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J Invest Dermatol. Author manuscript; available in PMC 2012 September 01.

Published in final edited form as:

J Invest Dermatol. 2012 March ; 132(3 0 1): 650–657. doi:10.1038/jid.2011.374.

Genetic Variants in Interferon Regulatory Factor 2 (*IRF2*) are Associated with Atopic Dermatitis and Eczema Herpeticum

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Abstract

Interferon regulatory factor 2 (*IRF2*) is a member of a family of transcriptional factors involved in the modulation of interferon induced immune responses to viral infection. To test whether genetic variants in *IRF2* predict risk of AD and ADEH, we genotyped 78 *IRF2* tagging single nucleotide polymorphisms (SNPs) in both European American (n=435) and African American (n = 339) populations. Significant associations were observed between AD and two SNPs (rs793814, P = 0.007, odds ratio (OR) = 0.52; rs3756094, P = 0.037, OR = 0.66) among European Americans and one SNP (rs3775572, P = 0.016, OR = 0.46) among African Americans. Significant associations were also observed between ADEH and five SNPs (P = 0.049-0.022) among European Americans. The association with ADEH was further strengthened by haplotype analyses, wherein a 5-SNP (CAGGA) haplotype showed the strongest association with ADEH (P = 0.0008). Eight *IRF2* SNPs were significantly associated with IFNγ production post-herpes simplex virus (HSV) stimulation (P = 0.048-0.0008), including an AD-associated SNP (rs13139310, P = 0.008). Our findings suggest distinct markers in *IRF2* may be associated with AD and ADEH, which may depend upon

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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ethnic ancestry, and genetic variants in *IRF2* may contribute to an abnormal immune response to HSV.

INTRODUCTION

Atopic dermatitis (AD) is a chronic skin disease affecting up to 15% of children in industrialized countries (Boguniewicz and Leung, 2010) (Homey *et al.*, 2006) (Barnes, 2009). A rare but serious complication of AD is eczema herpeticum (ADEH). We have recently reported that AD patients with ADEH have more severe Th2-polarized disease with greater allergen sensitization and more commonly have a history of food allergy, asthma, or both, compared to patients with AD only (Beck *et al.*, 2009). Although it is well-known that the primary predisposing factor for ADEH is herpes simplex virus-1 (HSV-1) exposure (Xu *et al.*, 2006) (Tay *et al.*, 1999), genetic susceptibility may be important. Indeed, our previous studies have demonstrated that a relatively uncommon null mutation in filaggrin (FLG, R501X), a major barrier protein, was 3 times more prevalent in patients with ADEH than those AD patients without EH (24% vs 8%, respectively) (Gao *et al.*, 2009b). In addition, skin barrier defects due to FLG mutations have been shown to play a crucial role in the development of AD (Nemoto-Hasebe *et al.*, 2009). However, the disease-associated allele is only present in 14% of ADEH cases among European Americans, and was less frequent in non-whites, suggesting additional risk variants in FLG or others are involved.

Our recent studies found that patients with ADEH have markedly reduced levels of interferon-gamma (IFNγ) compared with AD patients without EH, and the reduced IFNγ production may be due to the *IFNG* and *IFNGR1* SNPs (Leung *et al.*, 2011). IFNγ is a Th1 cytokine that plays a major role in the host innate and adaptive immune responses by activating macrophages, enhancing NK cell activation, promoting T cell differentiation as well as regulating B cell isotope switching to IgG2, and is implicated in the pathogenesis of allergic diseases (Gariboldi *et al.*, 2009; Herberth *et al.*, 2010; Wild *et al.*, 2000). Interestingly, infants with reduced frequencies of IFNγ-producing $CD4^+$ T cells in the cord blood (1^{st} quartile) had a higher risk of developing AD, suggesting that IFNγ is critical in controlling the development of AD.

Interferon regulatory factor 2 (*IRF2*) is a member of a family of transcriptional factors involved in the modulation of cellular responses to interferons (IFNs) and viral infection (Harada *et al.*, 1989). *IRF2*, a transcription repressor (Lace *et al.*, 2010; Matsuyama *et al.*, 1993), is induced by IFNγ and acts as an antagonist to IRF1 to block the IFNγ-mediated pathway (Kroger *et al.*, 2002). *IRF2* has been suggested to play a role in negative control of basophil expansion, which is critical for the regulation of Th1/Th2 balance (Hida *et al.*, 2005). Interestingly, Irf2 knockout mice show a defect in Th1 cell development and spontaneous development of an inflammatory skin disease with histologic evidence of epidermal thickening and keratinocyte proliferation similar to human AD (Hida *et al.*, 2000). All the studies suggest that IRF2 may be an important candidate gene for AD and ADEH.

Mutations in the gene encoding *IRF2* have been associated with psoriasis (Foerster *et al.*, 2004b) and AD (Nishio *et al.*, 2001) in Japanese subjects. However, the Japanese study was limited to a small number of subjects (N=24 cases and 24 controls). Rather than replicating

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the Japanese findings, we sought to test for associations between *IRF2* variants and AD using a comprehensive tagging SNP approach in ethnically diverse populations (European Americans, African Americans) participating in the NIH/NIAID sponsored, multicenter Atopic Dermatitis and Vaccinia Network (ADVN), and to explore the potential role of *IRF2* in a more severe form of AD, ADEH. We further tested for association between *IRF2* SNPs and IFNγ generation in mock- or HSV-infected PBMCs.

RESULTS

A total of 78 SNPs were genotyped in *IRF2* spanning a 98.7-kb region on chromosome 4q34.1–q35.1 (Figure S1). Genotype frequencies for all SNPs agreed with expectations under Hardy-Weinberg equilibrium. As shown in Figure S1, LD structure differed considerably between the two ethnic groups, with five LD blocks within *IRF2* for the African Americans and 10 LD blocks for the European Americans using the criteria of Gabriel *et al* (Gabriel *et al.*, 2002).

IRF2 variants are associated with AD in two independent North American populations

We first tested for association between genetic variants in *IRF2* and diagnosis of AD independently in the European American and African American samples. As summarized in Table 1, significant associations were observed for *IRF2* SNPs and AD in both European American and African American populations, albeit for different sets of SNPs (rs13139310, rs793814, rs12504466, rs3756094 [P = 0.045-0.002], and rs3775572, rs793794, rs793777, rs6831978 [P = 0.050-0.006], respectively) in different loci of the *IRF2* gene. Two SNPs remained significant after permutations correcting for multiple testing in the European American sample (rs793814, P = 0.007, Odds = 0.52, 95%CI =0.33–0.80; rs3756094, P = 0.037, OR = 0.66, 95%CI = 0.40–0.94) and one SNP remained significant in the African American sample (rs3775572, P = 0.016, OR = 0.46, 95%CI =0.25–0.83); all three markers were localized to intron 1. Haplotype analysis failed to identify any enhanced associations compared with single SNP analysis (Figure 1a).

IRF2 variants are associated with ADEH among European Americans

We next tested for association between European American AD patients with ADEH (N=112) compared to AD patients without ADEH (N=166), and observed significant associations for eight *IRF2* SNPs (P-value=0.008–0.043, OR range = 0.39–2.50, Table 1), with an intronic SNP rs809909 yielding the strongest association (P = 0.008, OR = 0.59, 95%CI=0.38–0.90). A synonymous SNP (rs3775543) in exon 9 that was previously associated with type 1 psoriasis (Foerster *et al.*, 2004a) was significantly associated with an increased risk of ADEH (P = 0.023, OR = 2.50, 95%CI =1.02–6.11). After correction for multiple testing, five SNPs remained significant (rs17488073, rs809909, rs11132242, rs1342852, and rs1124191, P = 0.049-0.022). In a sliding window haplotype analysis (2–5 SNP windows) (Figure 1B), additional associations were observed, with the strongest signal for a 5-SNP (CAGGA) haplotype spanning a region of 1.1kb on chromosome 4q34–35 that included marker rs809909 (minor allele frequency of 36.7% in ADEH+ and 27.2% in ADEH−, P = 0.0008, Table 2).

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IRF2 variants are associated with *IFNγ* ELISPOT values

To explore the role of *IRF2* in regulating IFNγ generation, we tested for association between the 78 *IRF2* SNPs and levels of IFNγ as determined by Spot Forming Units (SFU) in mock- or HSV-stimulated groups. Within the mock-stimulated group, association was observed for only one AD-associated SNP in the full sample (rs12504466, β = 0.260, *P* = 0.015, data not shown). However, when analyses were restricted to the HSV-stimulated group, significant associations were observed for 8 SNPs and the ELISPOT values in the full sample (N=64, *P*-value range of 0.047 to 0.0008, Table 3), of which SNP rs7677486 showed the strongest association with lower IFNγ ELISPOT values post HSV stimulation (β = −0.385, *P* = 0.0008). Of these, five SNP were also associated with IFNγ production among AD subjects (N=44, *P*-value range of 0.022 to 0.008, n=39). Interestingly, an AD-associated SNP rs13139310 was significantly associated with reduced levels of IFNγ (GG vs GA+AA, β = −0.326, *P* = 0.008, Figure 2).

Reduced IRF2 expression in ADEH patients

It is possible that a low amount of IFNγ in ADEH patients may be due to a lower IRF2 expression. To test the hypothesis, we specifically analyzed the IRF2 expression in non-lesional skin biopsies from ADEH, AD patients, and non-atopic patients in our recent geneChip profiling studies (Grigoryev *et al.*). A reduced expression was seen in skin biopsies from ADEH patients (n = 5) compared with AD patients (n=11) (ADEH *vs* AD, *P* = 0.048, Figure 3a). Of interest, the reduced expression in ADEH was further validated when RT-PCR was performed in additional sets of skin non-lesional biopsies from ADEH (n=8) and AD (n=10) patients (ADEH vs AD, *P* =0.029, Figure 3b).

DISCUSSION

The current study examined genetic variants in *IRF2* for association with AD and its serious clinical complication, ADEH. We selected 78 tagging SNPs covering the *IRF2* gene and tested for association in two independent North American populations. Our data demonstrated significant associations for *IRF2* SNPs and risk of AD and ADEH in both ethnic groups. Given the IFNγ generation we observed was significantly lower in PBMCs from AD patients, particularly ADEH patients, compared to non-atopic individuals after stimulation with HSV *ex vivo*, we further tested whether these genetic variants contribute to abnormal IFNγ levels in mock- and HSV-stimulated PBMCs. Indeed, significant associations were observed between *IRF2* SNPs and IFNγ production in HSV-stimulated PBMCs from a subset of ADVN subjects. Overall, our results suggest that genetic variants in *IRF2* are associated with risk of AD and ADEH, and they may contribute to an abnormal response to HSV exposure.

In this study, we selected 78 tagging SNPs to provide comprehensive coverage of the *IRF2* gene; among those, four SNPs are located in the promoter region flanking the SNP - 467G/A associated with AD in Japanese families (Nishio *et al.*, 2001), but none of these were associated with AD and its associated phenotypes. The exonic SNP rs3775543 [921G/A, (Gly/Gly)] that was previously associated with type I psoriasis (Foerster *et al.*, 2004b) showed significant association with ADEH among European Americans, with an effect size

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of 2.50. Because the marker rs3775543 is located at the +3 position of exon 9 and breaks a consensus splicing site sequence, we speculate that this mutation may lead to the abolishment of the splicing site, resulting in the intron remaining in mature mRNA and subsequently producing aberrant proteins. Indeed, mutations causing incorrect splicing of β-globin mRNA have been shown to be responsible for some cases of β thalassemia (Sierakowska *et al.*, 1996). Additionally, the 921A allele of the exonic SNP rs3775543 was predicted to bind to the SR (serine/arginine-rich) family member ASF/SF2, a pre-mRNA splicing factor playing a role in mRNA stability (Li and Manley, 2005) (Lemaire *et al.*, 2002), and this binding site was completely abolished in the 921G allele (Foerster *et al.*, 2004b). It would be of interest to determine whether marker rs3775543 (921G/A) genotypes contribute to the regulation of IRF2 expression.

We also observed evidence for an association between other *IRF2* SNPs and risk of ADEH. Haplotype analyses strengthened the evidence for association with ADEH+ among European Americans as compared to single marker analyses. In particular, a 5-SNP haplotype C-A-G-G-A spanning a region of 1.12 kb in intron 6 of *IFR2* (rs377552, rs809909, rs7655371, rs6812958, and rs2797507) showed the strongest association with risk of ADEH (ADEH+ *vs* ADEH−, 36.7% *vs* 27.2%, $P = 0.0008$). Furthermore, a 7.2-kb region within intron 6 centered on this haplotype contains three SNPs most strongly associated with ADEH+ (rs809909, rs11132242, and rs17488073), suggesting genetic variants in or around this region may influence ADEH susceptibility. Replication of these associations was performed in the smaller subset of African American ADEH patients, and significant associations between two SNPs were observed, which included SNP rs377552, a marker localized in the same region as the haplotype associated with ADEH among the European Americans (data not shown). Unfortunately, the modest African American sample renders these findings tentative until replication can be performed in a larger cohort.

Although we observed significant associations with AD in both ethnic groups, SNPs associated with AD in the European Americans did not overlap with those associated with AD in the African Americans. Failure to observe SNP-for-SNP replication in ethnically diverse populations is not uncommon, and may result primarily from variation i n allele frequencies, population admixture, heterogeneity of the phenotype, and environmental factors, as we have noted elsewhere (Mathias *et al.*, 2008). LD structure differed considerably between the two ethnic groups in this study, and different sets of tagging SNPs were determined to be optimal *a priori*, suggesting genetic heterogeneity. It is likely that variants other than those tested in this study are true causal variants, and the markers associated with disease in this study are presumably in strong linkage disequilibrium with the ungenotyped, "causative" SNP(s). We (Mathias *et al.*, 2008) and others (Neale and Sham, 2004) have suggested that a gene-based approach, rather than a SNP-for SNP approach, may provide evidence for genetic analysis at the functional level. In addition, to test for the possibility that associations observed were for manifestations of an HSV exacerbation in ADEH patients rather than the associated *IRF2* SNPs, we performed association analyses for HSV infection among controls, and found that none of the ADEH-associated SNPs showed associations with HSV infection, as defined by both HSV-1 and

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HSV-2 positivity (data not shown). The findings suggest these associations observed for AD and ADEH may be independent of HSV infection.

A major strength of our study is that we found significant associations between variants in *IRF2* and IFNγ production, particularly in HSV-stimulated PBMCs. To identify the possible mechanism, we explored whether genetic variants in *IRF2* contribute to the reduced levels of IFNγ. We observed significant associations for 8 *IRF2* SNPs in the full dataset and 5 SNPs remained significant among AD patients. Among these, SNP rs13139310, associated with IFNγ, was also associated with risk of AD. The results suggest that these *IRF2* SNPs may contribute to a defect in Th1 cell development by the down-regulation of IFNγ production. Additionally, we demonstrated a reduced IRF2 expression in ADEH patients as compared to AD. These current results are consistent with findings in the Irf2 knockout mice that showed a defect in Th1 cell development and spontaneous development of an inflammatory skin disease (Hida *et al.*, 2000). However, in this study, the sample size was relatively modest, and there was a lack of direct connection between *IRF2* SNPs, IRF2 expression, and IFNγ production, indicating that further studies are clearly warranted.

We recognize that the associations observed in this study between the *IRF2* SNPs and disease are not particularly robust, but we contend that the relatively modest *p*-values are the result of a limited sample size rather than a type-I error. The conventional Bonferroni correction is overly conservative and may miss real significant functional variants. Unfortunately, ADEH is a rare disease (~3%); the subjects used for this study have been recruited after a nearly five year effort from multiple-medical centers. Our power calculations demonstrated that the study population provided sufficient power (80%) to detect an OR of 2.06, even if the allele frequency with disease is only 10% (Figure S2). In addition, although we have attempted to replicate the findings in the African American population, the sample size is too small to make any informative conclusion. Although our sample size is limited, these findings, besides our recent report on *IFNG* and *IFNGR1*(Leung *et al.*, 2011), represent an important contribution to evidence that variants in *IRF2* may confer susceptibility to AD and its most severe complication, ADEH. More importantly, no interactions (Gene-gene interaction) were identified between *IRF2* and IFNG and IFNGR SNPs for either AD or ADEH (data not shown), indicating that the associations we observed for IRF2 are independent of the previous associations with IFNG and IFNGR1. As a next step, we will identify causal SNPs centered on the region showing the most significant associations with ADEH in a relatively large number of subjects with a comprehensive coverage and determine their functional relevance to the disease, including associations with IRF2 expression and IFNγ production, and establish their relationships with risk of ADEH.

Taken together, we have demonstrated evidence for an association between variants in *IRF2* and risk of AD, ADEH, and IFNγ production. To our knowledge this is previously unreported, we have provided evidence of association between ADEH and IFNγ production and genetic variants in *IRF2*. Our findings suggest *IRF2* may be a potential candidate gene and may play an important role in the pathogenesis of AD and ADEH by altering IFNγ production.

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MATERIALS AND METHODS

Study population and phenotypes

Subjects included 278 unrelated European American AD patients (of whom 112 had ADEH) and 157 healthy controls. For replication, we genotyped the same set of markers on 187 African American AD patients (of whom 32 had ADEH), and 156 healthy controls. Clinical characteristics of ADVN participants have been previously described (Beck *et al.*, 2009). Briefly, AD was diagnosed using the US consensus conference criteria (Eichenfield *et al.*, 2003). ADEH+ was defined as AD patients with at least one EH episode documented either by an ADVN investigator (or a physician affiliated with the same academic center) or diagnosis by another physician confirmed by HSV PCR, tissue immunofluorescence, Tzanck smear and/or culture (Hanifin *et al.*, 2001). Non-atopic, healthy controls were defined as having no personal history of chronic disease including atopy. All study participants were further evaluated by a detailed history and physical examination, as well as a questionnaire to assess history of cutaneous viral infections and concomitant medication use. In accordance with the Declaration of Helsinki Principles, the study was approved by the institutional review boards (IRB) at National Jewish Health, Johns Hopkins University School of Medicine, Oregon Health and Science University, University of California San Diego, Children's Hospital of Boston, and University of Rochester. All subjects gave written informed consent prior to participation.

Genotyping and quality control

A total of 78 *IRF2* SNPs were genotyped as presented in Table SI and approaches for SNPs selection was described in the Supplemental Materials. DNA was isolated from participants in the ADVN using standard protocols. *IRF2* SNPs were genotyped using a custom-designed Illumina (San Diego, CA, USA) oligonucleotide pool assay (OPA) for the BeadXpress Reader System (Gao *et al.*, 2009b). Detailed methods and quality control have been previously described (Gao *et al.*, 2009b).

IFNγ ELISPOT

The differential immune responses (*ex vivo*) to HSV have been investigated by measuring IFNγ production in isolated peripheral blood mononuclear cells (PBMCs) from a subset of the ADVN sample (64 subjects), and clinical characteristics of participants have been previously described (Leung *et al.*, 2011). IFNγ production was examined by using enzyme-linked immunosorbent spot (ELISPOT) adapted from the protocol as previously described (Janetzki *et al.*, 2005). Spot-forming cells were counted.

Quantitative real time RT-PCR

We have recently performed the geneChip profiling analyses in non-lesional skin biopsies from subjects with ADEH and AD (Grigoryev *et al.*). Briefly, skin biopsies from non-lesional areas were cultured in the presence of media alone (RPMI supplemented with 10% FCS) or 2.5×10^5 pfu VV for 24 hours, and then medium was removed, and biopsy specimens were submerged in Tri-Reagent (Molecular Research Center, Inc, Cincinnati, Ohio) for RNA isolation (Howell *et al.*, 2006). Detailed on gene expression profiling of

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sham-treated and VV-treated non-lesional skin biopsied from ADEH patients (n=5), AD patients (n=11), and non-atopic controls (NA, n=13) have been previously described (Grigoryev *et al.* 2010). Among those differentially expressed genes, IRF2 expression was significantly down-regulated in ADEH when compared with AD. We therefore validated the IRF2 expression by using quantitative PCR (RT-PCR). The isolated RNA from non-lesional skin biopsies was used to synthesize cDNA and then analyzed by real-time RT-PCR using an ABI Prism 7300 sequence detector (Applied Biosystems, Foster City, CA) as previously described (Gao *et al.*, 2009a). IRF2 expressions were normalized to the corresponding GAPDH and expressed as IRF2 mRNA expression relative to GAPDH ($2^{-\Delta Ct}$). The significance of the differences obtained was assessed using t-tests in which a P value of less than .05 was considered significant.

Statistical analyses

The Cochran–Armitage trend test was used to test for association between each individual SNP (under an additive model) and disease status using PLINK (Gabriel *et al.*, 2002). Haplotype analyses were performed with PLINK using sliding windows of 2–5 SNPs where empiric *P*-values for haplotype frequency differences were generated over 10,000 permutations. Departures from Hardy-Weinberg equilibrium at each locus were tested by using a chi-squared test separately for cases and controls in PLINK (Purcell *et al.*, 2007). Association tests were performed between individual genetic markers and log-transformed spot forming units (SFU)/10^6 PBMCs adjusted for age and gender using a linear regression analysis under a dominant model. Tests for association with a *P* value <0.05 were further adjusted by the PLINK permutation test (10,000 permutations), which provided a framework for correction for multiple testing (Gabriel *et al.*, 2002). Power calculation was performed using QUANTO version 1.1 program (Xu *et al.*, 2006).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We would like to acknowledge several groups without whom this study would not have been possible: ADVN coordinators (Patricia Taylor NP, Trista Berry BS, Susan Tofte RN, Shahana Baig-Lewis, Peter Brown BS, Lisa Heughan BA, CCRC, Meggie Nguyen BS, Doru Alexandrescu MD, Lorianne Stubbs RC, Deborra James RN, CCRC, Reena Vaid MD, Diana Lee MD), ADVN regulatory advisors (Judy Lairsmith RN and Lisa Leventhal, MSS, CIM, CIP), biological sample tracking (Jessica Scarpola, Muralidhar Bopparaju, Mary Bolognino MS, Lisa Latchney MS), NIAID-DAIT support (Marshall Plaut MD and Joy Laurienzo Panza RN, BSN, CCRC), DACI Laboratory (Robert Hamilton PhD) and all of the patients who participated in this study. A special thanks to Daniel Zaccaro MS, Jamie Reese BS and Susan Lieff PhD at Rho, Inc. for coordination of the study; Rasika Mathias (JHAAC) for her assistance in generating figure 2; and Patricia Oldewurtel (JHAAC) and Maureen Sandoval (NJH) for technical assistance.

Funding: This research was supported by The Atopic Dermatitis and Vaccinia Network NIH/NIAID contracts HHSN266200400029C and HHSN266200400033C. KCB was supported in part by the Mary Beryl Patch Turnbull Scholar Program.

ABBREVIATIONS

AD Atopic dermatitis

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ADEH	Atopic dermatitis complicated by eczema herpeticum
ADVN	Atopic Dermatitis and Vaccinia Network
EASI	Eczema Area and Severity Index
HSV	Herpes simplex virus
MAF	Minor allele frequency
SNP	Single nucleotide polymorphism
ELISPOT	Enzyme-linked immunosorbent spot
PBMCs	Peripheral blood mononuclear cells
SFU	Spot-forming units
IRF2	Interferon regulatory factor 2
LD	Linkage disequilibrium

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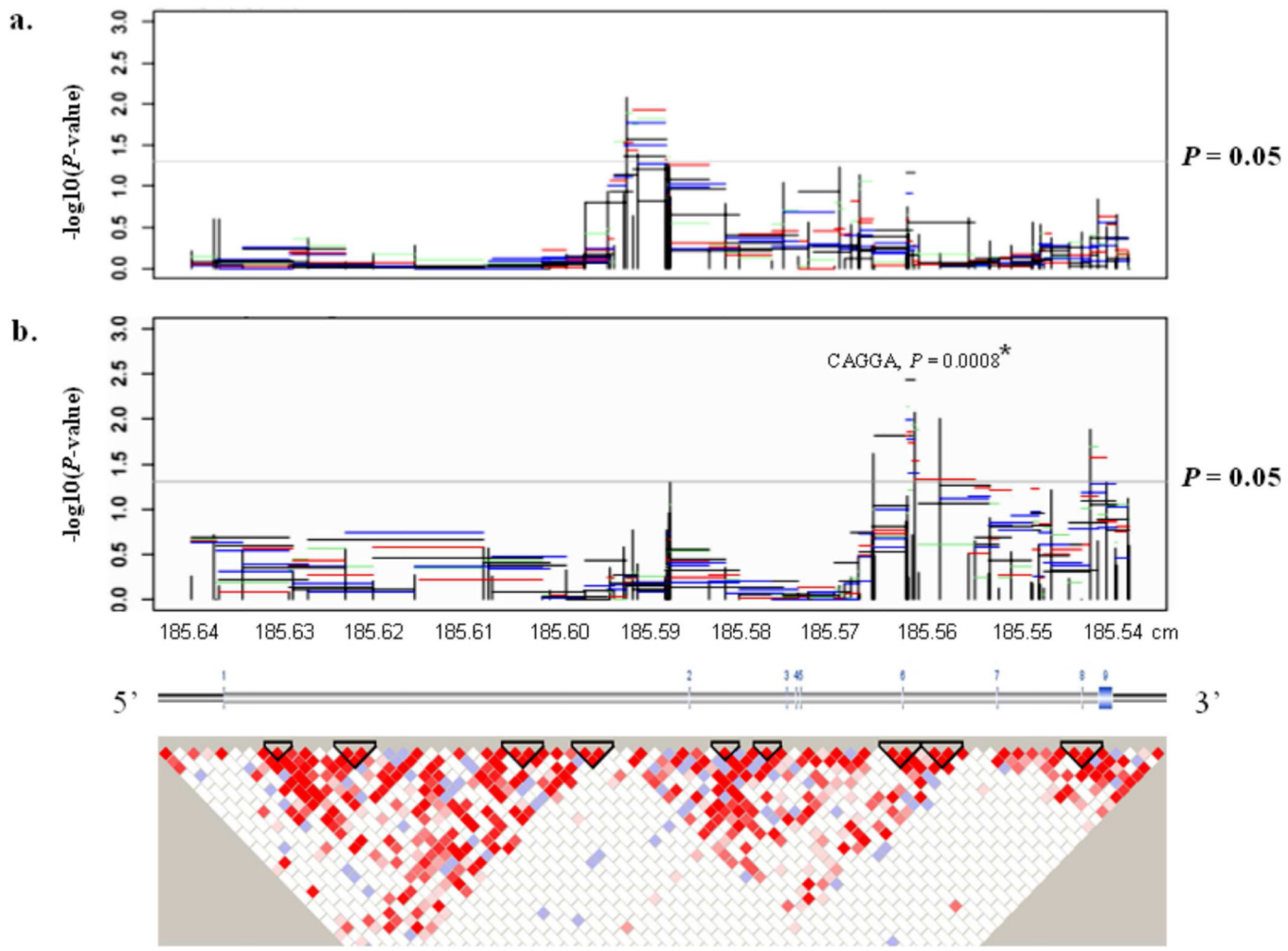

Figure 1.

Haplotype results showing Omnibus *P*-values constructed across sliding windows of sizes 2–5 for 78 *IRF2* SNPs and AD (a) and ADEH (b). *Black vertical lines* represent all individual SNP tests, and colored horizontal lines represent 2-, 3, 4, 5, haplotype tests. *See detailed data in Table 2. Lower plots illustrate patterns of LD (D') in these samples: red squares for strong LD, blue squares for non-significant LD, and white squares for little or no LD; numerical values were generated using HAPLOVIEW software.

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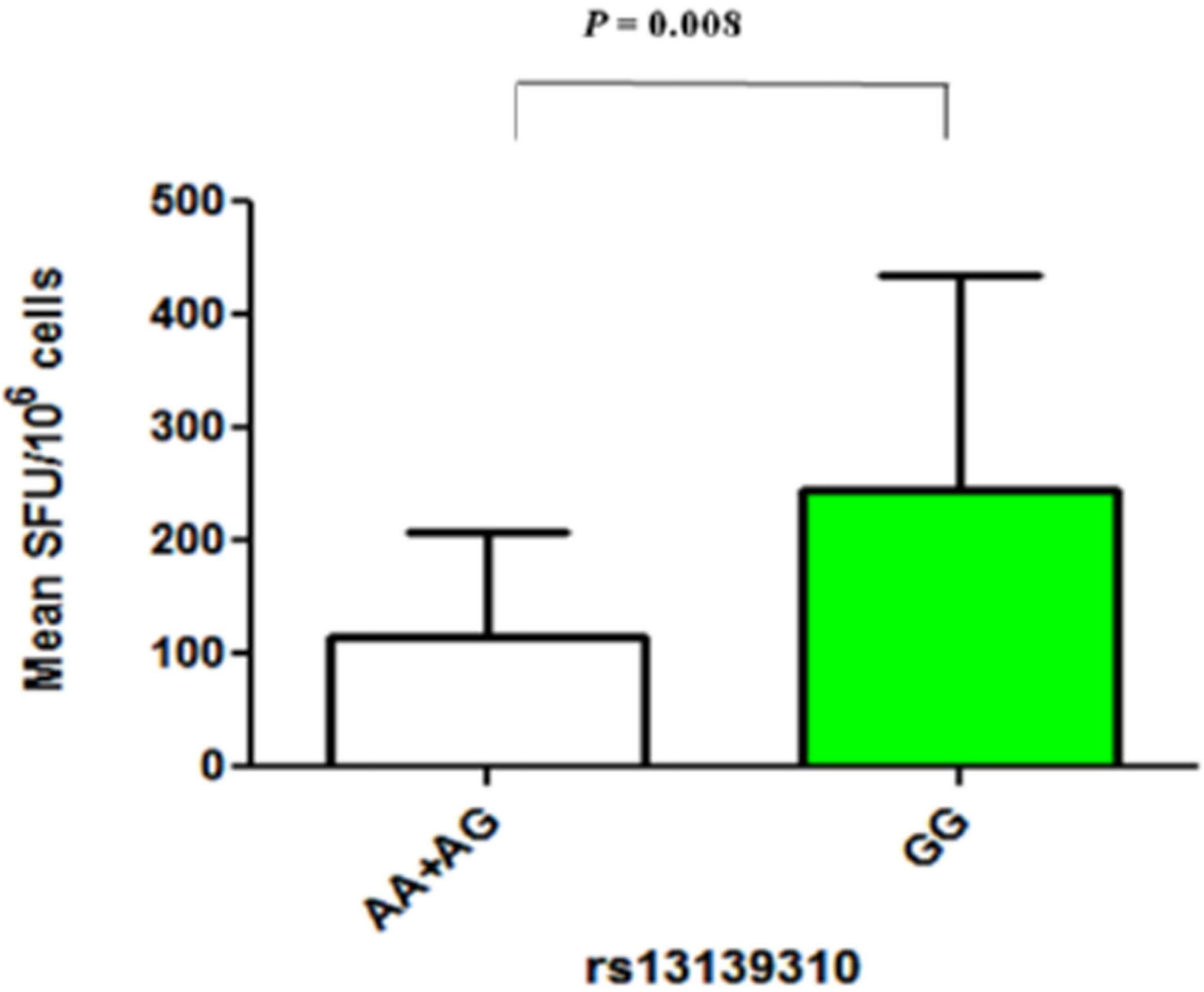

Figure 2.

Association of *IRF2* SNP rs13139310 with IFNγ production. IFNγ production was determined by the $\log_{10}$-transformed mean SFC/10^6 cells and expressed as Spot Forming Units (SFU). *IRF2* SNP rs13139310 was significantly associated with HSV-stimulated IFNγ levels (GG (n=44) vs GA+AA (n=12), $P = 0.008$).

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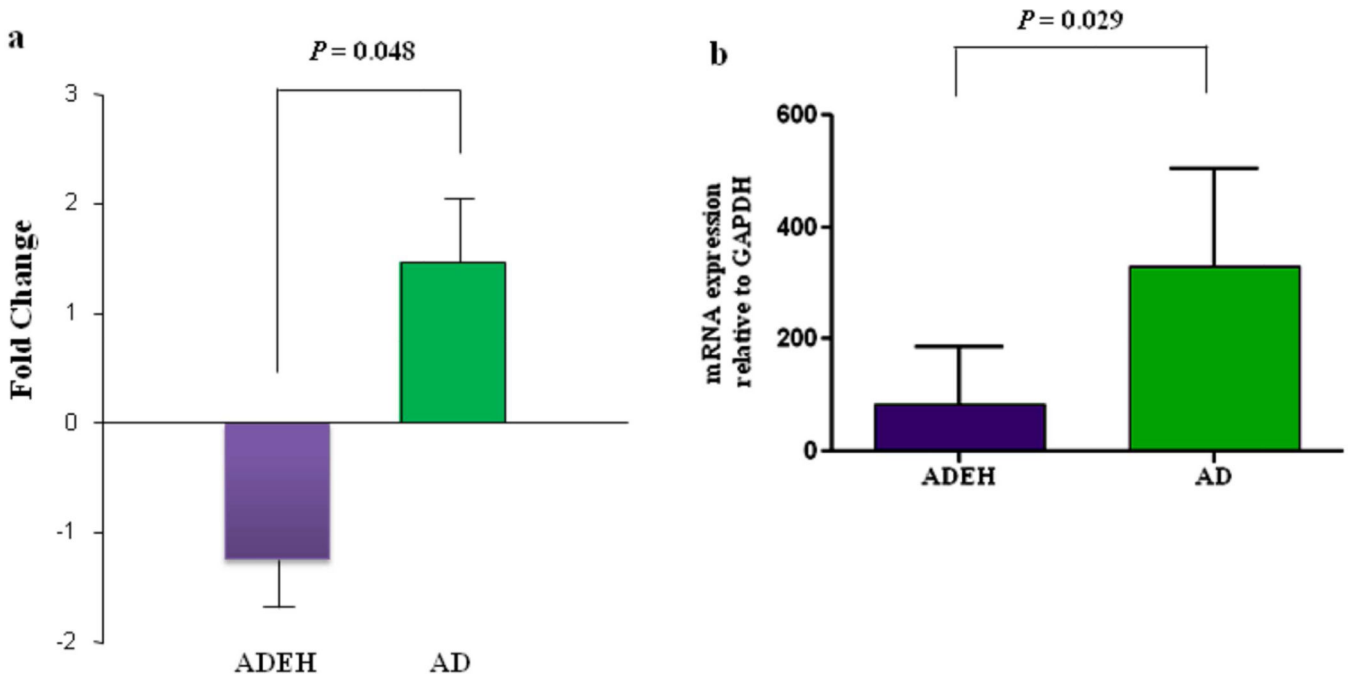

Figure 3.

IRF2 gene expression was decreased in VV-treated non-lesional skin biopsies from human subjects with ADEH compared with subjects with AD. a) IRF2 expression was detected by geneChip array in VV-treated non-lesional skin biopsies from ADEH patients (n = 5), AD patients (n=11), and non-atopic controls (NA, n=13). Y axis represents the fold changes for both AD and ADEH relative to NA. P value was determined by comparison made between mean fold changes for AD and ADEH. b) IRF2 expression was validated by qRT-PCR in VV-treated non-lesional skin biopsies from ADEH (n=8) and AD (n=10) patients. IRF2 expression was normalized to the corresponding GAPDH and expressed as IRF2 mRNA expression relative to GAPDH expression ($2^{-\Delta Ct}$).

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Table 1

IRF2 SNPs significantly associated (P<0.05) with AD and ADEH

SNP ID	Position	Role	Risk allele	Minor allele frequency		OR (95% CI)	P value	Adjusted P value*
				Cases	Controls			
European American (287 AD vs. 157 controls)								
rs13139310	185593905	Intron	A	0.38	0.48	0.65 (0.42–1.01)	0.045	0.153
rs793814	185591968	Intron	T	0.33	0.43	0.52 (0.33–0.80)	0.002	0.007
rs12504466	185590793	Intron	G	0.52	0.44	1.36 (1.02–1.83)	0.032	0.054
rs3756094	185587928	Intron	A	0.47	0.59	0.66 (0.40–0.94)	0.018	0.037
African American (187 AD vs. 156 controls)								
rs3775572	185606446	Intron	A	0.41	0.51	0.46 (0.25–0.83)	0.006	0.016
rs793794	185600031	Intron	A	0.46	0.59	0.61 (0.39–0.98)	0.030	0.068
rs793777	185551407	Intron	G	0.37	0.45	0.74 (0.53–1.02)	0.050	0.161
rs6831978	185540445	Downstream	G	0.04	0.09	0.36 (0.11–1.03)	0.035	0.104
European American population (112 ADEH+ vs. 166 ADEH−)								
rs7667268	185627070	Intron	A	0.75	0.17	0.39 (0.13–1.03)	0.043	0.104
rs807684	185587301	Intron	G	0.56	0.40	1.87 (0.99–3.51)	0.037	0.083
rs17488073	185565967	Intron	C	0.10	0.18	0.52 (0.27–0.95)	0.025	0.034
rs809909	185561604	Intron	A	0.27	0.39	0.59 (0.38–0.90)	0.008	0.022
rs11132242	185558935	Intron	G	0.53	0.41	1.60 (0.99–2.60)	0.009	0.049
rs3775543	185547212	Coding exon	A	0.18	0.08	2.50 (1.02–6.11)	0.023	0.061
rs1342852	185543111	Downstream	A	0.40	0.29	1.62 (1.07–2.44)	0.013	0.043
rs1124191	185539240	Downstream	A	0.33	0.50	0.49 (0.27–0.88)	0.010	0.022

*Associations that had a *P* value <0.05 were further adjusted using the PLINK permutation test (10,000 permutation).

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Table 2

A 5-marker haplotype covering a region of 1.17 kb showed the strongest association with ADEH among European Americans

Haplotype	rs3775552	rs809909	rs7655371	rs6812958	rs2797507	Frequency		*P*-value
	C / ***A***	**A** / **T**	**G** / **A**	**G** / **A**	**C** / **A**	**ADEH+**	**ADEH−**	
Haplotype 1	C	T	G	A	A	0.313	0.283	0.5099
Haplotype 2	C	A	G	G	A	0.102	0.236	**0.0008**
Haplotype 3	C	T	A	G	C	0.367	0.272	**0.0397**
Haplotype 4	A	A	G	G	C	0.054	0.101	0.0967
Haplotype 5	C	A	G	G	C	0.088	0.053	0.1503
Haplotype 6	C	T	G	G	C	0.075	0.055	0.4230
OMNIBUS: *P* = 0.0036								

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Table 3

Results for the strongest associations of *IRF2* SNPs with *IFN*-γ ELISPOT values

dbSNP	Position	Allele	AA	AB	BB	All subjects*		AD subjects	
						β	*P*-value	β	*P*-value
rs11723606	185596272	C/T	348±273 (5)	235±196 (39)	127+85(18)	0.271	0.027	0.323	0.022
rs13139310	185593905	A/G	129±149 (3)	111±74 (9)	245±192 (44)	−0.353	0.007	−0.326	0.008
rs1425551	185580052	A/C	129±88 (11)	170±137 (24)	308±219 (22)	−0.353	0.001	−0.245	0.008
rs7677486	185573846	C/T	125±82 (16)	178±121(27)	358±173(18)	−0.385	0.0008	0.215	0.022
rs10009261	185570024	C/T	129±108 (5)	142±105 (19)	272±203(35)	−0.307	0.004	−0.226	0.020
rs4862365	185548401	C/T	NA	291±205(22)	175±134 (37)	0.218	0.046	0.231	0.092
rs1342852	185543111	C/T	328±293 (7)	245±183 (25)	166±125 (27)	0.212	0.046	0.091	0.421
rs6831978	185540445	A/G	467 (1)	271±226 (13)	198±163 (45)	0.222	0.047	0.250	0.130

IFN-γ ELISPOT value was expressed as a mean spot forming units (SFU)±SD/10^6 PBMCs. The analysis was performed using a linear regression analysis under a dominant model (AA+AB *vs* BB) for a log-transformed and further age and gender adjusted SFU. AA: homozygote, AB: heterozygote, BB, wild type. β: Regression coefficient. MAF: Minor allele frequency.

*all subjects are HSV positive.