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LETTER TO THE EDITOR

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To the Editor,

Peanut allergy is a common food allergy and the main cause of anaphylaxis among children.[1] In recent years, oral immunotherapy has emerged as a promising treatment for children with different IgE-mediated food allergies, although safety issues must be considered.[2] The main aim of immunotherapy is to induce tolerance or desensitization to an allergen, which otherwise causes an allergic reaction. For oral immunotherapy, this means ingesting the allergen in a controlled manner with gradually increasing dosages. Specifically, peanut oral immunotherapy (pOIT) is able to induce tolerance/desensitization.[3] While the pathogenesis of food allergy is relatively well-studied,[4] mechanisms of OIT-induced tolerance are not well understood. Omalizumab (anti-IgE), which is used as treatment for severe allergic asthma and other IgE-driven allergies, can also facilitate OIT initiation[5]; however, little is known about the involved mechanisms, including possible changes at the transcriptional level. We therefore investigated transcriptional changes in whole blood using RNA-sequencing profiles during omalizumab treatment and pOIT in participants from the FASTX (Food Allergen Suppression Therapy with Xolair®) study previously described.[5,6]

17 peanut allergic adolescents (age 12–18 years) were included in this study (Table S1). Participants were sampled at treatment start (Baseline) and were then treated with omalizumab at recommended dosages for allergic asthma for an initial 8-week period and for some participants additional periods (total of 16 or 24 weeks) until basophil activation test (BAT/CD-sens)[7] analysis showed suppressed reactivity to peanut. All participants then underwent an open peanut challenge (pOIT start), before starting peanut OIT (pOIT) in combination with continued omalizumab treatment. The peanut dose was gradually increased until reaching a maintenance dose. Guided by a BAT/CD-sens after 8 weeks on the maintenance dose, participants decreased the omalizumab dose by 50% (Maintenance) and continued to decrease the dose if pOIT was tolerated. Eleven participants were able to tolerate pOIT without omalizumab for >8 weeks and then passed an open peanut food challenge (Final); six participants could not discontinue omalizumab; however, blood samples were obtained after 2–3 years on omalizumab (Final); six participants dropped out of the study (Figure S1). RNA-sequencing was performed on whole blood ($n = 17$) at Baseline, pOIT start, Maintenance and Final timepoints using the NovaSeq 6000 platform. DESeq2 was used for differential expression analysis of the omalizumab effect and a linear mixed-effect model for analyses during pOIT in combination with omalizumab (pOIT + O) after adjustment for treatment outcome and cell types estimates via deconvolution (Figure S2). For further details on the treatment protocol, methods and statistical analysis, see the Online Appendix and Table S1.

For study participant characteristics at Baseline, see Table S1. To elucidate whether omalizumab treatment alone induced alterations in peripheral blood gene expression, we investigated the two first timepoints, Baseline and pOIT start; however, no significant differences were observed (Figure S3). In the longitudinal analysis (pOIT start to Final), 680 genes associated with pOIT + O at nominal $p < .005$ (Table S2). The Gene Ontology (GO) biological process of these 680 genes is presented in Figure 1A,B. Upregulation of 337 genes was linked to GO terms 'protein regulation and modification', while 'neutrophil degranulation, immune response, phagocytosis and metabolic process' were among the top terms for the 343 downregulated genes. Out of the 680 genes, 16 were differentially expressed at false discovery rate (FDR) adjusted $p < .05$ (Table 1, Figure S4). The three genes with the largest negative and positive coefficients, respectively, are displayed in Figure 1C,D; downregulation of *ASGR2*, *GPBAR1* and *HM13*, and upregulation of *USP44*, *ICOS* and *CDKN2AIP*. Finally, we evaluated the overlap between our 680 pOIT + O-associated genes and peripheral blood gene expression changes associated with acute peanut allergic reactions in a recently published clinical study by Watson et al using the same *p*-value cut-off ($p < .005$).[8] Out of our 680 significant genes, 108 genes overlapped with differentially expressed genes found by Watson et al,[8] mostly with opposite direction, $P_{enrichment} = 0.0095$ (Figure 2).

Our results demonstrate that omalizumab treatment alone does not induce alterations in whole blood gene expression in patients with severe food allergy. This is not surprising given that these patients were unexposed to peanut allergen at the time of Baseline blood sampling, and any concomitant asthma, rhinitis or eczema were well controlled. However, since omalizumab can facilitate OIT, this drug likely allows for biological changes related to allergen tolerance during OIT. Our longitudinal analysis during pOIT + O identified up- and downregulation of several immune-related genes. CD278/ICOS (Inducible T-cell costimulatory) is expressed

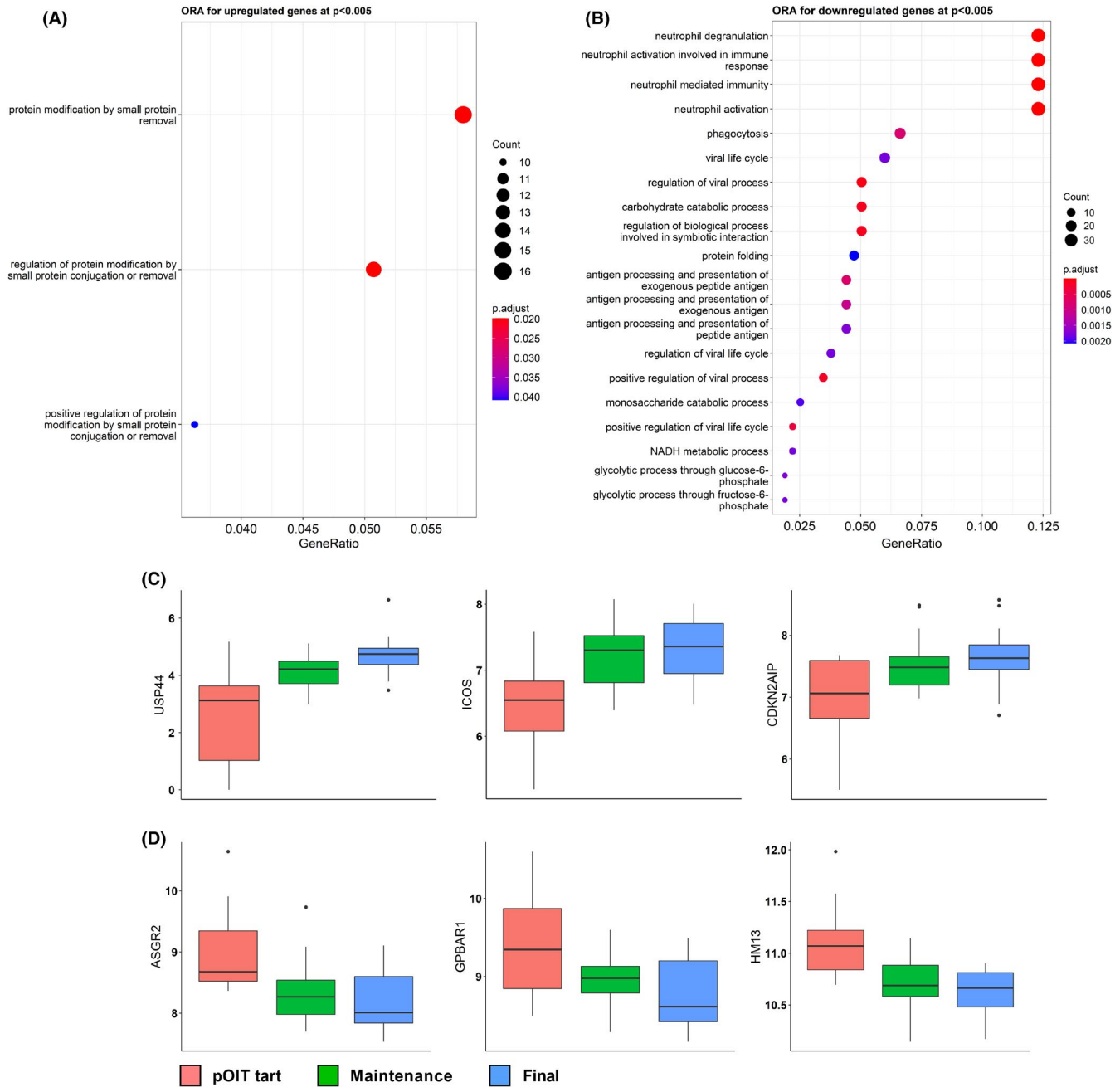

FIGURE 1 (A, B) Gene ontology (GO) biological process analysis of upregulated (A) and downregulated (B) genes. The x-axis shows the gene ratio of the overlapping genes of our gene list with the pathway gene set. The colour bar represents the adjusted *p*-value, and the circle size is the count of overlapping genes. (C, D) Boxplots display log2 expression values for six pOIT genes (C: upregulated, D: downregulated) throughout the treatment protocol at FDR $p < .05$. Red box: pOIT start, green box: Maintenance, blue box: Final. ORA = Over-representation analysis

on activated T cells and appears to play a role in directing effector T-cell differentiation and responses during inflammatory conditions.[9] ICOS expression on T regulatory cells and T follicular helper cells may be involved in the allergic disease mechanism.[10] In the pathway analyses, we observed significant enrichment for several GO biological process terms related to T-cell function and immune responses. Notably, we have previously described alterations in T-cell polyclonal *in vitro* activation during pOIT + O in the FASTX study,[11] and our cell fraction analyses also identified immunological changes (Figure S2). Comparing our findings with data described by Watson et al[8] suggests that pOIT + O may alter the expression level of genes that were found affected during an acute peanut allergic reaction.

The main limitations of this study are small sample size and lack of control subjects without omalizumab treatment, which may mask changes only associated with pOIT. Further, whole blood contains a highly heterogeneous cell population, which can only be partly accounted for by estimated cell fractions and our analysis may have

TABLE 1 FDR significant pOIT + O-related genes

Gene name	Full name	Ensembl.ID	Coefficient	Standard error	*p*-value	FDR
ASGR2	Asialoglycoprotein Receptor 2	ENSG00000161944	−0.3908	0.0809	4.68×10^{-05}	0.0496
GPBAR1	G Protein-Coupled Bile Acid Receptor 1	ENSG00000179921	−0.3264	0.0656	3.21×10^{-05}	0.0453
HM13	Minor Histocompatibility antigen H13	ENSG00000101294	−0.2385	0.0461	1.81×10^{-05}	0.0414
CALR	Calreticulin	ENSG00000179218	−0.2120	0.0370	3.67×10^{-06}	0.0386
RASSF4	Ras Association Domain Family Member 4	ENSG00000107551	−0.1865	0.0370	2.87×10^{-05}	0.0453
CFL1	Cofilin 1	ENSG00000172757	−0.1859	0.0331	5.85×10^{-06}	0.0386
KDELR1	KDEL Endoplasmic Reticulum Retention Protein Receptor 1	ENSG00000105438	−0.1680	0.0300	6.83×10^{-06}	0.0386
YWHAE	Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Epsilon	ENSG00000108953	−0.1576	0.0296	1.19×10^{-05}	0.0414
USP44	Ubiquitin Specific Peptidase 44	ENSG00000136014	1.0998	0.2224	3.48×10^{-05}	0.0454
ICOS	Inducible T-Cell Costimulator	ENSG00000163600	0.4596	0.0957	4.69×10^{-05}	0.0496
CDKN2AIP	Cyclin-dependent Kinase Inhibitor 2A Interacting Protein	ENSG00000168564	0.3603	0.0705	2.27×10^{-05}	0.0428
ATM	ATM Serine/Threonine Kinase	ENSG00000149311	0.2913	0.0563	1.95×10^{-05}	0.0414
ADD3	Adducin 3	ENSG00000148700	0.2619	0.0524	3.18×10^{-05}	0.0453
GNG2	G Protein Subunit Gamma 2	ENSG00000186469	0.2436	0.0497	4.01×10^{-05}	0.0486
SEPTIN11	Septin 11	ENSG00000138758	0.1897	0.0358	1.47×10^{-05}	0.0414
OGT	O-Linked N-Acetylglucosamine (GlcNAc) Transferase	ENSG00000147162	0.1859	0.0354	1.56×10^{-05}	0.0414

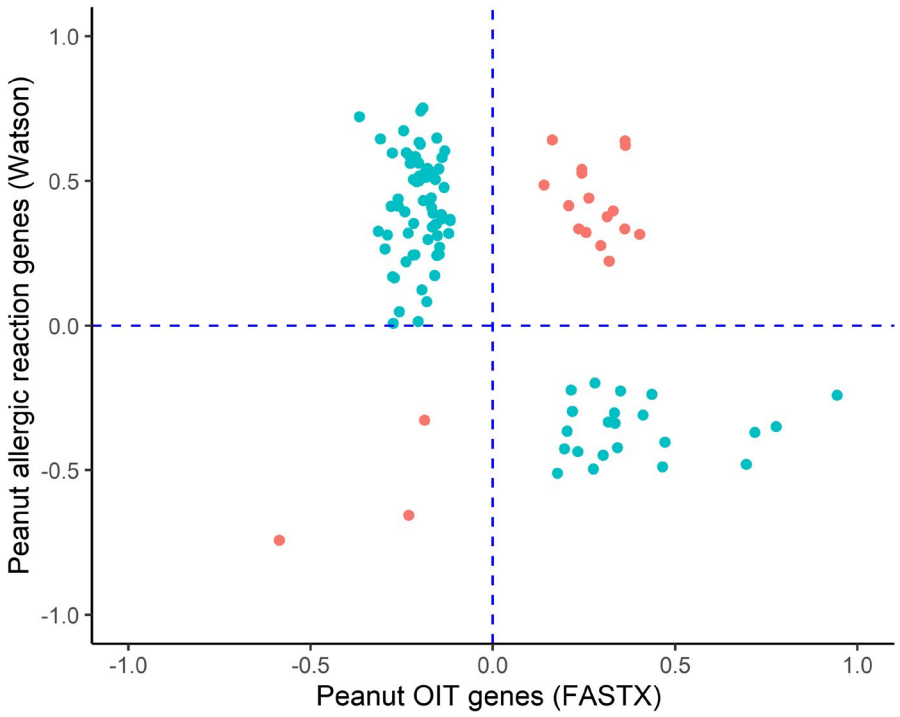

FIGURE 2 Overlap of 108 genes from the FASTX pOIT study with the peanut-related genes found in Watson et al. at nominal $p < .005$. The y-axis represents changes in gene expression between the means at baseline and the 4-h time point of the peanut challenge in Watson et al, and the x-axis is effect size estimates from the mixed-effect model in the FASTX pOIT study. The green colour shows opposite direction, and the pink same direction

failed to detect important treatment-related changes connected specifically to peanut antigen-specific cells. In conclusion, omalizumab treatment alone does not alter the transcriptional signature in peripheral blood of peanut allergic patients, but during pOIT + O, several immune-related signatures were observed. These results may provide insights into mechanisms of allergen tolerance.

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CONFLICT OF INTEREST

None.

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ETHICAL APPROVAL

The study was approved by the Ethics Committee in Stockholm: 2013/827-31/3, 2014/1980-32, 2016/1390-32, 2020–00807 and the Swedish Drug Agency: 5.1–2013–46183; the trial is registered at EudraCT: 2012–005625–78, ClinicalTrails.gov; NCT02402231. Patients and caregivers provided written informed consent.

PEER REVIEW

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SUPPORTING INFORMATION

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