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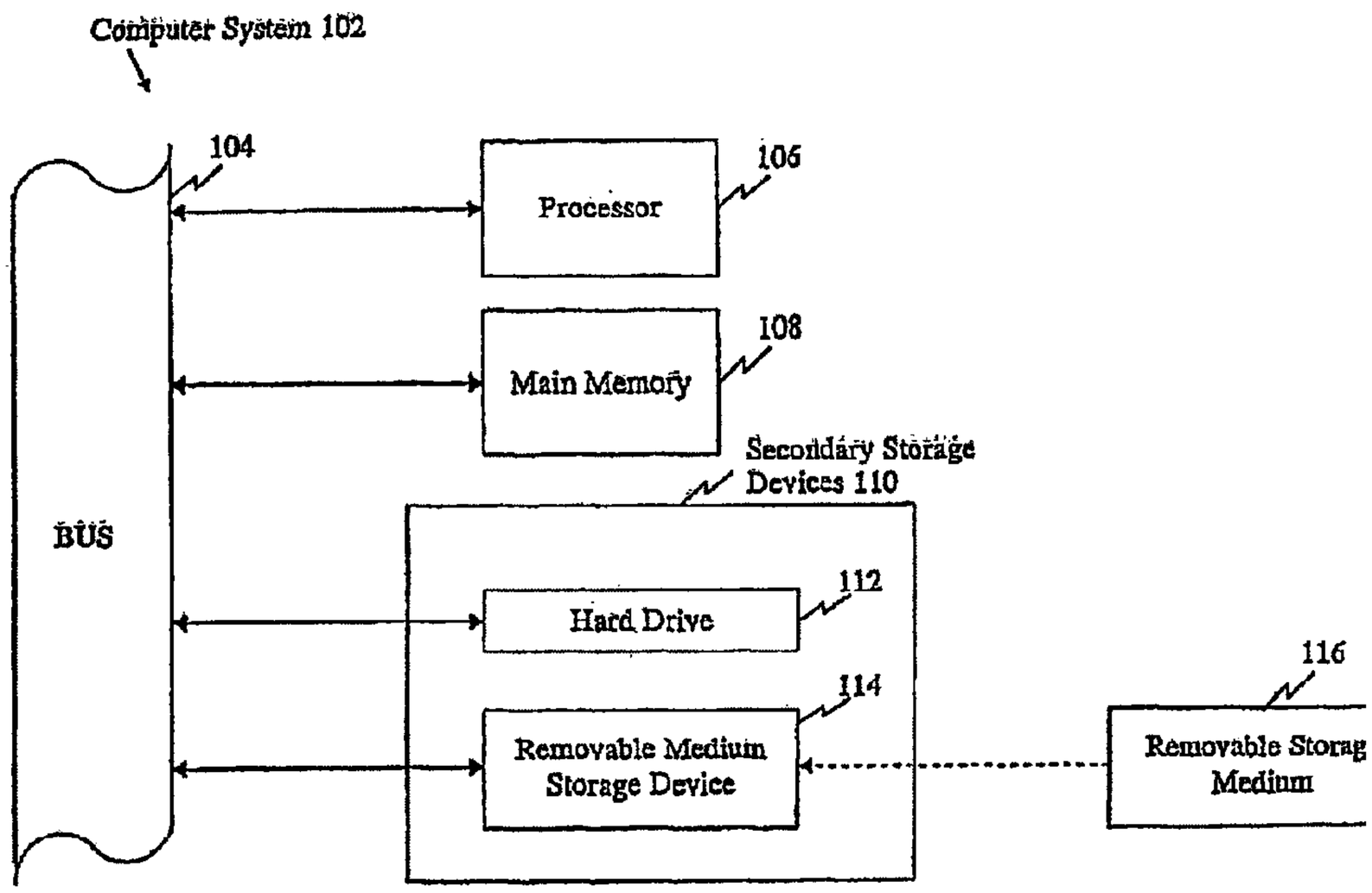
(71) **Demandeur/Applicant:**
CELERA CORPORATION, US

(72) **Inventeurs/Inventors:**
CARGILL, MICHELE, US;
HUANG, HONGJIN, US

(74) **Agent:** FETHERSTONHAUGH & CO.

(54) **Titre : POLYMORPHISMES GENETIQUES ASSOCIES A DES TECHNIQUES DE DETECTION DE CIRRHOSE DU FOIE ET UTILISATION DE CES POLYMORPHISMES**

(54) **Title: GENETIC POLYMORPHISMS ASSOCIATED WITH LIVER FIBROSIS METHODS OF DETECTION AND USES THEREOF**



(57) **Abrégé/Abstract:**
The present invention is based on the discovery of genetic polymorphisms that are associated with liver fibrosis and related pathologies. In particular, the present invention relates to nucleic acid molecules containing the polymorphisms, variant proteins encoded by such nucleic acid molecules, reagents for detecting the polymorphic nucleic acid molecules and proteins, and methods of using the nucleic acid and proteins as well as methods of using reagents for their detection.

Abstract

The present invention is based on the discovery of genetic polymorphisms that are associated with liver fibrosis and related pathologies. In particular, the present invention relates to nucleic acid molecules containing the polymorphisms, variant proteins encoded by such nucleic acid molecules, reagents for detecting the polymorphic nucleic acid molecules and proteins, and methods of using the nucleic acid and proteins as well as methods of using reagents for their detection.

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<212> DNA
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<210> 32206
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gagccctaat ggtgcaccct tccgacaatc cttcatcctt cccagacgtt tcatcagcca 180
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<211> 201
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<212> DNA
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<213> Homo sapiens

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<212> DNA
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<212> DNA
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<213> Homo sapiens

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<213> Homo sapiens

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<213> Homo sapiens

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<213> Homo sapiens

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<212> DNA
<213> Homo sapiens

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<210> 32360
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<211> 201
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<210> 32367
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<213> Homo sapiens

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<400> 32373
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<210> 32578
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<212> DNA
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<213> Homo sapiens

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<211> 201
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<213> Homo sapiens

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<213> Homo sapiens

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<212> DNA
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<400> 33001
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tgagcagtgt cctgctgttt t 201

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<210> 33003
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ccccttctct tgacagtggt gtttttgcct tcacactact gtatccctgg tccataccgt 180
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taatcagctt ttgcccagca aaaggaacag tcagcagagt aaacagacaa cccgcagagt 180
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<213> Homo sapiens

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<213> Homo sapiens

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<210> 33047
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<400> 33052
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gagttcagta tgtcaggact gctacattta tgagtgtggg tctttattga agtctctctt 180
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<210> 33090
<211> 201
<212> DNA
<213> Homo sapiens

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<211> 201
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<211> 201
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<210> 33097
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<210> 33099
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<212> DNA
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<210> 33101
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aaaaaaagtt gtaataaatt a 201

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gtctcaaaaa aaaaaaaaaa a 201

<210> 33104
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<212> DNA
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cgtctcaaaa aaaaaaaaaa a 201

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<211> 201
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tggttgtctg tgaggacaac agaaagtaat gttttctctg aaaagtttaa aagggtaaat 180
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gtctgtagag acaaaattgt tgttttcccc agttgtaaag tgtgcaatat tatccttgca 180
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<210> 33254
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<210> 33468
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taaagacata cccaagactg tgcaatttac aaaagaaaga ggtttaatga actcacattt 180
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<213> Homo sapiens

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<213> Homo sapiens

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<211> 201
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<212> DNA
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<213> Homo sapiens

<400> 33571
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<210> 33572
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<212> DNA
<213> Homo sapiens

<400> 33572
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<210> 33584
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catgctggat atttaccgtc agtcatttga ggaagttcac ttctctgctg gttttctgag 180
tgagggagcg atgagtagag g 201

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<212> DNA
<213> Homo sapiens

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tggaatttaa cacagttttc ccaggcccтt catggaattt raaaacatga ctctgaaagc 120
atgtggctga aatgagactc tttcacactg tttaaagatg catgtgcata tgttggtgtc 180
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<210> 33724
<211> 201
<212> DNA
<213> Homo sapiens

<400> 33724
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ctaccattcc ttgtggcaca gtgcatggtc tgcatggcac agtgtgtggt ctgtatggca 180
cagtgcatgg tccgcatggc a 201

<210> 33725
<211> 201
<212> DNA
<213> Homo sapiens

<400> 33725
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cattcaggag tcatattcta tctaagcttt aatggtgttg gtgttccctt tcctttggcc 180
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<210> 33726
<211> 201
<212> DNA
<213> Homo sapiens

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tctttaaccc tgtgaatgtc cgttttctct aaggcggggt gggctgaaac ataacataac 180
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<210> 33727
<211> 201
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<213> Homo sapiens

<400> 33727
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<211> 201
<212> DNA
<213> Homo sapiens

<400> 33728
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tctattatgc cagctgcaac tcaaatcaaa ggcactgttg mtttggtacc cgcatttaag 120
gagggaatac cccaaaaaac ttaaacttgt ctggctttgc aatggtggtg gagtcccaag 180
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<210> 33729
<211> 201
<212> DNA
<213> Homo sapiens

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aagaagggaa cctgcagagg cagaaacttc tgtgggttgc agttgtgttg agattacaca 180
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<210> 33730
<211> 201
<212> DNA
<213> Homo sapiens

<400> 33730
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<210> 33731

<211> 201
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<213> Homo sapiens

<400> 33731
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cacccagcac agtggcagca c 201

<210> 33732
<211> 201
<212> DNA
<213> Homo sapiens

<400> 33732
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atttgcattg tccccaaatc taacccaagc tgaaagacat yaggcctatc ttcttgcttt 120
atgcataatg gcagatctcc agggagggag aggatgtgtg ctggaagatt ctacaacttg 180
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<210> 33733
<211> 201
<212> DNA
<213> Homo sapiens

<400> 33733
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ggcttctgtt gctttatttc t 201

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<400> 33734
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<210> 33735
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<212> DNA
<213> Homo sapiens

<400> 33735
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gtttatttta gacaaatatt t 201

<210> 33736
<211> 201
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<400> 33736

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<210> 33737
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<213> Homo sapiens

<400> 33737
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tatggagcta gctggccttc cacaggttct ggagttaatg ctggagagaa gtaggggtgt 180
cagagcctac cctgtgttcc t 201

<210> 33738
<211> 201
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<213> Homo sapiens

<400> 33738
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<210> 33739
<211> 201
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<400> 33739
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gcagaagttg caaataactt gttgtgtttt cctaagtact gagctgtgtg aattggtttc 180
atgttgatgt tactgaagtc t 201

<210> 33740
<211> 201
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<400> 33740
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cgccccgagg ggtttcactt cccactgcct cttggtagcc rtgggcactt tggtgctctg 120
atgatgtaac atggagattg tccttgtctc catcgctgtc tttactggtg tatgcccatg 180
aggtatgtca aagtctgtat g 201

<210> 33741
<211> 201
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<400> 33741
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aacaagaatg tgaaaagcta tttattacca gatgtgggca saccctccag ccttttttтg 120
ttcttttaaa ccctaatctc tttttaaaat ttttttttatt tttaatgttt atgggtacat 180
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<210> 33742
<211> 201
<212> DNA
<213> Homo sapiens

<400> 33742
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tgttgcaaaa ttgagataat gataatagct aatgatacct stcccttact gataattgaa 120
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tatcctcatt ttggagatga a 201

<210> 33743
<211> 201
<212> DNA
<213> Homo sapiens

<400> 33743
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ttgtgggcaa tttcagcctc taaagtttga ttaaagatac ttcagatatt cctactgaga 180
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<210> 33744
<211> 201
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<400> 33744
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tgtattagtt tctgctggcc gctgtaacac attatcacca gtgtagcacc taaacaacac 180
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<210> 33745
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<400> 33745
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aaaatgttaa tacgttagtt ttctcactgt caatgtgttc tatgacatca tttatgcaaa 180
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<210> 33746
<211> 201
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<213> Homo sapiens

<400> 33746
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gcactgcctt gggtaaataa ttctctctcc acacatttcc tagtttataa tgaggagttt 180
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<210> 33747
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<400> 33747
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gtgccgttaa gggaattggc agtgttcaga cagaatggta acattctctc tgatcgtgtc 180
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<210> 33748
<211> 201
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<400> 33748
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<210> 33749
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<210> 33750
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<400> 33750
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<210> 33751
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<400> 33751
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atcttagaga gggacaccga tccctaccct gcatggggtt wttcacagag ccatggggac 120
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<212> DNA
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<400> 33752
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<210> 33753
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ggaaaaaggg gactgaaatt agtgctgata aaagcttgaa wtagacatta tagcactgaa 120
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<210> 33754
<211> 201
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gaggaacacc acctatcaga gttcacactg agtttgcaga ktcatccttt tgcttccagc 120
catcccatgg aatgctgttc ttggaagggt catctcatgg tccattgtcg gccctcttag 180
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<210> 33756
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<210> 33757
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<210> 33758
<211> 201
<212> DNA
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<210> 33759
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<210> 33761
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<212> DNA
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<212> DNA
<213> Homo sapiens

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<210> 33763
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ttgaaatatg tcataaagtt a 201

<210> 33764
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<213> Homo sapiens

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<213> Homo sapiens

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<213> Homo sapiens

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<211> 201
<212> DNA
<213> Homo sapiens

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gaatcaccaa tttagatatc caggcttcct aaaatataag taagtcagat ttagcattac 180
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<210> 33768
<211> 201
<212> DNA
<213> Homo sapiens

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caactcaaga cagttcaaag cagcttggag cctcttggat tttaaaaaga ctctgtggcc 180
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<210> 33769
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<213> Homo sapiens

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<213> Homo sapiens

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<210> 33771
<211> 201
<212> DNA
<213> Homo sapiens

<400> 33771
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ggctggaata gaagcaaaat t 201

<210> 33772
<211> 201
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<211> 201
<212> DNA
<213> Homo sapiens

<400> 33773
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<210> 33774
<211> 201
<212> DNA
<213> Homo sapiens

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<213> Homo sapiens

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<211> 201
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<213> Homo sapiens

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<210> 33831
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<212> DNA
<213> Homo sapiens

<400> 33831
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<210> 33832
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<212> DNA
<213> Homo sapiens

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<211> 201
<212> DNA
<213> Homo sapiens

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<211> 201
<212> DNA
<213> Homo sapiens

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<212> DNA
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<210> 33838
<211> 201
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<213> Homo sapiens

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<212> DNA
<213> Homo sapiens

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<210> 33841
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<400> 33841
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<210> 33842
<211> 201
<212> DNA
<213> Homo sapiens

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<210> 33843
<211> 201
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<210> 33844
<211> 201
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<210> 33845
<211> 201
<212> DNA
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<210> 33846
<211> 201
<212> DNA
<213> Homo sapiens

<400> 33846
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<210> 33847
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<210> 33848
<211> 201
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<213> Homo sapiens

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<210> 33849
<211> 201
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<210> 33850
<211> 201
<212> DNA
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<400> 33850
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<210> 33851

<211> 201
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<210> 33852
<211> 201
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<210> 33854
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<400> 33854
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<210> 33856
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<210> 33858
<211> 201
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<210> 33859
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<400> 33859
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<210> 33861
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<213> Homo sapiens

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<210> 33862
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<210> 33867
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<210> 33868
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<400> 33869
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<213> Homo sapiens

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<213> Homo sapiens

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<210> 33880
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<212> DNA
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<212> DNA
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<212> DNA
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<210> 33896
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<213> Homo sapiens

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<213> Homo sapiens

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<210> 33947
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<400> 33947
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gggctctgag tctttaaaag gcaacagaaa ggtctctgtt acgaaggggg aactagtttt 180
cattgtcact gtattaagca g 201

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<212> DNA
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<213> Homo sapiens

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<210> 33951
<211> 201
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<210> 33952
<211> 201
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<213> Homo sapiens

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cactttcaca gtcaagagtt a 201

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tagtgcatcc ttttaagatg gcttcctgca tttcgatata mgcataagct tgggtttcag 120
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<210> 33957
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<210> 33961
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<212> DNA
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What Is Claimed Is:

1. A method for identifying an individual who has an altered risk for developing liver fibrosis, comprising detecting a single nucleotide polymorphism (SNP) in any one of the nucleotide sequences of SEQ ID NOS: 1-261, 523-18973, and 18974-34256 in said individual's nucleic acids, wherein the presence of the SNP is correlated with an altered risk for liver fibrosis in said individual.

2. The method of claim 1 in which the altered risk is an increased risk.

3. The method of claim 1 in which the altered risk is a decreased risk.

4. The method of claim 1, wherein the SNP is selected from the group consisting of the SNPs set forth in Tables 6 and 7.

5. The method of claim 1 in which detection is carried out by a process selected from the group consisting of: allele-specific probe hybridization, allele-specific primer extension, allele-specific amplification, sequencing, 5' nuclease digestion, molecular beacon assay, oligonucleotide ligation assay, size analysis, and single-stranded conformation polymorphism.

6. An isolated nucleic acid molecule comprising at least 8 contiguous nucleotides wherein one of the nucleotides is a single nucleotide polymorphism (SNP) selected from any one of the nucleotide sequences in SEQ ID NOS: 1-261, 523-18973, and 18974-34256, or a complement thereof.

7. The isolated nucleic acid molecule of claim 6, wherein the SNP is selected from the group consisting of the SNPs set forth in Tables 3 and 4.

8. An isolated nucleic acid molecule that encodes any one of the amino acid sequences in SEQ ID NOS:262-522.

9. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:262-522.

10. An antibody that specifically binds to a polypeptide of claim 9, or an antigen-binding fragment thereof.

11. An amplified polynucleotide containing a single nucleotide polymorphism (SNP) selected from any one of the nucleotide sequences of SEQ ID NOS: 1-261, 523-18973, and 18974-34256 , or a complement thereof, wherein the amplified polynucleotide is between about 16 and about 1,000 nucleotides in length.

12. An isolated polynucleotide which specifically hybridizes to a nucleic acid molecule containing a single nucleotide polymorphism (SNP) in any one of the nucleotide sequences in SEQ ID NOS: 1-261, 523-18973, and 18974-34256.

13. The polynucleotide of claim 12, which is an allele-specific probe.

14. The polynucleotide of claim 12, which is an allele-specific primer.

15. The polynucleotide of claim 12, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of the primer sequences set forth in Table 5 (SEQ ID NOS:34257-34458).

16. A kit for detecting a single nucleotide polymorphism (SNP) in a nucleic acid, comprising the polynucleotide of claim 12, a buffer, and an enzyme.

17. A method for identifying an agent useful in therapeutically or prophylactically treating liver fibrosis, comprising contacting the polypeptide of claim 9 with a candidate agent under conditions suitable to allow formation of a binding complex between the

polypeptide and the candidate agent, and detecting the formation of the binding complex, wherein the presence of the complex identifies said agent.

18. A method for identifying an individual who has a risk for progressing rapidly from minimal fibrosis to bridging fibrosis/cirrhosis, comprising detecting a single nucleotide polymorphism (SNP) in any one of the nucleotide sequences of SEQ ID NOS: 1-261 and 523-34256 in said individual's nucleic acids, wherein the presence of the SNP is correlated with a risk for a rapid rate of progression to bridging fibrosis/cirrhosis in said individual.

19. A method for determining whether a hepatitis C virus-infected human has an increased risk for developing liver fibrosis, comprising:

a) testing nucleic acid from said human to determine the presence or absence of a polymorphism in gene PNPLA3 at position 101 of the nucleotide sequence defined by SEQ ID NO:8938 or its complement; and

b) correlating the presence of G at position 101 of SEQ ID NO:8938 or C at position 101 of its complement with said human having said increased risk for developing liver fibrosis.

20. A method for determining whether a hepatitis C virus-infected human has an increased risk for developing liver fibrosis, comprising:

a) testing nucleic acid from said human to determine the presence or absence of a polymorphism in gene LRP5 at position 101 of the nucleotide sequence defined by SEQ ID NO:18900 or its complement; and

b) correlating the presence of C at position 101 of SEQ ID NO:18900 or G at position 101 of its complement with said human having said increased risk for developing liver fibrosis.

21. A method for determining whether a human has an altered risk for developing liver fibrosis, comprising:

a) testing nucleic acid from said human to determine the presence or absence of a polymorphism in gene DDX5 at position 101 of the nucleotide sequence defined by SEQ ID NO:19179 or its complement; and

b) correlating the presence of the polymorphism at position 101 of SEQ ID NO:19179 or its complement with said human having said altered risk for developing liver fibrosis.

22. A method for determining whether a human has an altered risk for developing liver fibrosis, comprising:

a) testing nucleic acid from said human to determine the presence or absence of a polymorphism in gene MTP at position 101 of the nucleotide sequence defined by SEQ ID NO:22070 or its complement; and

b) correlating the presence of the polymorphism at position 101 of SEQ ID NO:22070 or its complement with said human having said altered risk for developing liver fibrosis.

23. The method of any one of claims 19 to 22, wherein said nucleic acid is a nucleic acid extract from a biological sample from said human.

24. The method of claim 23, wherein said biological sample is blood, saliva, or buccal cells.

25. The method of claim 23 or 24, further comprising preparing said nucleic acid extract from said biological sample prior to said testing.

26. The method of any one of claims 19 to 25, wherein said testing comprises nucleic acid amplification.

27. The method of claim 26, wherein said nucleic acid amplification is carried out by polymerase chain reaction.

28. The method of any one of claims 19 to 27, wherein said testing is performed using sequencing, 5' nuclease digestion, molecular beacon assay, oligonucleotide ligation assay, size analysis, single-stranded conformation polymorphism analysis, or denaturing gradient gel electrophoresis (DGGE).

29. The method of any one of claims 19 to 27, wherein said testing is performed using an allele-specific method.

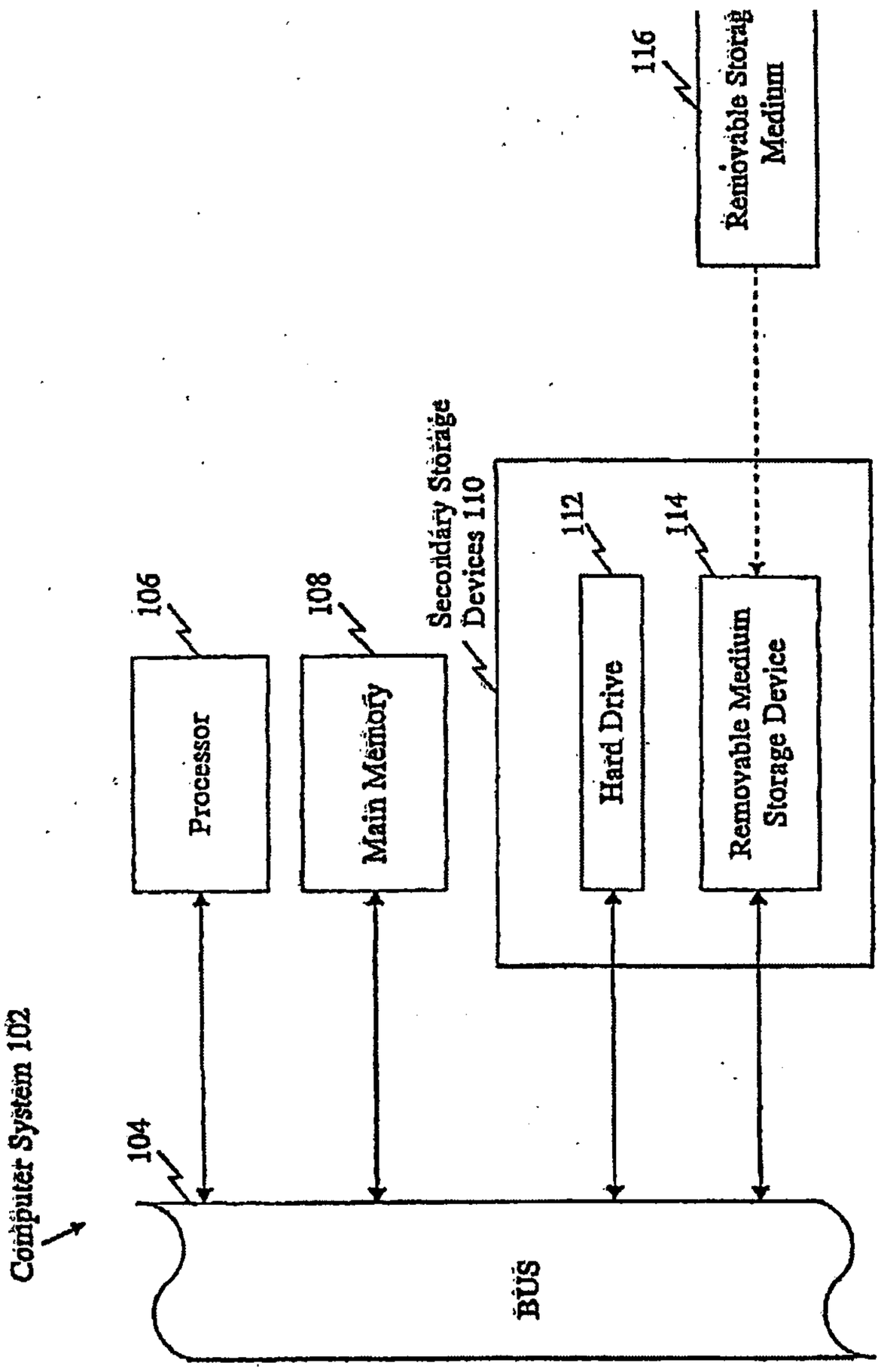
30. The method of claim 29, wherein said allele-specific method is allele-specific probe hybridization, allele-specific primer extension, or allele-specific amplification.

31. The method of claim 19 or 20, wherein said human is homozygous for said C or said G.

32. The method of claim 19 or 20, wherein said human is heterozygous for said C or said G.

33. The method of any one of claims 19 to 32, wherein said correlating is performed using computer software.

34. The method of any one of claims 19 to 33, which is an automated method.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Main Memory
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Secondary Storage Devices 110
Hard Drive
112
Removable Medium Storage Device
114
Removable Storage Medium
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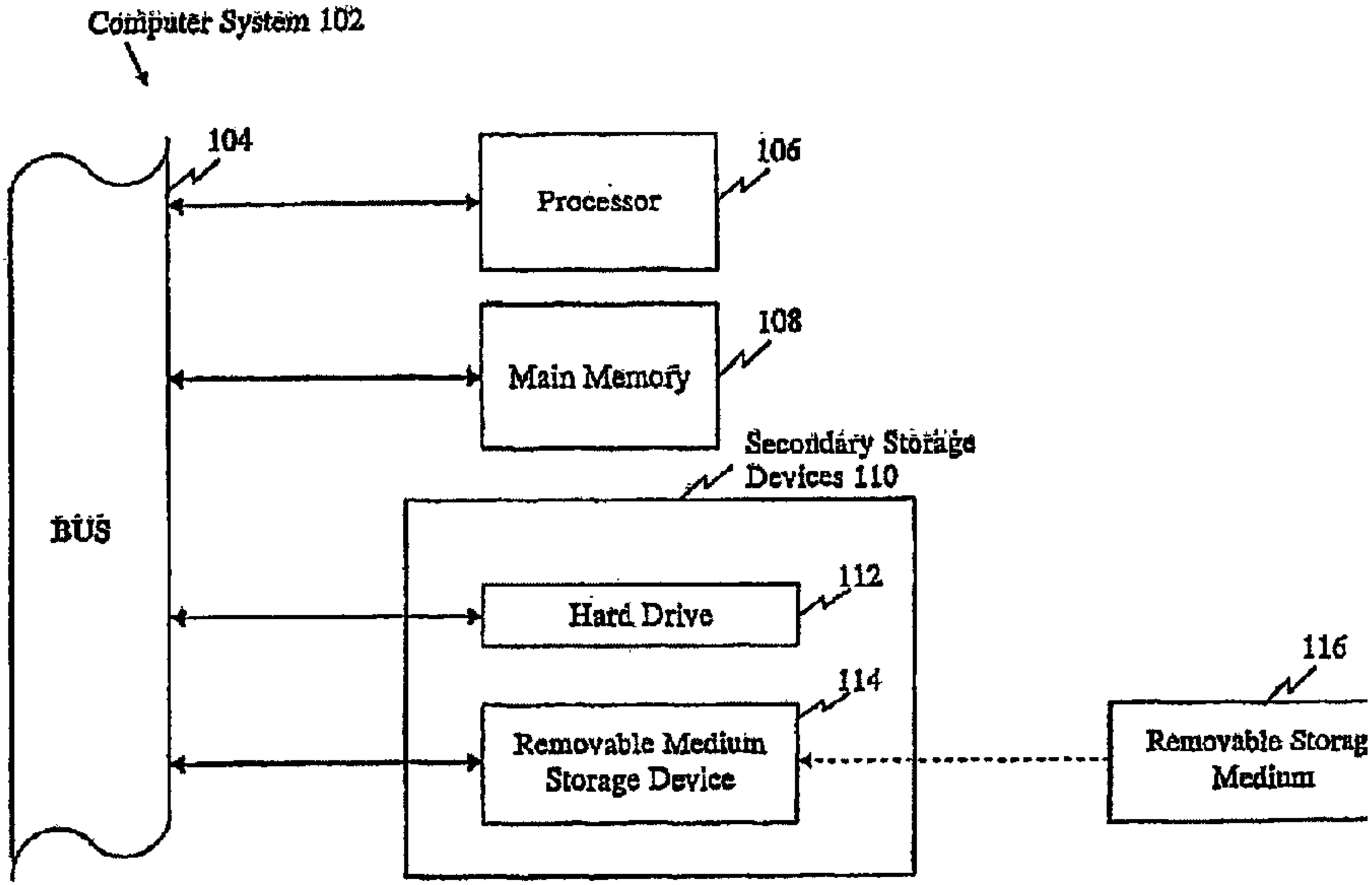
FIGURE 1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