

ORIGINAL ARTICLE

Atopic Dermatitis, Urticaria and Skin Disease

Omalizumab normalizes the gene expression signature of lesional skin in patients with chronic spontaneous urticaria: A randomized, double-blind, placebo-controlled study

Martin Metz¹ | Rebecca Torene² | Sergio Kaiser³ | Michael T. Beste² |
Petra Staubach⁴ | Andrea Bauer⁵ | Randolph Brehler⁶ | Janine Gericke¹ |
Martin Letzkus³ | Nicole Hartmann³ | Veit J. Erpenbeck³ | Marcus Maurer¹ 

¹Department of Dermatology and Allergy, Charité – Universitätsmedizin, Berlin, Germany

²Translational Medicine, Novartis, Cambridge, Massachusetts

³Translational Medicine, Novartis Pharma AG, Basel, Switzerland

⁴Department of Dermatology, University Medicine Mainz, Mainz, Germany

⁵Department of Dermatology, University Allergy Center, University Hospital Carl Gustav Carus, Technical University, Dresden, Germany

⁶Department of Dermatology, University Hospital Muenster, Muenster, Germany

Correspondence: Marcus Maurer, Dermatology and Allergy, Director of Research, Department of Dermatology and Allergy, Allergie-Centrum-Charité, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany (Marcus.Maurer@charite.de).

Present address

Janine Gericke, Novartis Pharma GmbH, Nuremberg, Germany.

Funding information

This work was supported by the Novartis Pharma AG, Basel, Switzerland.

Abstract

Background: Omalizumab, a humanized recombinant monoclonal anti-IgE antibody, proved to be effective in patients with chronic spontaneous urticaria (CSU), including severe and treatment-refractory CSU. Here, we report omalizumab's effect on gene expression in skin biopsies from CSU patients enrolled in a double-blind, placebo-controlled study.

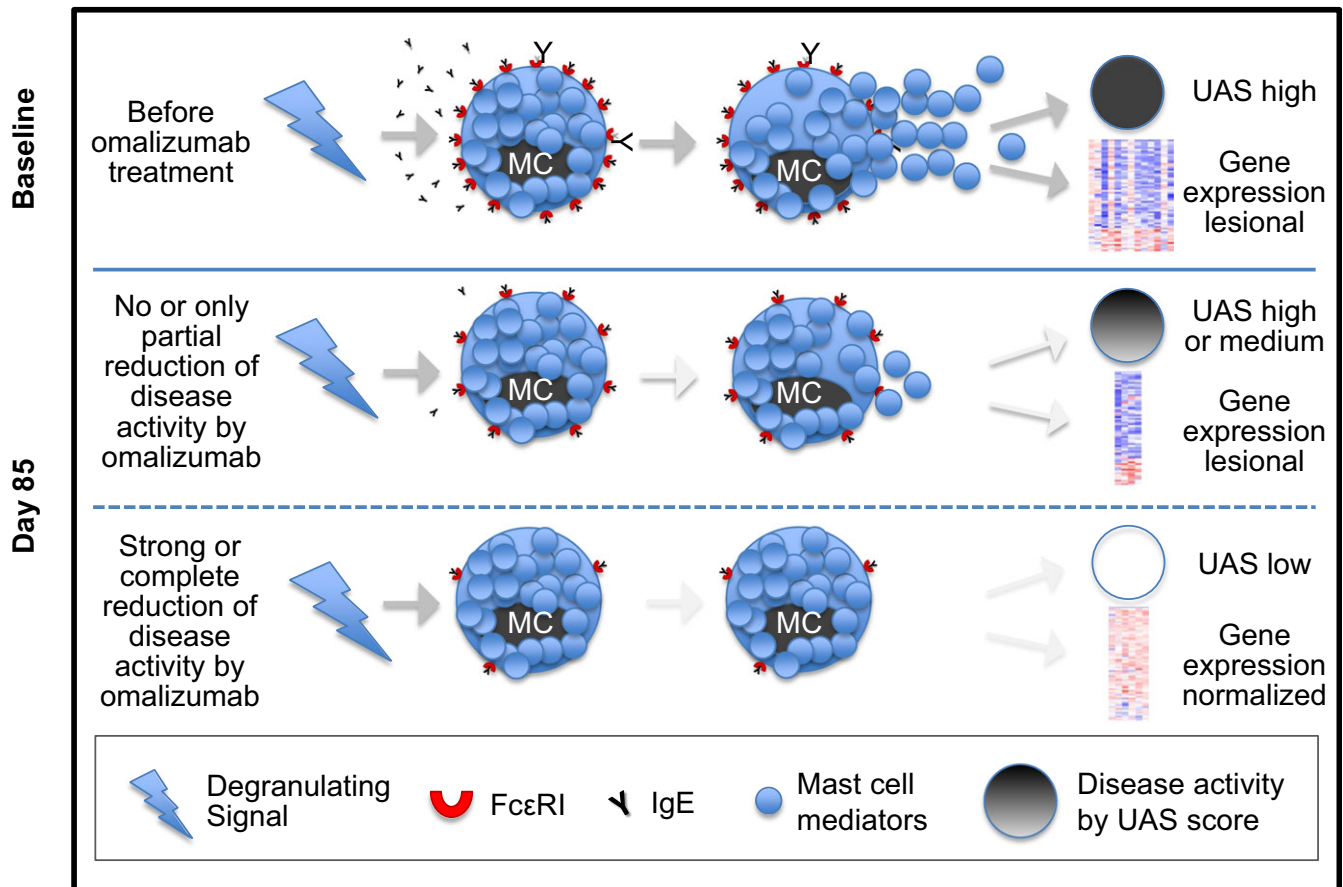
Methods: Chronic spontaneous urticaria patients (18–75 years) were randomized to either 300 mg omalizumab (n = 20) or placebo (n = 10) administered s.c. every 4 weeks for 12 weeks (NCT01599637). Lesional and nonlesional skin biopsies were collected from the same area of consenting patients and assessed at baseline and on Day 85 compared with skin biopsies from the same area of 10 untreated healthy volunteers (HVs). Gene expression data were generated using Affymetrix HG-U133Plus2.0 microarrays. Statistical analyses were performed using R packages.

Results: At baseline, 63 transcripts were differentially expressed between lesional and nonlesional skin. Two-thirds of these lesional signatures were also differentially expressed between lesional and HV skin. Upon treatment with omalizumab, >75% of lesional signatures changed to reflect nonlesional skin expression levels (different vs placebo, $P < 0.01$). Transcripts upregulated in lesional skin (vs nonlesional and/or HV skin) suggested increased mast cell/leukocyte infiltration (FCER1G, C3AR1, CD93, S100A8, and S100A9), increased oxidative stress, vascularization (CYR61), and skin repair events (KRT6A, KRT16). Lesional signatures were not modulated by treatment in nonresponders (defined based on UAS7 longitudinal changes ≥ 16).

Conclusion: Omalizumab, in treatment responders, reverted transcriptional signatures associated with CSU lesion phenotype to reflect nonlesional/HV expression levels; this is consistent with observed omalizumab-mediated clinical improvement observed in patients with CSU.

KEYWORDS

microarray affymetrix, mode of action, omalizumab, rna, UAS7



GRAPHICAL ABSTRACT

The gene expression of the lesional skin of patients with chronic spontaneous urticaria shows a distinct signature when compared to their non-lesional skin and the skin of healthy volunteers. The pattern of gene expression in the lesional skin of omalizumab-treated patients are different than before treatment, but not in their nonlesional skin. In treatment responders, omalizumab treatment shows reverted transcriptional signatures associated with CSU lesion phenotype to reflect nonlesional/healthy volunteer expression levels consistent with observed clinical improvement.

1 | INTRODUCTION

Omalizumab is a recombinant humanized monoclonal antibody that binds to IgE at its binding site to high-affinity IgE receptor (FcεRI).¹⁻³ It is effective in the treatment of chronic spontaneous urticaria (CSU),⁴⁻¹¹ including in patients who have relapsed after discontinuing treatment with omalizumab.¹² Omalizumab is approved for the treatment of CSU patients who remain symptomatic despite H₁-antihistamine treatment.^{13,14}

Urticaria is characterized by the development of hives and angioedema,¹⁵ both of which develop when skin mast cells get activated, degranulate, and release histamine and other proinflammatory mediators.¹⁶⁻²¹ Omalizumab is known to prevent the signs and symptoms of

CSU via its effects on IgE levels and FcεRI expression; this notion is based on several indirect and direct lines of evidence: (a) The pathology of CSU lesions resembles that of allergen-mediated late-phase skin reactions, suggesting involvement of FcεRI activation of mast cells and basophils;²² (b) CSU patients have been described to have histamine-releasing IgG autoantibodies against either FcεRI or IgE²³⁻²⁵ as well as mast cell-activating IgE autoantibodies against autoantigens such as thyroperoxidase;²⁶ (c) in patients with CSU, treatment with omalizumab reduces serum levels of IgE by >90% within the initial days after initiation of therapy;²⁷ and (d) in CSU patients, omalizumab treatment, over the course of initial 3 months, leads to reduction in FcεRI+ and IgE+ cells in the dermis.²⁷

Nevertheless, what is less clear is how omalizumab affects the skin of CSU patients following reduced mast cell degranulation and wheal

development. In fact, there is virtually no published information on how skin responses to mast cell degranulation and whealing differ in CSU patients who do or do not receive omalizumab treatment. Does omalizumab affect blood vessels, keratinocytes, fibroblasts, and other skin cells at sites of whealing? Does omalizumab reduce the influx of basophils, neutrophils, eosinophils, and other cells to sites of CSU wheal formation? Does omalizumab protect from the long-term consequences of recurrent whealing, such as increased number of skin blood vessels, mast cells, and eosinophils, all of which are seen in the nonlesional (postlesional and “ready-to-wheal”) skin of CSU patients?^{28–30} Answers to these questions could help us better understand the mechanisms of action of omalizumab in CSU as well as the pathogenesis of CSU. More importantly, answers to these questions may help optimize CSU treatment strategies.

To this end, we characterized the gene expression profiles of lesional skin (ie, wheals) and nonlesional skin (ie, skin not currently affected by wheals) of CSU patients before and after 12 weeks of treatment with omalizumab and of skin from healthy volunteers (HVs). Our analyses have been built on the results of three previous gene profiling studies of lesional and nonlesional skin in CSU patients who were not receiving omalizumab treatment.^{31–33}

2 | METHODS

This study was an exploratory, double-blind, parallel-group, randomized, placebo-controlled Phase II study (NCT01599637, EudraCT No. 2011-004216-31). Patients were enrolled at four clinical centers in Germany. The study was conducted in accordance with applicable local regulations (including European Directive 2001/20/EC, US Code of Federal Regulations Title 21, and Japanese Ministry of Health, Labor, and Welfare), the International Conference on Harmonization E6 Guideline for Good Clinical Practice, and the ethical principles of the Declaration of Helsinki. The study protocol and all amendments were reviewed by the independent ethics committee or institutional review board for each center, and written informed consent was obtained from each study participant before enrollment.

2.1 | Clinical study design

Details of the study design were reported previously.²⁷ In brief, the treatment period was 12 weeks; on Day 1 of the treatment period,

TABLE 1 Number of samples and analyzed microarray data from chronic spontaneous urticaria (CSU) patients

Treatment	Biopsy site	Baseline	Day 85
Omalizumab 300 mg	Lesional skin	17 (13)	10 (10)
	Nonlesional skin	18 (14)	18 (16)
Placebo	Lesional skin	8 (7)	7 (7)
	Nonlesional skin	9 (8)	7 (7)

Data are provided as number of available patient samples (and analyzed gene expression microarray data). In addition, gene expression data from 8 untreated, healthy volunteer skin biopsies were analyzed.

patients were randomized in a 2:1 ratio to omalizumab 300 mg or placebo (3 subcutaneous doses at 4-week intervals, ie, at Day 1, Week 4, and Week 8). After discontinuing the treatment, patients were followed for an additional 8 weeks (follow-up). Gene expression was analyzed from skin biopsies taken from CSU patients at baseline and Week 12 from nonlesional and lesional skin (available samples are summarized in Table 1). In addition, a single skin biopsy was collected from HVs. HVs were age- and gender-matched (± 2 years) male or female adults (18–75 years of age) in good health as determined by past medical history, physical examination, vital signs, 12-lead ECG, and laboratory tests.

2.2 | Patient inclusion/exclusion criteria

The study included CSU patients (18–75 years) who remained symptomatic despite H₁-antihistamine treatment at approved doses, characterized by the reoccurrence of itch and hives for >6 weeks before baseline, with a UAS7 score of at least 16 and an itch component of UAS7 of at least 8 during 2 weeks before randomization. Patients had to have had a CSU diagnosis for at least 6 months and be on an approved dose of an H₁-antihistamine for CSU before entering the study.

Thirty patients were randomized to treatment: 20 in the omalizumab group and 10 in the placebo group. In addition, 10 (age- and gender-matched) HVs participated in this study. Subject demographics and baseline characteristics are summarized in Table 2. The omalizumab and placebo groups of CSU patients were generally well matched for demographic characteristics. Details have been reported previously.²⁷

2.3 | Clinical efficacy data

Decreases in UAS7 after omalizumab treatment initiation were rapid (with separation of curves at Day 3) and sustained. At Week 12, the LS

TABLE 2 Demographic summary

Cohort	Omalizumab (300 mg s.c.) n = 20	Placebo n = 10	Healthy volunteers n = 10
Age (years)	37.5 (11.0)	41.1 (8.0)	36.4 (9.7)
Height (cm)	171.4 (7.3)	172.1 (10.8)	166.4 (6.3)
Weight (kg)	78.5 (16.0)	87.4 (21.6)	66.9 (13.4)
Female, n (%)	18 (90)	8 (80)	10 (100)
Caucasian, n (%)	20 (100)	10 (100)	10 (100)
BMI (kg/m ²)	26.8 (5.8)	29.3 (5.6)	24.2 (5.3)
Baseline IgE (ng/mL)	1037 (2474)	442 (581)	NA
Baseline UAS7	32.2 (8.0)	31.6 (7.7)	NA

BMI, body mass index; IgE, immunoglobulin E; NA, not applicable; UAS, urticaria activity score; UAS7, sum of the daily UAS scores over 7 d. Data are provided as mean (standard deviation) unless otherwise indicated. Age was calculated using date of screening and date of birth. Weight and height were taken from screening vital signs evaluations.

mean difference in UAS7 between treatment groups was -14.82 ($P = 0.0027$). Over 50% of patients from the omalizumab group were UAS7 responders at Week 12 (response to omalizumab treatment was defined as $UAS7 \leq 6$, while nonresponse was defined as $UAS7 \geq 16$). Approximately half of these had become responders by the end of Week 2. In contrast, $<15\%$ of patients receiving placebo had improved by Week 12, without any changes occurring by Week 2.²⁷

2.4 | Transcriptional assessment of skin biopsies

2.4.1 | Sample collection

Skin biopsies with a minimum diameter of 6 mm were taken at each scheduled time-point. Baseline biopsies were collected from one location with lesional skin and from another with nonlesional skin. Following treatment, biopsies were collected at locations close to the locations chosen at baseline. Skin biopsy samples were stored at -80°C until analyzed (see Table 1 for details).

2.4.2 | Microarray-based data generation

The effects on omalizumab on skin biopsies' gene expression were assessed using Affymetrix HG-U133Plus2.0 oligonucleotide microarrays (<http://www.affymetrix.com/>). After condensing to gene level analysis and filtering probesets, approximately 8050 transcripts remained in the dataset.

2.5 | Statistical analyses

All quality control and normalization of microarray-derived data were conducted in R: a language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>). QC and normalization using RMA of the transcriptional dataset were conducted using a series of packages downloaded from www.arrayanalysis.org. The "hgu133plus2hsentrezg" Chip Definition File (<http://brainarray.mbnl.med.umich.edu/>) was used for mapping probes to gene products. Affymetrix technical probesets as well as probesets showing low expression levels (ie, normalized and scaled expression ≤ 100) were filtered out. Subsequently, the resulting dataset was considered ready for downstream statistical analyses. In brief, linear models, as implemented in the R package limma, were used to assess the differences in gene expression: both longitudinal treatment-induced (referenced to baseline) and across CSU baseline phenotypes (ie, CSU lesional, CSU nonlesional, and healthy skin). The level of significance and absolute magnitude of change thresholds used to identify differentially expressed genes (DEGs) were set up at P values ≤ 0.05 and absolute fold change ≥ 1.50 , respectively. The false discovery rate (FDR) approach was used to correct nominal P values for multiple testing.

For analysis, first, baseline lesional and nonlesional data were compared. Second, both were compared to HV skin. As all baseline samples were untreated, both omalizumab and placebo groups were combined for these comparisons.

2.6 | IPA upstream regulator analysis

Upstream Regulator Analysis was performed using IPA (QIAGEN Inc., <https://www.qiagenbio-informatics.com/products/ingenuity-pathway-analysis>). The goal of the Upstream Regulator Analysis is to identify the cascade of upstream transcriptional regulators (TRs) that can explain the observed gene expression changes in a user's dataset.

In brief, upstream regulator analysis is based on prior knowledge of expected effects between TRs and their target genes stored in the Ingenuity Knowledge Base. In particular, this analysis examines how many known targets of each transcription regulator are present in the user's dataset and compares their direction of change (ie, expression in the experimental sample[s] relative to control) to what is expected from literature in order to predict likely relevant TRs. If the observed direction of change is mostly consistent with an activation state of the TR ("activated" or "inhibited"), then a prediction is made about that activation state. For each potential TR, two statistical measures, an overlap P value, and an activation z -score are computed. The overlap P value calls likely upstream regulators based on significant overlap between dataset genes and known targets regulated by a TR. The activation z -score is used to infer likely activation states of upstream regulators based on comparison with a model that assigns random regulation directions.

3 | RESULTS

3.1 | What is the gene expression profile of wheals, that is, the "lesional skin signature," in CSU patients?

The lesional skin of CSU patients showed a distinct gene expression signature in comparison with their nonlesional skin and the skin of HVs (Figure 1A): 63 and 106 genes were differentially expressed between CSU lesional and nonlesional skin (the "lesional" signature; Table 3) and between CSU lesional and HV skin (Table S1), respectively. Two-thirds of DEGs in CSU lesional vs nonlesional skin were also DEGs in the CSU lesional vs HV comparison. The lesional skin signature was characterized by transcripts linked to increased mast cells¹⁷ and leukocyte infiltration (FCER1G, C3AR1, CD93, S100A8, and S100A9), increased oxidative stress, vascularization (CYR61), and skin repair events (KRT6A, KRT16).

Upstream regulator analysis identified the potential involvement of several transcription factors/regulators (TP63, STAT3), kinases (EGFR), and cytokines (TNF, IL6, IL1A, IL1B) as regulators (Table 4) of the transcriptional changes underlying the transcriptional differences noted between lesional and nonlesional CSU skin. In brief, this analysis predicted all aforementioned regulators as activated in CSU lesion biopsies.

3.2 | What is the gene expression profile of nonlesional skin of CSU patients?

The nonlesional skin of CSU patients did not show a distinct gene expression signature (Figure 1A). Only 9 DEGs were differentially



FIGURE 1 Treatment-induced effects on chronic spontaneous urticaria (CSU) “lesional” signature. Differentially expressed genes between baseline nonlesional and lesional skin samples ($n = 63$), as listed in Table 3, were assessed for changes after treatment with omalizumab (A). Nonresponders (subjects with a UAS7 score ≥ 16 at Day 85) exhibit expression posttreatment profiles of lesional skin that resemble baseline CSU samples (ie, signature remains “lesion-like”), whereas responders’ profiles (UAS < 16 at Day 85) more closely resemble to those of HV (B). Some subjects are missing due to absence of lesion at Day 85

TABLE 3 Chronic spontaneous urticaria (CSU) “lesional” signature (n = 63 transcripts)

HUGO gene symbol	Gene name	P value	FDR-adjusted P value	Fold change
CCL2 ^{a,b}	C-C motif chemokine ligand 2	1.37E-06	2.14E-03	3.43
S100A8 ^{a,b}	S100 calcium binding protein A8	4.00E-04	1.73E-02	2.56
S100A9 ^a	S100 calcium binding protein A9	3.92E-04	1.73E-02	2.55
SERPINA3 ^a	Serpin family A member 3	5.13E-04	1.94E-02	2.50
THBS1 ^{a,b}	Thrombospondin 1	1.65E-04	1.23E-02	2.43
KRT16 ^a	Keratin 16	2.08E-05	4.95E-03	2.36
CYR61 ^{a,b}	Cysteine-rich angiogenic inducer 61	1.40E-05	4.09E-03	2.31
ICAM1 ^{ab}	Intercellular adhesion molecule 1	8.23E-05	9.72E-03	2.08
KRT6A ^a	Keratin 6A	8.15E-04	2.34E-02	2.07
ADAMTS1 ^{a,b}	ADAM metalloproteinase with thrombospondin type 1 motif 1	3.00E-06	2.14E-03	2.05
MT1M	Metallothionein 1M	1.95E-04	1.32E-02	2.00
C11orf96	Chromosome 11 open reading frame 96	3.45E-05	5.93E-03	1.98
BCL3	B-cell CLL/lymphoma 3	3.19E-06	2.14E-03	1.91
NAMPT ^{a,b}	Nicotinamide phosphoribosyltransferase	3.05E-03	4.45E-02	1.91
UAP1 ^b	UDP-N-acetylglucosamine pyrophosphorylase 1	2.49E-04	1.38E-02	1.91
NNMT ^b	Nicotinamide N-methyltransferase	6.92E-04	2.17E-02	1.87
EGR1 ^a	Early growth response 1	2.50E-06	2.14E-03	1.80
SLC20A1 ^b	Solute carrier family 20 member 1	6.08E-05	8.34E-03	1.79
MYC ^{a,b}	MYC proto-oncogene, bHLH transcription factor	1.49E-05	4.09E-03	1.77
GPX2	Glutathione peroxidase 2	1.40E-05	4.09E-03	1.76
ZFP36	ZFP36 ring finger protein	3.53E-07	1.42E-03	1.76
STEAP4 ^{a,b}	STEAP4 metalloredutase	4.24E-03	5.27E-02	1.75
KRT17	Keratin 17	2.65E-04	1.43E-02	1.75
SNHG15	Small nucleolar RNA host gene 15	2.58E-06	2.14E-03	1.70
GADD45B ^b	Growth arrest and DNA damage inducible beta	2.01E-06	2.14E-03	1.66
LDLR ^a	Low-density lipoprotein receptor	3.36E-05	5.93E-03	1.65
NFIL3	Nuclear factor, interleukin 3 regulated	6.15E-05	8.34E-03	1.64
SOD2 ^{a,b}	Superoxide dismutase 2	7.13E-04	2.21E-02	1.64
PI3 ^a	Peptidase inhibitor 3	3.23E-03	4.56E-02	1.64
CTSL ^b	Cathepsin L	2.46E-03	4.00E-02	1.62
JUNB ^b	JunB proto-oncogene, AP-1 transcription factor subunit	6.35E-08	5.11E-04	1.61
ID1	Inhibitor of DNA binding 1, HLH protein	3.06E-06	2.14E-03	1.61
ZNF593	Zinc finger protein 593	1.97E-05	4.95E-03	1.58
NOP16	NOP16 nucleolar protein	1.34E-04	1.19E-02	1.57
S100A3	S100 calcium binding protein A3	1.78E-02	1.12E-01	1.56
SERPINE2 ^b	Serpin family E member 2	1.24E-02	9.10E-02	1.56
PLSCR1 ^b	Phospholipid scramblase 1	1.95E-03	3.60E-02	1.55
MEDAG	Mesenteric estrogen-dependent adipogenesis	8.15E-03	7.45E-02	1.55
CCL13 ^b	C-C motif chemokine ligand 13	5.67E-03	6.15E-02	1.54
TIMP1	TIMP metalloproteinase inhibitor 1	6.56E-03	6.63E-02	1.54
OSMR ^b	Oncostatin M receptor	2.63E-04	1.43E-02	1.54
LYAR	Ly1 antibody reactive	9.72E-05	1.09E-02	1.53
SLC25A25 ^a	Solute carrier family 25 member 25	7.90E-06	3.97E-03	1.53
GALNT6	Polypeptide N-acetylgalactosaminyltransferase 6	1.16E-04	1.13E-02	1.53
PLAU	Plasminogen activator, urokinase	2.38E-04	1.36E-02	1.52
CSRNP1 ^a	Cysteine- and serine-rich nuclear protein 1	6.21E-06	3.33E-03	1.52

(Continues)

TABLE 3 (Continued)

HUGO gene symbol	Gene name	P value	FDR-adjusted P value	Fold change
C17orf96	Elongin BC and polycomb repressive complex 2-associated protein	7.51E-04	2.29E-02	1.52
FOS	Fos proto-oncogene, AP-1 transcription factor subunit	1.28E-03	2.82E-02	1.51
PANX1 ^b	Pannexin 1	8.04E-05	9.72E-03	1.51
CHRD1	Chordin-like 1	4.42E-03	5.37E-02	-1.50
HSPB2	Heat-shock protein family B (small) member 2	1.18E-03	2.71E-02	-1.51
SEPP1	Selenoprotein P	1.26E-03	2.82E-02	-1.51
SMAD5-AS1	SMAD5 antisense RNA 1	7.35E-03	7.10E-02	-1.51
FZD4	Frizzled class receptor 4	1.50E-03	3.07E-02	-1.52
GSTM5	Glutathione S-transferase mu 5	2.27E-03	3.86E-02	-1.54
PPARGC1A	PPARG coactivator 1 alpha	7.46E-03	7.11E-02	-1.54
ADH1B	Alcohol dehydrogenase 1B (class I), beta polypeptide	2.22E-02	1.24E-01	-1.54
CD34	CD34 molecule	4.16E-04	1.73E-02	-1.55
MIR29C	microRNA 29c	1.42E-03	2.97E-02	-1.57
PEAR1	Platelet endothelial aggregation receptor 1	3.19E-05	5.88E-03	-1.58
EMX2	Empty spiracles homeobox 2	7.35E-04	2.26E-02	-1.59
SCARA5	Scavenger receptor class A member 5	2.38E-03	3.93E-02	-1.62
OSR2	Odd-skipped related transcription factor 2	4.08E-05	6.85E-03	-1.83

Skin transcriptional signature identified from comparing CSU lesional vs nonlesional at baseline.

Rows sorted by fold change (decreasing).

^aTranscripts array identified and qPCR validated in Gimenez-Arnau et al reference as differentially upregulated in CSU lesional skin vs CSU nonlesional skin.

^bTranscripts array identified in Patel et al reference listed as differentially expressed between CSU lesional skin vs CSU nonlesional skin and HV skin.

expressed in nonlesional CSU skin compared with HV skin (Table S2). Upregulated transcripts were related to dendritic cells (CD209), complement (C1QB), cell cycling (MPEG1), and inflammation (IL10RA). Downregulated genes were related to a transporter (SLC47A2), a protein of the vitamin A family (CRABP1), and an endothelin receptor (EDNRB).

3.3 | Omalizumab treatment changes the lesional but not the nonlesional skin expression profile of CSU patients

After 12 weeks of treatment, the number of wheals/day had markedly decreased in most omalizumab-treated, but not placebo-treated, patients. In omalizumab-treated patients, but not placebo-treated patients, lesional skin signatures of patients were different than before treatment, as assessed using heatmap analyses (Figure 1A). In contrast, omalizumab did not change the nonlesional skin gene expression profile of CSU patients vs baseline and vs placebo (Figure 1A).

3.4 | Omalizumab makes the gene expression profile of lesional CSU skin look like that of nonlesional skin but only in patients with clinical benefits from treatment

Lesional skin gene expression profiles in most CSU patients who had received 12 weeks of omalizumab treatment closely resembled those of nonlesional CSU skin and HV skin. The proportion of lesional

signature genes (comprising 63 transcripts differentially expressed between lesional and nonlesional samples at baseline) resolving toward levels observed in HVs at Day 85 were contrasted between treated patients and placebos via a two-tailed Fisher exact test. Among omalizumab-treated patients, 50/63 (79%) signature genes moved toward HV levels, whereas among placebo-treated patients, 34/63 signature genes moved toward HV levels ($P = 0.0043$).

Of note, only patients who benefitted from omalizumab treatment exhibited normalization of their lesional skin signatures, whereas nonresponders did not (Figure 1B). Four of 10 omalizumab-treated patients were nonresponders, defined by a Week 12 UAS7 score of ≥ 16 . All these 4 patients had posttreatment lesional skin expression profiles that were very similar to those of baseline lesional skin.

4 | DISCUSSION

To our knowledge, this is the first study to show that omalizumab changes transcriptional responses of the skin of CSU patients. In line with previous observations, gene expression in the skin of CSU patients shows a distinct signature in lesional skin that is different from that in nonlesional or HV skin. Omalizumab reverts this signature to “normal,” and this is linked to its clinical effects.

Patel et al³¹ investigated gene expression in lesional and nonlesional skin of patients with CSU ($n = 8$) and in the skin of healthy controls ($n = 6$) using an Affymetrix Human Gene 1.0 ST Array; 506 and 51 genes were found to be upregulated and reduced in lesional

TABLE 4 “Lesional” signature: IPA upstream regulator analysis

Upstream regulator	Description	Molecule type	Predicted activation state	Activation z-score	P value of overlap	Target molecules in dataset
PDGF-BB	Platelet-derived growth factor-BB	Complex	Activated	3.316	2.24E-14	BCL3, CCL2, CYR61, EGR1, FOS, JUNB, MYC, NAMPT, PLAU, THBS1, ZFP36
TNF	Tumor necrosis factor	Cytokine	Activated	3.212	5.86E-12	BCL3, CCL2, EGR1, FOS, ICAM1, MYC, OSMR, PI3, PLAU, PLSCR1, PPARGC1A, S100A8, S100A9, SEPP1, SOD2, TIMP1
IL-6 ^a	Interleukin-6	Cytokine	Activated	3.070	1.26E-11	BCL3, CCL2, FOS, JUNB, LDLR, MYC, PLAU, SERPINA3, THBS1, TIMP1
IL-1A	Interleukin-1A	Cytokine	Activated	2.579	1.49E-09	CCL2, ICAM1, PI3, PLAU, S100A9, SERPINA3, SOD2
ERK	EPH receptor B2	Group	Activated	2.579	2.83E-08	CCL2, EGR1, FOS, ICAM1, JUNB, THBS1, ZFP36
IL-1B ^a	Interleukin-1B	Cytokine	Activated	2.750	3.39E-08	CCL13, CCL2, EGR1, ICAM1, LDLR, PPARGC1A, SERPINA3, SOD2
STAT3 ^b	Signal transducer and activator of transcription 3	Transcription regulator	Activated	2.383	4.82E-07	BCL3, CCL2, FOS, JUNB, MYC, NAMPT, TIMP1
NFKB ^b	Nuclear factor kappa B	Complex	Activated	2.580	5.35E-07	CCL2, ICAM1, JUNB, KRT17, MYC, NAMPT, PLAU, SOD2
TP63	Tumor protein p63	Transcription regulator	Activated	2.729	6.02E-07	ADAMTS1, CYR61, GPX2, KRT6A, MYC, PI3, PLAU, S100A8, THBS1
EGFR	Epidermal growth factor receptor	Kinase	Activated	2.382	2.44E-06	EGR1, FOS, ICAM1, KRT17, MYC, PLAU

Top 10 regulators predicted to be activated in the “lesional” signature (ie, CSU lesional skin vs CSU nonlesional skin at baseline).

Rows sorted by activation z-score (decreasing).

^aTranscripts array identified and qPCR validated in Gimenez-Arnau et al reference as differentially upregulated in CSU lesional skin vs CSU nonlesional skin.

^bTranscripts array identified in Patel et al reference listed as differentially expressed between CSU lesional skin vs CSU nonlesional skin and HV skin.

skin, respectively. The most upregulated genes were linked to cell adhesion (eg, selectin E), cell activation (eg, CD69), and chemotaxis (CCL2). These findings are consistent with our observation that CCL2 was the most strongly upregulated gene among the 63 genes differentially expressed in lesional skin vs nonlesional skin. From the 12 genes most differentially upregulated with the largest fold change in the study by Patel et al, 6 genes were also differentially expressed in our study (CCL2, THBS1, S100A8, STEAP4, CTSL, and UAP1), with similar but lower upregulation (Table 3). These genes are associated with inflammatory responses (predicted increase in NFKB, TNF, IL1B, and IL1A activity). Moreover, our findings of increased mast cell/leukocyte infiltration (FCER1G, C3AR1, CD93, S100A8, and S100A9), increased oxidative stress, vascularization (CYR61, also predicted increased PDGF-BB activity), and skin repair events (KRT6A, KRT16) are well in line with these previously published data, although not all previous studies support this conclusion.^{17,34,35}

Furthermore, upregulation of chemokine CCL2 and CXCL8 expression by monocytes has previously been reported in CSU.³⁶ CCL2, also referred to as MCP1 CCL2, is primarily secreted by monocytes, macrophages, and dendritic cells. It exhibits chemotactic activity for monocytes and basophils,³⁷ and it has been shown to induce histamine release from human basophils³⁸ and mast cells.³⁹ The upregulation of CCL2 in lesional skin points to the important role of basophils and mast cells in the development of CSU lesions.

A more recent study by Gimenez-Arnau et al³² reported 103 genes to be differentially expressed in lesional CSU skin (wheals) compared with nonlesional skin. The involved functional pathways

included cellular movement, inflammation, cellular growth, and differentiation, as well as cell-to-cell signaling. The functional networks related to more DEGs were built around IL1A, IL6, CCL2, and FBJ murine osteosarcoma viral oncogene homolog B (FOSB) (Table 3). This is in line with our IPA upstream regulator analysis, which revealed the potential activation of IL1A and IL6 among other cytokines involved in inflammation (Table 4).

Our findings suggest that there are only few long-term changes in skin gene expression in CSU, as nonlesional skin only showed 9 DEGs compared with the skin of HVs. Gimenez-Arnau et al, using Agilent whole human genome microarrays, quantitative real-time PCR, and an IPA, identified 31 differentially expressed (and confirmed) genes in nonlesional skin of CSU patients vs skin of healthy controls; these upregulated genes in nonlesional skin were related to epidermal homeostasis, inflammation, and dermal repair. In line with our findings, the number of regulated genes was lower than what they had observed by comparing lesional skin vs nonlesional skin, wherein 103 differentially expressed (and confirmed) genes were identified.³² On the other hand, Bankova et al³³ identified more DEGs in nonlesional CSU skin than we or Gimenez-Arnau et al did: 121 upregulated and 62 downregulated genes in CSU compared with nonatopic controls. In contrast, data from Patel et al³¹ did not reveal statistically significant gene expression differences between nonlesional skin and skin from healthy control subjects.

Taken together, the observations from previous studies by Patel and co-workers and Gimenez-Arnau and co-workers, together with

the findings in our study, support the concept that CSU, by nature, is not characterized by continued overt inflammatory changes of the skin. Rather, our results emphasize the difference in gene expression between lesional and nonlesional skin of CSU patients vs indicating baseline differences between nonlesional and healthy skin. Reasons for certain discrepancies between these gene expression studies may include differences in the techniques employed for skin sampling, sample analysis (gene expression analysis), and differences in the patient and healthy control populations, which were overall rather small.

4.1 | Omalizumab treatment changes gene expression in lesional but not nonlesional skin of CSU patients

Omalizumab treatment for 12 weeks did not significantly alter the expression of genes in nonlesional skin. However, it markedly changed the gene expression profile of whealing skin, while placebo treatment did not; this effect of omalizumab treatment on transcriptional response of the skin while whealing suggests that omalizumab, in addition to reducing wheal numbers, may change the quality of wheals. It would be interesting to study whether the wheals observed in CSU patients differ before and during omalizumab treatment in terms of duration, size, and other features; this might be addressed in future studies.

4.2 | Hypotheses on how omalizumab changes the gene expression profile of lesional CSU skin to look like that of nonlesional or HV skin

There may be several mechanisms involved by which omalizumab treatment could alter transcriptional skin response to wheals, that is, make the gene expression profile of lesional CSU skin appear as that of nonlesional skin.

Omalizumab reduces free IgE levels (and FcεRI expression) and, subsequently, wheal formation and disease activity.⁴⁰ When wheals do occur in omalizumab-treated CSU patients, the reduced levels of free IgE and FcεRI may result in reduced mast cell activation and less degranulation. This, in turn, may lead to reduced inflammatory responses, which fail to induce development of the lesional gene expression signature.

Another mechanism may be that the degranulation of MCs that induces wheals which lead to the CSU lesional signature requires two distinct signals: a degranulator and a degranulation modulator/modifier. Thus, omalizumab, by acting on IgE and FcεRI expression, two well-described modulators/modifiers of MC degranulation, may change how MCs respond to the induction of degranulation. IgE and FcεRI expression modulates the strength of degranulation and the production and release of cytokines and other mediators by mast cells, which may be needed to induce the distinct gene profile of lesional CSU skin. These mechanisms might be complementary, and other mechanisms, such as normalization of mast cell numbers in the skin of CSU patients by omalizumab, may be involved.

4.3 | Normalization of the lesional CSU skin signature by omalizumab is linked to its clinical efficacy

Of note, omalizumab only normalized the lesional gene expression in patients who benefitted from this treatment and not in nonresponders; these findings provide additional support that the normalization of short-term skin responses while whealing is due to the effects of omalizumab on the signs and symptoms of CSU. It shows that omalizumab, in patients who benefit from treatment, has effects that go beyond the reduction in wheal numbers and other markers of disease activity, because the wheals that develop in omalizumab treatment responders appear to be different and are unable to produce the gene expression profile that defines a “normal” wheal, that is, a CSU not influenced by anti-IgE treatment. In future studies, it would be of interest to compare the gene expression in CSU wheals (untreated and treated) to the gene expression of lesions in dermatographism or cold urticaria as there is mast cell secretion but no cellular infiltration. This would help to understand the underlying pathologic differences between the diseases and potentially reveal key mechanisms of omalizumab treatment in CSU.

4.4 | Limitations

Limitations of the study were the small sample size and the lack of additional documentation (eg, pictures) and characterization of the wheals which could have been linked to the gene expression data. We are not aware of any particular technical issue on the probes selected for the gene(s) under scope and therefore have not considered a confirmation by RT-PCR/immunohistochemistry as mandatory. Data from previous publications support our data and thereby validate our gene expression data to some extent.

5 | CONCLUSIONS

Omalizumab not only prevents the development of wheals but also downstream changes in the skin in response to wheals that occur despite treatment. Our findings, if confirmed, should prompt further investigations regarding the mechanisms that underlie these effects. Enhanced understanding of the mechanism of action of omalizumab on wheals and their downstream consequences in the skin of CSU patients can help better understand the pathogenesis of CSU, improve treatment efficacy of omalizumab in CSU patients, and develop new targeted treatment options for management of CSU.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of the investigators, clinical trial staff, and participants.

CONFLICTS OF INTEREST

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf. Marcus Maurer and Martin Metz have received a research grant to their institution from Novartis; Marcus Maurer has been a paid consultant for Novartis and Genentech, received grants from Novartis, and been paid by Novartis for lectures and development of educational presentations; Andrea Bauer has been paid by Novartis for educational lectures and has been a member of the Novartis urticaria board; Randolph Brehler has received a research grant to the institution and has been paid for lectures and consultancy; Petra Staubach has been a paid consultant for Novartis. She has received travel support and received payment to her institution as the study center, research grants, and payments for lectures and expert testimony; Janine Gericke is currently employed by Novartis Pharma GmbH; her contribution to the work in this publication was performed while she was employed by Charité - Universitätsmedizin Berlin, where she received research grants from Novartis; Veit J. Erpenbeck, Michael T. Beste, Sergio Kaiser, Martin Letzkus, and Nicole Hartmann have received no support from any organization for the submitted work, have had no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years, and are employees of Novartis. Rebecca Torene received no support from any organization for the submitted work, has had no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years, and was an employee of Novartis at the time the study was conducted.

AUTHOR CONTRIBUTIONS

M. Metz, M. Maurer, P. Staubach, A. Bauer, R. Brehler, J. Gericke, and V.J. Erpenbeck participated in design and/or conduct of the study. R. Torene, S. Kaiser, M.T. Beste, M. Letzkus, and N. Hartmann analyzed the data. All authors also participated in the interpretation of results and drafted and revised the manuscript. All authors approved the final version of the manuscript.

ORCID

Marcus Maurer  <http://orcid.org/0000-0002-4121-481X>

REFERENCES

1. Omalizumab: anti-IgE monoclonal antibody E25, E25, humanised anti-IgE MAb, IGE 025, monoclonal antibody E25, Olizumab, Xolair, rhuMAb-E25. *BioDrugs*. 2002;16:380-386.
2. Serrano-Candelas E, Martinez-Aranguren R, Valero A, et al. Comparable actions of omalizumab on mast cells and basophils. *Clin Exp Allergy*. 2016;46:92-102.
3. Eggel A, Baravalle G, Hobi G, et al. Accelerated dissociation of IgE-FcεpsilonRI complexes by disruptive inhibitors actively desensitizes allergic effector cells. *J Allergy Clin Immunol*. 2014;133:1709-1719.
4. Saini S, Rosen KE, Hsieh HJ, et al. A randomized, placebo-controlled, dose-ranging study of single-dose omalizumab in patients with H1-antihistamine-refractory chronic idiopathic urticaria. *J Allergy Clin Immunol*. 2011;128:567-573.
5. Maurer M, Altrichter S, Bieber T, et al. Efficacy and safety of omalizumab in patients with chronic urticaria who exhibit IgE against thyroperoxidase. *J Allergy Clin Immunol*. 2011;128:202-209.
6. Maurer M, Rosen K, Hsieh HJ, et al. Omalizumab for the treatment of chronic idiopathic or spontaneous urticaria. *N Engl J Med*. 2013;368:924-935.
7. Kaplan A, Ledford D, Ashby M, et al. Omalizumab in patients with symptomatic chronic idiopathic/spontaneous urticaria despite standard combination therapy. *J Allergy Clin Immunol*. 2013;132:101-109.
8. Saini SS, Bindslev-Jensen C, Maurer M, et al. Efficacy and safety of omalizumab in patients with chronic idiopathic/spontaneous urticaria who remain symptomatic on H1 antihistamines: a randomized, placebo-controlled study. *J Invest Dermatol*. 2015;135:67-75.
9. Staubach P, Metz M, Chapman-Rothe N, et al. Effect of omalizumab on angioedema in H1-antihistamine resistant chronic spontaneous urticaria patients: results from X-ACT, a randomised controlled trial. *Allergy*. 2016;71:1135-1344.
10. Zhao ZT, Ji CM, Yu WJ, et al. Omalizumab for the treatment of chronic spontaneous urticaria: a meta-analysis of randomized clinical trials. *J Allergy Clin Immunol*. 2016;137:1742-1750.
11. Kaplan AP, Joseph K, Maykut RJ, Geba GP, Zeldin RK. Treatment of chronic autoimmune urticaria with omalizumab. *J Allergy Clin Immunol*. 2008;122:569-573.
12. Metz M, Ohanian T, Church MK, Maurer M. Retreatment with omalizumab results in rapid remission in chronic spontaneous and inducible urticaria. *JAMA Dermatol*. 2014;150:288-290.
13. European Medicines Agency. Omalizumab summary of product characteristics. 2015.
14. Genentech USA I. XOLAIR prescribing information. 2017.
15. Zuberbier T, Aberer W, Asero R, et al. The EAACI/GA(2) LEN/EDF/WAO Guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. *Allergy*. 2014;69:868-887.
16. Zuberbier T, Asero R, Bindslev-Jensen C, et al. EAACI/GA(2)LEN/EDF/WAO guideline: definition, classification and diagnosis of urticaria. *Allergy*. 2009;64:1417-1426.
17. Elias J, Boss E, Kaplan AP. Studies of the cellular infiltrate of chronic idiopathic urticaria: prominence of T-lymphocytes, monocytes, and mast cells. *J Allergy Clin Immunol*. 1986;78(5 Pt 1):914-918.
18. Natbony SF, Phillips ME, Elias JM, Godfrey HP, Kaplan AP. Histologic studies of chronic idiopathic urticaria. *J Allergy Clin Immunol*. 1983;71:177-183.
19. Sabroe RA, Poon E, Orchard GE, et al. Cutaneous inflammatory cell infiltrate in chronic idiopathic urticaria: comparison of patients with and without anti-FcεpsilonRI or anti-IgE autoantibodies. *J Allergy Clin Immunol*. 1999;103(3 Pt 1):484-493.
20. Ying S, Kikuchi Y, Meng Q, Kay AB, Kaplan AP. TH1/TH2 cytokines and inflammatory cells in skin biopsy specimens from patients with chronic idiopathic urticaria: comparison with the allergen-induced late-phase cutaneous reaction. *J Allergy Clin Immunol*. 2002;109:694-700.
21. Kaplan AP, Horakova Z, Katz SI. Assessment of tissue fluid histamine levels in patients with urticaria. *J Allergy Clin Immunol*. 1978;61:350-354.
22. Grattan CE, Boon AP, Eady RA, Winkelmann RK. The pathology of the autologous serum skin test response in chronic urticaria resembles IgE-mediated late-phase reactions. *Int Arch Allergy Appl Immunol*. 1990;93(2-3):198-204.
23. Konstantinou GN, Asero R, Ferrer M, et al. EAACI taskforce position paper: evidence for autoimmune urticaria and proposal for defining diagnostic criteria. *Allergy*. 2013;68:27-36.

24. Hide M, Francis DM, Grattan CE, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med*. 1993;328:1599-1604.
25. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol*. 2010;125(2 suppl 2):S73-S80.
26. Altrichter S, Peter HJ, Pisarevskaja D, Metz M, Martus P, Maurer M. IgE mediated autoallergy against thyroid peroxidase—a novel pathomechanism of chronic spontaneous urticaria? *PLoS One*. 2011;6:e14794.
27. Metz M, Staubach P, Bauer A, et al. Clinical efficacy of omalizumab in chronic spontaneous urticaria is associated with a reduction of FcεpsilonRI-positive cells in the skin. *Theranostics*. 2017;7:1266-1276.
28. Kay AB, Ying S, Ardelean E, et al. Elevations in vascular markers and eosinophils in chronic spontaneous urticarial weals with low-level persistence in uninvolved skin. *Br J Dermatol*. 2014;171:505-511.
29. Gruber BL, Baeza ML, Marchese MJ, Agnello V, Kaplan AP. Prevalence and functional role of anti-IgE autoantibodies in urticarial syndromes. *J Invest Dermatol*. 1988;90:213-217.
30. Kikuchi Y, Kaplan AP. Mechanisms of autoimmune activation of basophils in chronic urticaria. *J Allergy Clin Immunol*. 2001;107:1056-1062.
31. Patel OP, Giorno RC, Dibbern DA, Andrews KY, Durairaj S, Dreskin SC. Gene expression profiles in chronic idiopathic (spontaneous) urticaria. *Allergy Rhinol (Providence)*. 2015;6:101-110.
32. Gimenez-Arnau A, Curto-Barredo L, Nonell L, et al. Transcriptome analysis of severely active chronic spontaneous urticaria shows an overall immunological skin involvement. *Allergy*. 2017;72:1778-1790.
33. Bankova LG, Sterba PM, Gober L, et al. Gene expression profiling of nonlesional skin from chronic idiopathic urticaria subjects is distinct from normal nonatopic skin. *J Allergy Clin Immunol*. 2009;123:S110:A378.
34. Smith CH, Kepley C, Schwartz LB, Lee TH. Mast cell number and phenotype in chronic idiopathic urticaria. *J Allergy Clin Immunol*. 1995;96:360-364.
35. Nettis E, Dambra P, Loria MP, et al. Mast-cell phenotype in urticaria. *Allergy*. 2001;56:915.
36. Santos JC, de Brito CA, Futata EA, et al. Up-regulation of chemokine C-C ligand 2 (CCL2) and C-X-C chemokine 8 (CXCL8) expression by monocytes in chronic idiopathic urticaria. *Clin Exp Immunol*. 2012;167:129-136.
37. Bischoff SC, Krieger M, Brunner T, Dahinden CA. Monocyte chemotactic protein 1 is a potent activator of human basophils. *J Exp Med*. 1992;175:1271-1275.
38. Kuna P, Reddigari SR, Rucinski D, Oppenheim JJ, Kaplan AP. Monocyte chemotactic and activating factor is a potent histamine-releasing factor for human basophils. *J Exp Med*. 1992;175:489-493.
39. Conti P, Boucher W, Letourneau R, et al. Monocyte chemotactic protein-1 provokes mast cell aggregation and [3H]5HT release. *Immunology*. 1995;86:434-440.
40. Saavedra MC, Sur S. Down regulation of the high-affinity IgE receptor associated with successful treatment of chronic idiopathic urticaria with omalizumab. *Clin Mol Allergy*. 2011;9:2.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Metz M, Torene R, Kaiser S, et al. Omalizumab normalizes the gene expression signature of lesional skin in patients with chronic spontaneous urticaria: A randomized, double-blind, placebo-controlled study. *Allergy*. 2019;74:141–151. <https://doi.org/10.1111/all.13547>

Table S1. Differentially expressed genes in CSU lesional skin versus HV skin at baseline (n=106 transcripts)

Rows sorted by fold change (decreasing)

HUGO gene symbol	Gene name	P value	FDR adjusted P value	Fold change
CCL2	C-C motif chemokine ligand 2	1.360E-04	5.967E-01	5.34
S100A8	S100 calcium binding protein A8	1.800E-03	6.283E-01	4.35
S100A9	S100 calcium binding protein A9	5.990E-03	6.283E-01	3.57
PLA2G2A	phospholipase A2 group IIA	1.800E-02	6.283E-01	3.13
CYR61	cysteine rich angiogenic inducer 61	1.100E-03	6.283E-01	2.96
THBS1	thrombospondin 1	1.270E-02	6.283E-01	2.76
STEAP4	STEAP4 metalloredutase	5.890E-03	6.283E-01	2.63
KRT6A	keratin 6A	1.430E-02	6.283E-01	2.54
IGKC	immunoglobulin kappa constant	2.170E-02	6.283E-01	2.53
MT1M	metallothionein 1M	5.940E-03	6.283E-01	2.43
UAP1	UDP-N-acetylglucosamine pyrophosphorylase 1	4.190E-03	6.283E-01	2.42

HUGO gene symbol	Gene name	P value	FDR adjusted P value	Fold change
ICAM1	intercellular adhesion molecule 1	6.120E-03	6.283E-01	2.42
NNMT	nicotinamide N-methyltransferase	7.750E-03	6.283E-01	2.37
LYZ	lysozyme	4.260E-03	6.283E-01	2.32
CD209	CD209 molecule	3.730E-03	6.283E-01	2.22
TIMP1	TIMP metalloproteinase inhibitor 1	5.580E-03	6.283E-01	2.20
SLC20A1	solute carrier family 20 member 1	1.910E-03	6.283E-01	2.19
KRT16	keratin 16	2.220E-02	6.283E-01	2.19
C11orf96	chromosome 11 open reading frame 96	5.940E-03	6.283E-01	2.18
ADAMTS1	ADAM metalloproteinase with thrombospondin type 1 motif 1	3.870E-03	6.283E-01	2.11
BCL3	B-cell CLL/lymphoma 3	2.650E-03	6.283E-01	2.03
ZFP36	ZFP36 ring finger protein	2.860E-04	5.967E-01	1.97
MEDAG	mesenteric estrogen dependent adipogenesis	2.140E-02	6.283E-01	1.96
SOD2	superoxide dismutase 2	9.710E-	6.283E-	1.95

HUGO gene symbol	Gene name	P value	FDR adjusted P value	Fold change
		03	01	
OSMR	oncostatin M receptor	2.030E-03	6.283E-01	1.90
PLSCR1	phospholipid scramblase 1	1.220E-02	6.283E-01	1.87
CTSL	cathepsin L	2.840E-02	6.283E-01	1.85
GPX2	glutathione peroxidase 2	5.740E-03	6.283E-01	1.85
KRT17	keratin 17	2.060E-02	6.283E-01	1.85
SPRR2G	small proline rich protein 2G	2.920E-02	6.283E-01	1.84
GCH1	GTP cyclohydrolase 1	1.610E-02	6.283E-01	1.79
GADD45B	growth arrest and DNA damage inducible beta	1.430E-03	6.283E-01	1.77
CCL13	C-C motif chemokine ligand 13	3.930E-02	6.349E-01	1.77
LDLR	low density lipoprotein receptor	5.750E-03	6.283E-01	1.77
MYC	MYC proto-oncogene, bHLH transcription factor	1.160E-02	6.283E-01	1.76
CTGF	connective tissue growth factor	1.530E-02	6.283E-01	1.72

HUGO gene symbol	Gene name	P value	FDR adjusted P value	Fold change
FCER1G	Fc fragment of IgE receptor Ig	2.100E-02	6.283E-01	1.70
IL4R	interleukin 4 receptor	9.320E-03	6.283E-01	1.69
EGR1	early growth response 1	1.260E-02	6.283E-01	1.68
TMEM2	transmembrane protein 2	1.880E-02	6.283E-01	1.68
HIF1A	hypoxia inducible factor 1 alpha subunit	4.530E-02	6.349E-01	1.67
IL10RA	interleukin 10 receptor subunit alpha	2.520E-03	6.283E-01	1.67
DDX21	DExD-box helicase 21	2.520E-02	6.283E-01	1.66
MIR22HG	MIR22 host gene	7.490E-03	6.283E-01	1.66
CRISPLD2	cysteine rich secretory protein LCCL domain containing 2	1.750E-02	6.283E-01	1.66
LRRC59	leucine rich repeat containing 59	1.690E-02	6.283E-01	1.65
SNHG15	small nucleolar RNA host gene 15	9.440E-03	6.283E-01	1.63
ID1	inhibitor of DNA binding 1, HLH protein	4.510E-03	6.283E-01	1.63
VMP1	vacuole membrane protein 1	1.150E-	6.283E-	1.62

HUGO gene symbol	Gene name	P value	FDR adjusted P value	Fold change
		02	01	
C3AR1	complement C3a receptor 1	8.910E-03	6.283E-01	1.62
CD93	CD93 molecule	2.210E-02	6.283E-01	1.60
CEBPD	CCAAT/enhancer binding protein delta	3.490E-03	6.283E-01	1.59
PLAU	plasminogen activator, urokinase	1.810E-02	6.283E-01	1.59
SOX7	SRY-box 7	4.220E-04	5.967E-01	1.59
NOP16	NOP16 nucleolar protein	2.140E-02	6.283E-01	1.59
PLA2G3	phospholipase A2 group III	5.610E-03	6.283E-01	1.59
CYBB	cytochrome b-245 beta chain	2.540E-02	6.283E-01	1.58
CDKN1A	cyclin dependent kinase inhibitor 1A	1.720E-03	6.283E-01	1.58
CKS2	CDC28 protein kinase regulatory subunit 2	3.520E-02	6.283E-01	1.58
PROCR	protein C receptor	1.110E-02	6.283E-01	1.57
FOS	Fos proto-oncogene, AP-1 transcription factor subunit	4.400E-02	6.349E-01	1.57

HUGO gene symbol	Gene name	P value	FDR adjusted P value	Fold change
JUNB	JunB proto-oncogene, AP-1 transcription factor subunit	2.050E-03	6.283E-01	1.56
IFITM1	interferon induced transmembrane protein 1	1.150E-02	6.283E-01	1.56
ZNF593	zinc finger protein 593	1.490E-02	6.283E-01	1.56
TNFRSF12A	TNF receptor superfamily member 12A	2.050E-02	6.283E-01	1.56
SCO2	SCO2, cytochrome c oxidase assembly protein	1.180E-02	6.283E-01	1.56
PTGIS	prostaglandin I2 synthase	4.110E-02	6.349E-01	1.56
MPEG1	macrophage expressed 1	1.050E-02	6.283E-01	1.55
THBD	thrombomodulin	4.790E-03	6.283E-01	1.55
CFB	complement factor B	4.780E-03	6.283E-01	1.55
LRRC32	leucine rich repeat containing 32	2.980E-02	6.283E-01	1.55
ZYX	zyxin	5.650E-03	6.283E-01	1.55
IFI16	interferon gamma inducible protein 16	2.910E-03	6.283E-01	1.54
ECSCR	endothelial cell surface expressed chemotaxis and	7.720E-	6.283E-	1.54

HUGO gene symbol	Gene name	P value	FDR adjusted P value	Fold change
	apoptosis regulator	03	01	
LYAR	Ly1 antibody reactive	2.120E-02	6.283E-01	1.54
ACTN1	actinin alpha 1	1.800E-02	6.283E-01	1.53
GALNT6	polypeptide N-acetylgalactosaminyltransferase 6	2.530E-02	6.283E-01	1.52
C1orf162	chromosome 1 open reading frame 162	2.720E-02	6.283E-01	1.52
SLC41A1	solute carrier family 41 member 1	1.580E-02	6.283E-01	1.52
SNHG17	small nucleolar RNA host gene 17	1.640E-03	6.283E-01	1.52
NOP2	NOP2 nucleolar protein	2.090E-02	6.283E-01	1.52
CSRNP1	cysteine and serine rich nuclear protein 1	7.380E-03	6.283E-01	1.52
SLC43A3	solute carrier family 43 member 3	1.010E-02	6.283E-01	1.51
DNAJA1	DnaJ heat shock protein family (Hsp40) member A1	1.500E-02	6.283E-01	1.51
PPIL1	peptidylprolyl isomerase like 1	3.810E-03	6.283E-01	1.51
ANGPTL4	angiopoietin like 4	1.710E-	6.283E-	1.50

HUGO gene symbol	Gene name	P value	FDR adjusted P value	Fold change
		02	01	
LRP4	LDL receptor related protein 4	7.630E-03	6.283E-01	-1.50
HSD11B2	hydroxysteroid 11-beta dehydrogenase 2	8.850E-03	6.283E-01	-1.50
MLPH	melanophilin	1.250E-03	6.283E-01	-1.51
TFCP2L1	transcription factor CP2 like 1	1.390E-02	6.283E-01	-1.54
WNT4	Wnt family member 4	1.050E-02	6.283E-01	-1.55
ADIRF-AS1	ADIRF antisense RNA 1	7.680E-03	6.283E-01	-1.55
LOC100049716	LOC100049716	3.460E-04	5.967E-01	-1.56
HSPB2	heat shock protein family B (small) member 2	4.020E-02	6.349E-01	-1.58
MAB21L3	mab-21 like 3	1.880E-02	6.283E-01	-1.59
KRT15	keratin 15	2.180E-02	6.283E-01	-1.62
ZNF273	zinc finger protein 273	5.610E-03	6.283E-01	-1.62
MIR210HG	MIR210 host gene	2.290E-02	6.283E-01	-1.65

HUGO gene symbol	Gene name	P value	FDR adjusted P value	Fold change
DCT	dopachrome tautomerase	9.630E-03	6.283E-01	-1.65
TNNI2	troponin I2, fast skeletal type	9.150E-03	6.283E-01	-1.67
CRABP1	cellular retinoic acid binding protein 1	3.160E-03	6.283E-01	-1.69
TNNC1	troponin C1, slow skeletal and cardiac type	4.470E-03	6.283E-01	-1.70
LINC00842	long intergenic non-protein coding RNA 842	4.730E-03	6.283E-01	-1.76
PPARGC1A	PPARG coactivator 1 alpha	4.510E-02	6.349E-01	-1.77
FMO1	flavin containing monooxygenase 1	3.020E-03	6.283E-01	-1.85
SLC47A2	solute carrier family 47 member 2	6.670E-04	5.967E-01	-2.07

Table S2. Differentially expressed genes in CSU nonlesional versus HV skin at baseline (n=9 transcripts)

Rows sorted by fold change (decreasing)

None of these transcripts were array-identified and qPCR validated in Gimenez-Arnau et al reference as differentially upregulated in CSU lesional skin versus CSU nonlesional skin

HUGO gene symbol	Gene name	P value	FDR adjusted P value	Fold change
CD209	CD209 molecule	3.160E-02	9.996E-01	1.78
C1QB	complement C1q B chain	2.800E-02	9.996E-01	1.71
MPEG1	macrophage expressed 1	5.340E-03	9.996E-01	1.62
IL10RA	interleukin 10 receptor subunit alpha	5.740E-03	9.996E-01	1.59
RNASE1	ribonuclease A family member 1, pancreatic	4.740E-02	9.996E-01	1.55
GPRIN2	G protein regulated inducer of neurite outgrowth 2	4.160E-03	9.996E-01	-1.52
EDNRB	endothelin receptor type B	6.110E-03	9.996E-01	-1.55
CRABP1	cellular retinoic acid binding protein 1	8.300E-03	9.996E-01	-1.59
SLC47A2	solute carrier family 47 member 2	3.400E-03	9.996E-01	-1.85

