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Title

The locus *C11orf30* increases susceptibility to poly-sensitisation

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Short title

C11orf30 associates with poly-sensitisation

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Abstract

A number of genetic variants have been associated with allergic sensitisation, but whether these are allergen-specific or increase susceptibility to poly-sensitisation is unknown. Using data from the large multicentre population-based European Community Respiratory Health Survey, we assessed the association between 10 loci and specific IgE and skin prick tests to individual allergens and poly-sensitisation. We found that the 10 loci associate with sensitisation to different allergens in a non-specific manner, and that one in particular, *C11orf30*-rs2155219, doubles the risk of poly-sensitisation (specific IgE/4 allergens: OR=1.81, 95%CI 0.80-4.24; skin prick test/4+ allergens: OR=2.27, 95%CI 1.34-3.95). The association of rs2155219 with higher levels of expression of *C11orf30*, which may be involved in transcription repression of interferon-stimulated genes, and its association with sensitisation to multiple allergens suggest that this locus is highly relevant for atopy.

Key words

Allergens; allergic sensitisation; genes for atopy; poly-sensitisation

Several studies have shown that genetic variants may play a role in allergic sensitisation [1-4], however the question of whether these are allergen-specific remains unanswered. Bonnelykke *et al.*, in a study of over 30,000 European children and adults, identified ten loci with genome-wide significance for 'allergic sensitisation' heterogeneously defined as either positive skin prick tests (SPT) or positive serum specific IgE (ssIgE) to at least one of a range of measured indoor, outdoor, and food allergens [4]. However, they did not assess the associations between genetic variants and sensitisation to individual allergens. This report explores these associations in more detail in adults from the multi-centre European Community Respiratory Health Survey (ECRHS), examining associations of 1) ssIgE and 2) SPT to individual allergens with the 10 variants identified by Bonnelykke *et al*.

Methods

Adults of European descent were randomly recruited from community-based sampling frames in the ECRHS I (1992-1994) [5]. Serum total and specific IgE were measured using the Pharmacia CAP System (Pharmacia Diagnostics AB, Uppsala, Sweden) [6], and subjects considered sensitised if allergen-specific IgE concentration was ≥0.35 kU/L. SPTs were conducted using Phazets (Pharmacia Diagnostics AB, Uppsala, Sweden), with a positive test being defined by a wheal diameter >0 mm [6]. SsIgE, but not SPTs, to specific food allergens were measured at first follow-up (ECRHS II: 2000-2002). Genotyping, on blood samples collected in 2000-2002, was performed with the Illumina 610K array (Illumina, Inc., Sand Diego, CA, USA), and missing genotypes imputed (MaCH algorithm using HapMap phase II CEU panel). This analysis includes subjects with measures of IgE and SPT, who were selected at random for genotyping (i.e. this sample is not enriched with asthmatics). Ethical approval from local research ethics committees and written consent from subjects were obtained.

Logistic regression models adjusted for age and gender were used to examine associations of each of the 10 single nucleotide polymorphisms (SNPs), under the additive mode of inheritance, with ssIgE to four aeroallergens [house dust mite (HDM), Timothy grass, cat, and *Cladosporium herbarum*] (controls negative to all) and five mixes covering 25 common food allergens (fx5, fx6, epcx1, epcx2, epcx3 [7]; controls negative to all) (Supplementary Figure E1). To control for population stratification, models were further adjusted for study centre and the two most informative ancestry principal components as in previous published analyses [8]. Similar models were used to assess SNP associations with positive SPT to nine aeroallergens (HDM, Timothy grass, cat, *Cladosporium herbarum*, birch, olive tree, *Alternaria alternata*, ragweed, and *Parietaria judaica*) (controls negative to all). Associations with sensitisation to two, three or more allergens, and with log-transformed total IgE were also examined. Statistical analyses were performed using R.3.0.3, and results considered significant when $P \leq 0.005$ (corrected for 10 SNPs; two sided).

Results and discussion

Characteristics of the 1554 subjects are presented in table 1. The prevalence of IgE sensitisation and positive SPT to at least one aeroallergen was 29.5% and 36.6%, respectively, and the prevalence of IgE sensitisation to at least one food allergen was 16.2%. As shown previously, the T allele (frequency 48%) of rs2155219, in *C11orf30*, increased risk of sensitisation to any allergen (sIgE: OR=1.30, 95%CI 1.09-1.54, *P*=0.003; SPT: OR=1.26, 95%CI 1.04-1.52, *P*=0.016). Furthermore, it was associated with sensitisation to each individual allergen and poly-sensitisation (sIgE/4 allergens: OR=1.81, 95%CI 0.80-4.24, *P*=0.16; SPT/4+ allergens: OR=2.27, 95%CI 1.34-3.95, *P*=0.003; Figure 1). These patterns were observed irrespective of whether sensitisation was measured by ssIgE or SPT. In a

previous report of ECRHS, agreement (kappa) statistics between ssIgE and SPT were 0.66, 0.56, 0.69, and 0.12 for HDM, cat, Timothy grass, and *Cladosporium herbarum*, respectively [9]. Adjusting the associations with sIgE for total IgE or using an SPT cut-off of 3 mm did not materially alter the effect estimates. We observed a strong and significant increased risk of sensitisation to cat, especially when considering mono-sensitisation to cat, as measured by ssIgE (P=3x10^{-5}), but this was not seen as clearly with sensitisation defined by SPT. Associations of sensitisation to foods with *C11orf30*-rs2155219[T] were less clear, but data were suggestive of an increasing risk with sensitisation to an increasing number of food allergens (Table 2 and Supplementary Figure E11). Although associations of sensitisation to the remaining 9 SNPs (*STAT6*-rs1059513, *SLC25A46*-rs10056340, *HLA-DQB1*-rs6906021, *IL1RL1/IL18R1*-rs3771175, *TLR1/TLR6/TLR10*-rs17616434, *LPP*-rs9865818, *MYC/PVT1*-rs4410871, *IL2/ADAD1*-rs17454584, *HLA-B/MICA*-rs6932730) did not always reach statistical significance (Table 2; Supplementary Figures E2-E20), effect estimates were, in general, in the same direction and of similar magnitude as those reported previously [4]. Using either ssIgE or SPT, these 9 SNPs associated with sensitisation to some individual allergens, but not consistently with increased susceptibility to poly-sensitisation. Finally, the magnitude of the associations between the 10 SNPs and total IgE was similar to that found for sensitisation to at least one allergen, with *C11orf30*-rs2155219[T] being the only one to show a statistically significant association with total IgE (Table 2). Excluding asthmatics from the analyses did not materially alter the effect estimates.

We show that *C11orf30*-rs2155219[T] increases susceptibility to poly-sensitisation, and that previously reported associations of 10 SNPs with ‘allergic sensitisation' are unlikely to be allergen specific, are observed with sensitisation to common indoor, outdoor and food allergens, and are present irrespective of whether measures are made by ssIgE or SPT. We

also show that only two of these 10 loci may associate with total IgE, suggesting that genetic regulation of total IgE is distinct from that for sIgE. However, our findings should be replicated before firm conclusions are drawn. The strengths of this European study are the population-based nature of the sample, the careful standardisation of measurement of atopy using both ssIgE and SPT [6], and the number and representativeness across Europe of the allergens tested. One limitation is the sample size, but we observed effect estimates for ssIgE and positive SPT similar to those reported by Bonnelykke *et al.* [4], even when they failed to reach statistical significance. Although the function of *C11orf30*-rs2155219[T] is unknown, its strong association with the expression of *C11orf30* [4], and its association with sensitisation to multiple allergens, whether measured by ssIgE or SPT, strengthen the evidence that this region is highly relevant for atopy. The protein encoded by *C11orf30*, thought to act as a transcription repressor of interferon-stimulated genes [10], shows medium to high expression levels in several organs, including the skin and the lung [11]. Our findings plus reported associations of *C11orf30* with other allergic and inflammatory diseases, such as atopic dermatitis [12], asthma [13], allergic rhinitis [2], and Crohn's disease [14], indicate that further elucidation of the biological function and regulation of this locus is warranted.

Author contributions

A.F.S.A. and D.L.J. designed the study, analysed the data, and drafted the manuscript. All authors critically revised the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Table 1. Characteristics of subjects from the random sample of the European Community Respiratory Health Survey with measures of specific IgE or skin prick tests and genotype data for the 10 single nucleotide polymorphisms* considered in the current analysis.

	N = 1554
Age in 1992 (years), median (interquartile range)	34.1 (27.9-40.1)
Sex (%)	
Females	51.3%
Males	48.7%
Country (%)	
Spain	21.0%
France	16.9%
Norway	14.7%
Sweden	13.6%
Switzerland	10.5%
Germany	10.3%
UK	9.5%
Estonia	3.5%
Physician diagnosed asthma, in 1992 (%)	5.7%
Hay fever or nasal allergies, in 1992 (%)†	24.6%
Total serum IgE in 1992 (kU/L), median (interquartile range)‡	28.1 (11.3-88.1)
Serum specific IgE to at least one aeroallergen, in 1992 (%)‡$	29.5%
Serum specific IgE to at least one food allergen, in 2002 (%)£	16.2%
Positive skin prick test to at least one aeroallergen, in 1992 (%)¥	36.6%

*****rs2155219, rs1059513, rs10056340, rs6906021, rs3771175, rs17616434, rs9865818, rs4410871, rs17454584, rs6932730. †Nine subjects had missing data for hay fever or nasal allergies. ‡One hundred and eighteen subjects did not provide serum. $Four allergens considered: house dust mite, Timothy grass, cat, and *Cladosporium herbarum*. £Five hundred and five subjects were not tested for food allergen serum specific IgE. ¥Nine allergens considered: house dust mite, Timothy grass, cat, *Cladosporium herbarum*, birch, olive tree, *Alternaria alternata*, ragweed, and *Parietaria judaica*. Seventy four subjects did not perform skin prick tests.

Table 2. Odds ratios (OR) and 95% confidence intervals (CI) for the association between ten single nucleotide polymorphisms (SNP) and IgE sensitisation, positive skin prick test, and total IgE.

				Bonnelykke *et al.*	**Present study**							
SNP	**Effect/ Alternative alleles**	**Effect allele frequency**	**Nearest gene**	**Allergic sensitisation* OR (95% CI)**	**Specific IgE to at least 1 aeroallergen† OR (95% CI)**	***P***	**SPT to at least 1 aeroallergen ‡ OR (95% CI)**	***P***	**Specific IgE to at least 1 mix of food allergens‖ OR (95% CI)**	***P***	**Total IgE β# (95% CI)**	***P***
rs2155219	T/G	0.48	*C11orf30*	1.18 (1.13-1.22)	1.30 (1.09-1.54)	0.003	1.26 (1.04-1.52)	0.016	1.17 (0.90-1.51)	0.249	0.08 (0.03, 0.12)	0.002
rs1059513	T/C	0.89	*STAT6*	1.30 (1.21-1.39)	1.34 (1.03-1.77)	0.035	1.29 (0.97-1.74)	0.081	1.26 (0.83-1.98)	0.290	0.10 (0.02, 0.17)	0.011
rs10056340	T/G	0.81	*SLC25A46*	0.83 (0.78-0.87)	0.77 (0.62-0.95)	0.015	0.84 (0.66-1.06)	0.134	0.79 (0.57-1.09)	0.148	-0.03 (-0.09, 0.03)	0.347
rs6906021	T/C	0.53	*HLA-DQB1*	0.87 (0.83-0.90)	0.86 (0.73-1.03)	0.102	0.90 (0.74-1.09)	0.287	1.01 (0.78-1.32)	0.920	-0.02 (-0.07, 0.03)	0.360
rs3771175	A/T	0.14	*IL1RL1/ IL18R1*	0.83 (0.78-0.88)	0.90 (0.70-1.14)	0.384	0.73 (0.55-0.97)	0.032	0.91 (0.61-1.33)	0.646	0.00 (-0.07, 0.07)	0.956
rs17616434	T/C	0.71	*TLR1/TLR 6/TLR10*	1.23 (1.18-1.29)	0.99 (0.82-1.21)	0.942	1.01 (0.81-1.25)	0.959	0.88 (0.66-1.19)	0.410	0.00 (-0.05, 0.06)	0.928
rs9865818	A/G	0.56	*LPP*	0.89 (0.86-0.92)	0.83 (0.70-0.99)	0.033	0.80 (0.66-0.96)	0.015	0.92 (0.61-1.20)	0.548	-0.03 (-0.08, 0.02)	0.251
rs4410871	T/C	0.28	*MYC/PVT 1*	1.14 (1.09-1.19)	0.95 (0.79-1.14)	0.599	0.88 (0.72-1.08)	0.226	0.82 (0.61-1.08)	0.165	0.00 (-0.05, 0.05)	0.981
rs17454584	A/G	0.77	*IL2/ADAD 1*	0.87 (0.83-0.91)	0.82 (0.67-1.00)	0.048	0.78 (0.63-0.96)	0.022	0.75 (0.56-1.00)	0.051	0.04 (-0.01, 0.10)	0.133
rs6932730	T/C	0.83	*HLA-B/ MICA*	1.14 (1.09-1.20)	1.06 (0.85-1.32)	0.607	1.06 (0.83-1.35)	0.660	1.34 (0.95-1.92)	0.107	0.00 (-0.07, 0.06)	0.898

*allergic sensitisation defined as IgE sensitisation and/or positive skin prick test to at least one allergen. Bonnelykke et al. Nature Genetics 2013;45(8):902-6.

†aeroallergens: house dust mite, Timothy grass, cat, and *Cladosporium herbarum*. IgE < 0.35 kU/L (n = 1011) vs IgE ≥ 0.35 kU/L (n = 424).

‡aeroallergens: house dust mite, Timothy grass, cat, *Cladosporium herbarum*, birch, olive tree, *Alternaria alternata*, ragweed, and *Parietaria judaica*. Wheal diameter = 0 mm (n = 796) vs wheal diameter > 0 mm (n = 460).

‖food allergens: fx5, fx6, epcx1, epcx2, epcx3. fx5: cow's milk, egg white, fish, soya bean, peanut, wheat; fx6: sesame, buckwheat, corn, rice; epcx1: hazelnut, walnut, celery, tomato, carrot; epcx2: mustard, shrimp, sunflower seed, poppy seed, lentil; epcx3: banana, kiwi, apple, peach, melon. IgE < 0.35 kU/L (n = 803) vs IgE ≥ 0.35 kU/L (n = 156).

#log-transformed total IgE, n = 1436.

244 **Figure legend**

245 **Figure 1.** Odds ratios (OR) and 95% confidence intervals for the association between
246 *C11orf30*-rs2155219[T] and: A) serum specific IgE to at least one of four common allergens
247 (house dust mite, Timothy grass, cat, and *Cladosporium herbarum*); B) positive skin prick
248 test to at least one of nine common allergens (house dust mite, Timothy grass, cat,
249 *Cladosporium herbarum*, birch, olive tree, *Alternaria alternate*, ragweed, and *Parietaria*
250 *judaica*. Numbers on the X axis correspond to number of sensitised participants.

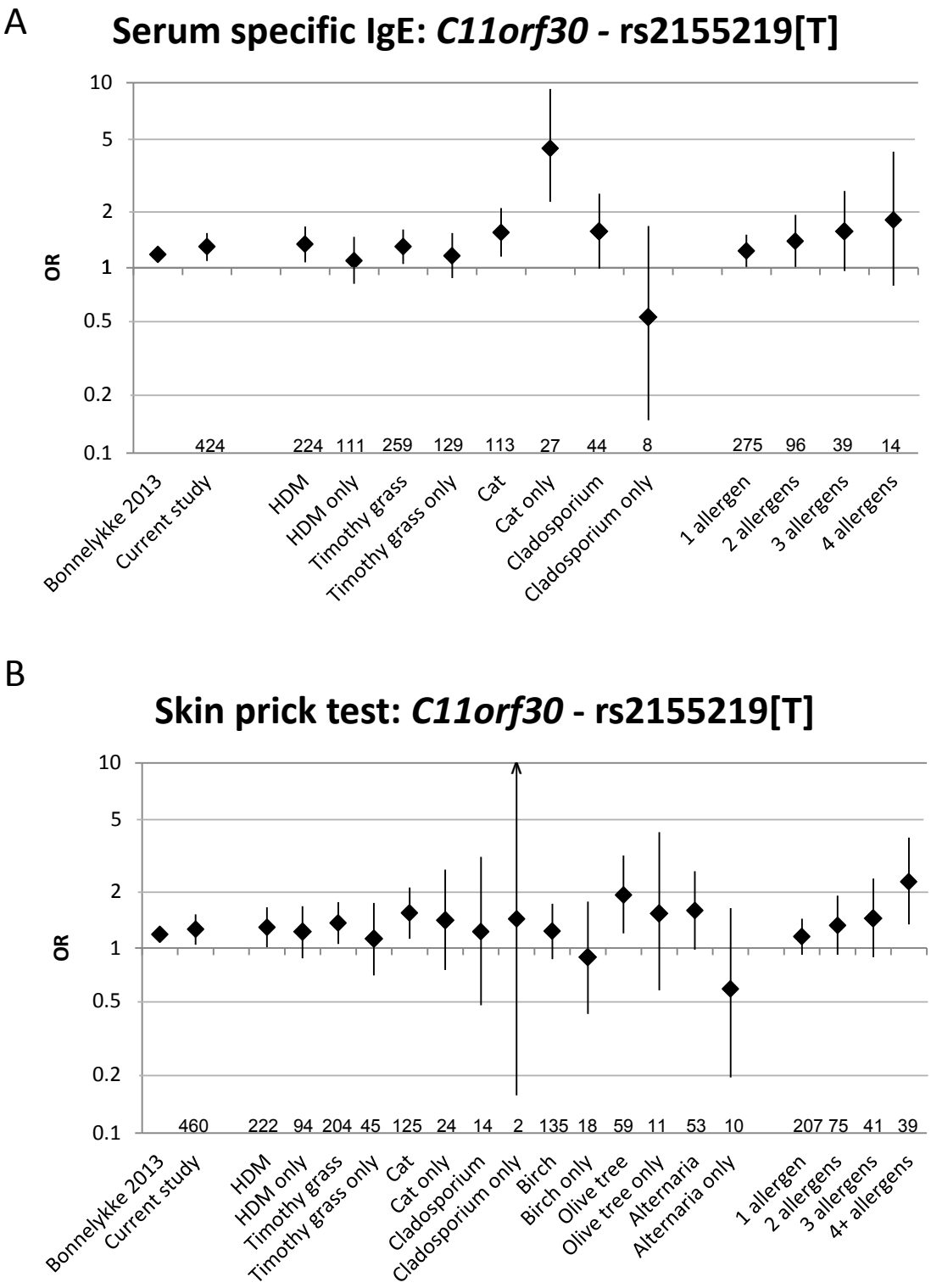

Figure 1. Odds ratios (OR) and 95% confidence intervals for the association between *C11orf30*-rs2155219[T] and: **A)** serum specific IgE to at least one of four common allergens (house dust mite, Timothy grass, cat, and *Cladosporium herbarum*). **B)** positive skin prick test to at least one of nine common allergens (house dust mite, Timothy grass, cat, *Cladosporium herbarum*, birch, olive tree, *Alternaria alternata*, ragweed, and *Parietaria judaica*). Numbers on the X axis correspond to number *of sensitised participants.*