SNPs talk to genes using landlines: long-range chromatin interactions link genetic risk with epigenetic patterns in Takayasu arteritis

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Systemic autoimmune/inflammatory conditions are complex both at the clinical and pathomechanistic level. While a number of rare conditions are caused by mutations in single genes, most autoimmune/inflammatory diseases are underlined by multiple genetic variants which, through poorly characterised or unknown processes, confer so-called 'genetic susceptibility'. The influence of environmental factors necessary for the expression of a clinical entity is usually mandated in the form of epigenetic changes.¹⁻³

Over the last decade, genome-wide association studies (GWAS) have identified a wealth of associations between genetic variants and autoimmune/inflammatory conditions. Studies have provided compelling evidence for germline polymorphisms and/or copy-number variants contributing to the risk for the development of disease. In most cases, genetic variants only increased the risk of developing disease, while the underlying disease-causing mechanisms remained unclear. Furthermore, healthy individuals may also carry risk alleles for inflammatory conditions while never developing symptoms.⁴ Understanding the contribution of genetic variants to disease expression is complex because most disease-associated gene variants are located in intergenic or intronic (non-coding) regions, limiting our understanding of their exact impact on genes, their influence on gene expression and underlying molecular events. The fact that a significant proportion of disease-associated polymorphisms are located distant

from genes, perhaps not even affecting neighbouring gene expression, led to the hypothesis that genetic variants may interfere with the regulation of distant genes on the same or even other chromosomes.

Indeed, interactions between genes and/or regulatory regions through DNA folding (allowing for physical contact between otherwise distant regions) and/or interactions between chromosomes mediating regulatory events has been suggested. Several genes linked to the immune response are regulated by long-distance chromatin interactions, including (but not limited to) cytokine genes in the Th2 cluster (*IL4*, *IL5* and *IL13* on chromosome 5 in humans, chromosome 11 in mice),⁵ the interferon- γ gene (*Ifng*: chromosome 10) and its receptor (*IfngR1*: chromosome 10) during Th1 subset determination,⁶ and antigen receptor genes during recombination in T and B lymphocytes.⁷⁻⁸ Several studies have provided insight into the involved mechanisms. The transcription of genes is promoted in 'transcription factories', that is, subnuclear compartments that contain DNA and clusters of RNA polymerases.⁹⁻¹¹ Coregulated genes, including those encoding for the aforementioned Th2 cytokines, can be found within the same transcriptional compartment, independent of their genomic location.⁵ The transcription factor CTCF, also known as 11-zinc finger protein or CCCTC-binding factor, associates primarily with regulatory regions and, in association with the cohesin complex, plays a key role in the three-dimensional organisation of genes, chromatin and chromosomes, thus regulating the interaction between (sometimes distant) regulatory elements and gene (co-) expression. The role of CTCF has particularly been studied in antigen receptor gene expression: B cells deficient in CTCF exhibit reduced DNA compaction at the *Igh* locus.¹²⁻¹⁵

Long-distance genomic interactions and the possibility of distant genetic variants affecting regulatory regions across the

genome has recently been discussed in the context of autoimmune/inflammatory conditions.¹⁶ Recent advances in large-scale laboratory analyses using extended population cohorts and small sample sizes, combined with bioinformatic approaches applying state-of-the-art technology, allow for the mapping of genetic data from GWAS to disease-associated epigenetic marks and transcriptional profiles. Furthermore, interactions between distant genomic regions can be monitored using chromatin interaction assays, enabling the functional analysis of the identified targets. Interactions between physically distant regions and the resulting recruitment of transcription factors and epigenetic modifiers to regulatory elements may furthermore explain the involvement of environmental triggers in transcriptional dysregulation at least in a subset of patients.^{2,16,17} Harley *et al*¹⁷ most recently reported that the EBNA2 protein (associated with the Epstein-Barr virus) occupies nearly half of systemic lupus erythematosus risk loci along with coclustered transcription factors demonstrating clearly the existence of gene-environment interactions. Similar EBNA2-anchored associations were recognised in patients with multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, type 1 diabetes, juvenile idiopathic arthritis and celiac disease. For the most part, however, the link between genetic association, effect on the expression of individual or groups of genes, and the underlying molecular mechanisms is yet to be determined.^{1,16}

Takayasu arteritis (TA) is a granulomatous system vasculitis affecting large vessels, most commonly the aorta, its major branches and/or pulmonary arteries.^{18,19} The molecular pathophysiology of TA remains unclear. Based on increased incidence in Asian when compared with Caucasian populations, the presence of genetic associations that increase an individual's risk without causing disease (eg, HLA-B*52), and the fact that women are significantly more frequently affected than men, a combination of genetic and environmental factors have been proposed.^{19,20} Treatment of patients with TA is largely empiric. Non-specific immune suppression is frequently used as first-line treatment and typically involves corticosteroids and steroid-sparing non-biological immunosuppressive agents, such as methotrexate, azathioprine, leflunomide, mycophenolate mofetil and/or cyclophosphamide. Beneficial effects of (partial) reconstitution of imbalanced cytokine expression (blockade of IL-6 or tumour necrosis factor α (TNF- α) signalling) and B cell

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Figure 1 Long-range interactions between *IL6* and *GPNMB* regulate gene expression. Recruitment of the transcription factor MEF2 and corecruitment of GATA to an enhancer element in the second intron of *IL6* allows for activation of the *GPNMB* gene through CTCF-mediated long-range chromatin interactions. Interaction of regulatory regions mediates increased *GPNMB* expression through assembly and recruitment of the transcriptional complex. The Takayasu arteritis risk allele rs2069837 (G>A polymorphism) results in increased recruitment of MEF2 to the intronic *IL6* regulatory element and resultant accumulation of HDAC proteins (namely HDAC1a, 4, 5 and 7) that alter enhancer function through histone deacetylation. Of note, IL-6 expression in monocytes/macrophages is not affected by suppression of enhancer activity. Reduced expression of *GPNMB* is associated with pro-inflammatory macrophage phenotypes (M1>M2) and increased pro-inflammatory cytokine expression (including IL-1 β , IL-6 and TNF- α). *GPNMB*, glycoprotein NMB; HDAC, histone deacetylase; MEF2, myocyte enhancer factor 2.

depletion have been reported, suggesting the involvement of these pathways in the pathophysiology of TA.¹⁹

In the accompanying manuscript 'Takayasu arteritis risk locus in *IL6* represses the anti-inflammatory gene *GPNMB* through chromatin looping and recruiting MEF2-HDAC complex', Sawalha *et al*²¹ connect the non-coding variant rs2069837 (G>A), located in an enhancer region within the second intron of the *IL6* gene, with altered expression of the anti-inflammatory molecule glycoprotein NMB (*GPNMB*) and, as a result, dysregulated cytokine expression. Using state-of-the-art bioinformatic and experimental

approaches, the authors have connected the disease-associated rs2069837 A alleles with increased recruitment of the myocyte enhancer factor 2-histone deacetylase (MEF2-HDAC) complex to an intronic enhancer element. Chromatin condensation initiated by the transcription factor CTCF allows for physical interactions between *IL6* and *GPNMB* regulatory elements, and associations with MEF2-HDAC in turn repress transcription of the anti-inflammatory molecule transmembrane *GPNMB* (figure 1), a regulator of inflammatory responses in macrophages. While promoting regulatory M2 polarisation, lack of *GPNMB* in animals results in increased and uncontrolled expression and release of pro-inflammatory IL-6, IL-1 β and TNF- α .^{22–24} This may explain why rs2069837 A alleles, which mediate reduced *GPNMB* expression and increased inflammatory responses in macrophages, are associated with protection against chronic hepatitis B virus infection, cervical cancer (a result of viral infection), as well as autoimmune inflammatory disease (TA).^{25–27} However, the situation may be complex since identical genetic variants and effects may be context-specific.²⁸ Further analysis of rs2069837 (G>A) across diseases (autoimmune/inflammatory vs infectious disease, malignancy and so on) may provide additional information on whether rs2069837 may be used for risk assessment and patient stratification across inflammatory conditions.

Taken together, in an exciting fashion, this work presents first evidence on how intronic non-coding genetic variants identified by GWAS contribute to disease pathogenesis in patients with TA. Experimental approaches applied in this study promise a 'new era' of biomedical research, testing both direct and indirect mechanistic involvement of genetic variants identified through GWAS approaches in the pathophysiology of autoimmune/inflammatory conditions. Indeed, identification of functional consequences of disease-associated genetic variants may allow for utilisation of genetic data from GWAS studies for identifying molecular targets and working towards personalised medicine. Based on molecular events involved in autoimmune/inflammatory conditions (as well as infections, cancers and so on), blockade of transcription factors (eg, with small molecules) or epigenetic modifiers (eg, HDAC inhibitors), delivery of molecules to cells or tissues (eg, through targeted delivery of microparticles)^{29–30} or future approaches to correct gene variants may result from such scientific approaches.

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