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

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**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 |
| rs3849266rs6691117rs7525160 | C to TA to G G to C | 1q321q321q32 | 0.130.080.14 | 0.090.950.42 | Intron variantCoding sequence variant, missense variantUpstream 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 |
| rs3849266rs6691117rs7525160 | ACGTTGGATGATAACTCGCCCCTCAATCTGACGTTGGATGACCCTACCATGACAAACCCGACGTTGGATGCAAAATCAAGGTTTAAAGTC | ACGTTGGATGGCAGGCAACTGTGTTTATACACGTTGGATGGAGTACCAGGAAACAGGAGTACGTTGGATGTCCTTCTGACATGTACTGCC | CCTCAATCTGCATTGATCCAGGCTGACATCTAAATCTGAAAGGTTTAAAGTCAAAATTATTTTAA |

**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 yearsGender Female MaleHepatitis BWithoutWithSmoking statusNeverEver/currentDrinking statusNeverEver/currentHBV DNA<102 ≥102Child-Pugh Class ABCMicrovascular invasionWithoutWithTumor stage (BCLC) 0/AB/Cα-fetoprotein level (ng/mL) <400 ≥400Tumor size (cm) ≤5cm ＞5cmTumor numberSingle MultipleTumor stage (TNM) I/II III/IVBackground cirrhosis Present AbsentPortal vein tumor thrombosis No YesDistant metastasis No Yes | 235644825161238138161166133168130228611202932287191208149150244552336688211265342972 | 1583936161411568411311186107890191612768148496912810394162351504760137170271961 | 772512902082544855476141295575258022228046568220831928749571011 | 0.4281.1980.9250.1160.7820.4610.1010.0950.6220.0240.2910.8160.3750.6840.1151.000 |

**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 yearsGender Female Male | 960273445788 | 44414295491 | 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\*0.9540.0780.080 | 0.042\*0.336 |
| Hepatitis BWithoutWithSmoking statusNeverEver/current | 648585781452 | 144442275311 | 1.38(0.89-2.14)1.69(1.13-2.52)1.42(0.98-2.08)1.57(0.97-2.55) | 0.1510.010\*0.0650.069 | 0.3240.695 |
| Drinking statusNeverEver/currentFamily history of liver cancerWithoutWith | 817416118548 | 32626056026 | 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\*0.1040.010\*0.148 | 0.8650.640 |

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) | 0.0670.004\* |
| Tumor number Single MultipleTumor 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\*0.0730.018\*0.024\* |
|  With WithoutPortal vein rumor thrombus With WithoutDistant metastasis With Withoutα-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\*0.2640.007\*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 |
| rs10494885rs2296160rs3737002rs3849266rs6691117rs7525160 | AAGG/AGAAGG/AGCCTT/CTCCTT/CTAAGG/AGGGCC/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) | 28.70628.53529.31028.45728.66728.44828.69128.43228.68428.46728.89128.350 | 26.835-30.57627.688-29.38227.428-31.19227.618-29.29527.516-29.81727.395-29.50127.553-29.82927.371-29.49227.631-29.73727.350-29.58427.669-30.11227.369-29.331 | 0.8760.3830.7300.6930.7180.356 |



**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.