OPEN ACCESS

University of Dundee

Candidate long-range regulatory sites acting on the IL17-pathway genes TRAF3IP2 and IL17RA are associated with psoriasis

Nititham, Joanne; Fergusson, Calum; Palmer, Colin; Liao, Wilson; Foerster, John

[Link to publication in Discovery Research Portal](#)

DR. JOHN P FOERSTER (Orcid ID : 0000-0002-8295-1867)

Candidate long-range regulatory sites acting on the IL17-pathway genes *TRAF3IP2* and *IL17RA* are associated with psoriasis.

Joanne Nititham[1], Calum Fergusson[2*], Colin Palmer[2], Wilson Liao[1&], John Foerster[2&]

[1]University of California at San Francisco, Department of Dermatology,

[2]University of Dundee, Medical School

[&]Corresponding authors: j.foerster@dundee.ac.uk, wilson.liao@ucsf.edu

*equal contribution

The authors declare no conflicts of interest

running head: IL17-pathway regulatory variants and psoriasis

Abstract

BACKGROUND: Drug-mediated disruption of IL17A, IL17F, and IL17RA proteins is effective in psoriasis. However, disruption of the IL17 pathway by functional mutations has so far only been shown to affect risk in *IL23R* and *TRAF3IP2*. It is unclear if this is due to rarity of disruptive mutations. OBJECTIVE: (i) to delineate the prevalence of mutations in key IL17-pathway genes; (ii) to identify candidate regulatory sites acting on *IL23R, IL17A, IL17RA, TRAF3IP2* from a distance. METHODS: Extraction of mutation frequencies from ExAc data, evolutionary sequence alignment; mapping of long-range-interacting (LRI) enhancers; genetic association testing in a novel psoriasis cohort. RESULTS: the prevalence of disruptive mutations in genes such as *IL17RA* is sufficient to have been detectable by existing datasets. Therefore, lack of their association with psoriasis indicates that genetic risk primarily resides in variants acting from a distance. We identify two LRI enhancer sites, regulating *IL17RA* and TRAF3IP2, respectively. The TRAF3IP2 regulator localises to the TRAF3IP2-antisense promoter, suggesting feedback-regulation. Both LRI sites are associated with psoriasis in a novel Scottish psoriasis cohort and the TRAF3IP2-LRI at rs71562294 replicates in the WTCCC cohort. CONCLUSION: Genetic risk for psoriasis may be encoded at LRI sites regulating IL17 pathway genes from a distance.

key words: psoriasis, *IL17A*, cohort, genetics, biologics

Background

Blocking the function of the proteins encoded by *IL17A/F, IL17RA,* and *IL23R* improves psoriasis. Accordingly, genetic associations between psoriasis and IL-17 pathway genes, including *IL23R* [1] and the key signal transducer *TRAF3IP2* (also called *ACT1*) [2] have been reported. Nevertheless, the overall risk conferred by these variants remains moderate (mean OR 1.98 for *IL23R* and 1.11 for *TRAF3IP2*, respectively [3]) and, despite acting as direct drug targets, no genetic association has been identified for *IL17F, IL17A, or IL17RA*. It remains unclear whether the frequency of disruptive mutations in these genes is too low to have been detectable by existing studies. Thus, present understanding of IL17 – pathway genetics in psoriasis does not account for the observation that close to 90% of patients show a major response to anti-IL17A or anti-IL17RA drugs [4-6].

Most disease-modifying variants do not reside in or near genes but instead in regulatory elements as far as 200 kb from the actual gene locus [7]. Such long-range-interaction (LRI) variants have been mapped by as DNA-hypersensitive sites (DHS), histone-marks indicating active transcriptional activity (H3K27Ac), or expression quantitative trait loci (eQTL). Although available databases do not fully represent key cell types driving psoriasis activity (γ/δ T-cells, Th17 subsets, dermal fibroblasts), they do allow preliminary mapping of putative LRI sites.

Questions Addressed

We here focussed on four key genes as follows: *IL23R, IL17A, IL17RA* as genes encoding direct drug targets and *TRAF3IP2*, since this gene exhibits the strongest genetic IL17 pathway association.

Blocking the activity of proteins including IL23R, IL17A, IL17RA inhibits the psoriasis phenotype. Therefore, blocking the activity of these same proteins through naturally occuring mutations should affect an individuals's risk and/or severity of being affected by psoriasis. However, this isn't fully borne out by existing genetic association studies. We therefore addressed these questions: First, have such associations not been found because relevant mutations are too infrequent or because they locate to distal regulatory sites, having thus escaped mapping to their target genes? Second: can we identify candidate distal variants regulating *IL23R, IL17A, IL17RA*, and *TRAF3IP2*? Third: can we detect genetic association of such variants with psoriasis?

Experimental Design

Due to space constraints, all Methods have been placed into the Supplement (section 'Supplementary Methods').

Results

Disruptive IL17-pathway variants are common but not universally associated with psoriasis risk. A large Chinese exome study reported association of the variant Gly149Arg in the *IL23R* gene with psoriasis [8]. A GWAS analysis of a similarly sized Caucasian cohort did not replicate this, but instead reported association of another variant in the same gene (IL23R:p.Arg381Gln) [9]. Conversely, the latter study reported association of two

coding variants in the *TRAF3IP2* gene not seen in the Chinese dataset [9]. By datamining of global exome variant data [10], we found that the Chinese and European datasets, respectively, detected those risk alleles most common in each population (Figure S3a), confirming that both datasets have sufficient statistical power to detect genetic associations of common variants (MAF > 0.1). Unexpectedly, we found that missense and loss-of-function (LOF) variants in IL17 pathway genes are in fact abundant in most global populations (Figure S3b, details in S1 datafile Exome Variants), and would have been detectable by the above-cited studies. Not all of these variants necessarily disrupt the gene product, but those likely to be deleterious can be identified by analyzing evolutionary protection. For example, more than 30 % of Europeans are carriers of the *IL17RA:p.*Ala691Thr missense mutation, which is 100 % conserved across a wide range of mammalian species (Figure S3c). The same goes for the *IL17RA*:p.Pro562Gln mutation, which is highly prevalent in East Asians (Figure S3c). In addition, Europeans, but not Asians exhibit two disruptive and evolutionary conserved mutations in *IL17F* that would have been detectable by [9] (Fig S3d). Of interest, the *IL17A* gene exhibits a notable scarcity of disruptive variants with the exception of one mutation relatively abundant in Hispanics, approximately 1%; (Fig. S3b). Independent of evolutionary protection, these mutations (exception: *IL17F:p.*Val155Ile) are predicted to be deleterious using the SIFT tool (http://sift.bii.a-star.edu.sg). Taken together, these data show that, while some variants directly disrupting *IL23R* and *TRAF3IP2* do show the expected genetic association with psoriasis, many frequent IL17-pathway mutations do not. Therefore, genetic risk affecting psoriasis may reside in LRI sites acting from a distance.

A LRI site regulating IL17RA expression. In order to detect LRI sites, we mined available datasets for LRI regions, scanning 200 kb intervals around *IL17A*, *IL17RA*, *IL23R*, and *TRAF3IP2*. As shown in Figure 1A, we identified evidence for two LRI's associated with *IL17RA* promoter activity in multiple cell lines based on CHIA-PET chromatin precipitation (labeled R1, R2, respectively). At higher resolution both R1 and R2 showed strong H3K27Ac histone marks as well as DNAse hypersenstivity, confirming active enhancer status (Figures 1B and 1C). Furthermore, both R1 and R2 contain SNPs independently identified as eQTL regulating *IL17RA* gene expression (see [11], for details S2 datafile eQTL). An additional independent eQTL dataset (GTex) also exhibits eQTL signals in R2, as well as an extensive cluster of eQTL signals within both R1 and R2 (bottom of Figure 1B, 1C, respectively, complete GTex data in Figure S1). Taken together, several lines of evidence suggest that two novel LRI regions, localized approximatly 80 kb downstream of the *IL17RA* promoter within the *CECR5* gene, regulate *IL17RA* expression.

Long-range interaction of the TRAF3IP2-antisense promoter with TRAF3IP2. We also found strong evidence for LRI between the *TRAF3IP2* promoter and the *TRAF3IP2-AS1* (antisense) promoter based on CHIA-PET (Figure 1D, region labelled "R3"). R3 is also highly enriched both for H3K27Ac and DHS marks (Figure 1E), consistent with active enhancer status. Again, two SNPs residing within R3, rs61269242 and rs1407644, have independently been identified as eQTL for TRAF3IP expression (S2 datafile eQTL). In further independent support, the TRAF3IP2 / TRAF3IP2-AS1 pair has been identified as a gene/natural-antisense pair in a recent study on breast cancer [12]. Taken

together, these data strongly suggest that TRAF3IP2 transcription is regulated by the abundance of its anti-sense product.

Psoriasis cohort. In order to test association of these novel putative regulatory sites with psoriasis we assembled a discovery cohort based on a North-East Scottish demographic. The clinical characteristics of the cohort are shown in Table S3. The Tayside cohort represents a mix of approximately 75% HLA-Cw6+ and 25 % HLA-Cw6- patients. Accordingly, the majority of patients (67%) exhibit age at onset > 40 y. The cohort is also marked by a high median age (63y), and high female percentage (54%). In addition, it is heterogeneous in terms of prior treatment (half of patients naïve with respect to systemic treatments). Taken together, the Tayside/Scotland psoriasis cohort is distinct from previously reported cohorts, thereby broadening the genetic coverage of psoriasis.

Genetic associations of IL17-signalling variants. We initially performed a single-point association analysis of the Tayside cohort spanning 200 kb to either side of the transcriptional start site of each of the candidate genes. As shown in table S4, we replicated the strong association previously observed with *TRAF3IP2*. We also identified association with the eQTL rs71562294 mapping to the region R3 within the *TRAF3IP2*-antisense (AS1) promoter (Table 1, S2 datafile eQTL). In addition, we identified two strongly associated SNPs located within 4 kb of the R1 LRI region regulating *IL17RA* (Table 1; Figure 1b marked by yellow lines and arrows). We did not detect association with the previously reported SNP rs11209026 at the *IL23R* gene locus [13]. We did identify significant association of SNPs in a putative enhancer 20kb upstream of *IL17A* which, however, only replicated in one out of three other cohorts (table S2, Fig.

S4) despite comparable allele frequencies (table S5), raising the possibility of an artifact. Thus, the present data identify novel associations at the distant *IL17RA* enhancer (R1) as well as a putative enhancer for *TRAF3IP2* located 200 kb downstream *TRAF3IP2-AS1* and confirm significant genetic association of single SNPs located within *TRAF3IP2*.

Conclusion

We here show that mutations disrupting *IL17RA, IL17A, IL23R* are common but do not necessarily affect psoriasis risk status, while drugs disrupting the proteins encoded by these same genes massively affect phenotype expression. This apparent paradox suggests that genetic risk for psoriasis may reside in LRI sites regulating these genes from a distance.

We identify regions R1 and R2 as novel LRI enhancers for *IL17RA*. In line with these findings, two SNPs within 5-8 kb of R1 are associated with psoriasis in our novel discovery cohort. We also identify a novel LRI site located within the *TRAF3IP2-AS1* promoter, 200 kb distant from the *TRAF3IP2* promoter, showing genetic association with psoriasis both in our discovery cohort, as well as in the large WTCCC replication cohort. Given the identification of this site as an eQTL for TRAF3IP2 and the recent identification of the *TRAF3IP-AS1* / *TRAF3IP2* pair by [12], rs71562294 located within the *TRAF3IP-AS1* promoter may well regulate *TRAF3IP2* protein abundance.

For *IL17A* itself, we observed a strikingly Th17-selective enhancer (Figure S2), which, in fact, exhibited association with psoriasis in the Scottish cohort, as well as a US-based replication cohort but not in additional replication cohorts (Results S1, S2). Comprehensive discovery of long-range interacting regions for

Th17 and γ/δ-T cell specific genes including *IL17A*, *IL17F*, and *IL23R* will require

DHS, histone, and eQTL data obtained in these cell types [14].

Acknowledgements

We are grateful for the expert assistance Fiona Carr in DNA preparation.

Conflict of Interest Statement:

The authors declare no conflict of interest.

Author Contributions Statement:

Designed the project: JF

Conducted experiments: CF, JN

Conducted data analysis: JF, CF, JN, WL

Contributed reagents/infrastructure/material: CP

Wrote the manuscript: JF

Edited/ commented on manuscript: JN, CF, WL, CP

References

[1] R.P. Nair, A. Ruether, J Invest Dermatol, 2008, 128,1653.

[2] U. Huffmeier, S. Uebe, Nat Genet, 2010, 42,996.

[3] B. Kisiel, K. Kisiel, PLoS One, 2017, 12,e0179348.

[4] M. Lebwohl, B. Strober, N Engl J Med, 2015, 373,1318.

[5] K.B. Gordon, A. Blauvelt, N Engl J Med, 2016, 375,345.

[6] R.G. Langley, B.E. Elewski, N Engl J Med, 2014, 371,326.

[7] F. Grubert, J.B. Zaugg, Cell, 2015, 162,1051.

[8] H. Tang, X. Jin, Nat Genet, 2014, 46,45.

[9] N. Dand, S. Mucha, Hum Mol Genet, 2017, 26,4301.

[10] M. Lek, K.J. Karczewski, Nature, 2016, 536,285.

[11] R. Joehanes, X. Zhang, Genome Biol, 2017, 18,16.

[12] S. Wenric, S. ElGuendi, Sci Rep, 2017, 7,17452.

[13] K.J. Zhu, C.Y. Zhu, Inflamm Res, 2012, 61,1149.

[14] M.R. Mumbach, A.T. Satpathy, Nat Genet, 2017, 49,1602.

Table 1. Replication of LRI TRAF3IP2 regulating region association with psoriasis[1]

		Scotland (Discovery)		US UKBB (Replication 1)		WTCCC (Replication 2)		Meta-analysis[2]	
GENE	SNP	OR	p	OR	p	OR	p	OR	p
TRAF3IP2	rs71562294	1.55	0.0043	1.14	0.39	1.62	1.0E-10	1.46	3.5E-04
IL17RA	rs1034858	0.75	0.0005	0.987	0.88	1.05	0.18	0.93	0.46
IL17RA	rs4819971	0.74	0.0005	0.989	0.89	1.05	0.19	0.92	0.45

Minor alle frequencies

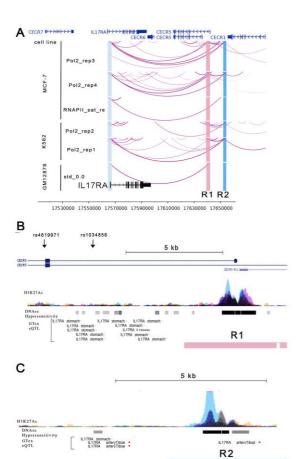|	allele	cases	controls	cases	controls	cases	controls
rs71562294	G	0.089	0.059	0.097	0.078	0.090	0.057
rs1034858	C	0.389	0.460	0.466	0.468	0.455	0.445
rs4819971	T	0.389	0.460	0.465	0.467	0.455	0.445

[1]OR- odds ratio, p- p-value. For details of the cohorts, see Methods. Size of replication cohort 1: 431 cases/ 838 controls; size of replication cohort 2: 2178 cases, 5175 controls.
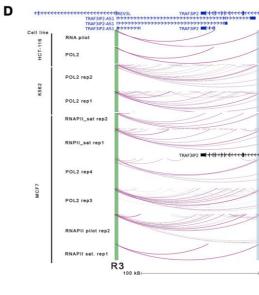
[2]Meta-analysis includes both discovery and replication cohorts, respectively. Both OR and P-values shown are for random models.

Legends to Figures

Figure 1. Long range interacting (LRI) regions connected with *IL17RA* and *TRAF3IP2* promoter activity. A, LRI were identified from the WashU epigenome browser (epigenomegateway.wustl.edu/browser/). The curved lines indicate significant correlation of transcription factor binding, as measured by CHIA-PET, with the active expression of a gene in the cell types shown. Tracks exhibiting LRI to the *IL17RA* promoter in cell types relevant to psoriasis are shown in the figure. Genomic position is labelled according to Hg19. The light blue shaded box denotes the *IL17RA* transcriptional start site (TSS). B, close-up of the R1 region, showing DNAse hypersensitive sites (black/grey bars), and histone H3K27Ac active enhancer marks (color code for each cell type: light blue: HUVEC; purple: NHEK, dark blue: K562, red: GM12878). eQTL marks linked to *IL17RA* expression at GTex are shown on the bottom (yellow dots with source tissue, respecively). The vertical arrows mark denote SNPs associated with psoriasis in the Tayside cohort (see Table S3). C, same as in B for region R2. D, LRI between the TRAF3IP2 promoter (light blue box on the right) and the TRAF3IP2-antisense (AS) promoter (marked R3). E, Close-up of the R3 region showing H3K27Ac enhancer as well as DNAse hypersensitivity marks.