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Human Leukocyte Antigen-Allelic Variations May Influence the Age at Cancer Diagnosis in Lynch Syndrome

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Abstract: Lynch syndrome (LS) is an inherited cancer predisposition disorder associated with an elevated risk of various epithelial cancers. Despite sharing the same pathogenic variant (PV), Lynch syndrome variant heterozygotes (LSVH) exhibit considerable phenotypic variability in cancer risk. The role of Human Leukocyte Antigen (HLA) in modifying cancer risk prompted our investigation into whether HLA variations act as genetic modifiers influencing age at cancer diagnosis in a unique cohort of LSVH carrying a PV in the *hMLH1* gene in South Africa. Within our extensive LS cohort, 426 individuals carried the same *hMLH1* PV (*MLH1*:c.1528C>T). We selected 100 individuals with the greatest diversity in age at cancer diagnosis and the oldest unaffected individuals for high-throughput HLA genotyping of 12 HLA class I and II loci using next-generation sequencing. Statistical analyses employed Kaplan-Meier survival analyses with Logrank tests and Cox proportional hazards. Following the robust application of statistical correction methods, six HLA alleles (3.2%) were significantly associated with a young age at cancer diagnosis. Notably, *HLA-B*15:17* and *HLA-DPB1*55:01* correlated significantly with very young colorectal cancer (CRC) diagnosis (Mean age: 21y [17–25]; HR = 71.59; q<0.001 and Mean age: 25y; HR = 54.05; q<0.001, respectively). Four *HLA-DPB1* alleles showed significant associations with a younger age at cancer diagnosis (*HLA-DPB1*04:02, -DPBI*20:01*, *-DPB1*55:01*, and *-DPB1*296:01*). HLA allele variations may influence the age at cancer diagnosis in LSVH with the same PV. Pending validation in a larger cohort, these high-risk HLA alleles could enhance cancer risk prediction models for personalized cancer screening in LSVH.

Keywords: lynch syndrome; colorectal cancer; germline pathogenic variant *MLH1*:c.1528C>T; Human leukocyte antigen (HLA); age at cancer diagnosis; genetic risk modifiers; personalized cancer screening

1. Introduction

Lynch syndrome (LS) is an inherited cancer-predisposing disorder caused by a germline pathogenic variant (PV) in one of the mismatch repair (MMR) genes or deletions in the 3′ region of the *EPCAM* gene [1]. LS is associated with a very high lifetime risk of developing primarily colorectal cancer (CRC) and extra-colonic cancers at a younger age, compared to the general population [1–3]. The lifetime risk of developing cancer in LS variant heterozygotes (LSVH) ranges from 30% to 80%

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depending on the mutated gene, cancer type, and other factors such as lifestyle, environmental exposure, epigenetic changes, and genetic risk modifiers [4–6]. This lifetime risk of developing cancer in LSVH differs in terms of age of cancer diagnosis (often used as a proxy for age of onset) and tumour site, even among individuals carrying the same PV [7–9]. Thus, the identification of additional specific genetic risk modifiers contributing to phenotype variations in cancer risk and age at cancer diagnosis in LSVH may assist in the implementation of highly personalized surveillance and screening interventions to reduce morbidity and mortality related to cancer in this at-risk population.

In LS cancer microenvironments, carcinogenesis is driven by immunoediting through the counter-selection of cell clones presenting frameshift neoantigens (produced as a consequence of MMR-deficiency), which depends mainly on the Human Leukocyte Antigen (HLA) alleles [10,11]. HLA alleles are responsible for presenting cellular antigens and eliciting antigen-specific immune responses [12]. While exogenous antigens are presented by HLA class II molecules, endogenous antigens are presented by HLA class I molecules, which interact with CD4-positive and CD8-positive T cells, respectively, which are among the most powerful mediators of anti-tumor immune responses [11,13,14]. HLA alleles are highly diverse among different individuals and populations. Each HLA allele has a unique shape and chemical properties in its antigen-binding groove, which enables it to fit with a specific antigen to a varying degree [11,15–17]. Thus, an individual's HLA typing is crucial in determining the binding of the antigens for presentation on the surface of the cell and eliciting antigen-specific immune response. However, it is not clear whether HLA allele variations may protect or influence cancer initiation in LSVH, hence the variability in cancer risk and age of cancer diagnosis/onset in these individuals [11]. This is because the crucial presentation of cancer antigens to the immune system by the HLA alleles may have a positive or negative impact on tumor initiation and progression, which can modify an individual's cancer onset risk [10,11,13,18,19]. For example, effective presentation of cancer antigens by HLA to the immune system has been found to play a significant role in the effectiveness of cancer immunotherapy, which aims to reactivate the impaired HLA-mediated anti-tumor immune response, such as immune checkpoint blockade [20,21].

The unique characteristics of cancer pathogenesis in LSVH present an opportunity to study the possible influence of HLA variations on cancer risk and the age of cancer onset. By investigating the impact of HLA allele variations on cancer incidence, cancer onset, and mutation profile in LSVH, we can gain new insights into the role of HLA alleles as modifiers of cancer risk. Several studies have investigated the influence of HLA allele variations on cancer susceptibility [22–26]. However, none of these studies investigated the influence of HLA allele variations on cancer risk or the age at cancer diagnosis in LSVH. We hypothesize that HLA allele variations may influence an individual's age at cancer diagnosis in LSVH.

In this study, we investigated our hypothesis using a unique cohort of LSVH carrying the same PV in the *hMLH1* gene (NM_000249.4(*MLH1*):c.1528C>T (p.Gln510Ter)) in South Africa.

2. Materials and Methods

2.1. Patients

In our large homogenous cohort of 426 genetically confirmed LSVH carrying the same *MLH1*:c.1528C>T South African founder PV, we selected 100 subjects for this study based on the following inclusion criteria: (i) patients exhibiting the greatest diversity in age at cancer diagnosis, i.e., patients who had 3SD below (youngest) and above (oldest) the mean age at cancer diagnosis from both extremities (n = 80, mean age 42.9, SD ±11.1 years), (ii) the oldest individuals who were not yet affected with cancer (n = 20), and (iii) the availability of blood genomic DNA sample with a minimum concentration of 20 ng/µl in our designated biorepository. All cancer patients were confirmed through pathology reports indicating the gender, age at the time of first diagnosis, presence or absence of malignancy, and the tumour site. Other demographics, such as ethnicity were retrieved from our in-house LS electronic database (Figure 1).

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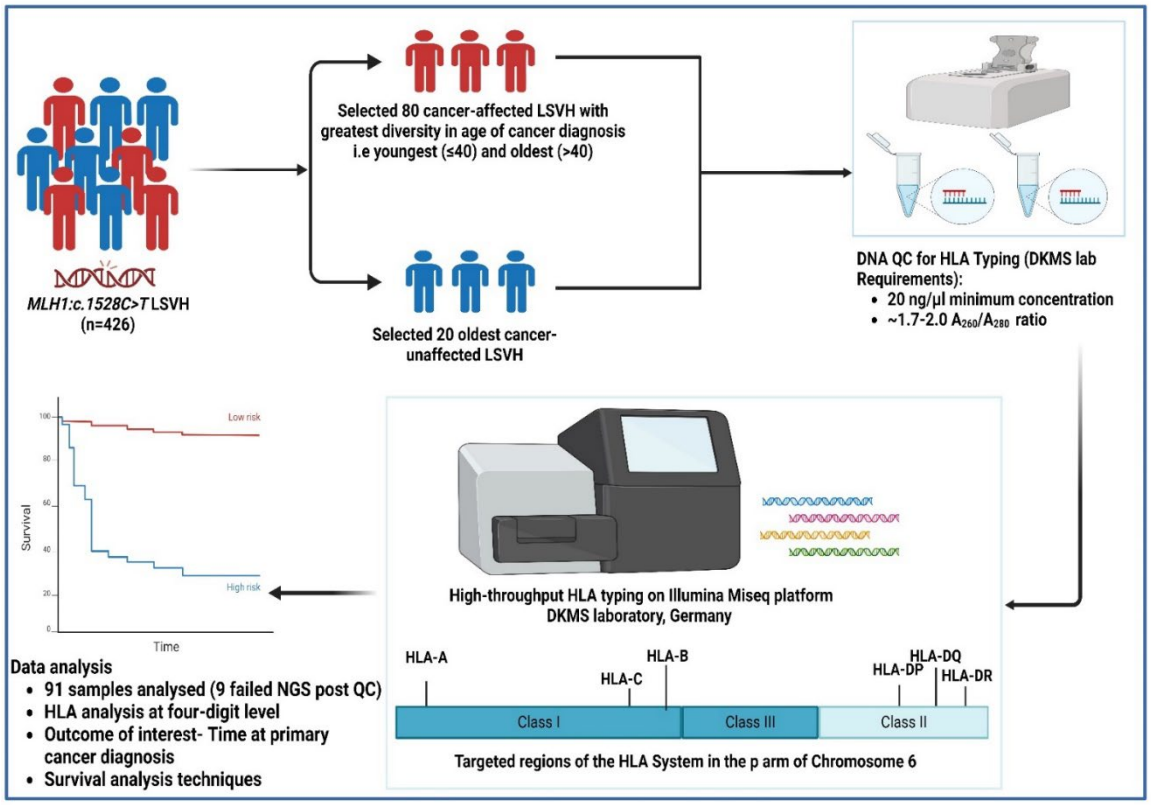

Figure 1. A schematic illustration of the study methodology.

2.2. DNA Samples

DNA samples of subjects meeting our inclusion criteria were retrieved from the -80°C biorepository at the Division of Human Genetics, University of Cape Town, South Africa. Genomic DNA was extracted from white blood cells (buffy coats). The DNA was quantified using the NanoDrop spectrophotometer and viewed using version 3.8.1 of the Nanodrop 1000 operating software (Thermo Fisher Scientific™, Johannesburg, South Africa). The integrity of the DNA samples was checked using 2.0% (w/v) gel electrophoresis, and the gel was visualized using the UVIpro Gold Transilluminator and through the UVPro software (UVItec, United Kingdom). DNA samples with the A_{260}/A_{280} ratio of 1.7–2.0 and a minimum of 20ng/ul concentration were selected for the downstream HLA high-throughput typing as per requirement by the Deutsche Knochenmarkspenderdatei (DKMS) laboratory, Germany (Figure 1).

2.3. HLA Typing

A high-throughput HLA genotyping targeting a total of twelve (12) HLA class I (-A, -B, -C, -E) and class II (DRB1, - DRB3, -DRB4, - DRB5, - DQA1, - DQB1, - DPA1, -DPB1) loci was performed at the DKMS Life Science Lab, Germany. The shotgun next-generation sequencing (NGS) technique was used on the Illumina MiSeq platform (Illumina, San Diego, California). All laboratory procedures and the sequencing strategy were performed similarly to a previous publication [27]. The amino acid sequence of the HLA protein distinguishes the biological effects of different HLA alleles, therefore, we limited our downstream analysis to a four-digit level to investigate the influence of HLA alleles on the age at cancer diagnosis [28] (Figure 1). The potential novel allele in the *DRB3* locus was characterized using the NGS-engine NGS-HLA typing software package (Version 2.15, GenDX) and the IPD-IMGT/HLA database (Version 3.54).

2.3. Statistical Analysis

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Statistical analysis was performed using the R statistical software (R Core Team, version 4.3.3). The outcome of interest was time at first cancer diagnosis (i.e., CRC or extra-colonic cancer). The risk of cancer diagnosis in one group relative to a reference group, at any age, was calculated using survival analysis techniques (Kaplan-Meier product limit method, with Logrank tests and Cox proportional hazards with 95% confidence intervals (CIs)), taking into account the fact that our research cohort included subjects who were cancer unaffected. Continuous data were presented as mean and standard deviation (SD) or median and interquartile range (IQR), whereas categorical data were presented as numbers (percentage). The immunotation R package (Version 1.10.0) was used to call the frequencies of HLA alleles from the IPD-IMGT/HLA Database (Version 3.54) [29]. All tests were two-tailed and p values were corrected for multiple comparisons according to the Benjamini-Hochberg method. Associations were considered significant if both the p- and q-values (adjusted p-value) <0.05 (Figure 1).

3. Results

Overall Demographic and Clinical Characteristics of the Patients

Demographics and clinical characteristics of two groups (cancer-affected and cancer-unaffected) of LSVH carrying the same PV (*MLH1*:c.1528C>T) in the *hMLH1* gene are summarized in Table 1. There were 78 cancer-affected and 13 cancer-unaffected LSVH. Of the 78 cancer-affected patients, 60 (78%) were diagnosed with CRC. As expected in LS, proximal colon tumours were the most common (58%) in this cohort (Table 1).

Table 1. Demographic and clinical characteristics of LS carriers investigated in this study.

Variable	Cancer unaffected-controls (LS carriers) (N=13)	Cancer affected -cases (N=78)		Total subjects (N=91)	*p-value
		Early-diagnosis (≤40) (N=35)	Late-diagnosis (>40) (N=43)		
Gender					
Male	**4 (30.8%)**	23 (65.7%)	19 (44.2%)	46	0.057
Female	9 (69.2%)	12 (34.3%)	24 (55.8%)	45	0.057
***Age at diagnosis (years)**					
Mean (SD)	69.2 (5.44)	31.9 (5.24)	53.1 (8.31)		
Median [Min, Max]	68.8 [62.7, 79.1]	32.0 [17.0- 39.4]	50.0 [39.8-72.3]		
Tumour site					
Proximal colon	*NA*	24	21	45	0.080
Distal colon	*NA*	5	5	10	0.726
Rectum	*NA*	2	1	3	0.441
Endometrium	*NA*	0	8	8	N/A
Breast	*NA*	0	3	3	N/A
Ovary	*NA*	1	0	1	N/A
Small intestine	*NA*	2	3	5	0.818
Bladder	*NA*	1	0	1	N/A
Kidney	*NA*	0	1	1	N/A
Skin	*NA*	0	1	1	N/A

*p-value for the difference between cancer cases with early-diagnosis (≤40) and late-diagnosis (>40) calculated using a Z-score test for two proportions with a two-tailed significance level of 0.05.

*Age at cancer diagnosis only for individuals with cancer and age at censoring for unaffected mutation carriers

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Effects of Gender and Cancer Site on Age at Cancer Diagnosis in LSVH

To find out whether the age at cancer diagnosis in this cohort was influenced by the gender of an individual and the type of cancer, we performed a survival analysis. We used age at cancer diagnosis as our outcome of interest to determine whether there is a difference in age at cancer diagnosis between males and females. We found that male LSVH were diagnosed with cancer at a younger age compared to female LSVH (Mean age: 40.7y [95% CI: 37.5-49.1] and 49.6y [95% CI: 46.0-57.1]), respectively, p=0.038) (Figure 2A). Our observations are consistent with previous findings on the effect of gender on cancer risk in LSVH [30–32]. We performed another analysis to compare the age at cancer diagnosis between CRC and extra-colonic cancers in this cohort. We found that LSVH with CRC were diagnosed ten-years younger than LSVH with extra-colonic cancers (Mean age: 39.0y [95% CI: 36.3 – 45.5] and 49.9y [95% CI: 32.8 -72], respectively, p=0.044) (Figure 2B). As the majority of these patients were actively undergoing endoscopic surveillance, we anticipate an earlier detection of CRC events compared to other types of LS-associated cancers. This finding was also similar to other cohorts of LSVH carrying different PVs in that the risk of developing CRC was typically higher and at a younger age compared to most extra-colonic cancers [33,34].

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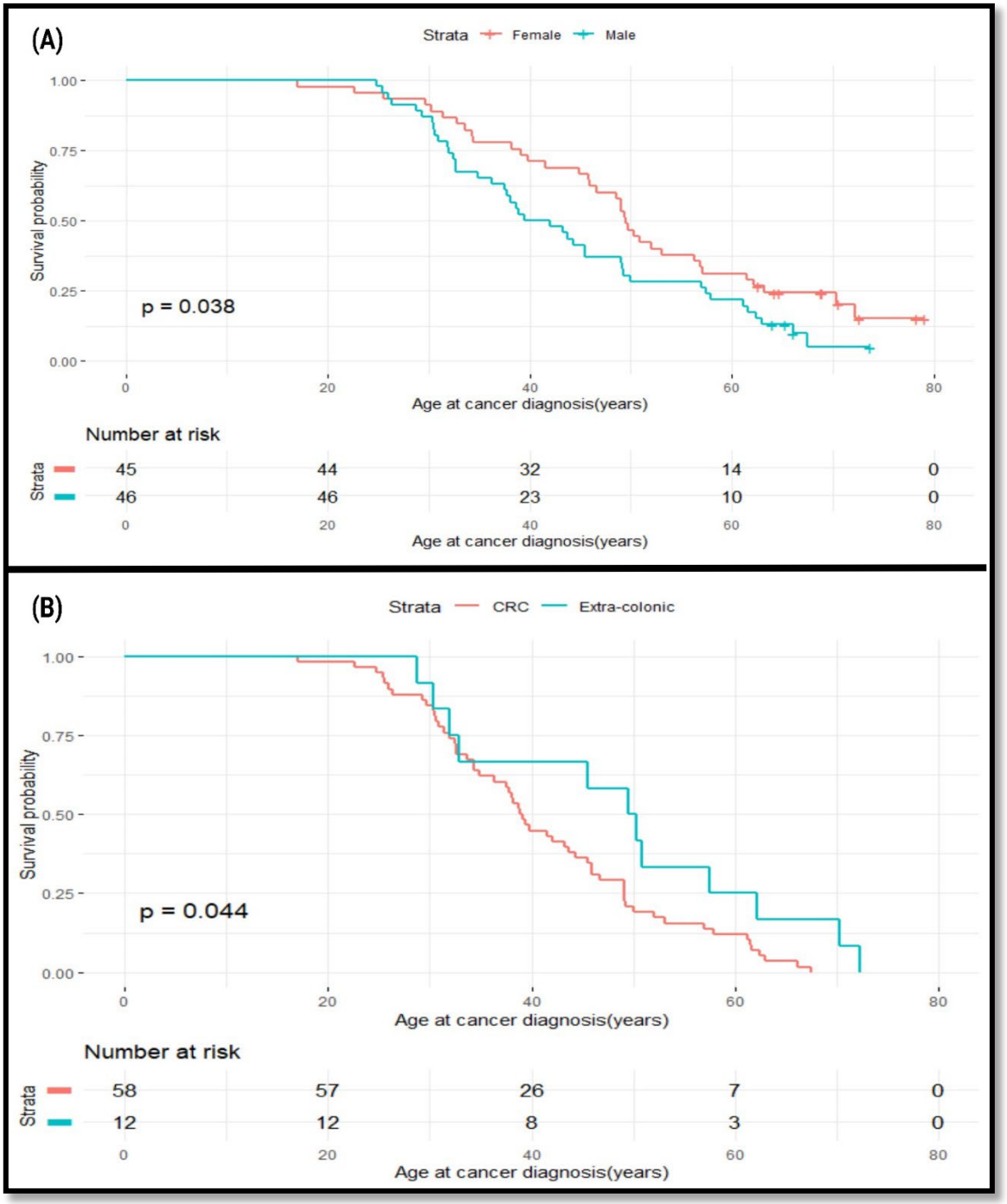

Figure 2. Survival analysis plots showing the number of LSVH at risk for cancer and age at cancer diagnosis stratified by gender and the site of cancer, showing: (A) that males developed cancer significantly earlier than females. (B) that CRC developed at a significantly younger age than extra-colonic cancers.

Effects of HLA Alleles on the Age at Cancer Diagnosis in LSVH

In order to study the effects of HLA allele variations on the age at cancer diagnosis in this cohort, we performed a Cox regression analysis to investigate whether HLA allele variations can influence age at cancer diagnosis in LSVH. We further adjusted our analysis by gender, to avoid the potential confounding effects of gender bias. Of 1785 HLA alleles typed from 12 HLA class I and II loci (187 unique HLA alleles), 78 individuals had primary cancers, and 13 remained unaffected. We summarised the details of different HLA alleles showing significant associations with age at any cancer diagnosis (either CRC or extra-colonic cancer) in Table 2.

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Table 2. Analysis of HLA alleles associated with age at cancer diagnosis in LS.

HLA Locus	HLA allele	Unadjusted			Gender Adjusted			Affected: Unaffected ratio	Mean age (range) for cancer affected
		HR (95% CI)	p-value	q-value	HR (95% CI)	P-value	q-value		
HLA-B	***B*15:17***	**94.65 (16.05-558.11)**	<0.001	<0.001	87.64 (14.94-514.03)	**<0.001**	**<0.001**	2:0	21(17, 25)
HLA-DRB3	*DRB3*03:XXX*	247.15 (14.16-4314.89)	<0.001	<0.001	301.04 (17.19-5270.18)	**<0.001**	**<0.001**	1:0	17
HLA-DPB1	*DPB1*04:02*	2.92 (1.22-7.02)	0.011	0.045	3.56 (1.46-8.65)	**0.005**	**0.029**	6:0	37 (25-50)
	*DPB1*20:01*	20.67 (2.60-164.55)	0.004	0.040	16.88 (2.11-134.75)	0.006	0.006	1:0	26
	*DPB1*296:01*	12.97 (1.68-100.14)	0.014	0.079	18.05 (2.31-141.30)	0.006	**0.029**	1:0	30
	*DPB1*55:01*	74.13 (1.68-100.14)	<0.001	<0.001	64.22 (12.26-366.25)	**<0.001**	**<0.001**	2:0	21 (17,25)

The following HLA class II alleles in the *HLA-DPB1* gene were significantly associated with a younger age at cancer diagnosis: *HLA-DPBI*04:02*, *HLA-DPBI*20:01*, *HLA-DPBI*55:01* and *HLA-DPBI*296:01* (Table 2). A potential novel allele in the *DRB3* locus detected in our youngest cancer patient diagnosed at the age of 17 years showed a significant association with young age at cancer diagnosis (Hazard ratio (HR) = 301.04, p<0.001, q<0.001). This potential novel allele bearing the *HLA-DRB3*:g.7953C>T variant is most similar to the *HLA-DRB3*03:01* allele (Supplementary Figure S1). There was no statistically significant association between all other HLA alleles observed and the age at cancer diagnosis in this cohort, as shown in a complete unadjusted and gender-adjusted comparison data for *HLA-A, -B, -C, -DRB1, - DRB3, -DRB4, - DRB5, - DQA1, - DQB1, - DPA1, -DPB1,* and *-E* alleles in Supplementary Tables S1 and S2, respectively. The relationship between the six HLA alleles associated with young age at cancer diagnosis and the cancer sites in our LSVH is depicted in Figure 3. The *HLA-DPB1*04:02* allele was linked to the age of CRC and extracolonic cancer diagnoses. Notably, it was the only allele associated with extracolonic cancer.

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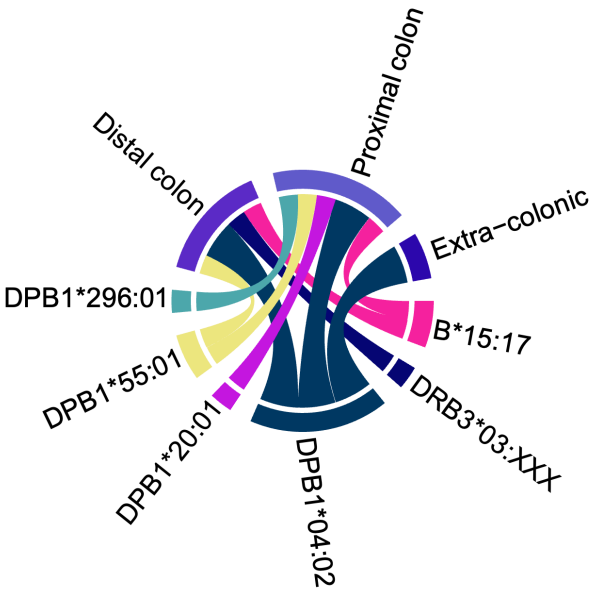

Figure 3. A chord diagram of the six HLA alleles associated with young age at cancer diagnosis and cancer sites in LSVH. Colour ribbons connecting the HLA alleles to cancer sites indicate potential associations.

Effects of HLA Alleles on the Age at CRC Diagnosis in LSVH

Considering that CRC is the most diagnosed cancer in LSVH and the most observed cancer in this study cohort, we investigated whether HLA allele variations could impact age at CRC diagnosis. All LSVH affected with extra-colonic cancers (n =20) were excluded from this analysis. Three (3) HLA alleles were significantly associated with age at CRC diagnosis (Table 3). Interestingly, two (2) LSVH carrying both *HLA-B*15:17* and *HLA-DPB1*55:10* developed CRC at a young age. There is no published evidence of linkage disequilibrium between these two HLA alleles [35]. These two alleles were significantly associated with young age at CRC diagnosis, with the mean age at cancer diagnosis of 21 years (Range 17-25, $p<0.001$, $q=0.003$ for *HLA-B*15:17* and $p<0.001$, $q<0.001$ for *HLA-DPB1*55:10*) (Table 3). Unadjusted and gender-adjusted association data of one hundred eighty-four (184) HLA alleles that had no significant association with age at CRC diagnosis are presented in Supplementary Tables S3 and S4, respectively.

Table 3. Analysis of HLA alleles associated with age at CRC diagnosis in LSVH.

HLA allele	Gender Adjusted-HR	P-value	q-value	95% CI	Affected: Unaffected ratio	Mean age (range) for cancer affected
B*15:17	71.59	<0.001	<0.001	11.68-438.65	2:0	21(17, 25)
DRB3*03:XXX	264.83	<0.001	0.001	14.96- 4689.40	1:0	17
DPB1*55:01	54.05	<0.001	<0.001	11.16-319.47	2:0	21 (17,25)

Different HLA Allele Frequencies between LSVH and the Previously Studied South African General Populations

South Africa has a diverse population with a unique genetic background, (ranging from indigenous African subpopulations, and immigrant European and Asian populations) and varying disease prevalence. This diversity could present unique HLA allele variations that are not found in other populations within the country (and internationally) [36–38]. Comparing HLA allele frequency

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(AF) between LSVH and different populations in South Africa, allows us to account for these population-specific factors. This information can aid in tailoring cancer screening and management approaches that are more effective and relevant to the South African context. Also, it can identify specific novel HLA alleles that may be associated with LS susceptibility or be likely protective, and unique amongst this cohort, compared to the general population. This information may contribute to early cancer risk assessments and highly personalized prevention strategies for LSVH in South Africa [39].

We compared the AF of the HLA alleles which were significantly associated with young age at cancer diagnosis in our LSVH cohort with the AF observed from other non-LS South African cohorts reported in the IPD-IMGT/HLA Database [35]. The *HLA-DPB1*04:02* allele was common in the LSVH cohort and South African general population (i.e., with AF greater than 0.05). Interestingly, *HLA-DPB1*20:01*, and *HLA-DPB1*296:01* alleles associated with a young age at cancer diagnosis in our LSVH cohort were observed for the first time in the South African study cohort (Figure 4).

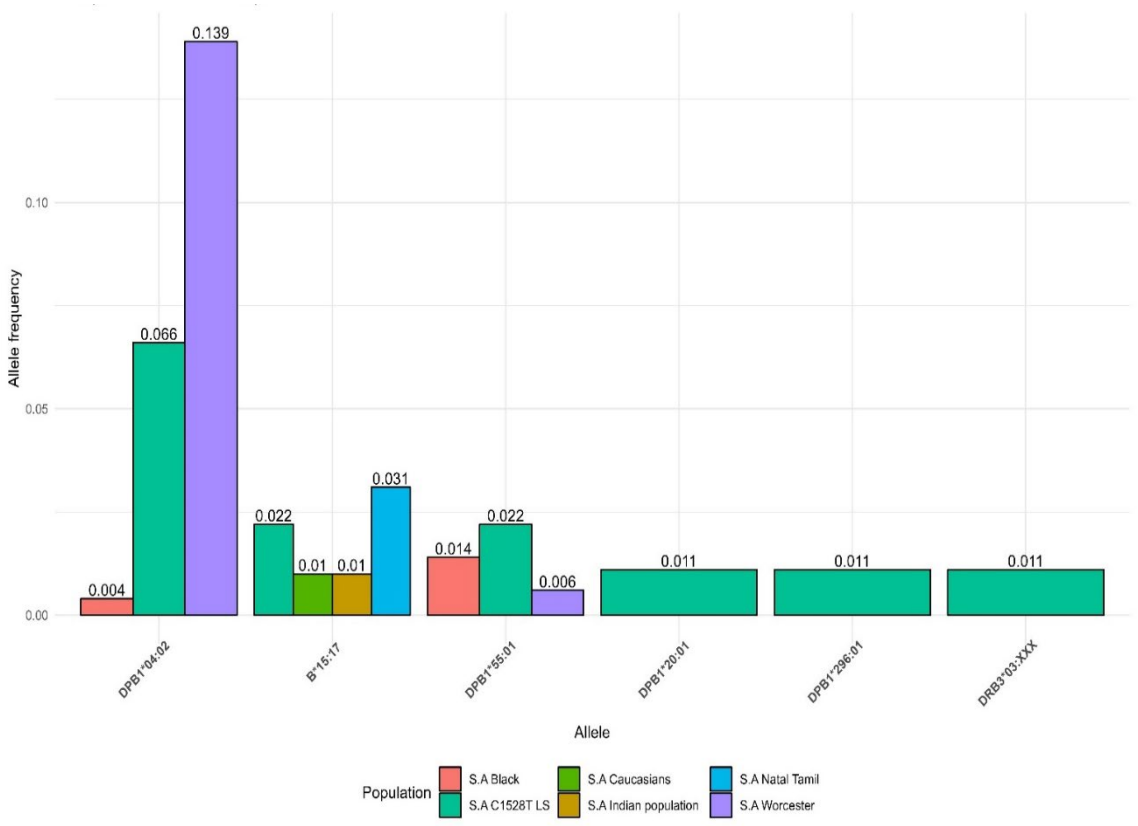

Figure 4. Comparison of HLA-allele frequencies of six (6) alleles associated with young age at cancer diagnosis in our LSVH cohort (Sea Green) and the previously studied South African general population (Red, Green, Blue, Brown, Purple).

4. Discussion

This is the first study aimed at identifying HLA class I and II alleles that may influence the age at cancer diagnosis in LSVH carrying the same PV in the *hMLH1* gene (*MLH1*:c.1528C>T). Our findings suggest that, once validated in a large cohort, the identification of high-risk HLA alleles could be factored into the risk prediction model calculations for offering tailored personalized cancer screening and surveillance strategies for LSVH.

Our study is part of an ongoing programme promoting the utility of personalized early cancer prevention in LSVH. In this instance, the strategy is to consider the effects of HLA allele variations as one of the potential genetic modifiers for cancer onset risk in a well-defined LSVH cohort.

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Importantly, personalized screening strategies will potentially reduce the overuse of invasive colonoscopies for CRC screening and (premature) cancer-preventive surgeries in LSVH [7,40].

The strongest association with a young age at cancer diagnosis in LSVH were conferred by the presence of the *HLA-DPB1*04:02* class II allele (Table 2). The *HLA-DPB1*04:02* allele was common in both LSVH cohort and non-LS South African general population, with the reported allele frequencies of 0.066 (6.6%) and 0.139 (13.9%), respectively (Figure 4). Therefore, once validated in a longitudinal study with a large cohort of LSVH, this potentially high-risk HLA allele may be considered as part of cancer risk assessment in LSVH, potentially promoting a more genetically-informed predictive testing and much more precisely targeted surveillance for cancer prevention strategies in South Africa.

Worldwide in non-LS populations, different HLA alleles have been reported to be associated with various cancers, such as cervical [26], leukaemia [41], hepatocellular [42], lung squamous cell [43], cutaneous T-Cell Lymphoma [44], and gastric carcinomas [45,46]. For instance, the HLA-DP gene polymorphisms (*HLA-DPB1*03:01*, and *HLA-DPB1*13:01*) have been significantly associated with an increased risk of cervical cancer in Chinese populations [47–50]. Furthermore, *HLA-DPB1*04:02:01:21* has been recently reported as a novel HLA allele in a patient with acute leukaemia in the UK [41]. In our LSVH cohort, the associations between HLA-DP alleles and cancer risk are consistent with previous associations in non-LS populations across various cancers, worldwide [41,47,51]. The HLA-DP locus forms part of the highly polymorphic HLA class II molecules, and genetic variations of the HLA alleles may lead to variations in the antigen-presentation on the specialised antigen-presenting cells, such as dendritic cells, thus potentially influencing or likely protecting against the development of cancer in LSVH [52,53].

Although we found statistically significant associations between different HLA alleles (*HLA-B*15:17*, *-DPB1*20:01*, *-DPB1*55:01*, *-DPB1*296:01* and the potential novel allele in the *DRB3* locus (most similar to *HLA-DRB3*03:01* allele)) and relatively young age at cancer diagnosis, the 95% confidence intervals were very wide due to our small sample size. However, additional investigation for these HLA alleles in patients with young age of cancer onset in a large cohort of LSVH can further complement and validate our findings. Generally, *HLA-B*15* has been widely reported to be likely protective against viral-associated cancers, such as cervical cancer ([OR], 0.64; $p = 1.56\times 10^{-9}$) [54] and Human papillomavirus-positive head and neck cancers ([OR], 0.31; $p = 0.015$) [55]. The HLA class I alleles play a crucial role in early infection and the mechanisms underlying early viral clearance [56], which may explain the protective effect against viral-associated cancers. However, in our cohort of LSVH, we found a significant association between the *HLA-B*15:17* allele and a young age at CRC diagnosis, which is a non-infection-attributable cancer. These different findings of *HLA-B*15* in infection and non-infection-attributable cancers, between the general population and our LSVH population, may be due to the low incidence of cancers attributable to infections in the LSVH. This was proposed to be due to a chronic hyper-immune response caused by the persistent production of neopeptides [57–59], as a result of a pathogenic germline defect in the post-replication DNA mismatch repair system (as occurs in LS).

Additionally, it is worth taking into account that HLA class I also plays a role in the presentation of tumor-derived peptides in a complex with the /beta 2 microglobulin (B2M) for recognition by cytotoxic CD8+ T lymphocytes to eliminate transformed cells. In this regard, any variation in this gene could favor cancer initiation and progression due to poor presentation of the cancer cells to the immune system, explaining the observed increased cancer risk in our LSVH cohort [10,19].

Our findings suggest that HLA allelic (amongst other genomic) variations could be potential factors influencing the age at cancer diagnosis in LSVH individuals. In this regard, it is worth considering that these findings, once confirmed in a large cohort of LSVH, could be used to implement more precise presymptomatic cancer surveillance programs as follows: (i) integrating HLA allele testing into routine cancer screening for LSVH, as those with certain low or high risk HLA alleles may require decreased or increased highly specialized screening and surveillance respectively, (ii) use of individual HLA allele information to stratify LSVH with the same PV into different risk groups for age at cancer onset (early or late), which could inform the frequency and intensity of their

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colonoscopic surveillance, (iii) consider HLA allelic information when deciding which cancer prevention strategy to recommend to LSVH individuals with known PVs, as those with certain high risk HLA alleles may benefit more from specific lifestyle changes or prophylactic treatments such as hysterectomy for endometrial cancer prevention or the use of aspirin and resistant starch for CRC prevention [40,60], (iv) develop predictive mathematical models that could take into account of both PVs and different HLA alleles to estimate an individual's life-time risk of developing cancer and the likely estimated age at cancer diagnosis in LSVH. However, HLA allelic variations could be just one of many genetic modifiers that can influence a person's risk of developing cancer in LSVH. Other already known cancer risk modifiers can affect the overall cancer risk in LSVH. These risk modifiers include polymorphisms in xenobiotic metabolism genes, epigenetic changes, lifestyle factors and strong family history of cancer [8,61–68].

The strengths of our study include: (a) the first research study to investigate the associations between HLA class I and II alleles and the age at cancer diagnosis using a genetically confirmed LS cohort, (b) the utilization of high-throughput HLA genotyping using NGS in this regard, (c) the homogenous nature of the study cohort as patients harbour the same LS-PV in the *hMLH1* gene and are originating from a population of a common ethnicity, and (d) new evidence suggesting that HLA allele variations may influence the age at cancer diagnosis in LSVH. The main limitations of our study are: (i) the relatively small sample size, mainly due to financial constraints to perform high-throughput NGS-based HLA typing in the whole cohort of LSVH, and (ii) we only performed a cross-sectional association study in LSVH without taking into account other possible cancer risk genetic and epigenetic modifiers, cofounders and the proved causality (causal-effect relationship). We are making efforts to recruit a longitudinal cohort of LSVH to overcome this limitation in our next study.

5. Conclusions

This study provides valuable insights into the potential role of HLA allele variations and the age at cancer diagnosis in LSVH. HLA allelic variations may influence the age at cancer diagnosis in LSVH carrying the same PV in the *hMLH1* gene (*MLH1*:c.1528C>T). As such, it is worth considering HLA allele information when recommending cancer prevention strategies for LSVH. High-throughput HLA allele typing can be included in LSVH routine cancer screening after it has been successfully validated in a large longitudinal cohort. This can be achieved by stratifying these individuals into different risk groups based on their at-risk HLA alleles. In this population of people at risk, all of these approaches have the potential to enhance personalized cancer screening.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: A snapshot of a potential novel allele in the *HLA DRB3* locus.; Table S1: Unadjusted comparison of HLA alleles and age at any cancer diagnosis in LSVH; Table S2: Gender-adjusted comparison of HLA alleles and age at any cancer diagnosis in LSVH; Table S3: Unadjusted comparison of HLA alleles and age at CRC diagnosis in LSVH; Table S4: Gender-adjusted comparison of HLA alleles and age at CRC diagnosis in LSVH.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Human Research Ethics Committee (HREC) of the University of Cape Town (*HREC 972/2021*).

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Informed Consent Statement: All patients gave informed consent for their data to be used for subsequent genetic studies linked to the previously approved study (*HREC R022/2019*).

Data Availability Statement: The data analyzed during the current study are available from the corresponding author upon request.

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