

## Letter to the Editor

**Genetic variants harbored in the forkhead box protein 3 locus increase hay fever risk**

To the Editor:

Disturbed regulation of T cells may lead to an imbalance between T<sub>H</sub>1 and T<sub>H</sub>2 cells and toward an elevated T<sub>H</sub>2 response, typically associated with allergy. Regulatory T cells are thought to be responsible for keeping T-cell populations and T-cell effects in balance, whereas modifications of regulatory T-cell signaling may lead to deviated immune responses. Recently, we had shown that T-cell-associated transcription factors influence the risk for asthma and allergy.<sup>1</sup> The transcription factor forkhead box protein 3 (FOXP3; forkhead/winged helix transcription factor) seems to specifically control regulatory T-cell function.<sup>2</sup> Deletions or change of function in the human *FOXP3* gene result in the development of severe immune dysregulation with polyendocrinopathic and enteropathic X-linked syndrome,<sup>3</sup> often associated

with high serum IgE levels.<sup>4</sup> Despite the prominent role T cells and T-cell regulation have in current asthma and allergy disease models, only 1 study has recently been published on *FOXP3* genetics in that field to our knowledge.<sup>5</sup>

We now investigated 40 kilo base pairs of genomic sequence harboring the *FOXP3* gene (Fig 1). On the basis of HapMap data (<http://www.hapmap.org>),<sup>6</sup> we identified 4 tagging polymorphisms (rs2294020, rs2294021, rs3761548, and rs3761549) with a minor allele frequency (MAF) >0.10 capturing all essential genetic information of the *FOXP3* gene locus using Haploview software.<sup>7</sup> We studied the influence of these single nucleotide polymorphisms (SNPs) on atopic diseases in a cross-sectional population sample of 3099 German children age 9 to 11 years from Dresden and Munich as part of the International Study of Asthma and Allergy in Childhood phase II. In that study, the prevalence of a doctor's diagnosis of asthma, hay fever, and atopic dermatitis was assessed by self-administered questionnaires and by

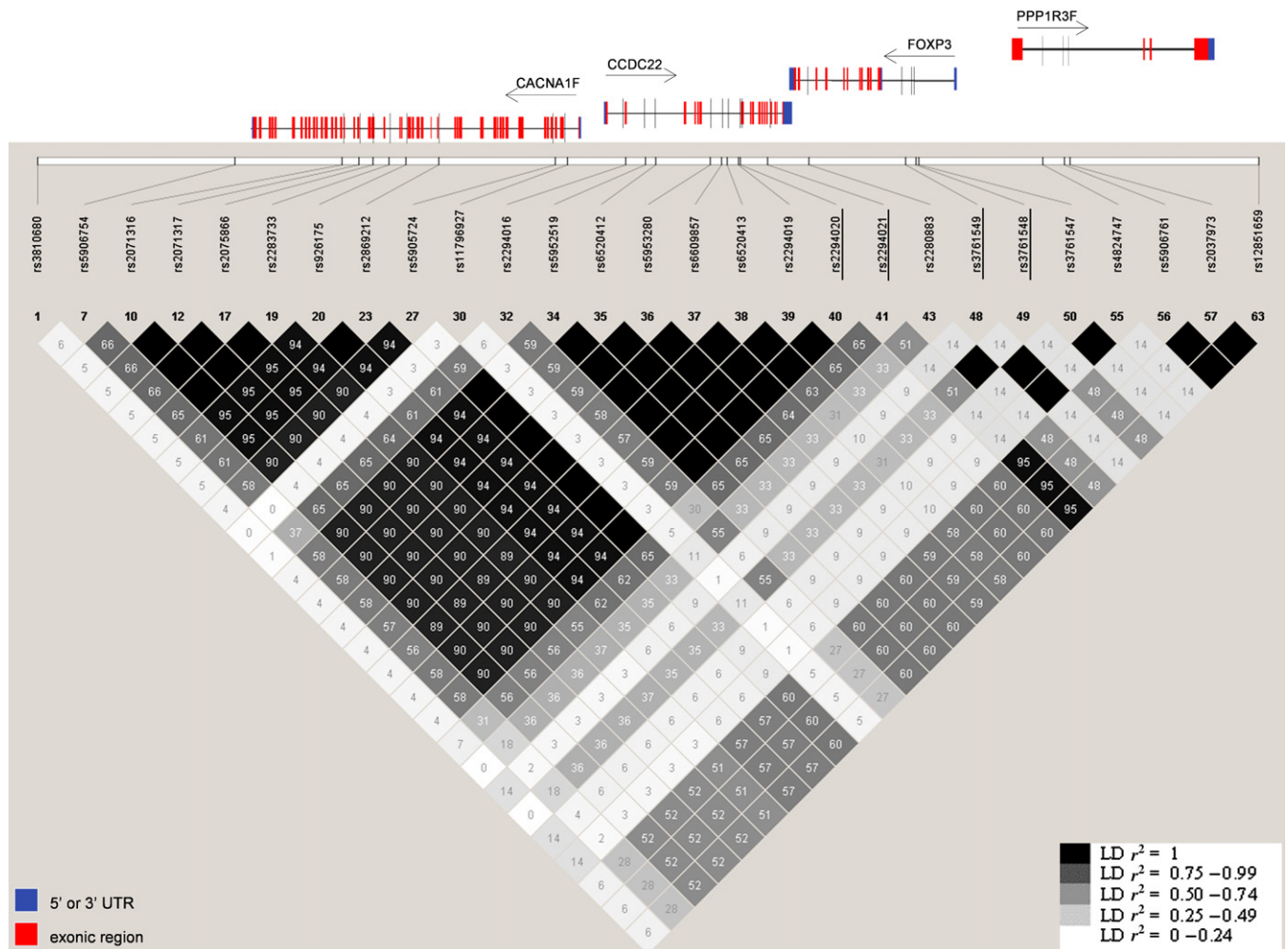


**FIG 1.** Depiction of gene structures on chromosome X, identified polymorphisms and LD ( $r^2$ ). The HapMap database was used to identify validated *FOXP3* SNPs. In addition, the screened region was extended around the *FOXP3* gene, thereby detecting SNPs in and around the neighbor genes *CACNA1F*, *CCDC22*, and *PPP1R3F*. Color coding for the linkage disequilibrium plot is given by Haploview: white ( $r^2 = 0$ ), shades of gray ( $0 < r^2 < 1$ ), and black ( $r^2 = 1$ ). Genotyped tagging SNPs are underlined. UTR, Untranslated region.

**TABLE I.** Associations between atopic phenotypes and tagging SNPs showing odds ratios, 95% CIs, and *P* values (given for Wald test)

| Tagging SNPs and MAFs                                 | rs 2294020 (MAF 0.31)                                          | rs 2294021 (MAF 0.45)                    | rs 3761548 (MAF 0.43)                    | rs 3761549 (MAF 0.12)                    |
|-------------------------------------------------------|----------------------------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| Atopic sensitization (SPT <sup>+</sup> ) <sup>†</sup> | 1.106<br>0.998-1.226<br><i>P</i> = .0546                       | 1.041<br>0.943-1.149<br><i>P</i> = .4236 | 0.927<br>0.839-1.024<br><i>P</i> = .1369 | 1.057<br>0.908-1.230<br><i>P</i> = .4774 |
| Asthma <sup>‡</sup>                                   | 1.100<br>0.942-1.286<br><i>P</i> = .2294                       | 1.072<br>0.924-1.245<br><i>P</i> = .3594 | 0.967<br>0.831-1.125<br><i>P</i> = .6607 | 0.951<br>0.749-1.208<br><i>P</i> = .6811 |
| Atopic dermatitis <sup>§</sup>                        | 1.028<br>0.914-1.157<br><i>P</i> = .6441                       | 0.928<br>0.828-1.039<br><i>P</i> = .1939 | 1.066<br>0.953-1.193<br><i>P</i> = .2644 | 1.022<br>0.86-1.216<br><i>P</i> = .8027  |
| Hay fever <sup>§</sup>                                | <b>1.256*</b><br><b>1.081-1.460</b><br><b><i>P</i> = .0030</b> | 1.131<br>0.975-1.313<br><i>P</i> = .1048 | 0.983<br>0.845-1.143<br><i>P</i> = .8234 | 0.793<br>0.609-1.032<br><i>P</i> = .0842 |
| Hay fever and atopic sensitization                    | <b>1.225</b><br><b>1.033-1.453</b><br><b><i>P</i> = .0196</b>  | 1.101<br>0.930-1.303<br><i>P</i> = .2635 | 1.006<br>0.848-1.193<br><i>P</i> = .9469 | 0.847<br>0.634-1.131<br><i>P</i> = .2605 |
| Current hay fever symptoms                            | <b>1.210*</b><br><b>1.069-1.371</b><br><b><i>P</i> = .0027</b> | 1.059<br>0.937-1.197<br><i>P</i> = .3582 | 0.962<br>0.851-1.089<br><i>P</i> = .542  | 0.981<br>0.809-1.191<br><i>P</i> = .8476 |

SPT, Skin prick test.

\*Significant after correction for multiple testing.

<sup>†</sup>Defined by skin prick test against 6 common aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria tenuis*, cat dander, and mixed grass and tree pollen).<sup>‡</sup>Defined by a parental report of a physician's diagnosis of asthma (at least once) or of spastic or asthmatic bronchitis (at least twice) in a self-administered questionnaire.<sup>§</sup>Defined by a parental report of a physician's diagnosis of the respective diseases. Significant associations are shown in boldface.

objective measurements such as lung function tests, skin prick tests, and serum IgE measurements as previously reported.<sup>8,9</sup> Further information on technical details concerning matrix-assisted laser desorption/ionization time-of-flight genotyping or population characteristics is available from the corresponding author on request.

Because of the X-chromosomal location, all SNPs were analyzed by fitting an additive effects-only logit model that equates the risks of male hemizygotes with female homozygotes. Hardy-Weinberg equilibrium did not deviate and was tested in females only. To correct for multiple testing, we used a Bonferroni correction in every phenotype. All tests were 2-sided, and the differences were considered significant with *P* < .05. Calculations were carried out with SAS (version 9.1.3) and SAS/Genetics software (SAS Institute, Inc, Cary, NC).

Tagging SNP rs2294020 downstream of *FOXP3* slightly increased the risk for atopy assessed by skin prick test in the study population and showed significant associations with hay fever (Table I). Also, when phenotypes related to the primary outcome hay fever were analyzed, significant associations between this SNP and hay fever with concomitant sensitization to inhalative allergens and current hay fever symptoms were observed. Associations for hay fever and current symptoms of hay fever remained statistically significant after correction for multiple testing. These results coincide with data from Zhang et al,<sup>5</sup> who recently observed associations between *FOXP3* SNPs (rs3761547 and rs3761548) and allergic rhinitis in a Chinese population.

Common polymorphisms and microsatellite markers had been described in the *FOXP3* gene and in its putative promoter and upstream untranslated region before. These gave conflicting association results with a number of complex immune and autoimmune diseases. Mutation events severely affecting *FOXP3* function are

rare and cause serious immune deficiency syndromes.<sup>3</sup> The SNPs that we studied and that are covered by the tagging SNPs genotyped here are located within and downstream of the *FOXP3* gene. When linkage disequilibrium (LD) analyses were extended, several other SNPs tagged by SNP rs2294020 (*r*<sup>2</sup> ≥ 0.9) were identified in and around neighboring genes coiled-coil domain containing 22 (*CCDC22*) and calcium channel, voltage-dependent,  $\alpha$ -1F subunit (*CACNA1F*; Fig 1). Whereas *CCDC22* is an unexplored gene with so far unknown biological function, *CACNA1F* is a voltage-gated calcium channel. Two polymorphisms in the allergic rhinitis associated LD block (rs2294020 located in exon 7 of *CCDC22*, and rs2075866 located in exon 28 of *CACNA1F*) were investigated further because of their position in exonic regions, putatively acting as exonic splicing enhancers. Indeed, using ESEfinder software, release 3.0 ([http://rulai.cshl.edu/cgi-bin/tools/ESE3/ese\\_finder.cgi](http://rulai.cshl.edu/cgi-bin/tools/ESE3/ese_finder.cgi))<sup>10,11</sup> allele-specific changes in score matrices for human serine/arginine-rich (SR) proteins were observed, which belong to a protein family essential for splicing (Fig 2). Also, *in silico* transcription factor binding analyses for promoter and intronic SNPs in the same LD block were performed that predicted numerous SNP-dependent alterations of transcription factor binding (Alibaba 2.1 software<sup>12</sup>). However, a comparative genomic analysis searching for conserved noncoding sequences revealed only poor phylogenetic conservation in intergenetic and intragenetic regions of the locus (VISTA Genome browser<sup>13</sup>). Thus, further functional analyses are needed to determine whether the associations of SNPs in this LD block observed with allergic rhinitis may be influenced by splicing alterations in genes close to *FOXP3* and/or locus regulatory elements in the area.

Although the causal SNP for this associated LD block is not identified yet, our data and those of Zhang et al<sup>5</sup> imply that genetic variations in the region of and around *FOXP3* may modify the risk

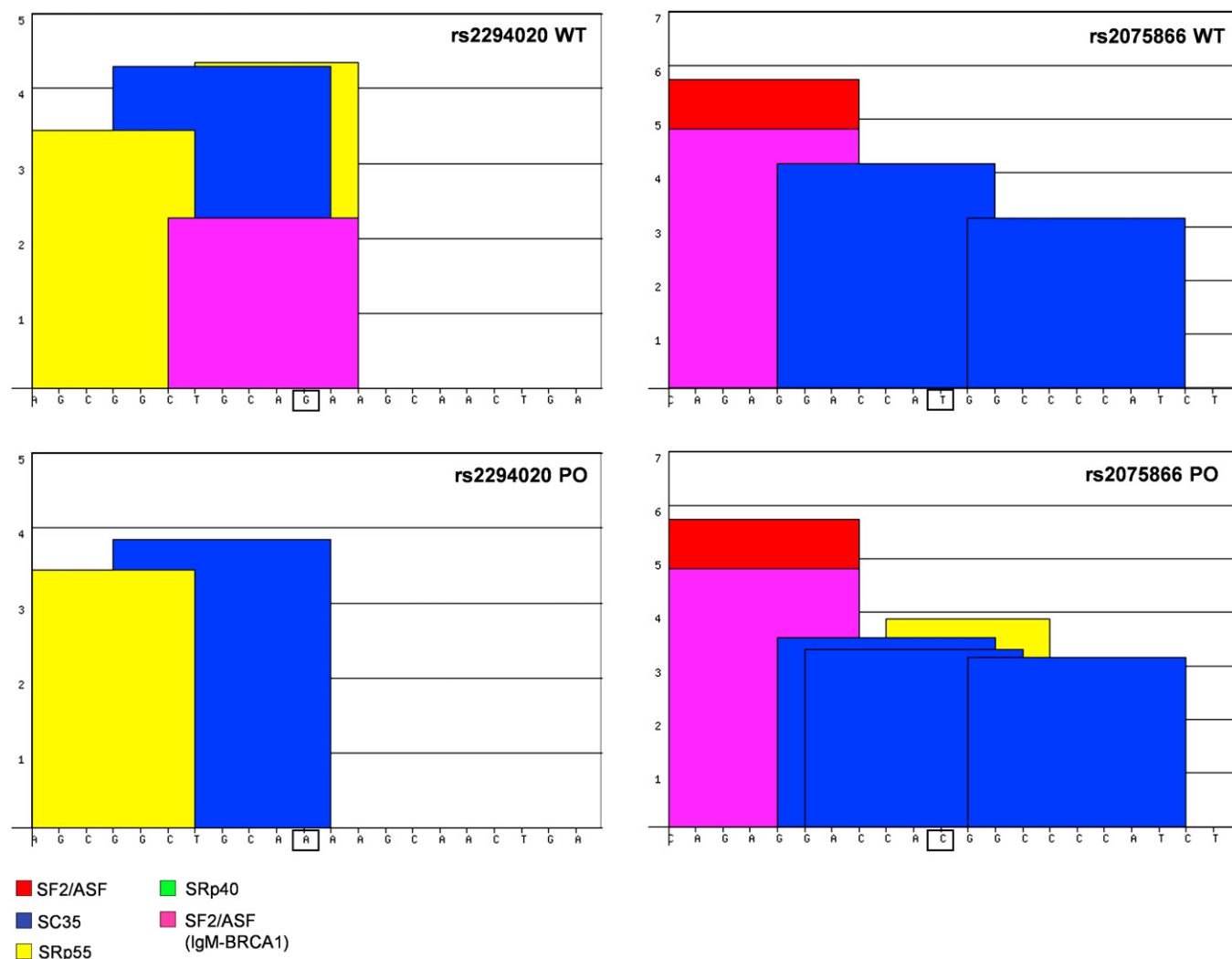


**FIG 2.** Allele-specific values for score matrices of human SR proteins (SF2/ASF, SC35, SRp40, or SRp55) for SNP rs2294020 and SNP rs2075866 using ESEfinder software (release 3.0).<sup>10,11</sup> Analyzed exonic sequence along the x-axis with respective SNP position highlighted. The *height of the bars* reflects the motif scores for the different SR proteins, and the *width of the bars* represents the length of the motif (6, 7, or 8 nucleotides). Only high score values above the selected threshold defined by the ESEfinder software are shown. *BRCA1*, Breast cancer 1 gene; *PO*, polymorphic allele; *WT*, wild-type.

for allergic rhinitis. The association with hay fever we observed is consistent in our population and a second report. Also, when objective measurements were included in the definition of the phenotype, the association was present and specific. Genetic association reports with hay fever are sparse despite the fact that the disease has a very well defined phenotype, is very frequent, and imposes a heavy burden on affected individuals and public health. Within the atopic phenotypes, it is the disease most clearly associated with IgE-specific sensitizations and may represent a good candidate disease for genetically driven variation in regulatory T-cell function.

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