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AUTHOR CONTRIBUTIONS

Conceptualization: SGK, SPP; Data Curation: SPP; Formal Analysis: SPP; Methodology: SGK, SK, SPP; Supervision: SGK, SK; Visualization: SPP, RK, MB; Writing - Original Draft Preparation: SPP, RK, MB; Writing - Review and Editing: SPP, RK, MB, SK, SGK.

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Pediatric to Adult Shift in Vitiligo Onset Suggests Altered Environmental Triggering

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TO THE EDITOR

Vitiligo is a common autoimmune disease in which the destruction of melanocytes results in patches of depigmented skin, sometimes in association with other concomitant autoimmune diseases (Picardo and Taïeb, 2019). Vitiligo heritability is 46% – 72%, underscoring the importance of both genetic and environmental contributions. However, although genome-wide association studies have identified 50 vitiligo risk loci, no environmental triggers are known with certainty (Roberts and Spritz, 2018).

Epidemiologic studies have shown that a number of autoimmune diseases are increasing in incidence and prevalence in industrialized countries (Lerner et al., 2016; Wang et al., 2015; Bach, 2018), sometimes paralleled by earlier disease onset (Karvonen et al., 1999; Wang et al., 2015). Hypotheses to explain these trends include altered timing and increasing frequency of exposures to infectious agents (Bach, 2018) and a wide variety of other possible environmental triggers (Cojocaru et al., 2008). There are no reported longitudinal prevalence or incidence data for vitiligo, so to detect clues to possible environmental triggers we analyzed historical trends in vitiligo age-of-onset.

Vitiligo consists of two major age-of-onset subgroups: early-onset (mean 10.3 ± 5.6 years; 38.4% of cases) and late-onset (mean 34.0 ± 14.5 years; 61.6% of cases) (Jin et al., 2019). The principal underlying genetic difference is the specific association of early-onset vitiligo with a major histocompatibility complex class II regulatory haplotype, rs145954018del-rs9271597A, that increases *HLA* gene expression and may accelerate the loss of immune tolerance. Here, we analyzed self-reported vitiligo age-of-onset in a cohort of 4,406 unrelated North American and European Caucasian cases incident from 1951-2013. As shown in Figure 1, over the study period vitiligo age-of-onset exhibited remarkable change, with mean age-of-onset more than doubling, from 14.7 ± 9.3 years in 1951 to 31.8 ± 20.2 years in 2013. The pattern of age-of-onset increase appeared generally similar in vitiligo cases from both North America and Europe (Supplementary Figure S1a and b).

Segmented linear regression indicated that almost all the change in vitiligo age-of-onset occurred over the period from 1970 to 2004, from a mean 14.6 ± 9.4 years in 1970 to 30.2 ± 17.3 years in 2004, an increase rate of

Abbreviation: GWAS, genome-wide association study

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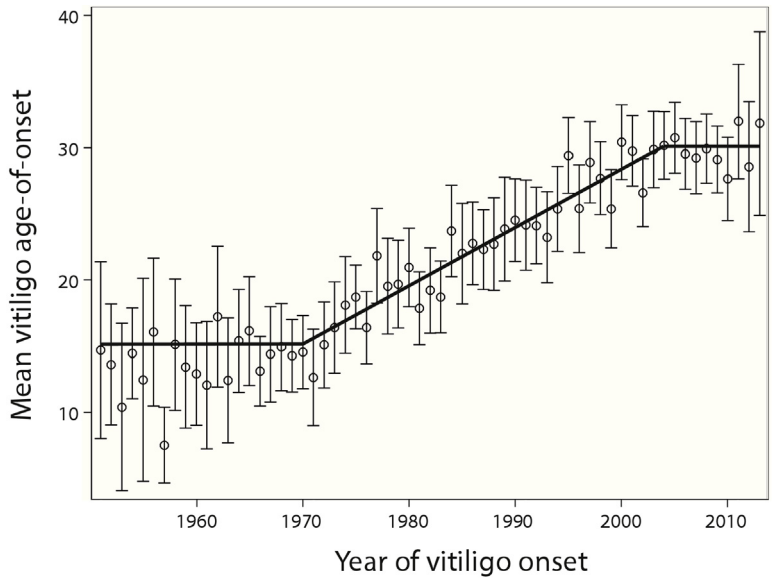

Figure 1. Mean vitiligo age-of-onset by calendar year of onset 1951-2013. Age-of-onset distribution in total Caucasian case sample ($n = 4,406$). The circles indicate means and the vertical bars 95% confidence intervals. Black segments indicate regression lines for the time periods 1951-1969, 1970-2004, and 2005-2013. The distributions appear generally similar in cases from North America and Europe (Supplementary Fig. S1).

approximately 0.44 years per year (95% confidence interval 0.38 - 0.50, $P = 2.10 \times 10^{-48}$). This does not merely reflect increasing lifespan, as the USA median age increased only by approximately 7.5 years over the same period (United States Census Bureau; www.census.gov). In contrast, the vitiligo age-of-onset was relatively constant both before (1951-1969; $P = 0.48$) and after (2005-2013; $P = 0.63$) this period (Figure 1).

As observed previously (Jin et al., 2019), the overall distribution of the vitiligo age-of-onset was bimodal, comprised of early-onset and late-onset subgroups, across the entire case series, within all three temporally-incident case cohorts, and in both males and females (Supplementary Figure S2). However, over time, the relative proportions of incident cases classified as early-onset versus late-onset reversed ($P = 9.36 \times 10^{-19}$). In the 1951-1969 incident cohort, most cases were classified as early-onset (53.7%), whereas in both the 1969-2004 and 2005-2013 incident cohorts, most cases were classified as late-onset (70.7% and 74.4%, respectively; Table 1). Furthermore, even within the late-onset subgroup, the mean age-of-onset differed over the cohorts, becoming much later over time (1951-1969, mean 25.0 ± 6.8 years; 1970-2004, mean 36.0 ± 10.3 years; 2005-2013, mean 40.5 ± 13.6 years; $P = 1.48 \times 10^{-61}$; Table 1). In contrast, there was far less change in age-of-onset among the cases classified as early-onset (1951-1969, mean 5.4 ± 2.7 years; 1970-2004, mean 5.7 ± 3.0 years; 2005-2013, mean 7.3 ± 2.7 years; $P = 1.21 \times 10^{-15}$). We observed similar trends in both males and females (Supplementary Table S1).

Genetic analysis of the rs145954018del-rs9271597A major histocompatibility complex haplotype that is associated with early-onset vitiligo (Jin et al., 2019) showed no significant change in effect size over time (Supplementary Table S2). Indeed, the proportion of cases with late-onset vitiligo increased across the three time periods regardless of whether or not those patients carried the haplotype (Supplementary Fig. S3). This result indicates that changing vitiligo age-of-onset over time does not result from a changed gene-environment interaction involving the rs145954018del-rs9271597A major histocompatibility complex class haplotype.

Thus, our findings show that, over the period of approximately 1970-2004, vitiligo age-of-onset by patient self-recall became later by more than two-fold. It is unlikely this change reflects altered patterns of public awareness and physician diagnosis of vitiligo, as these have become heightened over time and would thus serve to make self-recalled age-of-onset earlier, not later. Instead, it seems likely that, circa 1970 or earlier, one or more environmental changes altered vitiligo triggering, delaying disease onset, and effectively transforming vitiligo from a largely pediatric-onset to a largely adult-onset disease, both in North America and in Europe. While this apparently beneficial change provides an extraordinary inroad to discover vitiligo environmental triggers, the number of potential candidates is enormous. The 1960s saw many important positive environmental improvements, particularly in the USA, such as the Clean Air Acts of 1963 and 1970, the Nuclear Test Ban Treaty of 1963, the Water Quality Act of 1965, the National Environmental Policy Act of 1969, and the establishment of the Occupational Safety and Health Administration in

Table 1. Number of cases and mean vitiligo age-of-onset in each time period

	Early-Onset		Late-Onset		All Cases	
Period	No. (%)	Mean (SD)	No. (%)	Mean (SD)	No. (%)	Mean (SD)
1951-1969	147 (53.7)	5.4 (2.7)	127 (46.3)	25.0 (6.8)	395 (9.0)	14.0 (9.3)
1970-2004	621 (29.3)	5.7 (3.0)	1496 (70.7)	36.0 (10.3)	2787 (63.3)	24.4 (15.1)
2005-2013	271 (25.6)	7.3 (2.7)	788 (74.4)	40.5 (13.6)	1224 (27.8)	29.7 (18.4)

Abbrevation: SD, standard deviation.
Only cases with > 80% posterior probability of belonging to either the early-onset or the late-onset subgroup were included in the calculations of numbers in each subgroup.

1970, among others. More globally, in 1974 the "Sun Protection Factor" rating for sunscreens was introduced, leading to more effective sunscreens and consequent lower rates of UV exposure. The early 1970s even saw widespread acceptance of yogurt consumption (Fabricant, 1976), potentially altering the gut microbiome. Careful analysis of long-term trends in USA NHES, NHANES, and other serial biological data, as well as parallel data from non-Caucasian vitiligo cases from other continents, might provide important clues to environmental changes that underlie delayed autoimmune triggering and the shift of vitiligo onset toward later ages.

Data availability statement

Datasets related to this article can be found at www.ebi.ac.uk/gwas/search?query=30674883, hosted at the NHGRI-EBI GWAS Catalog (www.ebi.ac.uk/gwas/) (www.ebi.ac.uk/gwas/downloads/summary-statistics).

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CONFLICT OF INTEREST

The authors state no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: SAS, RAS; Data Curation: YJ; Formal Analysis: YJ; Funding Aquisition: RAS; Investigation: YJ, SAS, RAS; Project Administration: RAS; Software: YJ; Supervision: SAS, RAS; Validation: YJ; Writing – Original Draft Preparation: YJ, SAS, RAS.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org and at https://doi.org/10.1016/j.jid.2019.06.131

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Nanoparticle Targeting to Scalp Hair Follicles: New Perspectives for a Topical Therapy for Alopecia Areata

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TO THE EDITOR

Alopecia areata (AA) is a hair follicle (HF) disorder, in which the immune system attacks the HF and causes reversible hair loss. Even though AA is not a life-threatening disease, an association with psychosocial diseases and a severe drop in quality of life is common (Pratt et al., 2017). There is, as yet, no approved medicine in the therapy for AA. New potent drugs, such as Jak inhibitors, show promising results but incur severe adverse effects (Wang et al., 2018). For such drugs, targeted delivery to the site of action is essential.

The concept for follicular delivery of drug-loaded nanoparticles (NPs) for treatment of hair disorders shows potential. The key benefits of targeted biodegradable polymeric NP delivery into HFs include (i) protection of the encapsulated drug, (ii) minimization of drug exposure to the skin surface, as well as interfollicular permeation, (iii) maximization of the penetration into the HF compared with the free drug (Mathes et al., 2016), (iv) the possibility of building a drug depot in the upper part of the HF, creating possible protection of the NPs from external influences such as, textile contact, washing (Lademann et al., 2007), and (v) the concurrent ability of a sustained drug release from the depot to reduce the application frequency and enhance patient compliance (Hofmeier and Surber, 2017). Taking this into account, Jak inhibitor-loaded NPs could deposit in the upper part of the HF, release the drug in a controlled manner, which diffuses to the site of action (hair bulb), and be taken up by the follicular epithelial cells and immune cells (Divito and Kupper, 2014); thus reducing adverse effects with less systemic drug and skin exposure.

However, a hypothesized penetration mechanism for NP uptake into human HF postulates that, by the movement of the hair shaft, overlapping cuticle cells serve as a gear pump and push the NPs into the HF (Lademann et al., 2007; Radtke et al., 2017). Additionally, appropriate massage seems to be important in this context (Li et al., 2019). Thus,

Abbreviations: AA, alopecia areata; HF, hair follicle; NP, nanoparticle; PLGA, poly (lactic-co-glycolic acid)

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SUPPLEMENTARY MATERIALS AND METHODS

Patients and inclusion criteria

The subjects were 4,406 Caucasian USA, Canadian, and European cases from our previous vitiligo genome-wide association studies (Jin et al., 2016). All the cases met the clinical diagnostic criteria for vitiligo (Taïeb and Picardo, 2007) and provided self-reported age and age-of-onset. We included cases with onsets from 1951 to 2013 to ensure at least 10 incident cases per calendar year. Overall ethical oversight and approval was by the Colorado Multiple Institutional Review Board, with additional approval by the Institutional Review Boards of all the participating recruitment sites. All the subjects provided written consent.

Statistics and data analyses

The distribution of vitiligo age-of-onset over time showed two apparent breakpoints (Figure 1); accordingly, we fit a segmented linear model using the segmented (Muggeo, 2008) package in R, identifying breakpoints at 1969 and 2004. We split the cases into three temporally-defined incident cohorts: 1951-1969, 1970-2004, and 2005-2013. To define the underlying mixture of age-of-onset distribution in each case cohort, we performed goodness of fit analyses using a finite mixture model. We set component distributions as normal; the maximum number of mixture components as seven, fit the finite mixture model s using maximum likelihood, and chose the best fit model using the Bayesian information criterion. The cases per cohort were classified as early-onset or late-onset, including only cases with $\geq$ 80% posterior probability of belonging to either the early-onset (1951-1969, 0-9 years; 1970-2004, 0-10 years; 2005-2013, 2-12 years) or late-onset (1951-1969, 18-50 years; 1970-2004, 21-76 years; 2005-2013, 20-84 years) subgroups based on the best fit finite mixture model for each cohort. We used a chi-squared test to compare proportions of cases in each subgroup across the three cohorts. Because of unequal variances, we used Welch's analysis of varianceto compare the mean vitiligo age-of-onset in the early-onset subgroup and the late-onset subgroup across the three time periods. To test whether the effect of haplotype rs145954018del-rs9271597A on early-onset vitiligo differed across the three incident cohorts, we fit a logistic regression model with early-onset versus not as the response variable, the rs145954018del-rs9271597A haplotype, time period, and the interactions between the two as predictors. To control for population stratification, we derived significant eigenvectors for each genome-wide association study (GWAS) case cohort separately using GemTools (Klei et al, 2011) with the default parameter settings. For each GWAS case cohort, we used genotyped single nucleotide polymorphisms with the genotype missing rate (0.1%, minor allelic frequency) 0.01, the Hardy-Weinberg *P*-value $>$ 0.005, and chose tag single nucleotide polymorphisms with $r^2 <$ 0.01 using PLINK (Purcell et al., 2007) v1.9. The number of tag single nucleotide polymorphisms used for GWAS1, GWAS2, and GWAS3 were 13,459, 12,140, and 13,010, respectively. Significant eigenvectors for genetic ancestry were derived from the normalized graph Laplacian, with the number of significant eigenvectors estimated based on the eigengap heuristic and hypothesis testing (Lee et al., 2010). Significant eigenvectors were included as covariates in the haplotype analysis model. Analyses were performed using SAS v9.4 (https://www.sas.com) and R v3.52 (https://cran.r-project.org/bin/windows/base/rtest.html). All the tests were two-sided.

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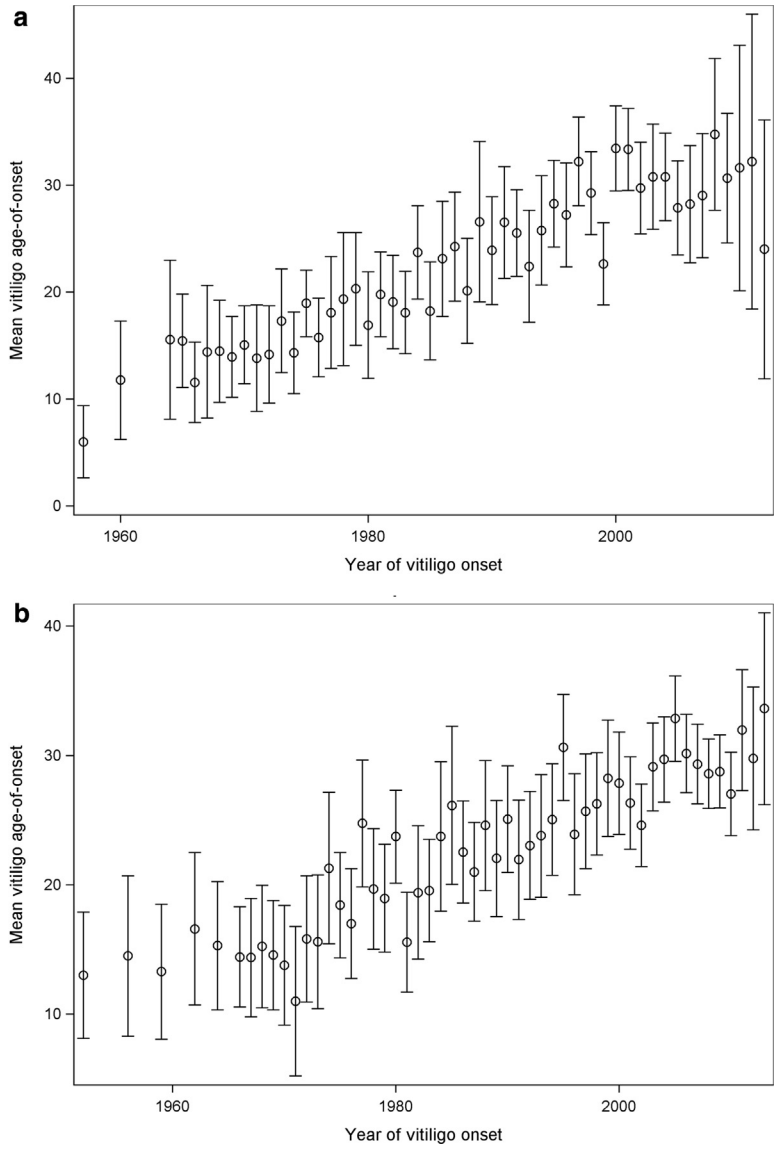
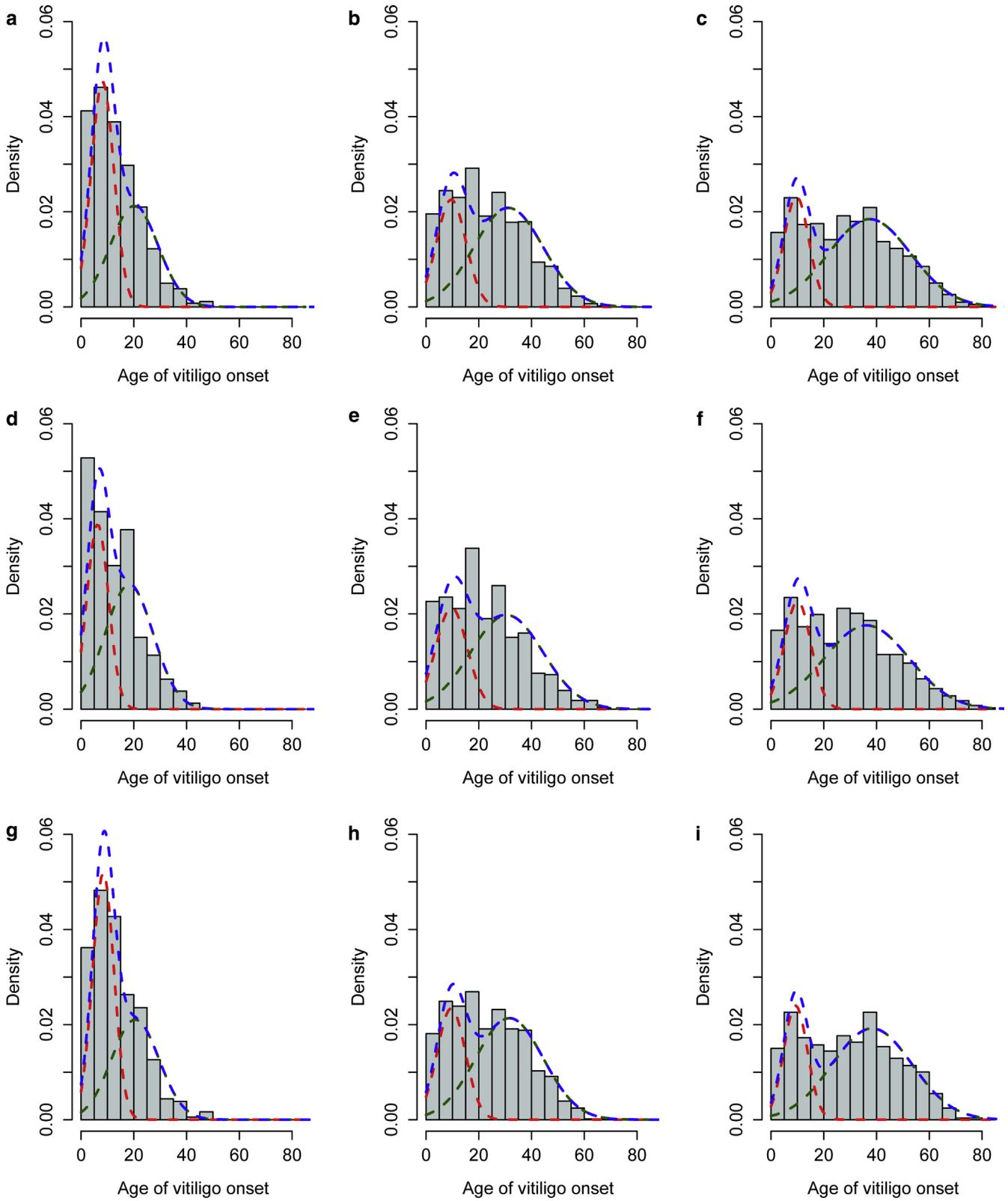
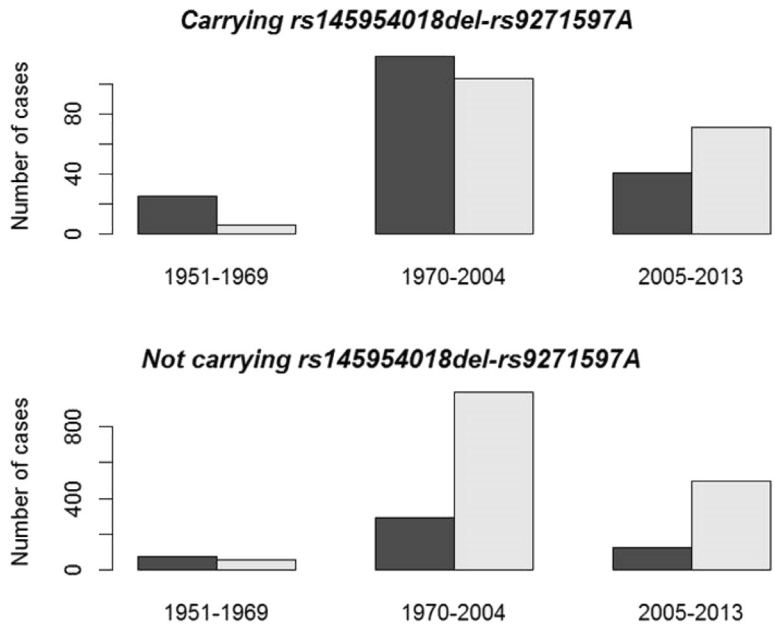


Supplementary Figure S1. Mean vitiligo age-of-onset by calendar year of onset 1951-2013 by geographic location. Age-of-onset distibution in (**a**) North America (n = 1,798); (**b**) Europe (n = 2,608). The circles indicate means and the vertical bars 95% confidence intervals. Black segments indicate regression lines for the time periods 1951-1969, 1970-2004, and 2005-2013.



Supplementary Figure S2. Distribution of vitiligo age-of-onset in all cases in males and in females during the 1951-69, 1970-2004, and 2005-2013 incident time periods. Best fit models from goodness of fit analyses were carried out by the finite mixture model procedure in SAS. Blue lines show the full distributions, red lines the early-onset distributions, and green lines the late-onset distributions. Best fit models for distribution of vitiligo age-of-onset in (**a**) all cases with onset 1951-1969; (**b**) all cases with onset 1970-2004; (**c**) all cases with onset 2005-2013; (**d**) male cases with onset 1951-1969; (**e**) male cases with onset 1970-2004; (**f**) male cases with onset 2005-2013; (**g**) female cases with onset 1951-1969; (**h**) female cases with onset 1970-2004; (**i**) female cases with onset 2005-2013.



Supplementary Figure S3. Increasing proportion of vitiligo cases classified as late-onset among patients both carrying and not carrying the rs145954018del-rs9271597A major histocompatibility complex class II enhancer haplotype in the 1951-1969, 1970-2004, and 2005-2013 incident time periods. Black boxes, cases classified as early-onset; gray boxes, cases classified as late-onset.

Supplementary Table S1. Number of cases and mean vitiligo age-of-onset in each time period in the early-onset and late-onset subgroups in males and females

	Males				Females			
	Early-Onset		Late-Onset		Early-Onset		Late-Onset	
Period	No. (%)	Mean (SD)	No. (%)	Mean (SD)	No. (%)	Mean (SD)	No. (%)	Mean (SD)
1951-1969	47 (55.3)	4.8 (3.0)	38 (44.7)	24.4 (7.0)	100 (52.9)	5.7 (2.5)	89 (47.1)	25.3 (6.7)
1970-2004	219 (31.2)	5.4 (3.1)	484 (68.8)	36.1 (10.7)	402 (28.4)	5.8 (2.9)	1012 (71.6)	36.0 (10.1)
2005-2013	123 (26.3)	7.2 (2.6)	345 (73.7)	39.7 (14.0)	148 (25.0)	7.4 (2.8)	443 (75.0)	41.1 (13.2)

Abbrevation: SD, standard deviation.

Only cases with >80% posterior probability of belonging to either the early-onset or the late-onset subgroup were included in the calculations of numbers in each subgroup.

Supplementary Table S2. Association between early-onset and rs145954018del-rs9271597A, time period

Characteristics	df	Chi-Square	*P*-value > Chi-Square	Coefficient (OR)	95% confidence interval
Joint Tests					
rs145954018del-rs9271597A	1	5.14	0.02	–	–
period	2	73.59	1.05×10^{-16}	–	–
*rs145954018del-rs9271597A*period*	2	3.84	0.15	–	–
Analysis of Maximum Likelihood Estimates					
Intercept	1	2.90	0.09	0.30	-0.05 – 0.65
rs145954018del-rs9271597A	1	5.14	0.02	1.11	0.13 – 2.08
period1970-2004	1	64.80	8.28×10^{-16}	-1.51	-1.89 – -1.14
period2005-2013	1	68.29	1.41×10^{-16}	-1.67	-2.08 – -1.27
*rs145954018del-rs9271597A*period1970-2004*	1	0.23	0.63	0.24	-0.78 – 1.26
*rs145954018del-rs9271597A*period2005-2013*	1	0.27	0.6	-0.28	-1.35 – 0.79

Abbrevations: df, degrees of freedom; OR, odds ratio.

The results were obtained from a logistic regression model with the age-of-onset subgroup as the response variable (coded 1 for cases assigned to early-onset subgroup, 0 for late-onset subgroup), the rs145954018del-rs9271597A haplotype (coded 1 for carrying at least one haplotype, and 0 otherwise), time period, the interactions between the two as predictors, and the signficant eigenvectors from each GWAS as covariates. The period from 1951 to 1972 was used as the reference group, and therefore, it was not included in the model.