REVIEW

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Significant SNPs Related to Telomere Length and Hepatocellular Carcinoma Risk in Chronic Hepatitis B Carriers

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Abstract

Chronic hepatitis B virus (HBV) infection increases the risk of developing cirrhosis and hepatocellular carcinoma (HCC) with suspected interactions between virus replication and host immune responses. A number of reports have suggested that telomerase function may be involved in chronic hepatitis B (CHB) pathogenesis, but positive or negative associations with HCC risk remain for discussion. Mean telomere length is an indicator of biological aging and it has been reported that reduction in NBV carriers compared to normal individuals. In somatic cells, telomeres contain simple, tandemly repeated G-rich sequences that frequently are reduced by 50 to 200 base pairs at each cell division. Several genome-wide association studies (GWAS) in diverse ethnic populations have revealed eleven single nucleotide polymorphisms (SNPs) linked to telomere length. Two of these, rs398652 and rs621559, have prognostic value and could be used as genetic markers. This review describes current knowledge concerning telomerase activity and telomere length as well as significant polymorphisms in HBV-related HCC patients. In particular, to cast light on genotype-phenotype interactions, we used SNPnexus to evaluate effects of the two SNPs on risk of disease and complex disorders.

Keywords: Chronic hepatitis B (CHB)- hepatocellular carcinoma (HCC)- SNPs- telomere length- SNPnexus

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Introduction

Hepatitis B virus (HBV) infection leads to chronic hepatitis B (CHB), which is one of the most common causes of hepatocellular carcinoma (HCC) (McMahon, 2008). About 15-40% of people developing chronic HBV infection should progress to cirrhosis and end stage liver disease (Poustchi et al., 2008; Wanich et al., 2016). Various viral factors associated with HCC are the HBV genotype, mutations of the basal cell promoter, and high viral load (Ghadir et al., 2012; Poustchi et al., 2008). Liver inflammation and cirrhosis favor the cancer process. Polymorphisms in cytokine genes are also potential host factors related to HCC (Mohamadkhani et al., 2015). The integration of the HBV genome is probably an initial carcinogenic event. The integrated HBV genome can activate neighboring cell genes to develop a selective growth advantage for liver cells (Tamori et al., 2003). This virus evolved several strategies to evade immune defenses and the production of hepatitis B proteins can act as transactivators on various cellular genes for tumor development (Mohamadkhani et al., 2011b; Moradzadeh et al., 2013). Telomere is a well-organized complex at the end of linear eukaryotic chromosomes, comprising the tandem repeat DNA sequences TTAGGG and related proteins. The study of telomere length in association with aging and human disease has been paid more attention during recent years. Telomere function is indispensable for keeping the physical integrity of linear chromosomes in addition to healthy human aging, which can suppress tumor development in way of an irreversible cell cycle arrest (Klapper et al., 2001). Therefore, the ability of virus-infected cells to avoid the senescence process is essential for long-term survival and proliferation of infected cells and the probability of transformation. The activity of telomerase and telomere length in HCC-related HBV has been also investigated in several reports (Fu et al., 2012; Liu et al., 2014; Ma et al., 2016; Pan et al., 2014). This review recaps on recent finding on telomerase genotype and telomere length-related carcinogenesis in patients with chronic hepatitis B.

Pathogenesis of Hepatitis B

It is estimated that 400 million people worldwide are HBV carriers. The Hepatitis B virus (HBV) accounts for about 780,000 deaths every year worldwide, mainly due to chronic hepatitis. In highly endemic countries in Asia, most infections are contracted after birth or perinatal. Chronic hepatitis B is not a static disorder and the natural history of the disease is influenced by both viral and host factors. The natural history of hepatitis B is complex and is influenced by many factors including age at infection,

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viral factors such as HBV genotype, viral mutations, HBV replication level, host factors such as sex, age and immune status (McMahon, 2008; Mohamadkhani et al., 2015; Mohamadkhani et al., 2011a). Chronic infection of hepatitis B increases the risk of developing cirrhosis and hepatocellular carcinoma (Mohamadkhani et al., 2017b; Poustchi et al., 2008; Shahnazari et al., 2014). Cohort studies of East Asian countries indicated annually HCC incidence rates of 0.2 per 100 in inactive carriers with HBV infection, 0.6 for 100 people with chronic HBV without cirrhosis and 3.7 for 100 people with compensated cirrhosis (El-Serag and Kanwal, 2014; Somboon et al., 2014).

Liver injuries in chronic infection are considered to be associated with the activity of exhausted HBV specific T cells. The immune response of HBV-specific CD8 + T cells plays a significant guarding role against the virus (Mohamadkhani et al., 2015). T cell exposure to high antigenic loads is a determining factor of T cells dysfunction, and consequently the reduced reactivity of these cells present in chronic infection is probably related to the high level of antigen in these patients (Besharat et al., 2016; Schuch et al., 2014). Nonetheless, other mechanisms may also contribute to the inhibition of T cells, including the tolergenic respose of the liver and Treg cells function through the production of Transforming growth factor-β (TGF-β) (Yang et al., 2014). Viral infections typically cause cytokine-induced inflammatory reactions in which the release of chemokine leading to cancer development. Kupffer cells in continuous inflammation and oxidative stresses induce activation stellate cells by producing NFκB and AP1 leading to cirrhosis, fibrosis and development of HCC. Furthermore, HBsAg may inhibit the production of interferon (IFN) which is important for resolution of HBV infection (Mohamadkhani et al., 2017a; Mohamadkhani et al., 2014; Schuch et al., 2014).

Integration of the HBV genome, commonly in partial form, has occurred in patients with chronic hepatitis to activate proto-oncogenes with the rapid onset of HCC (Tamori et al., 2003).

Overview of Telomerase Activity and Telomere Length

Telomerase (terminal transferase) is a RNA-dependent DNA polymerase that contains of a long stretch of hexameric (TTAGGG)n DNA repeats, encoded by the same gene in chromosome 10. This structure generally organizes the molecular basis for unlimited proliferation. The end of the telomere cannot be fully replicated by formal DNA-dependent DNA polymerases and consequently in the absence of mechanisms to counteract this effect, telomeres become too short and inefficient to protect chromosomes with each round of cell division or with increasing the age (Bojovic et al., 2015; Kong et al., 2014). Telomere shortening and consequently losing capping function at the chromosomal ends, restricted the life span of human cells. When telomeres reach a critical short length, DNA damage checkpoints are activated and limit cell survival by induction of senescence or apoptosis (Yang et al., 2001).

An efficient RNA component that functions as a template for DNA telomere synthesis in humans, known as hTR or the human telomerase RNA gene (hTERC), together with another part, a catalytic protein telomerase reverse transcriptase (hTERT) with reverse transcriptase activity, are two necessary fragments of Telomerase. Regardless of the telomerase activity, hTR is enormously synthesized in all human cells, obviously with fivefold upsets in tumor cells. While hTERT mRNA expresses less than 1 to 5 copies per cell and is entirely associated with telomerase activity. The hTERT expression and subsequent telomere synthesis are regulated at the transcriptional level by the hTERT promoter, which includes a series of E boxes plus five rich GC elements that can bind c-Myc and Sp1 respectively (Klapper et al. 2001; Xu and Goldkorn 2016; Yang et al., 2001).

Genes and ecological factors determine the length of telomere, thought, blacks have longer telomere length in leukocyte than white and women than men. Likewise, Homo sapiens exhibit shorter telomere than most other mammalian species. Recent findings revealed that polymorphisms related to telomerase and telomere length are associated with aging and age-dependent pathological features. Monogenic diseases such as dyskeratosis congenita and idiopathic pulmonary fibrosis have revealed mutations in genes regulating telomere length and consequently induce a decrease in leukocyte telomere length that increases the risk of aplastic anemia (Jemielity et al., 2007; Prescott et al., 2011). The impact of telomerase activity and the telomere length on protection against progressive liver fibrosis in chronic liver diseases shown that shorter telomere of immune cells is associated with their poorer activity against viral infection (Helby et al., 2017). Hepatocytes transduced with a human TERT gene obtain a prolonged lifespan in culture and maintain permissive to the hepatitis B virus by constitutive expression of hTERT that is regulated by tumor virus protein x (Lv et al., 2013). Human tumor viruses mainly increase the transcription of telomerase that can extend telomeric ends in immortal tumor cells (Cheng et al., 2017; Helby et al., 2017).

Polymorphisms Related Leukocyte Telomere Length

The effects of change in telomeres length, which generally form the life expectancy of humans, linked to the higher risk of many aging-related diseases and increased susceptibility to various malignant tumors

Table 1. Genomic Coordinates and External Links of SNPs rs398652 and rs621559

SNP	Chromosome	chrom Position	Genes	Allele1	Allele2	Contig	contig Position	Band	Trait	dbSNP	HapMap
rs398652	chr14	56525569	PELI2	G	A	GL000116.1	37525569	q22.3	Telomere length	rs398652	rs398652
rs621559	chr1	43645411	WDR65	G	A	GL000006.1	13617329	p34.2	Telomere length	rs621559	rs621559

(Klapper et al., 2001; Yang et al., 2001). Telomeres that are involved in cellular functions are regulated by multiple genes for their length. It has been reported that telomere length is genetically heritable, classical twin studies have shown that genetic factors can contribute up to 80% of the heritability of telomere length (Gu et al., 2011; Klapper et al., 2001; Sameer et al., 2014). Therefore, the genetic variants of shortened telomere length contribute in increased risk of cancer (Pourahmadi et al., 2015). Genome-wide association studies (GWAS) are a powerful tool to explain the genetic basis of complex disorders and has recently provided novel insights into the genetic architecture of many common diseases and traits (Liu et al., 2014; Mohamadkhani and Poustchi, 2015; Wolpin et al., 2014). These studies and the validation efforts of candidate genes have shown that eleven single nucleotide polymorphisms (SNPs) related to the telomere length in various ethnic populations recorded as: rs4452212, rs7235755, rs6028466, rs398652, rs621559, rs654128, rs12696304, rs3772190, rs4387287, rs2162440, and rs16847897 (Gu et al., 2011; Levy et al., 2010; Pan et al., 2014). Gu et al., (2011) in a multistage genome association study of 300,000 single nucleotide polymorphisms (SNPs) in healthy controls, showed that four SNPs rs6028466 on 20q11.22, rs621559 on 1p34.2, rs398652 on 14q21 and rs654128 on 6q22.1 were significantly associated with the length of telomere in all populations. The association of these SNPs with bladder cancer risk in a powerful case control study showed that protective genotypes (GA, AA) of rs398652 was inversely associated to the risk of bladder cancer but with longer telomeres length. In fact, individuals with the variant alleles of rs398652 significantly reduced risk of bladder cancer (GG, the major genotype) (Gu et al., 2011). Further studies showed the impact of genetic variants rs621559 and rs398652 on telomere length and susceptibility to esophageal squamous cell carcinoma (ESCC) in Chinese (Shi et al., 2013). In addition, analysis of the average length of leukocytes telomeres in 2,917 individuals revealed an association with locus rs12696304 on 3q26. Each of the minor allele copies of rs12696304 was correlated with a discount of about 75 pairs of telomere lengths, equal to 3.6 years of telomere-length linked to age (Codd et al., 2010).

The meta-analysis of four observational GWAS presented two associated SNPs rs4387287 and rs4452212 belonging to OBFC1 (for oligonucleotide / oligosaccharide-binding) and CXCR4 (chemochin (CXC pattern) receptor 4) genes with the length of telomere (Levy et al., 2010). The results of six independent GWAS and validation study by Mangino et al., (2012) also confirmed associations of above loci with telomere length. In addition, they recognized two new genomic regions in association with telomere length on chromosome 17p13.1 (complex telomere maintenance component 1 (CTC1)) and 19p12 (Zinc 676 zinc protein (ZNF676)). SNPs evidenced by CTC1; rs3027234 and ZNF676; rs412658, established the effect of common genetic variants with telomere length. Similarly, two novel variants rs2162440 and rs7235755 belong to the coding region for VPS34/PIKC3C on chromosome 18q12.2 was associated with short telomere length in two independent European ancestors (Mangino

Table 2. Genotypes and Allele Frequencies Hapmap population

SNP	Genotype1	Count	Frequency	Genotype2	Count	Frequency	Genotype3	Count	Frequency	Allele1	Count	Frequency	Allele2	Count	Frequency	population
rs398652	AA	3	0.0183	GG	122	0.7439	AG	39	0.2378	A	45	0.1372	G	283	0.8628	CEU
rs621559	AA	0	0	GG	144	0.8727	AG	21	0.1273	A	21	0.0636	G	309	0.9364	CEU
rs398652	AA	29	0.1737	GG	54	0.3234	AG	84	0.503	A	142	0.4251	G	192	0.5749	YRI
rs621559	AA	27	0.1617	GG	63	0.3772	AG	77	0.4611	A	131	0.3922	G	203	0.6078	YRI
rs398652	AA	35	0.4167	GG	12	0.1429	AG	37	0.4405	A	107	0.6369	G	61	0.3631	CHB
rs621559	AA	5	0.0595	GG	50	0.5952	AG	29	0.3452	A	39	0.2321	G	129	0.7679	CHB
rs398652	AA	36	0.4186	GG	14	0.1628	AG	36	0.4186	A	108	0.6279	G	64	0.3721	JPT
rs621559	AA	6	0.0698	GG	39	0.4535	AG	41	0.4767	A	53	0.3081	G	119	0.6919	JPT

et al., 2009). The inverse association of minor allele variant C at rs16847897 in TERC locus is also reported with telomere length (Prescott et al., 2011).

In the study of Pan et al., (2014) in two independent CHB Han Chinese populations, of eleven telomere length-related SNPs, two loci (14 q22.3, rs398652 and 1p34.2, rs621559) were significantly related to the HBV-related HCC risk. These results demonstrate that SNPs rs398652 and rs621559 could be common genetic components that increase the risk of various types of malignancy.

Telomerase Activity and Telomere Length in HBV-Related HCC

The impact of hTERT mutation and genotypes related to telomere length that could have implicated both in the pathogenesis of CHB and in the risk of HCC-related HBV have been studied in several studies. Mutations in the core promoter of the hTERT gene were also determined to be the most frequent noncoding somatic mutations in the different cancers and HCCs. It has been shown that polymorphisms in telomerase-related genes such as rs1713449 at telomerase associated protein 1 (TEP1) and rs1469557 at the PIN2/TERF1-interacting telomerase inhibitor 1 (PINX1), with Hardy-Wienberg equilibrium, are related to HCC risk (Sriprapun et al., 2016). Kong et al., (2014) showed an increase in the expression level TERT in cell lines and HCC tumors with rs2853669 G allele (at the ATG site located on -245 bp upstream of the TERT gene) compared with rs2853669 A allele. In addition, Cheng et al., (2017) showed that the genotypes CT / CC of rs2736098 in TERT were significantly associated with CHB risk and cirrhosis compared with TT genotype. The rs2736100 variants did not show differences between healthy controls and patients with CHB, so telomere length was not different between cases and controls.

Liver biopsies from HBV-positive patients, regardless of the stage of disease, exhibit strong telomerase activity and short telomeres compare to normal liver tissue. Telomeres have even found to be shorter in liver HCC tissues than in noncancerous liver (Bellon and Nicot, 2008). Sequencing analysis of HBV-positive and HBV-negative HCCs and adjacent normal tissues revealed frequent integration of HBV genome in about 86% of tumors. There were also increased copy-number variations (CNVs) of enhancer, X and core of HBV in tumor cells (Sung et al., 2012). Further studies revealed the integration of HBV genome into the promoter of hTERT gene that correlated with increased expression of TERT in HCC and were significantly associated with less differentiated tumors. Sanger sequencing validated the recurrent HBV integration in TERT gene with unregulated expression in tumor versus normal tissue (Sung et al., 2012). HBX has been shown to increase the expression of telomerase and telomerase activity in hepatoma cells. The transfection of the preS2 genome in a cell line of hepatocellular carcinoma has also been associated with increased expression and activity of telomerase. Transfection and luciferase assay to examine the effects of HBx on telomerase showed that HBx could

Table 3. Genetic Association of Complex Diseases and Disorders (GAD)

SNP	GAD Id	Association	Phenotype	Disease Class	Gene	Reference	Pubmed	SNP reported	Associated SNPs	Entrez gene
rs398652	726415	Y	Stroke	NEUROLOGICAL	ANG		0	N	rs9322855	283
rs398652	746287	Y	Phospholipids	METABOLIC	ABCB1	Rozenn N Lemaitre et al. PLoS genetics 2011	21829377	N	rs12587311	5243
rs621559	719751	Y	Heart Failure	CARDIOVASCULAR	SPEN		0	N	rs10927873	23013
rs621559	721327	Y	Insulin	METABOLIC	EPHB2		0	N	rs10799771	2048
rs621559	721474	Y	Insulin Resistance	METABOLIC	EPHB2		0	N	rs10799771	2048
rs621559	727342	Y	Tunica Media	CARDIOVASCULAR	PTP4A2		0	N	rs563978	8073
rs621559	739706	Y	Respiratory Function Tests	CARDIOVASCULAR	PDE4DIP	Jemma B Wilk et al. BMC medical genetics 2007	17903307	N	rs41440844	
rs621559	745461	Y	Liver Cirrhosis, Biliary	METABOLIC	IL12RB2	George F Mells et al. Nature genetics 2011	21399635	N	rs17129789	3595
rs621559	745526	Y	Telomere	CANCER	WDR65	Jian Gu et al. Cancer prevention research (Philadelphia, Pa.) 2011	21460395	Y	rs621559	149465
rs621559	747900	Y	Hypothyroidism	METABOLIC	MTF1	Nicholas Eriksson et al. PloS one 2012	22493691	N	rs3748682	4520

stimulate hTERT promoter in a dose-dependent manner in several cells. Truncated and mutated reporter assays revealed that Sp1 binding sites, mapped on -132 to +5 nt of hTERT promoter, were important for HBx-mediated upregulation of hTERT (Liu et al., 2010; Sung et al., 2012). However, the study of HBx isoform in hepatoma cell lines has shown that HBx isoform reduced the expression of human telomerase which caused the telomere shortening. HBx transcriptionally suppressed human telomerase by increasing myc-associated zinc finger protein (MAZ) binding to its consensus sequence in telomerase promoter through physical association with MAZ (Su et al., 2007). In addition, evaluation of hTERT in plasma of HCC patients revealed higher levels of hTERT than healthy controls and those with CHB that presenting its potential to be an appropriate tumor marker for common tumors (Yang et al., 2011).

Due to the understanding the genotype-phenotype interactions, we used SNPnexus to evaluate the genotype frequencies and genomic matches of SNPs of rs398652 at 14 q22.3 and rs621559 at1p34.2 with the risks of disease and complex disorders. SNPnexus was developed to evaluate the potential impact of novel SNPs through GWAS results on the potent transcriptome, proteome and regulatory patterns of genomic variations (Chelala et al., 2009; Dayem Ullah et al., 2013).

In silico analysis by entering the rs398652 and rs621559 queries in the SNPnexus query options, we showed the Genomic Coordinates and External Links as well as genotype-phenotype associations in Tables 1 to 5. The locus of rs398652 locates around 60 kb from the PELI2 gene, regulates encoding pellino2 (a key protein in inflammation and production of cytokines). Unlike, the gene of WDR65 that encompasses the locus of rs621559 is not informative. However, SNPnexus showed that both SNPs falls into an intronic zone and are in association with telomere length and carcinogenesis.

The Genomic Organization and analysis of both loci is presented in Table 1. Genotypes and allele frequencies from the International HapMap project in the CEU population (Northern and Western European ancestry), YRI (Yoruba in Ibadan, Nigeria), CHB (Han Chinese in Beijing, China) and JPT (Japanese in Tokyo, Japan) are available in Table 2. The genotype frequencies of all SNPs correspond to Hardy–Weinberg equilibrium, however as indicated in this table, the frequencies of genotypes and allele of both loci are not similar in the four populations. The association of loci rs398652 and rs621559 with complex diseases and disorders with related references are indicated in Table 3. The data indicated that both loci rs398652 and rs621559 are involved in metabolic disorders, however the rs398652 SNP is responsible for stroke and rs621559 in heart failure and liver cirrhosis.

In conclusion, several SNPs all over the genome are expected to be associated with telomere length, however few of common genetic variation in candidate genes of human genome have been shown to related to HBV-related HCC within different global populations. The average telomere length of circulating leukocytes is shorter in patients with CHB than in healthy subjects. Two loci (14 q22.3, rs398652 and 1p34.2, rs621559) were significantly related to the HBV-related HCC risk and their potential effects were evaluated by SNPnexus for genotype-phenotype associations. Taken together, these findings indicate that telomerase plays an important role in HBV-related HCC risk, however, additional studies will be helpful in understanding the regulatory patterns of genomic variations related to telomere length.

Conflict of interest

The authors declare that they have no conflict of interest.

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