**Genetic polymorphisms in CD35 Gene contribute to the susceptibility and prognosis of hepatocellular carcinoma**

Li-Mei luo1, Qin Li2, Zhen-Zhen Su1, Li-Xin Li1, Bei Cai1, Yu-Fu Peng2, Yang-Juan Bai1 and Fei Liu2,**\***

1 Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China

2 Department of Liver Surgery, Liver Transplantation Center, West China Hospital of Sichuan University, Chengdu, China

**\*Corresponding author:** Fei Liu, *E-mail*: [liufei8306@163.com](mailto:liufei8306@163.com)

**Supplementary table S1. SNPs of CD35 gene evaluated in this study**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| SNP ID | Base change | Chromosome | MAF in controls | *P* for HWE | Functional Consequence |
| rs10494885 | A to G | 1q32 | 0.14 | 0.10 | Downstream transcript variant |
| rs2296160 | A to G | 1q32 | 0.12 | 0.61 | Missense variant, coding sequence variant |
| rs3737002 | C to T | 1q32 | 0.13 | 0.15 | Missense variant, coding sequence variant |
| rs3849266  rs6691117  rs7525160 | C to T  A to G  G to C | 1q32  1q32  1q32 | 0.13  0.08  0.14 | 0.09  0.95  0.42 | Intron variant  Coding sequence variant, missense variant  Upstream transcript variant |

Abbreviations: SNP, single nucleotide polymorphism; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium

**Supplementary table S2. Primer sequences used in the selected tag SNPS of CD35 genotyping**

|  |  |  |  |
| --- | --- | --- | --- |
| **SNP ID** | **PCR primer 1 (5’ to 3’)** | **PCR primer 2 (5’ to 3’)** | **Extension Primer (5’ to 3’)** |
| rs10494885 | ACGTTGGATGCCAGCCAACTGACCTTTATG | ACGTTGGATGTGATGTGTAATGCCACAGAC | cGTCTGATTTTCTTTCCTGTTAC |
| rs2296160 | ACGTTGGATGAGATGCCAGAATTCCTCAGC | ACGTTGGATGCCAGAGTGATGTTTTGTGAC | aAACTATGAGAGCATCATGTG |
| rs3737002 | ACGTTGGATGAAATACCCAGGACGGCATTC | ACGTTGGATGCAGAGCAGTTTCCATTTGCC | aaaaTCAAAGTCATTAATTGGGATC |
| rs3849266  rs6691117  rs7525160 | ACGTTGGATGATAACTCGCCCCTCAATCTG  ACGTTGGATGACCCTACCATGACAAACCCG  ACGTTGGATGCAAAATCAAGGTTTAAAGTC | ACGTTGGATGGCAGGCAACTGTGTTTATAC  ACGTTGGATGGAGTACCAGGAAACAGGAGT  ACGTTGGATGTCCTTCTGACATGTACTGCC | CCTCAATCTGCATTGATCCA  GGCTGACATCTAAATCTGA  AAGGTTTAAAGTCAAAATTATTTTAA |

**Supplementary table S3. Clinical and pathological features of HCC patients treated with hepatectomy**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Covariate | n | rs7525160 genotype | | *P*-value |
| CG/CC | GG |
| Age  <65 years  ≥65 years  Gender  Female  Male  Hepatitis B  Without  With  Smoking status  Never  Ever/current  Drinking status  Never  Ever/current  HBV DNA  <102  ≥102  Child-Pugh Class  A  B  C  Microvascular invasion  Without  With  Tumor stage (BCLC)  0/A  B/C  α-fetoprotein level (ng/mL)  <400  ≥400  Tumor size (cm)  ≤5cm  ＞5cm  Tumor number  Single  Multiple  Tumor stage (TNM)  I/II  III/IV  Background cirrhosis  Present  Absent  Portal vein tumor thrombosis  No  Yes  Distant metastasis  No  Yes | 235  64  48  251  61  238  138  161  166  133  168  130  2  286  11  202  93  228  71  91  208  149  150  244  55  233  66  88  211  265  34  297  2 | 158  39  36  161  41  156  84  113  111  86  107  89  0  191  6  127  68  148  49  69  128  103  94  162  35  150  47  60  137  170  27  196  1 | 77  25  12  90  20  82  54  48  55  47  61  41  2  95  5  75  25  80  22  22  80  46  56  82  20  83  19  28  74  95  7  101  1 | 0.428  1.198  0.925  0.116  0.782  0.461  0.101  0.095  0.622  0.024  0.291  0.816  0.375  0.684  0.115  1.000 |

**Supplementary table S4. Stratified analysis of CD35 rs7525160 genetic variation and HCC risk**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variable | n | HCC (n) | Adjusted OR (95% *CI*) a | *P*-value | *P* for interaction |
| Age  <65 years  ≥65 years  Gender  Female  Male | 960  273  445  788 | 444  142  95  491 | 1.85(1.30-2.62)  0.98(0.56-1.74)  1.68(0.94-2.97)  1.37(0.96-1.94) | 0.001\*  0.954  0.078  0.080 | 0.042\*  0.336 |
| Hepatitis B  Without  With  Smoking status  Never  Ever/current | 648  585  781  452 | 144  442  275  311 | 1.38(0.89-2.14)  1.69(1.13-2.52)  1.42(0.98-2.08)  1.57(0.97-2.55) | 0.151  0.010\*  0.065  0.069 | 0.324  0.695 |
| Drinking status  Never  Ever/current  Family history of liver cancer  Without  With | 817  416  1185  48 | 326  260  560  26 | 1.50(1.03-2.18)  1.50(0.92-2.45)  1.49(1.10-2.02)  3.04(0.67-13.75) | 0.034\*  0.104  0.010\*  0.148 | 0.865  0.640 |

a adjusted for gender, smoking, Hepatitis B and drinking status. \**P*<0.05, statically significant.

**Supplementary table S5. Subgroup analyses of CD35 rs7525160 genetic variation and HCC risk**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Characteristics | GG [n (%)] | CC/CG [n (%)] | Adjusted OR (95% *CI*) a | *P*-value |
| Tumor size (cm)  ≤5cm  ＞5cm | 112(62.20)  68(37.80) | 223(54.90)  183(45.10) | 1.37(0.98-1.92)  1.75(1.19-2.56) | 0.067  0.004\* |
| Tumor number  Single  Multiple  Tumor stage (TNM)  I/II  III/IV  Liver cirrhosis | 117(65.00)  63(35.00)  131(72.80)  49(27.20) | 273(67.20)  133(32.80)  283(69.70)  123(30.30) | 1.55(1.11-2.15)  1.45(0.97-2.17)  1.47(1.07-2.03)  1.64(1.07-2.52) | 0.009\*  0.073  0.018\*  0.024\* |
| With  Without  Portal vein rumor thrombus  With  Without  Distant metastasis  With  Without  α-fetoprotein level (ng/mL)  <400  ≥400 | 104(57.80)  76(42.20)  17(9.40)  163(90.60)  5(2.80)  175(97.20)  137 (76.10)  43(23.90) | 244(60.10)  162(39.90)  61(15.00)  345(85.00)  14(3.40)  392(96.60)  281(69.20)  125(30.80) | 1.50(1.05-2.15)  1.45(1.01-2.08)  2.30(1.24, 4.27)  1.44(1.06, 1.95)  1.86(0.63，5.50)  1.50(1.12，2.02)  1.40(1.02, 1.92)  1.94(1.24, 3.04) | 0.026\*  0.042\*  0.008\*  0.018\*  0.264  0.007\*  0.035\*  0.004\* |

a adjusted for gender, smoking, Hepatitis B and drinking status. \**P*<0.05, statically significant

**Supplementary table S6. CD35 genetic variation on overall survival of hepatectomy HCC patients**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| CD35  SNP ID | Genotype | Frequency [n (%)] | Death [n (%)] | MST(months) | 95%CI(months) | *P*-value |
| rs10494885  rs2296160  rs3737002  rs3849266  rs6691117  rs7525160 | AA  GG/AG  AA  GG/AG  CC  TT/CT  CC  TT/CT  AA  GG/AG  GG  CC/CG | 46(15.4)  253(84.6)  39(13.0)  260(87.0)  135(45.2)  164(54.8)  136(45.5)  163(54.5)  155(51.8)  144(48.2)  102(34.1)  197(65.9) | 4(14.8)  23(85.2)  2(7.4)  25(92.6)  11(40.7)  16(59.3)  11(40.7)  16(59.3)  13(48.1)  14(55.2)  7(25.9)  20(74.1) | 28.706  28.535  29.310  28.457  28.667  28.448  28.691  28.432  28.684  28.467  28.891  28.350 | 26.835-30.576  27.688-29.382  27.428-31.192  27.618-29.295  27.516-29.817  27.395-29.501  27.553-29.829  27.371-29.492  27.631-29.737  27.350-29.584  27.669-30.112  27.369-29.331 | 0.876  0.383  0.730  0.693  0.718  0.356 |



**Supplementary figure S1.** MALDI-TOF genotyping and direct sequencing map for CD35 rs7525160. (A). The three genotypes of CD35 rs7525160 polymorphism detected by MALDI-TOF. (B). Sequencing map for genotypes of CD35 rs7525160 GG genotype. (C). Sequencing map for genotypes of CD35 rs7525160 CG genotype.