775 was also strongly associated with AA in our sample although the *P*-values were less significant and the ORs were lower than those for rs3087243. Furthermore, conditional analysis revealed that rs1427678 explained the entire association signal at the locus.

In conclusion, our results provide strong support for the hypothesis that *CTLA4* is a susceptibility gene for AA, and they also suggest that it has the strongest effect in patients with a severe form of the disorder. Given the low *P*-values observed in our study and the genome-wide association study by Petukhova *et al.* (2010), we consider *CTLA4* a proven susceptibility gene for AA.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank the patients and controls for their participation in this study. Silke Redler is the recipient of a BONFOR fellowship from the Medical Faculty of the University of Bonn. Markus Nöthen is the recipient of a grant from Alfried Krupp von Bohlen und Halbach-Stiftung. Regina C. Betz is a past recipient of an Emmy-Noether fellowship.

Karsten K.-G. John[1,12], Felix F. Brockschmidt[1,2,12], Silke Redler[1], Christine Herold[3], Sandra Hanneken[4], Sibylle Eigelshoven[4], Kathrin A. Giehl[5], Jozef De Weert[6], Gerhard Lutz[7], Roland Kruse[8], Hans Wolff[5], Bettina Blaumeiser[9], Markus Böhm[10], Tim Becker[3,11], Markus M. Nöthen[1,2] and Regina C. Betz[1]

[1]Institute of Human Genetics, University of Bonn, Bonn, Germany; [2]Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany; [3]Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany; [4]Department of Dermatology, University of Düsseldorf, Düsseldorf, Germany; [5]Department of Dermatology, University of Munich, Munich, Germany; [6]Department of Dermatology, University Hospital of Gent, Gent, Belgium; [7]Hair & Nail, Wesseling, Germany; [8]Dermatological Practice, Paderborn, Germany; [9]Department of Medical Genetics, University of Antwerp, Antwerp, Belgium; [10]Department of Dermatology, University of Münster, Münster, Germany and [11]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany
E-mail: regina.betz@uni-bonn.de
[12]These authors contributed equally to this work.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Anjos S, Nguyen A, Ounissi-Benkalha H *et al.* (2002) A common autoimmunity predisposing signal peptide variant of the cytotoxic T-lymphocyte antigen 4 results in inefficient glycosylation of the susceptibility allele. *J Biol Chem* 277:46478–86

Betz RC, König K, Flaquer A *et al.* (2008) The R620W polymorphism in PTPN22 confers general susceptibility for the development of alopecia areata. *Br J Dermatol* 158:389–91

Betz RC, Pforr J, Flaquer A *et al.* (2007) Loss-of-function mutations in the filaggrin gene and alopecia areata: strong risk factor for a severe course of disease in patients comorbid for atopic disease. *J Invest Dermatol* 127: 2539–43

Blaumeiser B, van der Goot I, Fimmers R *et al.* (2006) Familial aggregation of alopecia areata. *J Am Acad Dermatol* 54:627–32

Brunet JF, Denizot F, Luciani MF *et al.* (1987) A new member of the immunoglobulin superfamily—CTLA-4. *Nature* 328:267–70

Kristiansen OP, Larsen ZM, Pociot F (2000) CTLA-4 in autoimmune diseases—a general susceptibility gene to autoimmunity? *Genes Immun* 1:170–84

Maier LM, Anderson DE, De Jager PL *et al.* (2007) Allelic variant in CTLA4 alters T cell phosphorylation patterns. *Proc Natl Acad Sci USA* 104:18607–12

Petukhova L, Duvic M, Hordinsky M *et al.* (2010) Genome-wide association study in alopecia areata implicates both innate adaptive immunity. *Nature* 466:113–7

Plenge RM, Padyukov L, Remmers EF *et al.* (2005) Replication of putative candidate-gene associations with rheumatoid arthritis in >4000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. *Am J Hum Genet* 77:1044–60

Redler S, Brockschmidt FF, Forstbauer L *et al.* (2010) The TRAF1/C5 locus confers risk for familial and severe alopecia areata. *Br J Dermatol* 162:866–9

Safavi KH, Muller SA, Suman VJ *et al.* (1995) Incidence of alopecia areata in Olmsted County, Minnesota, 1975 through 1989. *Mayo Clinic Proc* 70: 628–33

Tobin DJ (2003) Characterization of hair follicle antigens targeted by the anti-hair follicle immune response. *J Investig Dermatol Symp Proc* 8:176–81

Ueda H, Howson JM, Esposito L *et al.* (2003) Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423:506–11

Erythropoietic Uroporphyria Associated with Myeloid Malignancy Is Likely Distinct from Autosomal Recessive Congenital Erythropoietic Porphyria

Journal of Investigative Dermatology (2011) **131,** 1172–1175; doi:10.1038/jid.2011.5; published online 17 February 2011

TO THE EDITOR

Congenital erythropoietic porphyria (CEP; MIM 263700) is a rare autosomal recessive disease caused by mutations in uroporphyrinogen III synthase (*UROS*) or, rarely, in *GATA1* genes, leading to UROS deficiency (Fritsch *et al.*, 1997; de Verneuil *et al.*, 2003; Phillips *et al.*, 2007). The resulting overproduction of type I porphyrin isomers by erythroid cells causes severe photosensitivity and hemolytic anemia.

Abbreviations: BFU, burst-forming unit; CEP, congenital erythropoietic porphyria; MDS, myelodysplastic syndrome; UROS, uroporphyrinogen III synthase