Unraveling the immune signature of herpes zoster : insights into the pathophysiology and human leukocyte antigen risk profile

Unravelling the immune signature of herpes zoster: Insights into pathophysiology and the HLA risk profile

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SUMMARY

Using gene expression and association studies, our research uncovered the MHC locus as a major risk factor for the development of herpes zoster. Additionally, a clear type I interferon and adaptive immune signature was identified in individuals with HZ.

SHORT TITLE

The immune signature in herpes zoster

ABSTRACT

The varicella-zoster virus (VZV) infects over 95% of the population. VZV reactivation causes herpes zoster (HZ), known as shingles, primarily affecting the elderly and immunocompromised individuals. However, HZ can also occur in otherwise healthy individuals. We analyzed the immune signature and risk profile in HZ patients using a genome-wide association study across different UK Biobank HZ cohorts. Additionally, we conducted one of the largest HZ HLA association studies to date, coupled with transcriptomic analysis of pathways underlying HZ susceptibility. Our findings highlight the significance of the MHC locus for HZ development, identifying five protective and four risk HLA alleles. This demonstrates that HZ susceptibility is largely governed by variations in the MHC. Furthermore, functional analyses revealed the upregulation of type I interferon and adaptive immune responses. These findings provide fresh molecular insights into the pathophysiology and the activation of innate and adaptive immune responses triggered by symptomatic VZV reactivation.

KEYWORDS

HLA association, MHC locus, Herpes Zoster, Type I interferon response, Gene expression analysis, Genome-wide association study

BACKGROUND

Herpes zoster (HZ, shingles) is caused by symptomatic reactivation of the varicella-zoster virus (VZV) and typically presents as a painful dermatomal rash, frequently accompanied by mild symptoms such as fever, headache, and fatigue [1]. Post-herpetic neuralgia (PHN), i.e. long-lasting neuropathic pain, is the most common complication of HZ and significantly adds to the disease burden [2, 3]. Naturally, over 95% of the population gets infected with VZV, resulting in chickenpox (varicella) [1, 4]. Upon resolution of a primary infection with VZV, the VZV particles gain access to neural ganglia where latency is established [1]. At a later age, VZV can reactivate to cause HZ [1].

The lifetime risk of developing HZ is between 25% and 30%, rising to 50% in those aged at least 80 years [2, 5]. Indeed, increasing age is a well-known risk factor for the development of HZ, thought to be a result of a decline in cellular immunity, i.e. immunosenescence [6, 7]. Yet, HZ also frequently occurs in immunocompromised patients [8] and otherwise healthy individuals too regardless of age [2, 9]. Besides waning cellular immunity due to aging, the composition of the T cell receptor (TCR) repertoire could also be a determining factor for the development of HZ. The TCR repertoire reflects the major histocompatibility complex (MHC) background of an individual [10]. Thus, depending on an individual's MHC constitution encoded by human leukocyte antigen (HLA) genes, each individual will be able to recognize a different set of (virus-derived) peptides and will generate a different immune response [10]. As such, an individual's HLA genetic profile could be a risk factor for the development of HZ [10]. The nine so-called classical MHC genes are *HLA-A*, *-B, -C* belonging to MHC class I, and *HLA-DPA1, -DPB1, -DQA1, -DQB1, -DRA*, and *HLA-DRB1* belonging to MHC class II [10]. A previous pilot study including 50 Belgian individuals with a history of HZ and a control population of 25,000 Belgians obtained via the Red Cross, already showed that *HLA-A*11* was protective, whereas *HLA-B*37* was a risk allele for the development of HZ [11]. In addition, a meta-analysis showed that *HLA-A*02* and *HLA-B*40* were protective, whereas *HLA-A*33* and *HLA-B*44* were risk alleles for PHN in Japanese patients [12].

Besides HLA genetic variation, other genetic polymorphisms, especially related to immune system genes, can impact an individual's likeliness to develop HZ. Following VZV infection, VZV is immediately sensed by

the innate immune system via pattern-recognition receptors (PRRs) like RNA Polymerase III [13, 14]. Subsequent activation of downstream pathways leads to the production of type I interferons (IFNs) and proinflammatory cytokines that inhibit viral replication and recruit inflammatory cells to the site of infection [13, 15]. Binding of these type I IFNs to their receptor ultimately leads to the induction of interferon-stimulated genes (ISGs) with direct antiviral effector functions [16]. The importance of an adequate type I IFN response to control VZV infection is illustrated by several studies reporting that mutations in *POLR3, TLR3, STAT1, STAT2, TYK2* and *NEMO* lead to increased susceptibility to VZV infection or even VZV viral encephalitis [17-20]. In addition to direct antiviral effector functions, type I IFNs also promote T cell expansion and activation [21]. CD4+ and CD8+ T effector cells are essential for recovery, additionally memory T cells that develop during a primary infection are hypothesized to help prevent VZV reactivation [22]. Ultimately, to identify potential biomarkers and functional mechanisms that dictate HZ susceptibility and its associated risk profile, a dual approach of genomic studies and functional transcriptomic analyses in HZ patients is essential.

METHODS

Participants

A total cohort of twenty-six herpes zoster patients aged between 18 and 70 years (median age 51 years; 13 men, 13 women) were prospectively recruited during an active HZ episode, as confirmed by a positive VZV PCR on skin swab or saliva (n=24) or by significantly elevated (> 4 times) VZV IgG serum titers (n=2). The Supplementary Methods give a detailed description of the included samples.

Neutralizing autoantibody assay

Serum from HZ patients and controls were co-incubated with either IFNα2, IFNβ or IFNꞶ at different doses (high dose 10ng/mL, lower dose 100pg/mL) in 10% plasma. Next, a neutralization assay using a luciferase-based system was used as described in Science Immunology, Bastard et al. 2021 [23]. The results were expressed as either positive (1) or negative (0) for the individual cytokines and doses and raw values were expressed as a percentage of the non-neutralizing samples of the day.

UK Biobank data collection

The whole-exome sequencing (WES) data was obtained from the population level exome OQFE variants (200k release) in the UK Biobank [24]. This data was used to perform a genome-wide association study (GWAS) (Figure 1) and HLA association study on multiple subsets of this data. Inclusion and exclusion criteria as well as a detailed description of the statistical analysis procedure can be found in the Supplementary Methods. Figure 1 gives an overview of the processed data used in the GWAS.

[Figure 1]

RNA extraction, 3' mRNA sequencing and NGS data processing

Whole blood RNA was extracted using the PaxGene blood RNA extraction kit (Qiagen) following the manufacturer's instructions. RNA samples were prepared with the QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen GmbH) for a NextSeq 500/550 sequencing run (high output v2.5 kit, 150 cycles, single read, Illumina). Raw data from the NextSeq was demultiplexed and further processed through an in-house developed 3'mRNA sequencing pipeline, after which differential gene expression and gene ontology analyses were performed. All experimental protocols and the full NGS analysis pipeline can be found in the Supplementary Methods.

RESULTS

HZ patients show no neutralizing autoantibodies against IFNα2, IFNβ or IFNϖ

Since autoantibodies against cytokines, have previously been associated with VZV central nervous system vasculopathy [25] and PHN [26] and anti-interferon antibodies have been shown to be present in a significant proportion of severe COVID-19 patients [23, 27], we determined neutralizing autoantibodies against IFNα2, IFNβ and IFNϖ in HZ patients during the HZ episode and one year later, and in controls. We did not detect autoantibodies against the interferons tested here, in any of the participants (Supplementary Table S3, Additional File 2).

Genome-wide association analysis shows MHC locus involvement in HZ pathophysiology

Given that the neutralizing autoantibodies did not show a host susceptibility for HZ, we conducted an extensive genome-wide association study (GWAS) on the 'white' subpopulation of the UK Biobank data. This GWAS included 2,684 HZ patients and 125,698 control participants with a total of 48,015 genetic variants that passed quality control. The results revealed a diverse variant distribution across the exome (Figure 2A). A distinct peak was observed on chromosome 6, containing a concentration of highly associated variants within the MHC locus (Figure 2C). Within this locus, eight variants exceeded the significance threshold of 1e-05, of which six remained significant even after Benjamini-Hochberg multiple testing correction (FDR $\leq$ 0.05).

The highest associated variant (p-value=2.521e-07) is located in the MHC class I polypeptide-related sequence B (*MICB*) gene. Furthermore, a cluster of intron variants and synonymous mutations for the *HLA-G* gene were observed (Figure 2C). The QQ plot shows no population stratification (λ = 1.066; Figure 2B), which suggests a complex inheritance mechanism and provides compelling evidence for the role of HLA genes as a potential risk factor for HZ development.

Moreover, our analysis was extended to the Asian and black subpopulations (Supplementary Figure S1, Additional File 1). Despite revealing several significantly enriched variants, the limited number of HZ patients in both groups (Asian=59 HZ; black=29 HZ) restricts the power of these analyses. While the Asian subpopulation showed no population stratification (λ = 1.006), the lambda value of the black subpopulation suggested multiple genetic backgrounds within the cohort (λ = 1.357). Therefore, although these findings hold promise for future research, their current reliability is limited due to the modest HZ sample sizes.

[Figure 2]

HLA association analysis reveals several protective and risk alleles for HZ

The GWAS analysis revealed an enrichment of variants in the MHC locus, prompting the analysis of HLA allele frequencies between HZ patients (n=2,826) and healthy controls (n=135,875) (Table 1). We identified four risk HLA alleles that were significantly enriched (p-value $\leq$ 0.05) in HZ patients: *HLA-A*01:01, HLA-B*07:02, HLA-C*07:02* and *HLA-B*40:01*. Additionally, five protective HLA alleles were found to be significantly depleted (p-value $\leq$ 0.05) in HZ patients: *HLA-A*33:03, HLA-DRB1*11:01, HLA-A*02:01, DRB3*02:02* and *HLA-DQB1*03:01*.

Furthermore, the analysis was repeated for the three subpopulations separately to analyze the effect of population stratification on these results (Supplementary Table S4, Additional File 2). Only three unique HLA alleles were consistently depleted in all three different cohorts, including *HLA-A*33:03, DRB1*11:01* and *DRB3*02:02* while none were consistently enriched in the three subpopulations. However, the Asian and black populations only consist of a small fraction of HZ patients compared to control participants (1.51% and 1.14% HZ patients respectively) resulting in decreased statistical power to show significant differences.

[Table 1]

Upregulation of interferon-stimulated genes dominates the immune response during HZ

Our findings point towards a complex inheritance pattern with unknown heritability for HZ. To uncover the functional mechanisms behind this complexity it is imperative to conduct functional genomics (i.e., transcriptomics). We first compared the gene expression profile from whole blood of HZ patients taken during the HZ episode with those one year after the HZ episode and found 841 DEGs (596 upregulated and 245 downregulated genes; Supplementary Table S5, Additional File 2) (Figure 3A). Seven of the most upregulated genes, *IFI44, IFI44L, IFI27, RSAD2, ISG15, SERPING1* and *SIGLEC1*, are ISGs produced by the innate immune system upon virus encounter.

In addition, *BATF2*, involved in the differentiation of CD8+ thymic conventional dendritic cells following infection, was also significantly upregulated during the acute HZ episode. Two genes associated with the antibody response: *MZB1* and *IGHG4* were significantly upregulated too, as well as RNA polymerase III (*POLR3*) subunit D (*POLR3D*) and GL (*POLR3GL*) and several additional ISGs: *Mx1, IFIT5, OASL, IFI35, IFI27*

and *STAT2* (Supplementary Table S5, Additional File 2). Finally, *CXCR3* which is primarily expressed on activated T lymphocytes and NK cells, was also significantly upregulated during HZ (Supplementary Table S5, Additional File 2). No notable downregulated genes related to viral or immunological functions were found. The heatmap shows clustering of gene expression profiles from blood taken during the acute HZ episode (HZ_XX, light blue) and clustering of those one year after HZ (HZ_XX.1Y, pink/red) (Figure 4).

[Figure 3]

[Figure 4]

Next, we compared the gene expression profiles of HZ patients during the active HZ episode with those of control participants and found 485 DEGs (341 upregulated and 144 downregulated genes: Supplementary Table S6, Additional File 2) (Figure 3B). Ten of the most upregulated genes including *IFI27, IFI44L* and *IGHG4*, were also found in the top upregulated genes when comparing acute HZ versus one year after HZ. Interestingly, *C4BPA*, an inhibitor of the classical and lectin pathways of the complement system, was upregulated by 2 log folds. The relationship between the logFC in the different conditions is shown in Figure 3C and 3D.

Finally, when the gene expression profiles of blood from HZ patients during convalescence were compared with controls, no DEGs were found (Supplementary Table S7, Additional File 2). In addition, as a control, we performed DGE analyses on samples from control participants taken one year apart from each other. Six DEGs were found which were all related to ribosomal processes and are unlikely to reflect biologically significant differences.

Functional enrichment analysis shows activation of host immunity to viral infection

When we compared gene expression profiles from patients during HZ with those one year after HZ, we found 843 DEGs. GO enrichment analysis of these DEGs revealed the involvement of 290 significant GO categories (top 200, Supplementary Table S8, Additional File 2) including several pathways related to viral processes and host immune responses, also those specific to viral infection. Table 2 shows the first 20

significant enriched GO categories that were related to viral processes and host immune responses. These results clearly indicate that viral processes were ongoing and that host immunity to viral infection was activated. Interestingly, six of these categories also came up in the GO enrichment analysis of the DEGs during HZ onset versus those of control participants (Table 3, Supplementary Table S9, Additional File 2). Not surprisingly, viral transcription is one of the top GO terms in both analyses.

[Table 2]

[Table 3]

DISCUSSION

In this project we set out to understand why otherwise healthy individuals might develop symptomatic VZV reactivation, i.e. herpes zoster. To achieve this goal, we applied a multidisciplinary approach. Initially, no autoantibodies against IFNα2, IFNβ, and IFNϾ were found. Therefore, we explored germline susceptibility as a cause for HZ development and identified an enrichment of genetic variants within the MHC locus of HZ patients. Their presence holds important value since the MHC locus harbors the HLA genes [28] which play a critical role in the adaptive immune response [29]. These results align with previous GWAS findings that also discovered genetic variation in the HLA region to be associated with HZ, including variants in the non-coding HLA Complex P5 (*HCP5*) gene and the *HLA-B* gene [30, 31]. Hence, it is possible that these variants modulate immune responses, thereby significantly influencing an individual's susceptibility to HZ.

The highest associated variant was located in *MICB* gene and an increase in *MICB* expression in herpesvirus-infected cells can lead to an enhanced T cell response [32]. Moreover, herpesvirus proteins can intracellularly retain *MICB*, thereby helping the infected cells evade NK and T cell responses [33]. These findings provide further evidence that variations in *MICB* expression or function could significantly impact the immune system's ability to mount an effective response against VZV reactivation [34]. Similarly, we discovered a cluster of genetic variants in the *HLA-G* gene, which has been associated with immune evasion of viral-infected cells through inhibition of host immune responses [35]. Interestingly, the clustering of variants within the MHC region suggests a collective contribution to the elevated risk of developing HZ through a complex inheritance pattern.

Next, we identified four enriched and five depleted HLA alleles in HZ patients, highlighting the importance of an individual's HLA background on HZ susceptibility. Prior smaller studies have linked some of the identified alleles to PHN in HZ patients [12, 36, 37]. Their findings show an enrichment of both *HLA-A*33* and *HLA-B*44* in PHN patients compared to those without PHN and control groups, including a significant depletion of *HLA-A*33* in PHN- HZ patients compared to PHN+ HZ patients. This supports our findings showing a significant depletion of *HLA-A*33* in our HZ cohort. Furthermore in these prior studies, *HLA-A*02* and *HLA-B*40* were significantly depleted in the PHN group, while displaying enrichment in HZ patients, similar to our findings. Since these studies primarily focused on PHN+ associated alleles, rather than examining the overall risk for HZ development, it is possible that these nuanced associations indicate alleles that may confer protection against HZ while increasing the risk of developing PHN, and vice versa, as has been reported in other studies [12, 38]. Further, our findings are based on one of the largest predominantly 'white' Caucasian cohorts to date, which differs significantly from these prior studies which were largely conducted in Asian populations. This is further supported by the fact that our analysis aligns remarkably with HLA associations identified in a European-focused study, where alleles *HLA-A*02:01* and *HLA-DRB1*11:01* were also found to be depleted in HZ [39]. Our research highlights the specificity of risk and protective alleles for different genetic backgrounds [40, 41]. Furthermore, it is vital to recognize that these results do not prove causality, nor unveil underlying biological mechanisms, proving the need for future studies to validate these findings. Studies aimed at assessing the impact of variants on HLA expression, antigen presentation machinery and the broader immune response will shed light on the precise (multifaceted) molecular mechanisms underlying their association with HZ. Additionally, expanding the sample sizes and using whole-genome data will increase statistical power and offer valuable insights into the functional consequences of these genetic variants.

Incorporating functional studies to help decipher the risk profile is necessary for as comprehensive understanding of the underlying biological mechanisms dictating HZ susceptibility. The pre-HZ to peri-HZ period can span several weeks, leading to a certain degree of heterogeneity in immune response among HZ samples. Despite this variability, we are still able to identify significant differences between the gene expression profiles of the different cohorts. Notably, ISGs such as *IFI44, IFI44L, IFI27, ISG15, RSAD2,*

SERPING1 and *SIGLEC1* showed distinct upregulation during the active HZ episode compared to one year after HZ. All of which are involved in modulation of the viral immune response through a variety of mechanisms, including negative feedback loop [42], type I IFN-induced apoptosis [43], regulation of the complement cascade and other pathways. Besides ISGs-upregulation we found that *POLR3D* and *POLR3GL*, were significantly upregulated during HZ. Mutations in *POLR3A, POLR3C, POLR3E* and *POLR3F* have been associated with susceptibility to VZV-induced encephalitis and pneumonitis [14, 44]. However, such associations for *POLR3* subtypes D or GL have not been described so far. It is interesting to note that although the upregulation of POLR3D and POLR3GL might not directly indicate an increased HZ susceptibility, it does signify the importance of this pathway in controlling HZ once reactivation has been initiated.

Furthermore, a recent report investigated the involvement of cellular calcium disorder in the development of PHN, revealing different DEGs [45]. In our cohort, several DEGs related to the GO term 'negative regulation of cytosolic calcium ion concentration' were upregulated during the symptomatic HZ episode: *CAB39, CACNA1D, CAMK1D* and *CACNB4*. Conversely, only one gene related to calcium signaling (*CIB1*) was upregulated one year after HZ. In addition, two genes related to calcium signaling (*S100B* and *CALHM6*) were upregulated during acute HZ compared to controls. Thus, our data suggest that indeed calcium signaling pathways are involved in HZ pathogenesis.

Whilst we found several DEGs during HZ compared to one year after HZ, we did not find any DEGs one year after HZ versus control participants, indicating that the patients have returned to baseline. However, some HZ patients had a very high expression of *C4BPA* (although overall not significant) compared to controls. Indeed, a recent study showed that direct binding of C4BP to influenza A virus subtype H1N1 suppressed viral infection, whereas binding to H3N2 subtype promoted viral infection [46]. Since we see upregulation of *C4BPA* in patients during HZ and 1 year after the HZ episode compared to the control group, further investigation is needed to determine whether C4BP may play a (pro- or anti-viral) role in VZV infection or reactivation. Analysis of these patterns reveals a compelling similarity in expression profiles shared between HZ and other viral infections (Supplementary Table S10, Additional File 1). Although this comparison is not exhaustive, it highlights that the HZ immune signature we identified is also present

across multiple viral infections. This is not unexpected but highlights the importance of these genes and pathways in the overall anti-viral immune response. However, to uncover the specific effects of these differentially expressed genes on the individual infections, further targeted studies are required.

GO enrichment analysis revealed upregulation of the type I IFN signaling pathway, ISG15-protein conjugation and complement activation during HZ. Interestingly, upregulation of the type I IFN response in HZ patients is in line with our data that showed no neutralizing autoantibodies against IFNα2 or IFNβ (nor IFNω) in HZ patients (or controls), implying a functional type I IFN response. Of note, a previous study in which autoantibodies against IFNα, INFγ, GM-CSF and IL-6 were determined in sera of HZ patients, found neutralizing autoantibodies in the HZ group suffering from PHN but not in the HZ group without PHN [26]. GO analysis also revealed enrichment of the interleukin-1 (IL-1) and -12 (IL-12) pathway during HZ, involved in inflammatory cell recruitment [47] and differentiation of naive T cells as well as enhancement of NK-and CD8+ T cells cytotoxicity [48, 49]. Related to the latter, the T cell receptor signaling pathway and the MHC Class I and Class II antigen presentation pathways were significantly enriched during HZ. Finally, B cell receptor signaling, and pathways involved in phagocytosis were also significantly enriched during HZ. This accentuates the critical role of ISGs and antigen processing pathways linked to B-cells and T-cells in driving susceptibility to HZ.

Recognizing one limitation of our approach would be the bulk sequencing approach, whereas a single cell RNA sequencing approach would allow the identification of cell-type specific transcriptional changes that occur. While fully unraveling the intricate mechanisms that underlie HZ development remains beyond our current capabilities, this research has already demonstrated several crucial components tied to the genetic background and biological pathways that play an important role in the risk profile and pathophysiology of HZ development and a multitude of other viral infections in both immunocompromised and healthy individuals.

DECLARATIONS

Ethics approval and consent to participate

This study was approved by the ethics board of the Antwerp University Hospital and University of Antwerp (reference number: 17/48/546). All methods were performed in accordance with the relevant guidelines and regulations when applicable. Written informed consent was obtained from all study participlksants.

Consent for publication

Not applicable.

Competing interests

JLC is an inventor on patent application PCT/US2021/042741, filed July 22, 2021, submitted by The Rockefeller University that covers diagnosis of susceptibility to, and treatment of, viral disease and viral vaccines, including COVID-19 and vaccine-associated diseases. BO, KL, and PM are employees of and/or stockholders of Immunewatch. Immunewatch is a spin-off company focusing on the development of AI-based models that give insights into the T cell response.

Availability of data and materials

The RNA sequencing data generated and analyzed during the current study is available in the Gene Expression Omnibus (GEO) repository, under GEO accession GSE242252. DGE and GO results are included in this published article (and its supplementary information files).

The whole-exome sequencing data that supports the GWAS and HLA findings of this study is available from the UK Biobank, but restrictions apply to the availability of this data, which was used under license for the current study, and so is not publicly available.

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Authors' contributions

The data used in this project was generated by MB, JS, EB, NM, OA, JLe, AB, JLa, EL, JW, KP, MPE, HJ, JLC, PB, AS. Data analysis was performed by RV, MB, KM, PB, PM; under the supervision of VVT, PP, PD, BO, PM, KL. The manuscript was written by RV, MB, EB, KM, BO, PM, KL and manuscript was edited by all.

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FIGURES AND FIGURE LEGENDS

UK Biobank data				
Ethnicity	**Variants**	**Participants**	**Cases**	**Controls**
White	**48,015**	**59,700**	**1,067**	**58,633**
		68,682	**1,617**	**67,065**
Asian	**43,187**	**2,078**	**22**	**2,056**
		1,889	**37**	**1,852**
Black	**72,336**	**1,075**	**11**	**1,064**
		1,469	**18**	**1,451**
		134,893	**2,772**	**132,121**

Figure 1: Overview of the UK Biobank data used for the genome-wide association studies. *The filtered dataset was divided into three ethnicity groups, based on the metadata included in the UK Biobank. The table shows the number of variants per group as well as the gender distribution over the control and HZ participants. Figure made using BioRender.*

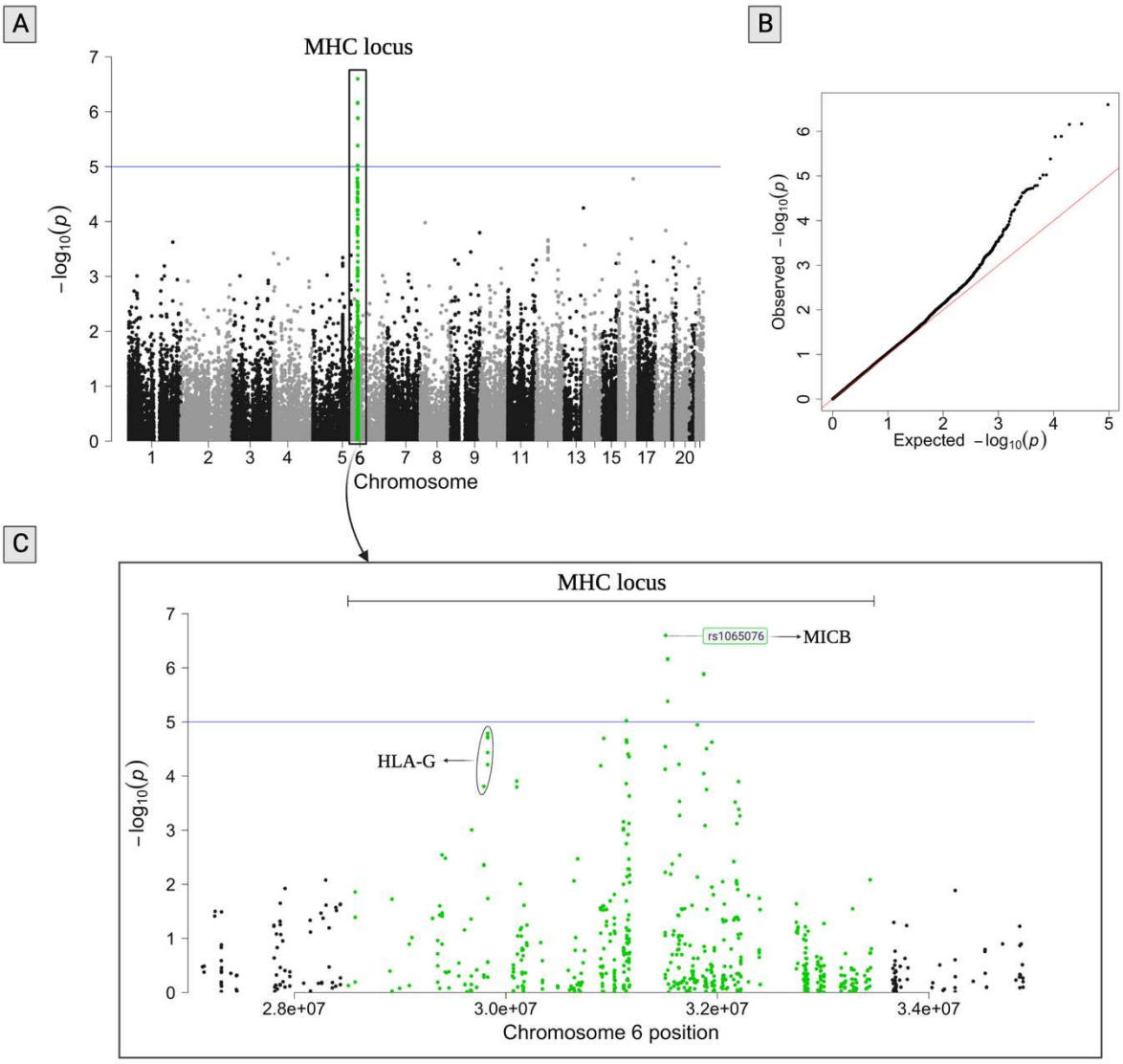

Figure 2: Manhattan plot of the 'white' subpopulation identified an association of the MHC locus with HZ. (A) *The Manhattan plot depicts different genetic variants (n=48,015) and their association with the development of herpes zoster. The plot was made using the 'qqman' R package.* ***(B)*** *QQ plot for the*

association analysis, depicting the expected versus the observed p-values. ***(C)*** *Manhattan plot of the zoomed in MHC locus on chromosome 6 identifies variants in HLA-related genes including MICB. The most highly associated variant (p=2.521e-07) is annotated using dbSNP. Green = MHC locus; MHC = Major Histocompatibility Complex; MICB = MHC class I polypeptide-related sequence B.*

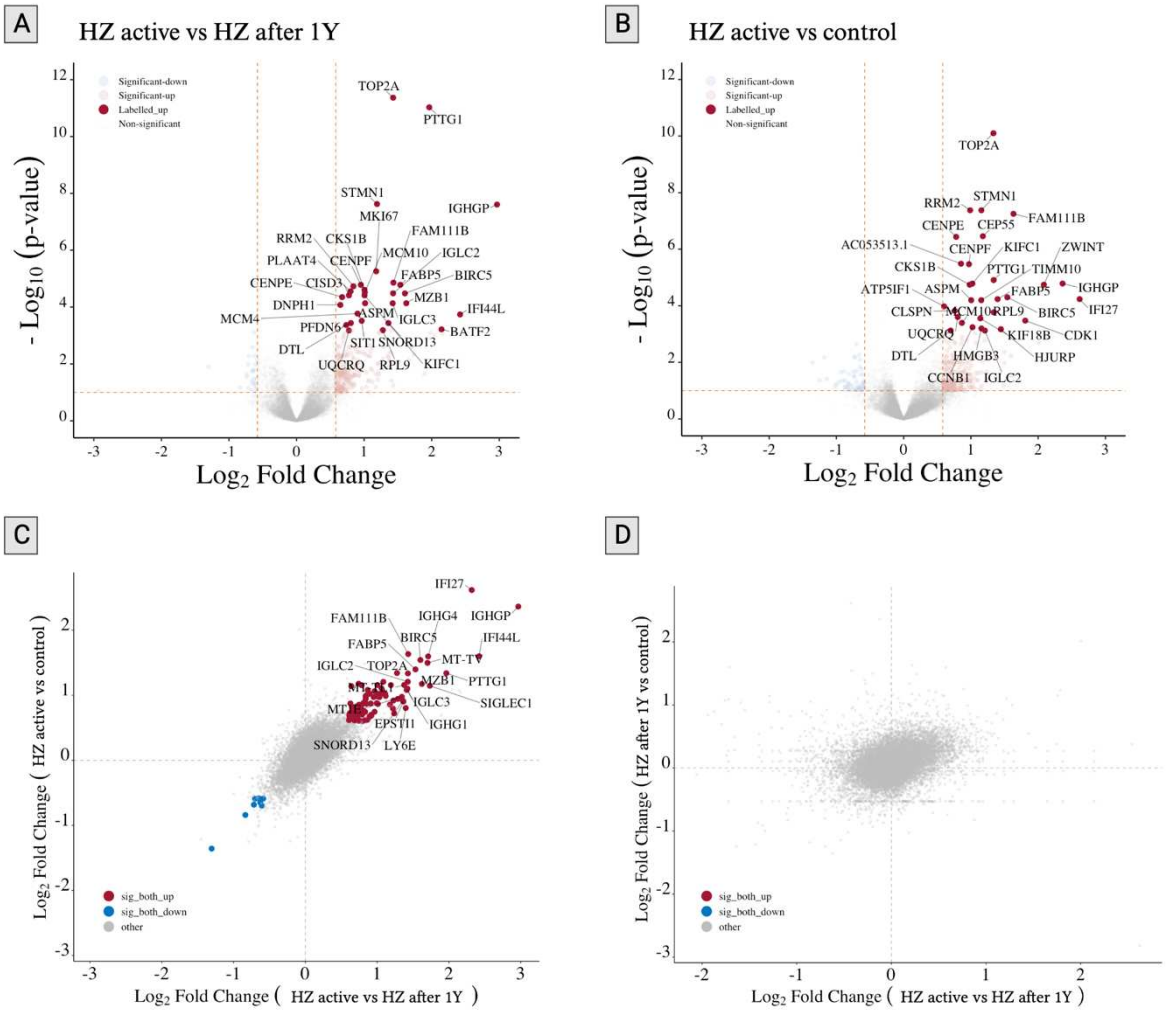

Figure 3: Differential expression analysis identifies overexpression of innate and adaptive immune transcripts in active HZ disease. *(A, B) Volcano plots represent top differential expression transcripts of (A) HZ Active vs HZ after 1Y and (B) HZ Active vs control. Dotted orange lines represent the FDR=0.1 and logFC±0.58 cut-off. (C,D) Scatter plot of logFC for (C) HZ Active vs HZ after 1Y compared to HZ Active vs*

control and (D) HZ Active vs HZ after 1Y compared to logFC of HZ after 1Y vs control. Significantly upregulated in both (red), significantly downregulated in both (blue); significantly dysregulated in both with opposite logFC (orange). Non-significant in at least one analysis (grey). Dotted grey lines represent x=0 and y=0 logFC. Grey dots denote non-significant transcripts (FDR>0.1), colored dots were significantly dysregulated. Figures were made using ggVolcanoR. HZ = Herpes Zoster; FDR = False discovery rate; logFC = Log2 fold change.

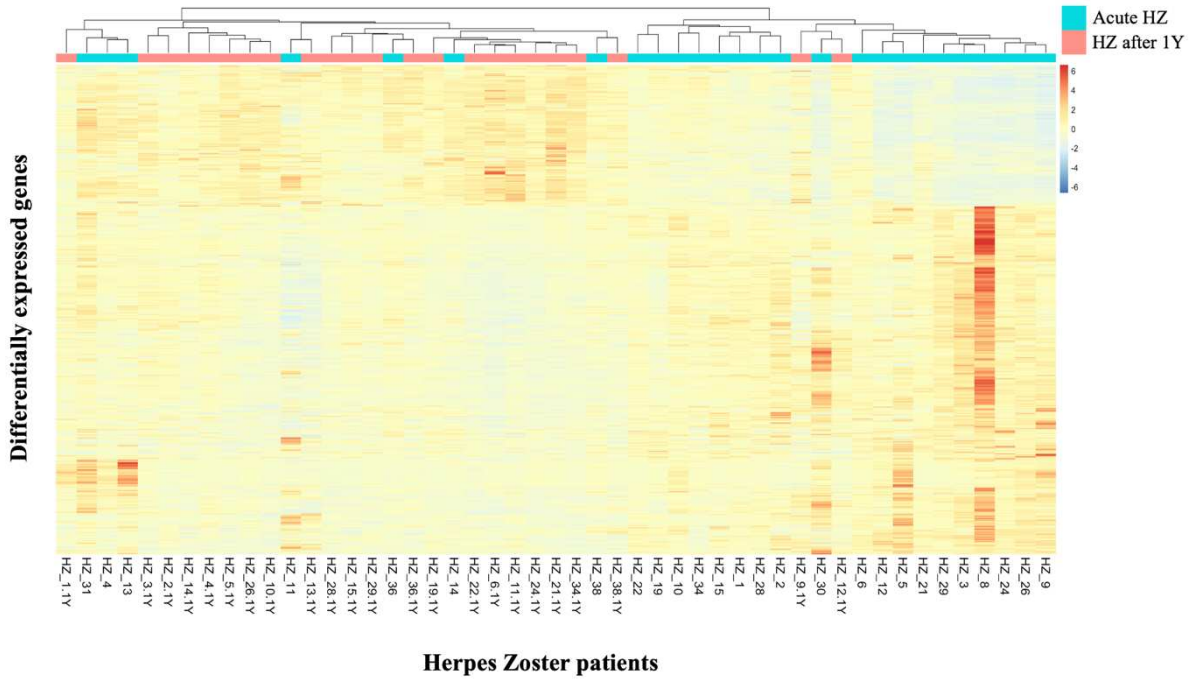

Figure 4: Heatmap of DEGs from blood taken during HZ compared to one year after HZ. *Heat map shows clustering of gene expression profiles from blood taken during the acute HZ episode (HZ_XX, light blue) and clustering of those one year after the HZ episode (HZ_XX.1Y, pink/red).*

TABLES AND TABLE LEGENDS

Table 1: All significant HLA alleles related to HZ development in HZ patients versus healthy participants.

	Adj. P values

HLA alleles	Odds ratio	95% CI	Enrichment	Depletion
A*01:01	1.170	1.082 – 1.264	0.0130	/
B*07:02	1.175	1.081 – 1.276	0.0130	/
C*07:02	1.163	1.072 – 1.261	0.0153	/
B*40:01	1.234	1.098 – 1.384	0.0195	/
A*33:03^	0.351	0.186 – 0.602	/	0.0021
DRB1*11:01^	0.736	0.621 – 0.867	/	0.0122
A*02:01	0.874	0.810 – 0.943	/	0.0264
DRB3*02:02^	0.860	0.787 – 0.939	/	0.0264
DQB1*03:01	0.873	0.803 – 0.948	/	0.0381

All significantly enriched or depleted HLA alleles in the complete UK Biobank dataset with their odds ratio and 95% confidence intervals. CI = Confidence Interval, ^ = significantly depleted in all three subpopulations

Table 2: Top GO categories related to viral processes and host immune responses of DEGs during HZ versus those one year after HZ.

GO.ID	Term	P value
GO:0019083	viral transcription	1.00E-20
GO:0002479	antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent	0.00014
GO:0050852	T cell receptor signaling pathway	0.0002
GO:0050853	B cell receptor signaling pathway	0.00029
GO:0035722	interleukin-12-mediated signaling pathway	0.00042
GO:0070498	interleukin-1-mediated signaling pathway	0.00056
GO:0060337	type I interferon signaling pathway	0.00094

GO:0051607	defense response to virus	0.00095
GO:0016032	viral process	0.00102
GO:0032020	ISG15-protein conjugation	0.00165
GO:0038096	Fc-gamma receptor signaling pathway involved in phagocytosis	0.00459
GO:0006955	immune response	0.01661
GO:0045059	positive thymic T cell selection	0.01864
GO:0006910	phagocytosis, recognition	0.02094
GO:0019886	antigen processing and presentation of exogenous peptide antigen via MHC class II	0.02287
GO:0006958	complement activation, classical pathway	0.02994
GO:0002230	positive regulation of defense response to virus by host	0.0316
GO:0046596	regulation of viral entry into host cell	0.03664
GO:0019064	fusion of virus membrane with host plasmamembrane	0.03672
GO:0050871	positive regulation of B cell activation	0.03989

Table 3: GO categories related to viral processes and host immune responses during HZ versus control participants

GO.ID	Term	P value
GO:0019083	viral transcription	7.10E-15
GO:0035722	interleukin-12-mediated signaling pathway	0.01785
GO:0016032	viral process	0.02103
GO:0002479	antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent	0.02176

GO:0006910	phagocytosis, recognition	0.02291
GO:0070498	interleukin-1-mediated signaling pathway	0.03111

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