Increased herpes zoster risk associated with poor HLA-A immediate early 62 protein (IE62) affinity

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Abstract

Around 30% of individuals will develop herpes zoster (HZ), caused by the varicella zoster virus (VZV), during their life. While several risk factors for HZ, such as immunosuppressive therapy, are well known, the genetic and molecular components that determine the risk of otherwise healthy individuals to develop HZ are still poorly understood. We created a computational model for the Human Leukocyte Antigen (HLA-A, -B and -C) presentation capacity of peptides derived from the VZV Immediate Early 62 (IE62) protein. This model could then be applied to a HZ cohort with known HLA molecules. We found that HLA-A molecules with poor VZV IE62 presentation capabilities were more common in a cohort of 50 individuals with a history of HZ compared to a nationwide control group, which equated to a HZ risk increase of 60%. This tendency was most pronounced for cases of HZ at a young age, where other risk factors are less prevalent. These findings provide new molecular insights into the development of HZ, and reveal a genetic predisposition in those individuals most at risk to develop HZ.

Keywords

Varicella zoster virus

Herpes zoster

HLA association

MHC peptide affinity

Introduction

Primary varicella-zoster virus (VZV) infection causes the common childhood disease chickenpox (varicella), which is a vaccine-preventable relatively benign disease in children with a small incidence rate of severe complications (Marin et al. 2008). After resolution of chickenpox, VZV remains dormant in the neural ganglia of its host. Reactivation of this dormant VZV can lead to the development of herpes zoster (HZ, also known as shingles), which is characterized by a painful rash following a dermatomal distribution. In most cases HZ resolves within a two to three week time period. However, in some cases the pain can persist for months or years, a debilitating condition known as post-herpetic neuralgia (PHN) (Drolet et al. 2010). The average burden of disease and cost related to HZ are estimated to be much higher than the burden of disease related to chickenpox (Bilcke et al. 2012). The incidence of HZ increases with age and HZ has been observed to occur more frequently in individuals taking immunosuppressive medications, malignancy, depression, diabetes mellitus, rheumatologic diseases, asthma, chronic cytomegalovirus infection and/or HIV infection (Thomas and Hall 2004; Ogunjimi et al. 2014b; Ogunjimi et al. 2015b; Ogunjimi et al. 2015a). Both ageing and the aforementioned risk factors are commonly expected to increase the risk of HZ by reducing the host's VZV-specific cellular immunity (Ouwendijk et al. 2013). Although it is very difficult to obtain data on VZV-specific cellular immunity preceding the onset of HZ, a prospective HZ-vaccination trial found lower VZV-specific immunity in participants who would later develop HZ compared to controls (Levin et al. 2008).

Another predisposing factor that has been investigated for HZ and other infectious diseases is the variation contained within the Human Leukocyte Antigen (HLA) molecules (Crosslin et al. 2015). HLA molecules are encoded in the Major Histocompatibity complex

(short arm chromosome 6p21.3). The three classical HLA class I molecules are encoded by the *HLA-A*, *HLA-B* and *HLA-C* loci. The proteins encoded by these loci are expressed on the surface of all nucleated cells and present short peptides, typically with a length of around nine amino acids, digested from intracellular proteins (Yewdell 2006). Upon presentation by a HLA-A, -B or -C molecule, the epitope can be recognized by the T-cell receptor of a CD8+ T-cell, which will in turn trigger an immune response. In the present study, we focus on these HLA molecules, as these are highly relevant for the defense against intracellular viral infections, such as latent VZV. Studies in Asian HZ patients have shown that certain *HLA* alleles are associated with a higher risk of PHN (Sato et al. 2002; Sumiyama et al. 2008; Meysman et al. 2015; Chung et al. 2016). The most common found association is that of increased risk with HLA-A*33:03 and HLA-B*44:03, with an odd ratio (OR) of 4.27 and 6.14 and a corrected P-value of 0.0007 and 0.0005 respectively within a single study (Sumiyama et al. 2008). So far no data on the association between *HLA* alleles and the risk of HZ or PHN in Caucasian patients have been published.

Various computational models exist to predict the binding affinity of a peptide to a specific HLA molecule with high accuracy (Lundegaard et al. 2007; Calis et al. 2013). In a previous study, we developed a framework to use such computational models to study and compare the presentation of pathogen-derived epitopes by various HLA molecules in a clinical context (Meysman et al. 2015). By using this framework, we showed that the HLA molecules that are associated with an increased risk for PHN in Asian populations seem to have a lower epitope affinity for the VZV proteome than those that decrease the risk for PHN (Meysman et al. 2015). However, this framework assumes equivalence among all VZV proteins. This is a fairly large assumption as different proteins are expressed during different stages in the VZV life cycle and therefore some proteins are more readily available for inducing an immune response. In a previous study, we

have shown that high affinity epitopes for the majority of common HLA molecules are not uniformly spread across VZV proteins (Meysman et al. 2016). Those proteins that are expressed during the immediate-early (IE) stage of the life cycle were found to be most depleted for immunogenic epitopes. The most prominently epitope-depleted of these IE proteins is the transcription factor Immediate Early 62 (IE62) protein (Meysman et al. 2016), which is encoded by two identical VZV genes, *ORF62* and *ORF71*. The IE62 protein is the largest VZV protein and it has been the subject of a large number of molecular and immunological studies. From these studies, it is known that the IE62 protein plays an important role in the VZV infection cycle and is found in high abundance in infected cells (Baudoux et al. 1995; Yang et al. 2008). In addition, IE62 has been identified as a major contributor to the CD8+ T-cell-mediated host response to VZV (Arvin et al. 1991; Bergen et al. 1991; Sabella et al. 1993; Arvin et al. 2002; Frey et al. 2003; van der Heiden et al. 2009; Ogunjimi et al. 2014a). Genomic analysis has also shown that the IE62 protein has acquired the bulk of mutations between varicella strains and is thus under a different evolutionary pressure than the other VZV proteins (Gomi et al. 2002; Yamanishi 2008). This protein also shares a high amino acid homology to the IE3 protein in herpes simplex (Kinchington et al. 1992). Thus within the context of HZ, the IE62 protein is the prime candidate for further investigation in the context of HLA affinity predictions.

In this study, we investigated the molecular relationship between the *HLA* alleles and the occurrence of HZ at an individual level. Specifically, the VZV IE62 presentation capabilities of the HLA-A, -B, and -C molecules for a large HZ cohort were modeled and compared to that of the population background. The Belgian population serves as an ideal testing ground, as the varicella vaccine is not part of the recommended vaccination program. Estimates place the

number of children vaccinated against VZV at less than 2.5% (Blumental et al. 2016), thus it can be expected to not have a great effect within the population.

Results

Poor IE62 epitope presentation by HLA-A molecules

The capacity for each HLA-A, HLA-B and HLA-C molecule to bind VZV IE62 peptides was modeled and converted into a single score. The resulting IE62 rank affinity score (RAS) is a measure for each HLA molecule and each individual to present epitopes derived from the IE62 protein, with larger ranks denoting worse IE62 presenters. The IE62 ranking was calculated for 203 HLA molecules, and can be found in Online Resource 1. Around 28% of all HLA molecules had an IE62 rank of less than 10, which indicates the presence of a strong binding peptide within the protein for this HLA molecule. The majority of these strong binders were from the *HLA-B* locus. In addition, less than 15% of molecules had an IE62 rank worse than 55, which corresponds to HLA molecule with lower binding IE62-derived peptides, but the majority of these were from the *HLA-A* locus. These values can be directly compared to a VZV-wide RAS, which represents the presentation capacity for all VZV peptides and has been found to follow PHN risk in some cases (Meysman et al. 2015). The correlation between the IE62 rank and the VZV-wide rank was poor (Pearson's correlation R=-0.05, p-value=0.457), which suggests that these models are capturing different aspects of the HLA epitope binding capacity.

Traditional HLA association analysis has a low specificity

HLA-A, -B, and -C allele typing was performed in 50 individuals with a history of HZ. In addition, *HLA-A, -B, and -C* allele data from 26 644 volunteer donors from the Belgian region with an unspecified history of HZ was used for the control "background" population. An exploratory comparison of *HLA* distributions between the cohort and the background population suggests an enrichment of *B*37* in the HZ group, while *A*11* has been depleted (see Table 1). However this analysis has a poor specificity like most *HLA* associations studies due to the large

variety in *HLA* types compared to the sample size. Therefore no strong statistical conclusions can be drawn from these associations. These results are further confounded by the fact that nothing is known about the HZ-status in the control population. A significant proportion of the general population was/is at risk for HZ (either in the past or future) implying that the "true" *HLA*-associated genetic biases could actually be more significant than our analysis shows.

HZ incidence correlates with HLA-A IE62 affinity

Given the important role of the IE62 protein within the VZV life cycle and recognition by the host's immune system, it is our hypothesis that the IE62 RAS values play a role within HZ incidence. Fitting a logistic regression model to discriminate the HZ cohort from the background population using the IE62 RAS values revealed a strong contribution from the HLA-A IE62 rank (df=1046 z=2.365 p-value=0.0180), but little to no contribution from the HLA-B (z=1.568 p-value=0.117) and HLA-C IE62 rank (z=-0.865 p-value=0.387). The model showed that the HZ cohort had a worse HLA-A IE62 rank than the background. The distribution of the HLA-A IE62 rank on the individual level is distinctly bimodal, as shown in Figure 1. Some individuals have HLA-A molecules that feature a target IE62 peptide with high affinity and thus a likely candidate for a CD8+ mediated-immune response. The remaining HLA-A molecules have markedly poor IE62 presentation capacity. Comparing the HLA-A IE62 rank does indeed reveal that there was a higher frequency of individuals with a high rank in the HZ cohort than in the background, as suggested by the fitted regression model. If we set the cut-off at 55 between good and poor IE62 ranks (based on the gap in the bimodal distribution of the scores), we find that 19 HZ patients exceed it while the background (including about 20-30% individuals who will develop HZ during lifetime) would suggest that we only find on average 12 for this sample size (n=50, binomial test p-value=0.0288). These results show that individuals with HLA-A molecules that

poorly present IE62-derived epitopes are enriched in the HZ group. If these results indicate a global trend, then individuals with a HLA-A IE62 rank higher than 55 have a 60% higher chance to develop HZ in their lifetime than a random individual [Bayes' Rule, 95% confidence interval: 4%-122%].

VZV-wide affinity is a poor fit for HZ risk

The same trend cannot be found when the HLA-derived VZV-wide RAS values are used, where all the VZV peptides are treated as equivalent and whose values matched with risk/protection alleles for PHN. Fitting a logistic regression model to discriminate the HZ cohort based on these VZV-wide RAS values revealed a poor fit with little to no contribution from any of the three studied loci (HLA-A coefficient z=0.145 p-value=0.884; HLA-B z=0.047 p-value=0.963; HLA-C z=-0.582 p-value=0.561; df=1046).

The HLA-A IE62 rankings themselves provide insight into why an IE62-focused model is a better fit than the VZV-wide RAS model. A good example is HLA-A*11:01, which was infrequent within the HZ cohort. The VZV-wide RAS value for this allele was 92.76. This rank signifies that there are only a handful of VZV peptides that HLA-A*11:01 can bind with a high affinity when compared to the expected human cellular protein composition. However one of these high affinity peptides is the nonamer SALNQFYQK from residue 554 to 562 in the IE62 protein. It is the 20th highest affinity nonamer in the entire VZV proteome for HLA-A*11:01 and was assigned a rank of 28 based on the human peptide background. Further, while the affinity model used here did not explicitly use this information, the SALNQFYQK nonamer is also predicted to have a high cleavage probability, namely 0.97 (Stranzl et al. 2010). This signifies that this peptide is a likely product from the proteosomal cleavage of IE62, allowing it to be captured by the MHC complex and subsequently presented on the cell surface. These results

therefore suggest that HLA-A*11:01 is a poor presenter of VZV epitopes in general, but very good at presenting specific epitopes for IE62, which may decrease HZ risk.

Poor HLA-A IE62 presentation is most prominent in young HZ patients

Age is a known strong risk factor for the development of HZ, thus we can expect that any genetic factor will have a higher contribution in young and healthy individuals. To validate this hypothesis and the previous findings, the HZ cohort was split into two groups, those that were younger than 35 years when HZ first occurred (7 individuals) and those that were 35 years or older (43 individuals). Comparative analysis showed that the younger group of the HZ cohort had a worse HLA-A IE62 rank (average = 67) than those that were older (average = 43), visualized in Figure 2. Despite the small sample size of young volunteers in this study, statistical tests showed a significant difference (t=-3.516 df=13.462 p-value = 0.0036). These results suggest that poor VZV presentation by the HLA-A molecule increases the risk of HZ occurring at a younger age.

Discussion

About 20-30% of individuals will develop HZ during their lifetime. Although medical conditions and therapies that suppress the cellular immunity are known to be associated with a higher risk for HZ, it remains difficult to predict in otherwise healthy individuals who will develop HZ. In this study, we identified a molecular basis for the genetic susceptibility to develop HZ in otherwise healthy Belgian individuals.

VZV immediate-early protein 62 (IE62) is known to be important for the CD8+ mediated immunity against VZV (Frey et al. 2003) and recent studies have suggested that VZV evolution has led to a profound depletion of high-affinity MHC-I restricted epitopes within this protein (Meysman et al. 2015; Meysman et al. 2016). In the present study, we were able to show that HZ patients were more prone to have HLA-A molecules that are poor presenters for the VZV IE62 protein than the baseline Belgian population. Indeed our results showed that individuals with poor IE62 presentation capacity have a 60% higher risk to develop HZ in their lifetimes. Furthermore, this genetic bias could be validated by considering those individuals that developed HZ at a relatively young age (<35y), where other age-related HZ risk factors are not a factor. The trend that younger HZ cases are more often associated with poor IE62 presentation fits well in our hypothesis. However a larger follow-up with more HZ cases at a young age should be a future priority to exclude the possibility of spurious correlation. This found HZ-IE62 association does contrast with the previously described association between PHN and HLA presentation capacities for the complete VZV proteome. These findings match previous observations that the *HLA* alleles associated with PHN risk in Asian populations do not seem to impact HZ risk (Sumiyama et al. 2008; Chung et al. 2016). Taken all results together, we hypothesize now that sudden VZV reactivation is controlled, at least in part, by VZV IE62 specific CD8+ T-cells,

whereas protection from ongoing inflammation (causing PHN), once VZV reactivation has occurred, is best controlled by a broader VZV-specific CD8+ T-cell immunity.

Contribution from either the HLA-B or HLA-C rank affinity score to HZ risk was undetected in this study, which might have one of three possible explanations. Firstly, the contribution to the individual variance that exists in the immune response against HZ from these two alleles may be limited. In this case, these loci indeed play an important role in the defense against HZ but it is the same regardless of the specific variant that is present. However this is not supported by those HLA-B alleles that have been found associated with VZV-causative diseases in this and other studies (Ozawa et al. 1999; Sumiyama et al. 2008). Secondly, the RAS model may be insufficient to capture the diversity of these alleles and an alternative model might have more success. Thirdly, the contributions of these loci may be smaller than that of the HLA-A locus and this study may not have had sufficient power to discern them.

The advantage of the presented approach is that the rank affinity scores (RAS) values based on the HLA presentation capacity puts forward a clear molecular explanation why some individuals are more susceptible to HZ than others. Individuals are more at risk if their HLA-A molecule is a poor presenter of IE62-derived epitopes. As only 37% of the test volunteers with a HZ history were typed as a poor IE62 presenter, this does still leave room for other factors that influence the symptomatic reactivation of VZV in otherwise healthy individuals. This includes factors not included in this study, such as exogenous boosting of the cellular immunity (after natural exposure to chickenpox patients), which has recently been shown to last for up to a year in 25% of elderly Belgian individuals (Ogunjimi et al. 2017). In addition, there is no guarantee that the presentation tendency we found in these individuals is entirely causal. In this case, IE62 presentation capability may be a proxy for the presentation of immediate-early epitopes in

general, i.e. not limited to that of IE62. For example, those alleles with a high IE62 affinity may feature other high affinity epitopes in the same protein or other immediate-early proteins as they feature similar amino acid composition. The presence of a single high affinity epitope does not preclude the presence of others, yet the absence of such a peptide does. However offering a proteome wide ranking of high affinity epitopes, as with the VZV-wide rank, may dilute the importance of specific proteins or epitopes and thus may offer a poor model for the epitopes that actually trigger an immune response. The relationships we observed fit well with the current knowledge on VZV and the importance of the IE62 protein for the immune response (Bergen et al. 1991; Frey et al. 2003). Thus in lieu of a different model that better explains the data or conflicting data sources, the assumption that the model captures a molecular process holds.

HLA disease associations are ubiquitously studied and well known for a large variety of conditions. However thus far there is a lack of knowledge concerning the molecular basis of increased or decreased risk of disease in the presence of some HLA molecules. Most HLA association analyses conclude at identifying those common *HLA* alleles that may be enriched or depleted in patient cohorts, without a clear testable hypothesis on how these alleles impact disease risk. The presented study has managed to expand upon traditional *HLA* association studies by directly modeling the epitope presentation capabilities of each HLA molecule. By directly summarizing the presentation capability into a single measure, HLA-dependent associations can be tested and identified with a fraction of the samples needed for a more traditional *HLA* association study, coupled with greater insight. The presented approach thus has great potential for use in other infectious diseases.

Materials and Methods

Volunteer recruitment and study design

For this study, 50 unrelated Belgian individuals (median age 56 years – range [20-73] – 31/50 women) with a history of HZ during adulthood were recruited for *HLA* typing. The study was designed so that doubling the HZ risk with poor RAS scores could be picked up at 5% significance with 95% power. None of the recruited individuals reported a history of malignant disease, none were undergoing treatment with immunosuppressive drugs and none were vaccinated against VZV. This study was approved by the ethical committee of the Antwerp University Hospital. Informed consent was obtained from all individual participants included in the study.

HLA typing

HLA-A, -B, and -C types of the study participants were determined using SeCore® Sequencing Kits (Thermo Fisher Scientific, Waltham, MA). Capillary electrophoresis was performed using 3130xl Genetic Analyzer® (Applied Biosystems, Foster City, CA). HLA SBT uType software, linked to the IMGT® database (International ImMunoGeneTics information system® http://www.imgt.org), was used for the *HLA* typing from the electropherograms. The full *HLA* typings can be found in Online Resource 2.

HLA background population

The *HLA* distribution of the Belgian population was provided by HILA, the HLA laboratory of the Belgian Red Cross-Flanders and is based on the typing of 26 644 individuals from the registry for candidate stem cell donors (CSCD) enrolled from September 2008 until April 2015. The upper age for the registry was 50 years; the mean age for the candidates in the registry was

34.7 years and the median age 33.7 years. This population can be considered as a genetic representative for the Belgian population as it included candidate donors from all regions. The *HLA* typing was done up to the 2nd field for all major *HLA* alleles, and haplotype frequency data was derived up to 1st field level. These data formed the basis of the reported population frequencies used to calculate the enrichment of specific alleles in the HZ cohort based on a two-sided binomial test in an exploratory analysis.

HLA-epitope affinity rank

The framework to predict the affinity of a given HLA molecule for the VZV proteome is similar to that described in our previous publication (Meysman et al. 2015). It is based on the principle that each VZV epitope is competing with other peptides within the intracellular environment for HLA presentation. As intracellular background, we used a set of 109 highly abundant human proteins based on proteomics data collected from the PRIDE database (Martens et al. 2005). The HLA binding affinity for each nonamer with the VZV proteome and the set of 109 human proteins was predicted using NetCTLpan (Stranzl et al. 2010). The human-derived nonamers were sorted and assigned a rank. Each VZV nonamer was then scored based on the ranking it would receive when compared to the human nonamer set, so that:

$$RAS_i = rank(v_i)\ in\ \{Aff(H)\ \cup\ Aff(v_i)\}$$

Where RAS_i is the assigned rank affinity score of the VZV peptide v_i. The function *rank()* is the index of the ordered set starting at index 0. The function *Aff()* is the HLA binding affinity prediction of a given peptide. The set *H* is the set of peptides contained in the highly abundant human proteins.

For the IE62 rank score, the best ranking nonamer within the IE62 protein was taken as representative for the IE62 presentation capacity. This is based on the principle that the presence of a single high-affinity epitope does not preclude the presence of other high-affinity epitopes, yet the absence of such an epitope does. To compare to the presentation capacity of the entire VZV proteome, we also assigned a VZV-wide rank affinity score for each HLA variant equal to the average of the top 200 high affinity epitopes. For both the VZV-wide affinity rank and the IE62 rank, the best ranking allele of the two alleles in an individual were taken as the score for the entire locus (*HLA-A*, *HLA-B*, or *HLA-C*). Inclusion of the worst ranking alleles in the regression models did not increase their information content or fit, and thus were excluded from the main analysis.

Statistical testing

To analyse the contribution of the three studied *HLA* loci on the occurrence of HZ, we used a logistical regression. As background, a set of 1000 randomized individuals was constructed from the Belgian population haplotype and allele frequencies. Repeated randomizations showed that this procedure is a robust background that delivered similar results each time. The used logistical regression is defined by the following formula:

$$HZ = 1/(1 + e^{-(c_a A + c_b B + c_c C + c_0)})$$

With *HZ* the binary outcome variable that distinguishes the HZ cohort (1) from the background (0), and *A*, *B*, *C* the respective IE62 rank affinity scores for respectively the HLA-A, HLA-B, and HLA-C proteins. The contributions of the coefficients to the model fit (c_a, c_b, and c_c) were estimated through the ANOVA test. Individuals were divided into three age categories based on the known observation that HZ risk starts increasing at age 50 (Kawai et al. 2014) and an additional division halfway in the lower-bound group at age 35.

References

Arvin AM, Sharp M, Moir M, et al (2002) Memory Cytotoxic T Cell Responses to Viral Tegument and Regulatory Proteins Encoded by Open Reading Frames 4, 10, 29, and 62 of Varicella-Zoster Virus. Viral Immunology 15:507–516. doi: 10.1089/088282402760312377

Arvin AM, Sharp M, Smith S, et al (1991) Equivalent recognition of a varicella-zoster virus immediate early protein (IE62) and glycoprotein I by cytotoxic T lymphocytes of either CD4+ or CD8+ phenotype.

Baudoux L, Defechereux P, Schoonbroodt S, et al (1995) Mutational analysis of varicella-zoster virus major immediate-early protein IE62. Nucleic Acids Research 23:1341–1349. doi: 10.1093/nar/23.8.1341

Bergen RE, Sharp M, Sanchez A, et al (1991) Human T cells recognize multiple epitopes of an immediate early/tegument protein (IE62) and glycoprotein I of varicella zoster virus. Viral Immunol 4:151–166. doi: 10.1089/vim.1991.4.151

Bilcke J, Ogunjimi B, Marais C, et al (2012) The health and economic burden of chickenpox and herpes zoster in Belgium. Epidemiology and infection 140:2096–109. doi: 10.1017/S0950268811002640

Blumental S, Sabbe M, Lepage P (2016) Varicella paediatric hospitalisations in Belgium: a 1-year national survey. Archives of Disease in Childhood 101:16–22. doi: 10.1136/archdischild-2015-308283

Calis JJ a., Maybeno M, Greenbaum J a., et al (2013) Properties of MHC class I presented peptides that enhance immunogenicity. PLoS computational biology 9:e1003266. doi: 10.1371/journal.pcbi.1003266

Chung HY, Song EY, Yoon JA, et al (2016) Association of human leukocyte antigen with postherpetic neuralgia in Koreans. APMIS 124:865–871. doi: 10.1111/apm.12575

Crosslin DR, Carrell DS, Burt A, et al (2015) Genetic variation in the HLA region is associated with susceptibility to herpes zoster. Genes and immunity 16:1–7. doi: 10.1038/gene.2014.51

Drolet M, Brisson M, Schmader KE, et al (2010) The impact of herpes zoster and postherpetic neuralgia on health-related quality of life : a prospective study. Canadian Medical Association Journal 182:1731–1736. doi: 10.1503/cmaj.091711

Frey CR, Sharp MA, Min AS, et al (2003) Identification of CD8+ T cell epitopes in the immediate early 62 protein (IE62) of varicella-zoster virus, and evaluation of frequency of CD8+ T cell response to IE62, by use of IE62 peptides after varicella vaccination. The Journal of infectious diseases 188:40–52. doi: 10.1086/375828

Gomi Y, Sunamachi H, Mori Y, et al (2002) Comparison of the complete DNA sequences of the Oka varicella vaccine and its parental virus. Journal of virology 76:11447–59. doi: 10.1128/JVI.76.22.11447-11459.2002

Kawai K, Gebremeskel BG, Acosta CJ (2014) Systematic review of incidence and complications of herpes zoster: towards a global perspective. BMJ open 4:e004833. doi: 10.1136/bmjopen-2014-004833

Kinchington PR, Hougland JK, Arvin AM, et al (1992) The varicella-zoster virus immediate-early protein IE62 is a major component of virus particles. Journal of virology 66:359–66.

Levin MJ, Oxman MN, Zhang JH, et al (2008) Varicella-zoster virus-specific immune responses in elderly recipients of a herpes zoster vaccine. The Journal of infectious diseases 197:825–35. doi: 10.1086/528696

Lundegaard C, Lund O, Kesmir C, et al (2007) Modeling the adaptive immune system: predictions and simulations. Bioinformatics (Oxford, England) 23:3265–75. doi: 10.1093/bioinformatics/btm471

Marin M, Meissner HC, Seward JF (2008) Varicella Prevention in the United States: A Review of Successes and Challenges.

Martens L, Hermjakob H, Jones P, et al (2005) PRIDE: the proteomics identifications database. Proteomics 5:3537–45. doi: 10.1002/pmic.200401303

Meysman P, Fedorov D, Van Tendeloo V, et al (2016) Immunological evasion of immediate-early varicella zoster virus proteins. Immunogenetics 68:483–486. doi: 10.1007/s00251-016-0911-4

Meysman P, Ogunjimi B, Naulaerts S, et al (2015) Varicella-zoster virus-derived major histocompatibility complex class I-restricted peptide affinity is a determining factor in the HLA risk profile for the development of postherpetic neuralgia. Journal of virology 89:962–9. doi: 10.1128/JVI.02500-14

Ogunjimi B, Buntinx F, Bartholomeeusen S, et al (2015a) Herpes zoster is associated with herpes simplex and other infections in under 60 year-olds. The Journal of infection 70:171–177. doi: 10.1016/j.jinf.2014.08.016

Ogunjimi B, Hens N, Pebody R, et al (2015b) Cytomegalovirus seropositivity is associated with herpes zoster. Human Vaccines & Immunotherapeutics 11:1394–1399. doi: 10.1080/21645515.2015.1037999

Ogunjimi B, Smits E, Heynderickx S, et al (2014a) Influence of frequent infectious exposures on general and varicella-zoster virus-specific immune responses in pediatricians. Clinical and

vaccine immunology : CVI 21:417–26. doi: 10.1128/CVI.00818-13

Ogunjimi B, Theeten H, Hens N, Beutels P (2014b) Serology indicates cytomegalovirus infection is associated with varicella-zoster virus reactivation. Journal of medical virology 86:812–9. doi: 10.1002/jmv.23749

Ogunjimi B, Van den Bergh J, Meysman P, et al (2017) Multidisciplinary study of the secondary immune response in grandparents re-exposed to chickenpox. Scientific Reports 7:1077. doi: 10.1038/s41598-017-01024-8

Ouwendijk WJ, Laing KJ, Verjans GM, Koelle DM (2013) T-cell immunity to human alphaherpesviruses. Current Opinion in Virology 3:452–460. doi: 10.1016/j.coviro.2013.04.004

Ozawa a, Sasao Y, Iwashita K, et al (1999) HLA-A33 and -B44 and susceptibility to postherpetic neuralgia (PHN). Tissue antigens 53:263–8.

Sabella C, Lowry PW, Abbruzzi GM, et al (1993) Immunization with the immediate-early tegument protein (open reading frame 62) of varicella-zoster virus protects guinea pigs against virus challenge. Journal of virology 67:7673–6.

Sato M, Ohashi J, Tsuchiya N, et al (2002) Association of HLA-A*3303-B*4403-DRB1*1302 haplotype, but not of TNFA promoter and NKp30 polymorphism, with postherpetic neuralgia (PHN) in the Japanese population. Genes and immunity 3:477–81. doi: 10.1038/sj.gene.6363890

Stranzl T, Larsen MV, Lundegaard C, Nielsen M (2010) NetCTLpan: pan-specific MHC class I pathway epitope predictions. Immunogenetics 62:357–68. doi: 10.1007/s00251-010-0441-4

Sumiyama D, Kikkawa EF, Kita YF, et al (2008) HLA alleles are associated with postherpetic

neuralgia but not with herpes zoster. The Tokai journal of experimental and clinical medicine 33:150–3.

Thomas SL, Hall AJ (2004) What does epidemiology tell us about risk factors for herpes zoster? The Lancet infectious diseases 4:26–33.

van der Heiden PLJ, de Boer R, van der Steen DM, et al (2009) Identification of varicella-zoster virus-specific CD8 T cells in patients after T-cell-depleted allogeneic stem cell transplantation. Journal of virology 83:7361–4. doi: 10.1128/JVI.02662-08

Yamanishi K (2008) Molecular Analysis of the Oka Vaccine Strain of Varicella-Zoster Virus. The Journal of Infectious Diseases 197:S45–S48. doi: 10.1086/522122

Yang M, Hay J, Ruyechan WT (2008) Varicella-zoster virus IE62 protein utilizes the human mediator complex in promoter activation. Journal of virology 82:12154–63. doi: 10.1128/JVI.01693-08

Yewdell JW (2006) Confronting complexity: real-world immunodominance in antiviral CD8+ T cell responses. Immunity 25:533–43. doi: 10.1016/j.immuni.2006.09.005

Tables

Table 1. HLA allele counts for the HZ cohort.

HLA	% in population	% in HZ+	Odds ratio [95% Confidence interval]	P-value
A*01	15.38	18	1.17 [0.717, 1.752]	0.49
A*02	30.35	27	0.89 [0.613, 1.213]	0.52
A*03	15.95	23	1.442 [0.951, 2.037]	*0.074*
A*11	5.45	1	0.183 [0.005, 0.999]	*0.045*
A*23	2.03	3	1.478 [0.307, 4.196]	0.46
A*24	8.92	9	1.009 [0.471, 1.838]	1
A*25	1.38	2	1.449 [0.176, 5.1]	0.4
A*26	2.38	2	0.84 [0.102, 2.957]	1
A*29	3.49	2	0.573 [0.07, 2.017]	0.59
A*30	2.21	3	1.357 [0.282, 3.854]	0.49
A*31	2.72	1	0.368 [0.009, 2.002]	0.53
A*32	3.77	3	0.796 [0.165, 2.259]	1
A*33	1.12	1	0.893 [0.023, 4.862]	1
A*68	4.29	5	1.166 [0.383, 2.63]	0.62
B*07	12.78	16	1.252 [0.738, 1.931]	0.37
B*08	10.17	13	1.278 [0.699, 2.085]	0.32
B*13	2.26	2	0.885 [0.108, 3.114]	1
B*14	2.55	1	0.392 [0.01, 2.136]	0.53
B*15	8.62	9	1.044 [0.487, 1.902]	0.86
B*18	5.44	6	1.103 [0.411, 2.317]	0.82
B*27	3.58	3	0.838 [0.174, 2.379]	1
B*35	9.77	12	1.228 [0.651, 2.049]	0.4
B*37	1.22	5	4.098 [1.347, 9.249]	*0.0078*
B*38	1.57	2	1.274 [0.155, 4.483]	0.67
B*39	2.6	1	0.385 [0.01, 2.095]	0.53
B*40	7.4	8	1.081 [0.475, 2.048]	0.71
B*44	13.17	7	0.532 [0.217, 1.055]	*0.075*
B*50	1.11	3	2.703 [0.561, 7.674]	0.1
B*51	7.2	7	0.972 [0.397, 1.929]	1
B*53	0.51	1	1.961 [0.05, 10.678]	0.4
B*55	1.84	1	0.543 [0.014, 2.96]	1
B*56	0.61	1	1.639 [0.041, 8.928]	0.46

B*57	3.5	1	0.286 [0.007, 1.556]	0.27
B*58	0.89	1	1.124 [0.028, 6.119]	0.59
C*01	2.83	6	2.12 [0.789, 4.453]	*0.065*
C*02	5.16	6	1.163 [0.433, 2.442]	0.65
C*03	14.97	13	0.868 [0.475, 1.416]	0.68
C*04	12.11	15	1.239 [0.714, 1.943]	0.36
C*05	8.09	7	0.865 [0.354, 1.717]	0.86
C*06	9.12	9	0.987 [0.46, 1.798]	1
C*07	30.53	33	1.081 [0.783, 1.412]	0.59
C*08	2.51	1	0.398 [0.01, 2.17]	0.53
C*12	5.17	5	0.967 [0.318, 2.182]	1
C*14	1.76	1	0.568 [0.014, 3.094]	1
C*15	3.12	2	0.641 [0.078, 2.256]	0.77
C*16	3.95	2	0.506 [0.062, 1.782]	0.44

Figure legends

Fig. 1 Density plot of the predicted HLA-A IE62 rank by volunteer or background. Higher values indicate that the strongest bound IE62 peptide by a HLA-A molecule has a comparatively poor affinity. The solid line shows the distribution for the background, the dashed line for the HZ cohort. A dashed vertical line denotes the location of IE62 rank affinity score 55. All HLA molecules higher than this value are noted as a poor presenter for IE62.

Fig. 2 Violin plots indicating the variation of the HLA-derived IE62 RAS for different age categories. A larger RAS value indicates a worse IE62 presenter. From left to right, adults younger than 35 (7 volunteers), adults between 35 and 50 (15 volunteers), and adults 50 or older (28 volunteers).

Supplemental materials

Online Resource 1 Calculated IE62-specific and VZV-wide RAS values for all tested HLA alleles. (Online Resource 1.pdf)

Online Resource 2 HLA genotype of all recruited volunteers. (Online Resource 2.xlsx)