

Letter to the Editor

Age-specific profiling of cutaneous allergy at high temporal resolution suggests age-related alterations in regulatory immune function

To the Editor:

Aging is associated with complex alterations in T-cell-mediated immune function. The overall incidence of delayed-type cutaneous hypersensitivity increases with age and this is paralleled by a higher prevalence of autoimmune disease. Conversely, there is an age-related decline in the ability to mount an effective immune response to viral infection and vaccination. Here, we report a comprehensive evaluation of the age dependency of sensitization for allergens in the baseline series in a single-center cohort of 45,110 patients undergoing patch testing over a 30-year period. This is the largest reported series to date permitting the evaluation of this phenomenon at a higher temporal resolution than previously described.

Patch test records of all patients undergoing testing with a modified European baseline series between 1985 and 2014 were retrieved from a computerized database at St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London. Reactions were read on days 2 and 4 according to the Menne and White criteria. For each allergen, it was recorded whether the allergen was tested and whether the result was positive or negative. Reactions recorded as +/++/+++ were regarded as positive. Questionable or irritant reactions were regarded as negative. Statistical analysis and visualization was undertaken with the R language for statistical computing (see this article's [Methods](#) section in the Online Repository at www.jacionline.org).

In our center, the age distribution of patients presenting for patch testing increases until the early 20s and then decreases thereafter; this pattern is broadly consistent across 3 decades of testing ([Fig 1, A](#)). The overall prevalence of patients sensitized to at least 1 allergen increases throughout the lifespan; however, the rate of increase is most rapid before the age of 20 years, with peak incidence in individuals of age 40 to 60 years and a modest decline subsequent to this ([Fig 1, B](#), red line). Patients reacting to 1 or 2 allergens (blue, green lines) exhibit an increase in prevalence until the age of 20 years followed by a plateau; in contrast, reactions to 3 or more allergens (purple, orange lines) show a steady increase throughout the lifespan.

We next wished to examine allergen-specific age sensitization profiles. We calculated unadjusted (see [Fig E1](#) in this article's Online Repository at www.jacionline.org) and adjusted (see [Fig E2](#) in this article's Online Repository at www.jacionline.org) age-specific prevalence for each of 37 allergens in the baseline series. Allergens were then hierarchically clustered according to the age-specific prevalence profile of positive reactions ([Fig 1, C](#); see [Table E1](#) in this article's Online Repository at www.jacionline.org). The overall trend of an increase in the prevalence of positive reactions with age is apparent; however, it is clear that there are substantial differences between allergens. These differences are particularly apparent when groups identified by hierarchical clustering are visualized separately ([Fig 1, D-I](#)). For example, cobalt and nickel exhibit peak prevalence in the early 20s with

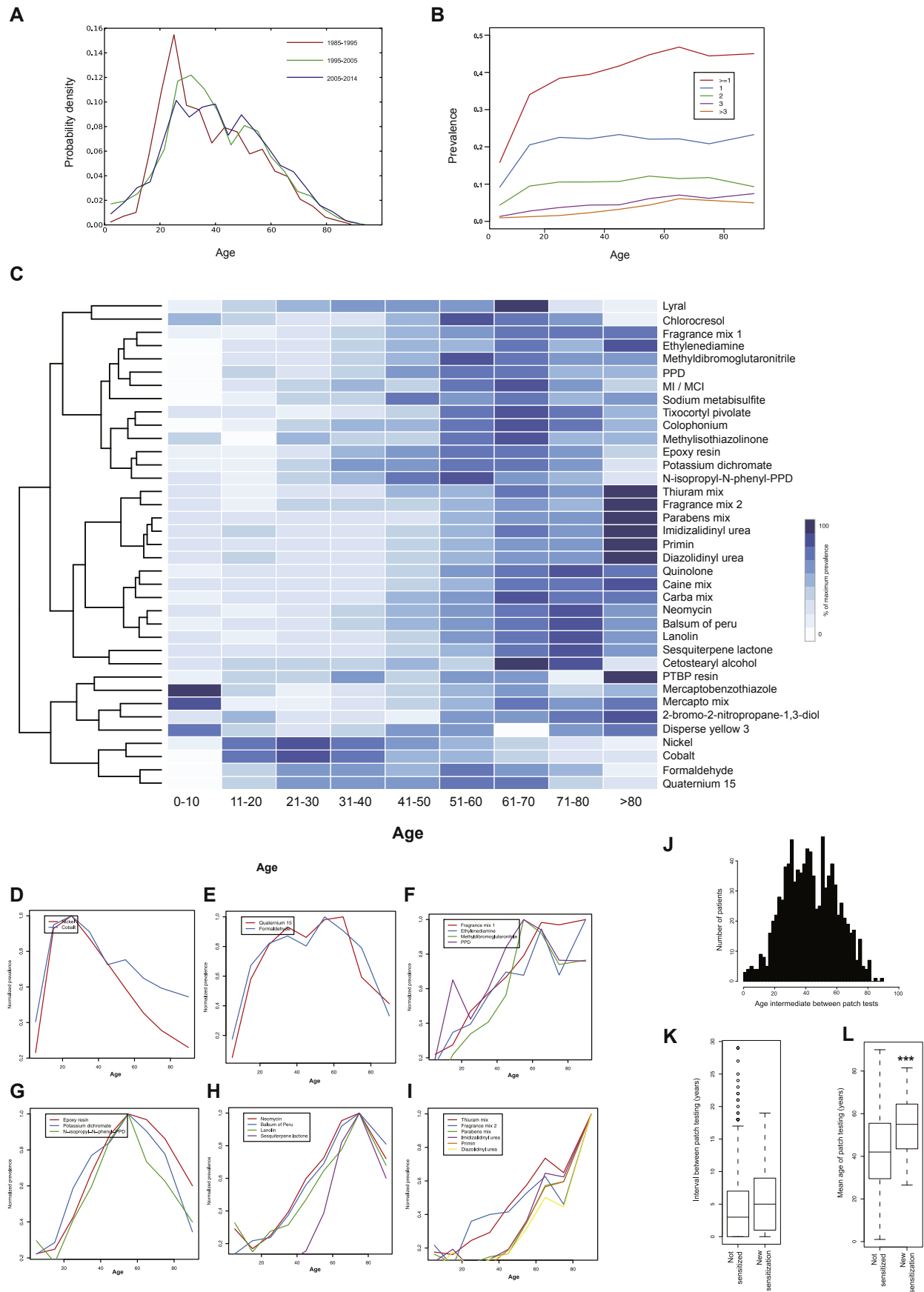
subsequent decline ([Fig 1, D](#)), whereas quaternium 15 and formaldehyde exhibit peak incidence in the early 20s with plateau until early 60s and then subsequent decline ([Fig 1, E](#)). The peak incidence for allergens including fragrance mix 1 and p-Phenylenediamine is in the late 50s ([Fig 1, F](#)). In contrast, a group of allergens including thiuram mix, fragrance mix 2, and Primin exhibit a progressive increase throughout the lifespan ([Fig 1, I](#)). Importantly, these patterns are conserved when patients are stratified according to decade of testing, sex, and atopy status (see [Fig E3](#) in this article's Online Repository at www.jacionline.org).

The ability to sensitize individuals to dinitrochlorobenzene declines concomitant with age with the decline commencing as early as age 40 years.¹ In contrast, the overall prevalence of cutaneous allergy and the prevalence of sensitization to specific allergens including fragrance mix 1,² topical medicaments,³ and methylisothiazolinone⁴ is higher in older individuals. However, a full understanding of this phenomenon has been constrained by the limited temporal resolution of previous studies. Our analysis reveals 3 broad patterns of age-specific sensitization.

First, a cohort of allergens exhibits a peak prevalence of sensitization in early life with a progressive decline at older ages. Preeminent among these is nickel. This age dependence of nickel sensitization has been previously recognized and, in principle, could reflect age-specific alterations in exposure patterns, the delayed induction of tolerance, or immunosenescence. Interestingly, the age profile of cobalt sensitization closely parallels that of nickel. Because exposure patterns will differ, this suggests that a common biological mechanism operates in the sensitization to these metal ions and would be consistent with a role for age-related alterations in immune function "immunosenescence." The latter reflects a complex interplay of factors including the production of peripheral lymphocytes and thymic involution.

Second, the prevalence of sensitization to allergens such as fragrance mix 1 and 2, ethylenediamine, thiuram mix, paraben mix, primin, imidazolidinyl urea, and diazolidinyl urea increase progressively to the oldest ages. To determine whether this reflects a greater intrinsic risk of sensitization at older ages or simply the effects of cumulative exposure over the lifespan, we identified 940 patients who underwent patch testing to fragrance mix 1 on more than 1 occasion over this 30-year period ([Fig 1, J](#)). Of these, 41 had an initial negative patch test result with a subsequent positive test result implying *de novo* sensitization. To explore the factors governing sensitization in this context, we performed logistic regression with interval between testing and age of patient as predictor variables ([Fig 1, K and L](#)). There was no significant relationship to test interval ($P = .16$); however, there was a highly significant relationship with patient age ($P = .0001$), implying that older individuals have a higher intrinsic risk of sensitization. This conclusion is supported by the observation that a similar age profile can be observed for allergens newly introduced to the market.^{5,6} We propose that this higher risk of sensitization may result from impaired efficiency of regulatory immune function in the elderly.

Finally, a large cohort of allergens including formaldehyde, quaternium 15, epoxy resin, potassium dichromate, and methylisothiazolinone/methylchloroisothiazolinone exhibit the highest prevalence of sensitization between the ages of 40 and 70 years with a subsequent decline at older ages. A change in allergen



prevalence, for example, due to the introduction or withdrawal of allergens from the market is unlikely to be causative because these profiles are conserved when examined across 3 separate decades (Fig E3, B-D). These observations may instead reflect the superposition of alterations in T-cell-mediated immunity and regulatory immune function.

Previous work provides a potential mechanistic basis for these observations. CD25⁺ regulatory T cells from older mouse donors had impaired function in a delayed-type hypersensitivity assay with associated dysregulation of activatory cytokine production.⁷ CD25⁺ T cells from aged mice also exhibited an impaired ability to suppress IL-17⁺ T cells.⁸ Consistent with these findings, a human study identified suppression of the immunoregulatory cytokine IL-10 for T cells derived from elderly individuals.⁹

In summary, we have reported the age-specific prevalence of sensitization for a wide range of allergens implicated in delayed-type cutaneous hypersensitivity. Our analysis reveals complex allergen-specific age profiles for sensitization that may reflect alterations in T-cell-mediated immunity and regulatory immune function. Limitations of our study include a lack of mechanistic data; furthermore, we cannot exclude a contributory role for age-related changes in skin barrier function, systemic medical conditions, or pharmacological therapy. Nevertheless, this is the largest reported single-center series to date and the first longitudinal study of this nature and as such is uniquely suited to the *in vivo* dissection of age-related alterations in T-cell-mediated immunity.

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FIG 1. A, Age distribution of patients undergoing patch testing in 1985-1995 (red), 1995-2005 (green), and 2005-2014 (blue). To facilitate comparison, patients were subdivided according to age groups of a decade, with individuals older than 80 years grouped together because of smaller numbers. **B,** Age-specific prevalence of patients exhibiting sensitization to 1 or more allergens (red), exactly 1 allergen (blue), 2 allergens (green), 3 allergens (purple), and 3 or more allergens (orange). Data are normalized for the number of patients in this age interval undergoing testing. **C,** Hierarchical clustering of age-specific prevalence of sensitization for 37 allergens. The age-specific prevalence of positive test results for each allergen are plotted as a heat map, with white representing the lowest and dark blue the highest prevalence. **D-I,** Visualization of individual groups of allergens identified by hierarchical clustering analysis. **J,** Age distribution of patients (n = 940) undergoing patch testing to fragrance mix 1 on more than 1 occasion. For each patient, the age corresponding to a time point intermediate between the 2 patch tests is plotted. **K and L,** Factors governing the acquisition of sensitization to fragrance mix 1 in serial testing. A total of 940 patients underwent patch testing to fragrance mix 1 on more than 1 occasion over the 30-year period. Of these 41 patients had negative results in the first test and positive results in the subsequent test. **K,** Relationship between interval of testing between 2 patch tests and probability of *de novo* sensitization. **L,** Relationship between age of patient and probability of *de novo* sensitization (****P* < .0001). Significance levels are calculated with logistic regression with interval between tests and patient age as predictor variables and *de novo* sensitization as the response variable. *MCI*, Methylisochlorothiazolinone; *MI*, methylisothiazolinone; *PPD*, p-Phenylenediamine; *PTBP*, para-tertiary butylphenol formaldehyde resin.

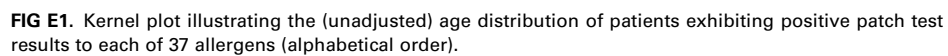
METHODS

Patch testing was performed with aluminum Finn Chambers (Bio-Diagnostics) with TROLAB allergens. A total of 45,110 consecutive patients were recorded between 1985 and 2014. All patch test results were read by a dermatologist in a consistent manner and recorded in a standard proforma with subsequent entry into a computerized database.

Data analysis was performed with the R language for statistical computing. Unadjusted age-specific prevalence is defined as the number of positive patch test results at each age and was computed by fitting the data to a Gaussian kernel density estimator. A limitation of this analysis is that the rate of patch testing differs according to age and therefore to compute the adjusted age-specific prevalence, patients were divided by age into decades. Because of the smaller number of patients, patients older than 80 years were grouped

together. Adjusted age-specific prevalence was defined for each age group as the number of positive test results divided by the number of tests performed to give a vector for each allergen. To compare allergen-specific clustering relationships, vectors were normalized to the peak prevalence observed for each allergen and the Euclidean distance between vectors was computed. Hierarchical clustering was subsequently performed with the R *hclust* function using standard parameters.

For patients who underwent patch testing on more than 1 occasion and who exhibited negative testing to fragrance mix 1 in the first test, logistic regression was performed with interval of testing and mean age (ie, age at the midpoint in time between the 2 tests) as predictor values and *de novo* sensitization as the outcome variable. *P* values are calculated via the Wald *z* statistic.



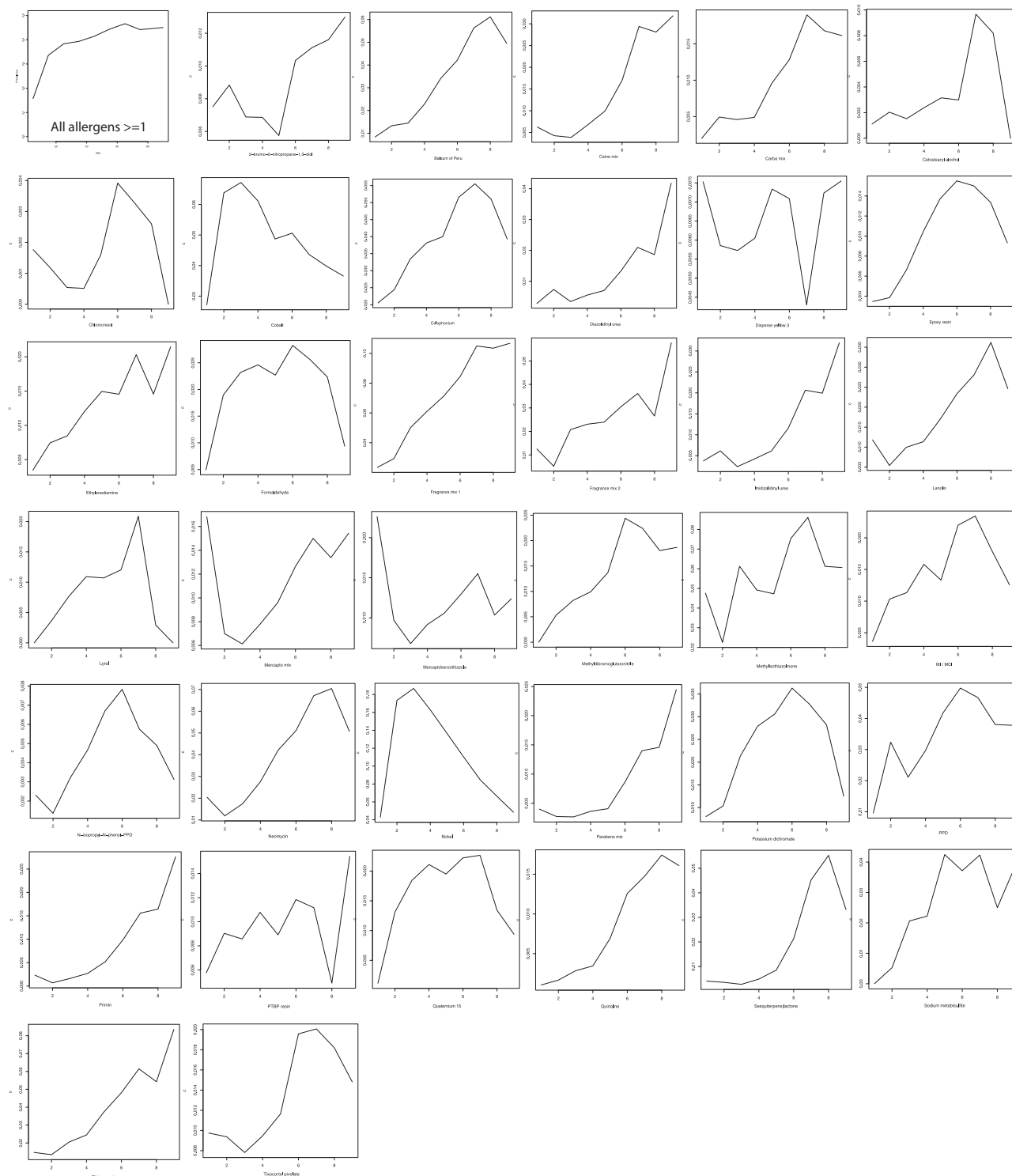


FIG E2. Histogram illustrating age-specific prevalence of positive reactions for patients undergoing patch testing to each of 37 allergens (alphabetical order).

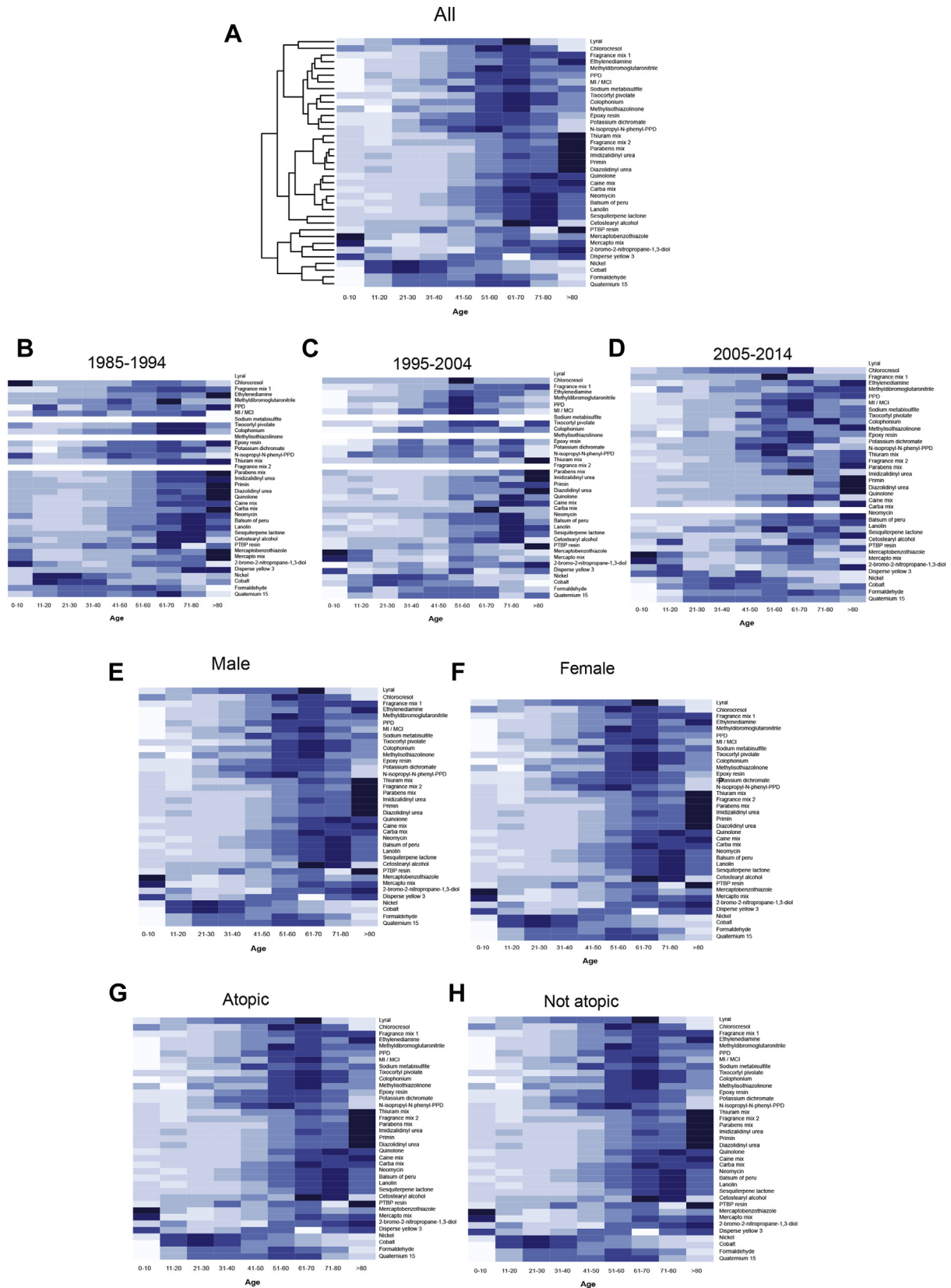


FIG E3. Stratification of age-specific prevalence of sensitization according to date of testing, age, sex, and atopy status. **A**, All patients. **B-D**, Stratification according to date of testing, 1985-1995 (Fig E3, **B**), 1995-2005 (Fig E3, **C**), and 2005-2014 (Fig E3, **D**). **E** and **F**, Stratification according to sex, male (Fig E3, **E**) and female (**F**). **G** and **H**, Stratification according to atopy status, atopic (Fig E3, **G**), not atopic (Fig E3, **H**). *MCI*, Methylisochlorothiazolinone; *MI*, methylisothiazolinone; *PPD*, p-Phenylenediamine; *PTBP*, triethyl butylphenol formaldehyde resin.

TABLE E1. Overall prevalence of positive patch test results according to allergen and age of patient

Allergen	Age (y)								
	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	>80
Balsum of Peru	0.008	0.013	0.014	0.023	0.034	0.042	0.056	0.061	0.050
2-Bromo-2-nitropropane-1,3-diol	0.008	0.009	0.007	0.007	0.006	0.010	0.011	0.012	0.013
Caine mix	0.006	0.004	0.004	0.007	0.010	0.017	0.029	0.028	0.032
Carba mix	0.002	0.005	0.005	0.005	0.010	0.013	0.019	0.017	0.016
Cetostearyl alcohol	0.001	0.002	0.002	0.002	0.003	0.003	0.010	0.008	0.000
Chlorocresol	0.002	0.001	0.001	0.001	0.002	0.004	0.003	0.003	0.000
Cobalt	0.027	0.064	0.067	0.061	0.049	0.051	0.044	0.040	0.037
Colophonium	0.021	0.024	0.033	0.038	0.040	0.052	0.056	0.051	0.039
Potassium dichromate	0.008	0.010	0.021	0.028	0.031	0.036	0.033	0.028	0.013
Quaternium 15	0.001	0.013	0.018	0.021	0.020	0.022	0.023	0.013	0.009
Epoxy resin	0.003	0.004	0.007	0.010	0.014	0.016	0.015	0.013	0.009
Ethylenediamine	0.003	0.007	0.008	0.012	0.015	0.015	0.020	0.015	0.021
Methyldibromoglutaronitrile	0.000	0.005	0.008	0.010	0.014	0.024	0.022	0.018	0.019
Fragrance mix 2	0.013	0.005	0.021	0.023	0.024	0.030	0.036	0.027	0.058
Formaldehyde	0.005	0.019	0.023	0.025	0.023	0.028	0.026	0.022	0.009
Imidizalidinyl urea	0.004	0.006	0.002	0.004	0.006	0.012	0.021	0.020	0.032
Diazolidinyl urea	0.003	0.007	0.003	0.006	0.007	0.013	0.021	0.019	0.042
MI/MCI	0.004	0.010	0.011	0.016	0.013	0.022	0.023	0.018	0.013
Sesquiterpene lactone	0.004	0.004	0.003	0.005	0.009	0.021	0.045	0.055	0.033
Lyrar	0.000	0.004	0.008	0.011	0.011	0.012	0.021	0.003	0.000
Mercaptobenzothiazole	0.023	0.010	0.007	0.009	0.011	0.013	0.016	0.010	0.012
Mercapto mix	0.017	0.007	0.006	0.008	0.010	0.013	0.015	0.013	0.015
Methylisothiazolinone	0.048	0.023	0.061	0.049	0.047	0.075	0.086	0.061	0.061
Neomycin	0.020	0.012	0.017	0.028	0.042	0.051	0.067	0.070	0.051
Nickel	0.043	0.173	0.187	0.163	0.137	0.110	0.085	0.066	0.048
Parabens mix	0.004	0.003	0.003	0.004	0.004	0.009	0.014	0.015	0.024
Fragrance mix 1	0.023	0.029	0.050	0.061	0.071	0.084	0.105	0.103	0.107
PPD	0.010	0.032	0.021	0.030	0.042	0.050	0.047	0.038	0.038
<i>N</i> -isopropyl- <i>N</i> -phenyl-PPD	0.002	0.001	0.003	0.005	0.007	0.008	0.006	0.005	0.003
Primin	0.002	0.001	0.002	0.003	0.005	0.010	0.016	0.016	0.028
PTBP resin	0.006	0.009	0.009	0.011	0.009	0.012	0.011	0.005	0.015
Quinoline	0.001	0.002	0.003	0.003	0.007	0.013	0.015	0.017	0.016
Sodium metabisulfite	0.000	0.005	0.021	0.022	0.042	0.037	0.042	0.025	0.038
Thiuram mix	0.015	0.013	0.020	0.024	0.037	0.048	0.061	0.054	0.084
Tixocortyl pivalate	0.010	0.009	0.008	0.009	0.012	0.020	0.020	0.018	0.015
Lanolin	0.012	0.005	0.010	0.011	0.017	0.023	0.028	0.036	0.025
Disperse yellow 3	0.008	0.006	0.006	0.006	0.007	0.007	0.004	0.007	0.008

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