



4-19-2011

# Methods and Animal Model for Analyzing Age-Related Macular Degeneration

Jayakrishna Ambati

University of Kentucky, jayakrishna.ambati@uky.edu

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Ambati, Jayakrishna, "Methods and Animal Model for Analyzing Age-Related Macular Degeneration" (2011). *Ophthalmology and Visual Science Faculty Patents*. 4.

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US007928284B2

(12) **United States Patent**  
**Ambati**

(10) **Patent No.:** **US 7,928,284 B2**  
(45) **Date of Patent:** **Apr. 19, 2011**

(54) **METHODS AND ANIMAL MODEL FOR ANALYZING AGE-RELATED MACULAR DEGENERATION**

(75) Inventor: **Jayakrishna Ambati**, Lexington, KY (US)

(73) Assignee: **University of Kentucky Research Foundation**, Lexington, KY (US)

(\* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 98 days.

(21) Appl. No.: **12/382,702**

(22) Filed: **Mar. 23, 2009**

(65) **Prior Publication Data**

US 2009/0260091 A1 Oct. 15, 2009

**Related U.S. Application Data**

(63) Continuation of application No. 10/685,705, filed on Oct. 16, 2003, now Pat. No. 7,595,430.

(60) Provisional application No. 60/422,096, filed on Oct. 30, 2002.

(51) **Int. Cl.**

- A01K 67/00** (2006.01)
- A01K 67/027** (2006.01)
- A01K 67/033** (2006.01)
- A61K 49/00** (2006.01)
- G01N 33/00** (2006.01)

(52) **U.S. Cl.** ..... **800/3**; 800/13; 800/14; 800/18; 424/9.1

(58) **Field of Classification Search** ..... None  
See application file for complete search history.

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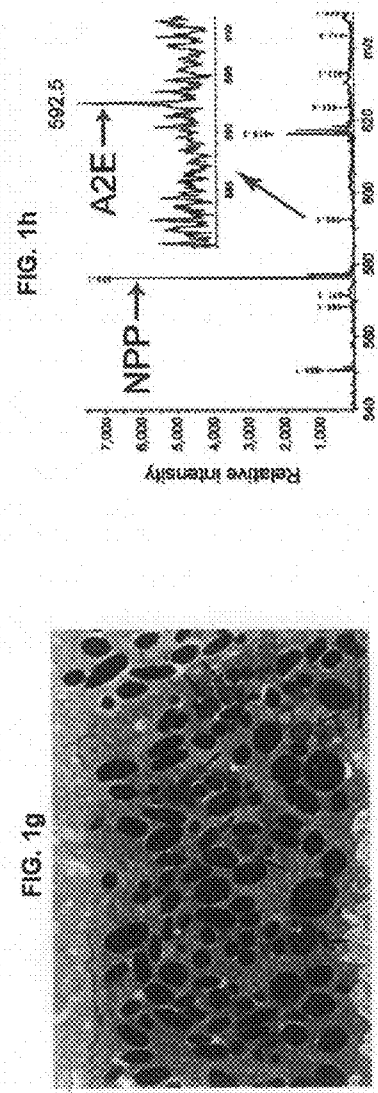
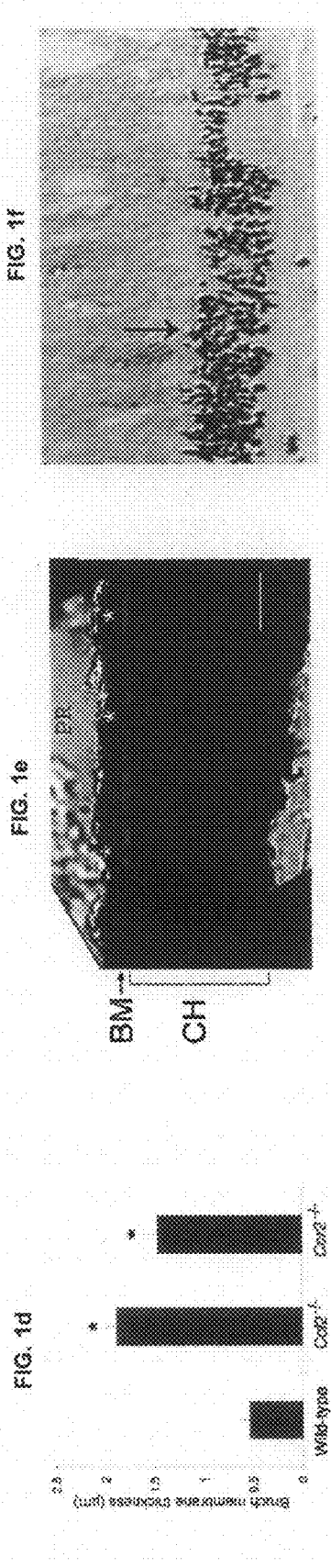
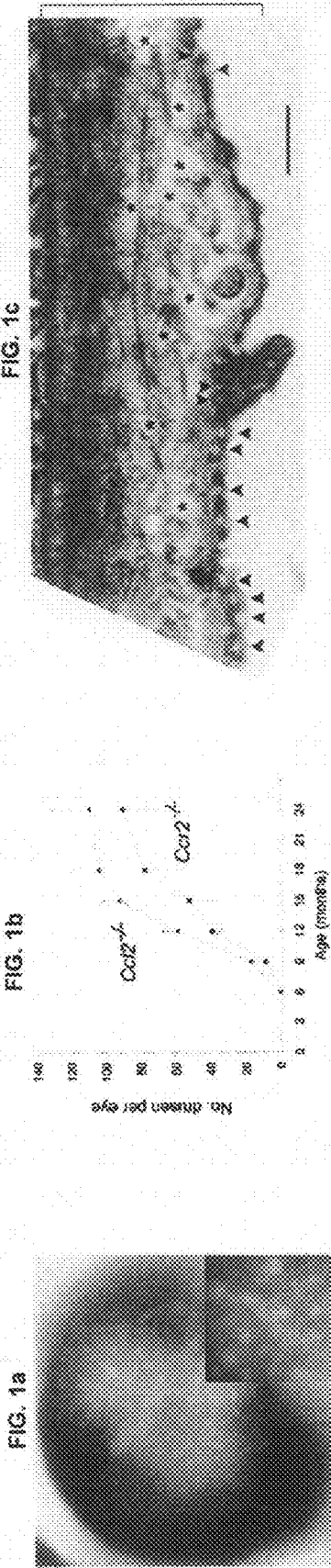
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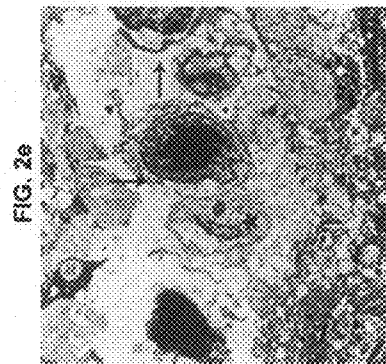
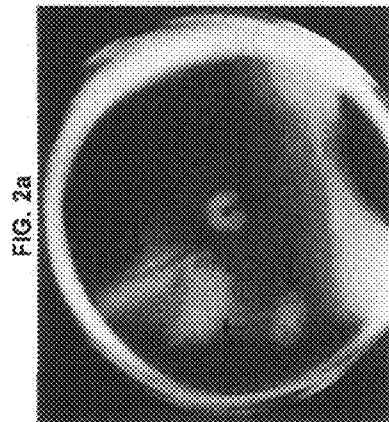
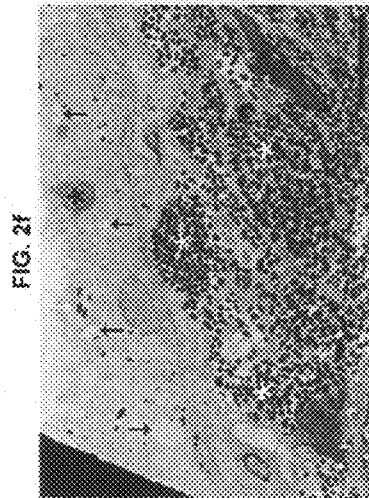
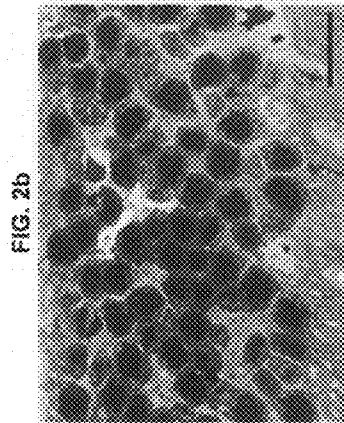
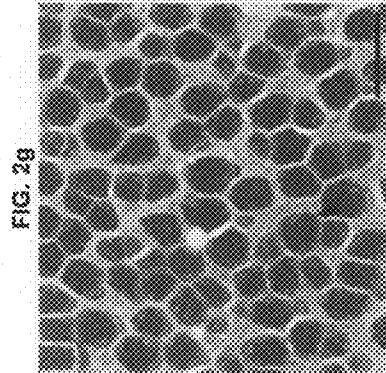
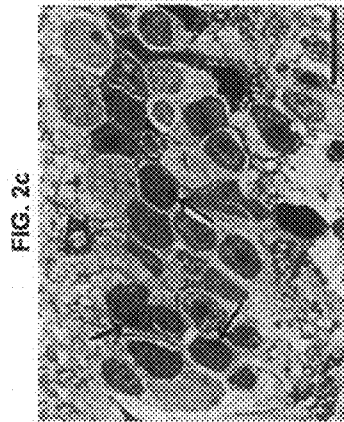
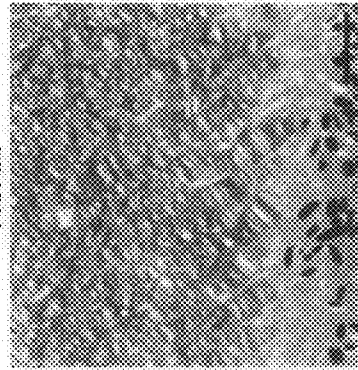
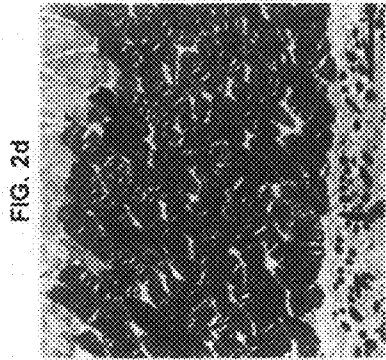
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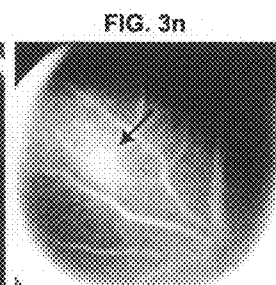
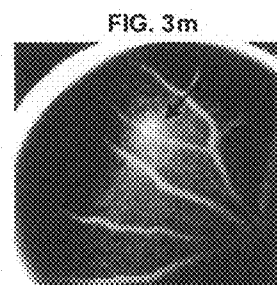
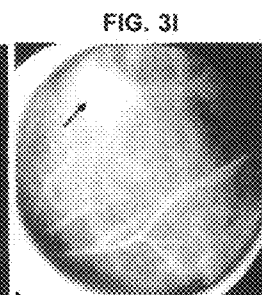
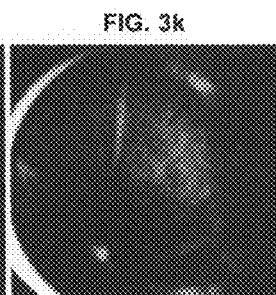
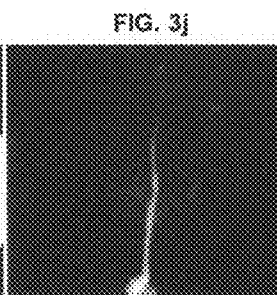
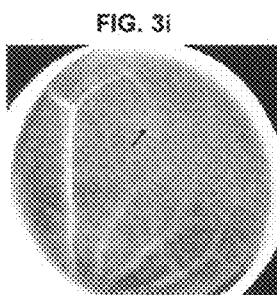
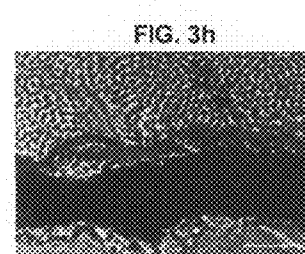
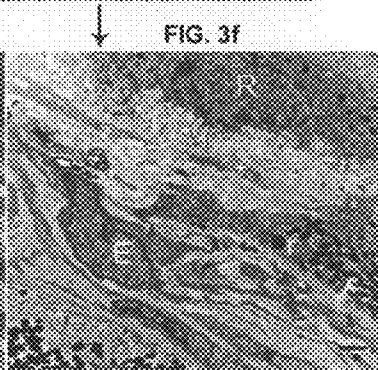
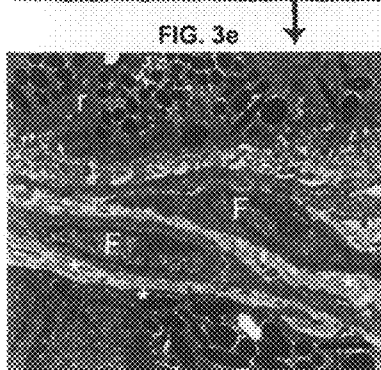
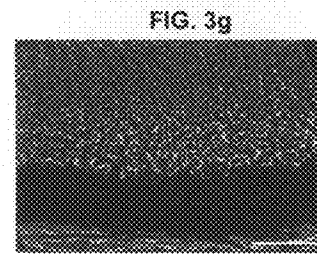
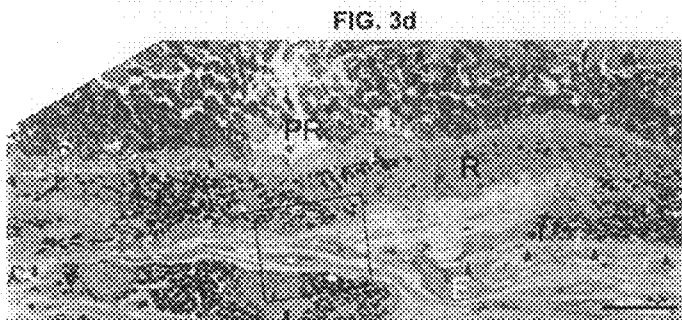
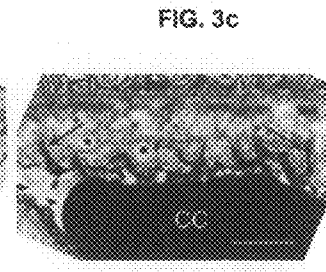
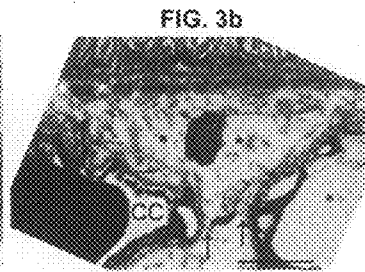
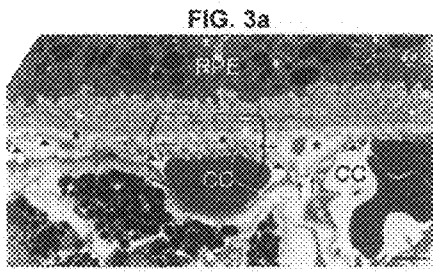
(57) **ABSTRACT**

Methods for testing candidate drugs for treatment of age-related macular degeneration are provided. Ccl2-deficient, and Ccr2-deficient mice are used to determine the effect of candidate drugs and treatments on development of age-related macular degeneration. Also provided is a Ccl2-deficient, Ccr2-deficient dual knockout mouse, which is a useful animal model for age-related macular degeneration.

**28 Claims, 37 Drawing Sheets**  
**(6 of 37 Drawing Sheet(s) Filed in Color)**







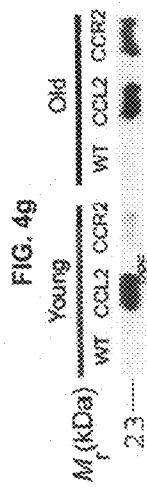
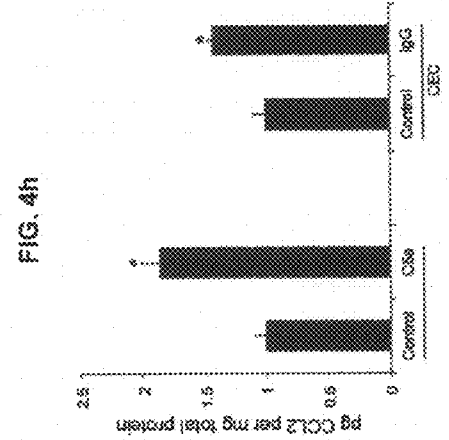
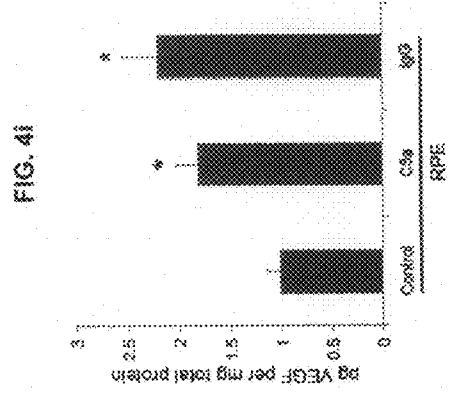
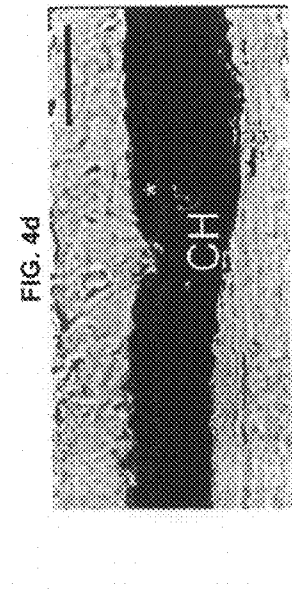
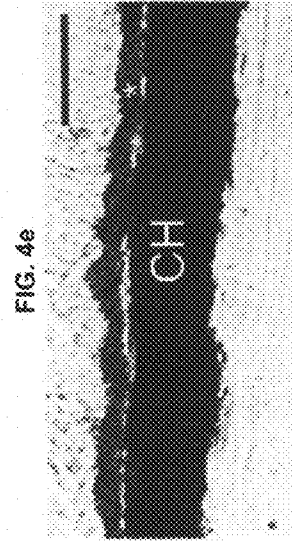
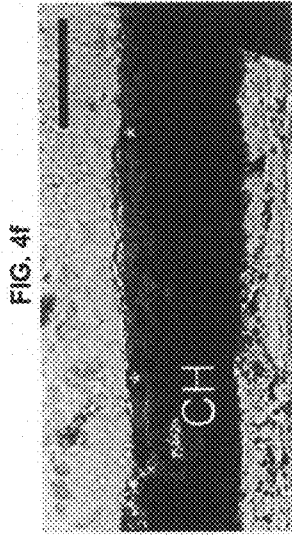
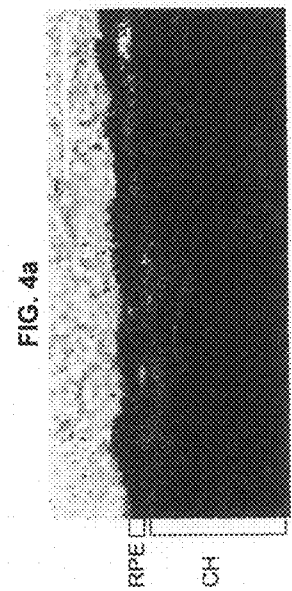
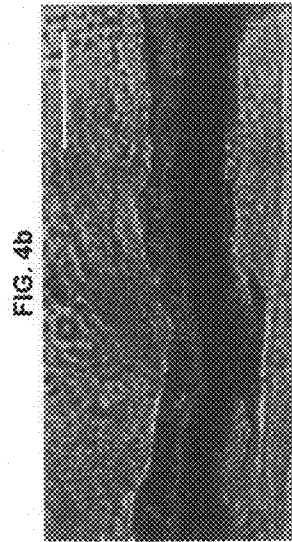
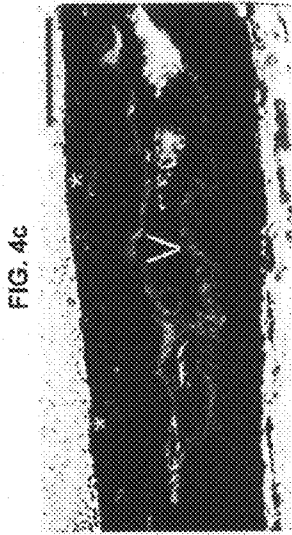




FIG. 5b

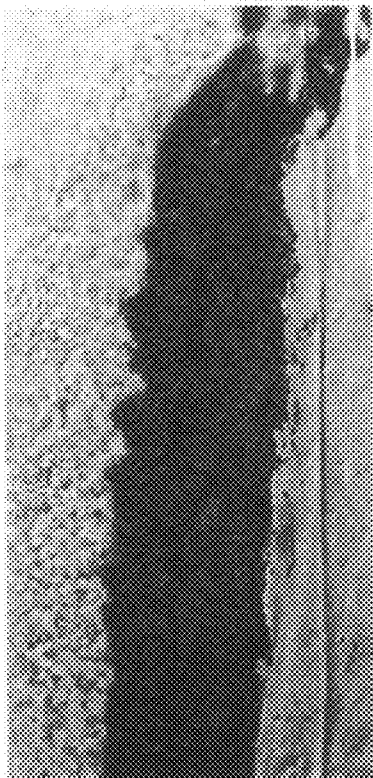


FIG. 5a

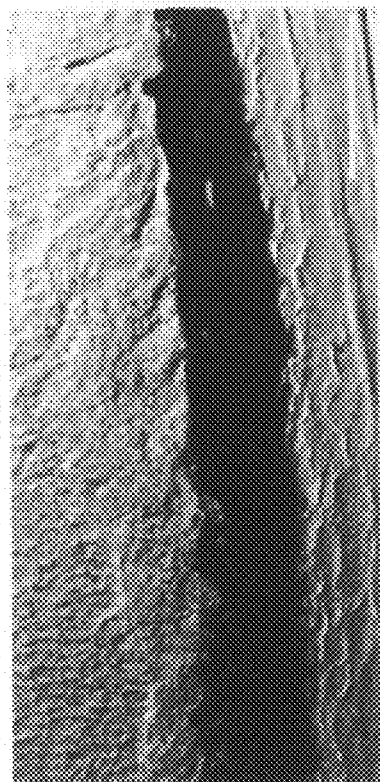


FIG. 5e

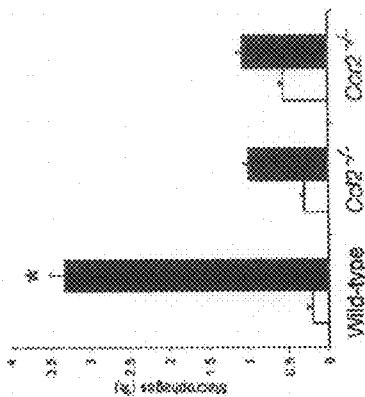


FIG. 5d

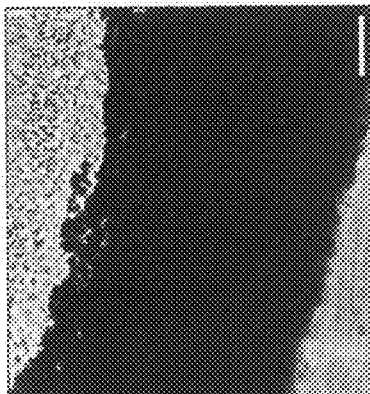


FIG. 5c

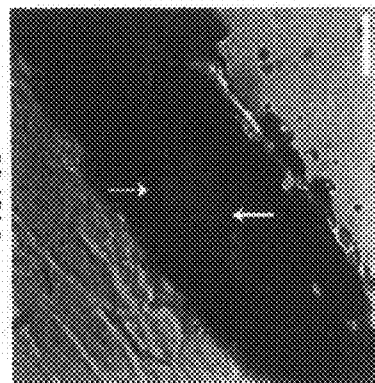
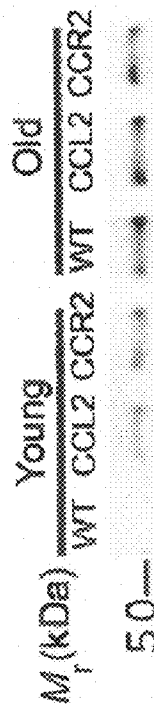


FIG. 5f



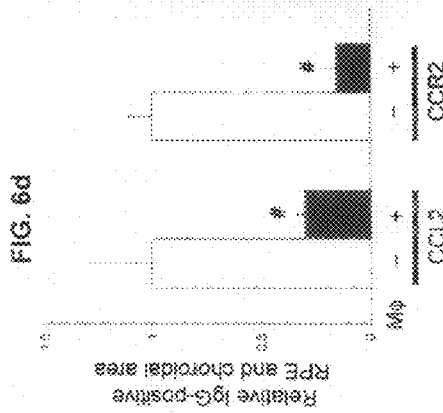
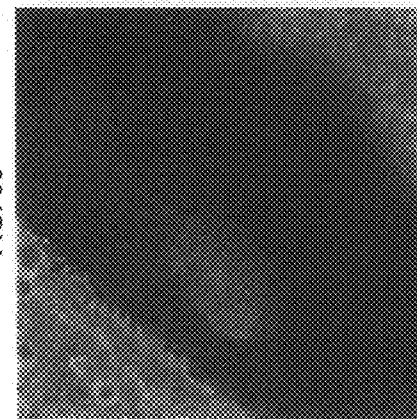
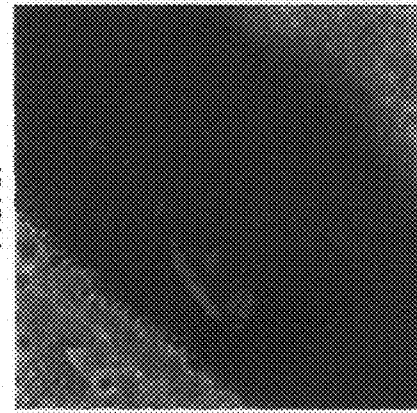
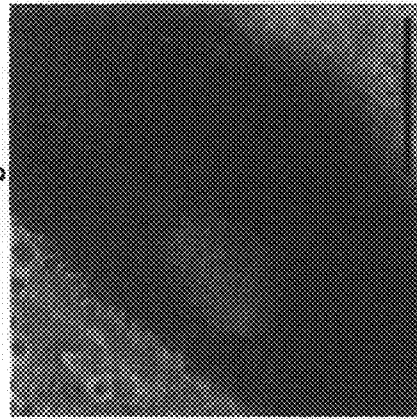
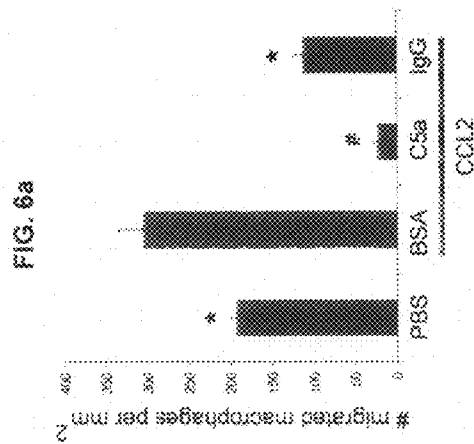
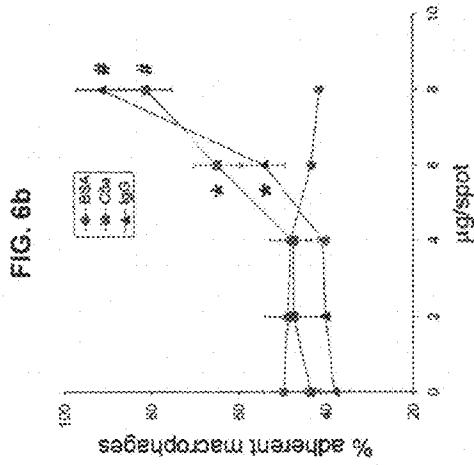
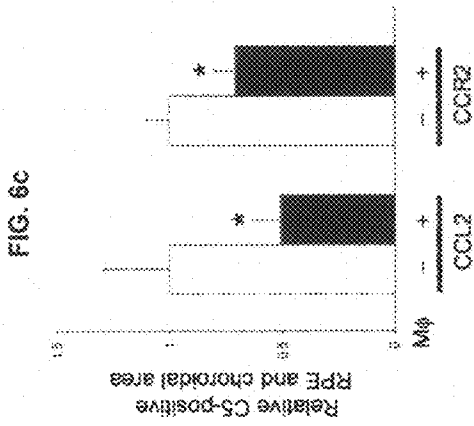




FIG. 7A

SEQ ID NO: 1 Human Ccl2 gene

Sequence 1:

```

ggaaccgaga ggctgagact aaccagaaa catccaattc tcaaactgaa gctcgcactc      60
tcgcctccag catgaaagtc tctgccgcc ttctgtgect gctgctcata gcagccacct      120
tcattcccca agggctcgct cagccagatg caatcaatgc cccagtcacc tgctgttata      180
acttcaccaa taggaagatc tcagtgcaga ggctcgcgag ctatagaaga atcaccagca      240
gcaagtgtcc caaagaagct gtgatcttca agaccattgt ggccaaggag atctgtgctg      300
acccaagca gaagtgggtt caggattcca tggaccacct ggacaagcaa acccaaactc      360
cgaagacttg aacctcact ccacaacca agaactcgcg gctaacttat tttcccctag      420
ctttcccag acaccctgtt ttatatttatt ataatgaatt ttgtttgttg atgtgaaaca      480
ttatgcotta agtaatgtta attcttattt aagttattga tgttttaagt ttatctttca      540
tggtactagt gttttttaga tacagagact tggggaaatt gcttttcctc ttgaaccaca      600
gttctacccc tgggatgttt tgagggctct tgcaagaatc attaatacaa agaatttttt      660
ttaacattcc aatgcattgc taaaatatta ttgtggaaat gaatattttg taactattac      720
accaaataaa tatatttttg tacaaaaaaaa aaaaaaa                                757
    
```

FIG. 7B

SEQ ID NO: 2 Human Ccl2 gene variant

Sequence 2:

```

agactaacc agaaacatcc aattctcaaa ctgaagctcg cactctcgcc tccagcatga      60
aagtctctgc cgcccttctg tgccctgctgc tcatagcagc caccttcatt cccaagggc      120
tcgctcagcc agatgcaatc aatgccccag tcacctgctg ttataacttc accaatagga      180
agatctcagt gcagaggctc gcgagctata gaagaatcac cagcagcaag tgtcccaaag      240
aagctgtgat cttcaagacc attgtggcca aggagatctg tgetgacccc aagcagaagt      300
gggttcagga ttccatggac cacctggaca agcaaaccca aactccgaag acttgaacac      360
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ttagatacag agacttgagg aaattgcttt tcctottgaa ccacagttct acccctggga      600
tgttttgagg gtctttgcaa gaatcattaa tacaagaat tttttttaac attccaatgc      660
attgctaaaa tattattgtg gaaatgaata ttttgtaact attacaccaa ataaatatat      720
ttttgtacaa aaaaaaaaaaaa aaa                                           743
    
```

FIG. 7C

SEQ ID NO: 3 Human Ccl2 promoter region

Sequence 3:

ccgagatggt	cccagcacag	ccccatgtga	gagctccctg	gctccgggcc	cagtatctgg	60
aatgcaggct	ccagccaaat	gcattctctt	ctacgggata	tgggaacttc	caaagctgcc	120
tcttcagagt	gggaatttcc	actcacttct	ctcacgccag	cactgacctc	ccagcggggg	180
agggcattct	ttcttgacag	agcagaagtg	ggaggcagac	agctgtcact	ttccagaaga	240
ctttcttttc	tgattcatac	ccttcacctt	ccctgtgttt	actgtctgat	atatgcaaag	300
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agcatgactg	gattataaag	ggaaattgaa	tgcggtccac	caagttcatg	gtaaaggatg	660
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gtttcaacac	tggttgggga	gaaaaggagt	aactagtgag	attcaggcag	aacaagaata	900
aggctcctca	agaggcacia	gcaaagcagg	gctcagattg	atttgttctc	tcttcactct	960
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tggaaatggaa	acatcctggg	tgggagtctc	agcacatcta	ctattctgtc	tgagttactg	1140
gacaaataac	ttcagtttta	acctaocgaa	agctggggtg	gttggaggac	tgggcaggca	1200
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gccattaaca	tgctcaagt	actcctatca	tatttgtaag	agacaacagt	tactgaaat	1560
gaattctaag	gtctttgggt	ttttatcagt	gtgcttctgt	agtttctgag	gaaatctaag	1620
gcacaactga	ggaatgaagt	caggctttcc	aattcccgaa	atactcctcc	actgcttaact	1680
catgtccctt	ggaaattaag	aaggaagcca	ggagaatagc	tgccataacc	agggatgaac	1740
ttcttgctca	ctgctgctg	ctatgctagc	aacagcctcc	taactcataa	tgacttagcc	1800

## FIG. 7C (continued)

SEQ ID NO: 3 Human Ccl2 promoter region

Sequence 3:

atgaggaatg	tttctagatt	ctccttttagc	tgtctgceca	tttggagat	gctgaggaca	1860
gagagaggac	ccaagcaggc	aactagttgg	aggacttgta	cacgtttcct	tccagcagta	1920
tgtcagagag	gtgagcagcc	cactggggac	agggctgcct	gggttctgtg	ctcgagggga	1980
ccttgagcag	gctatattaac	ccttctgtgc	ctcagttgcc	tgatctataa	catgaaaatt	2040
agcaatccct	actagataaa	gttggggaat	ttacagagtt	aatatttgta	aaggctcgag	2100
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ctgggaggcc	ccttggaatg	tggcctgaag	gtaagctggc	agcgagcctg	acatgctttc	2400
atctagtttc	ctcgcttcct	tccttttctg	cagttttcgc	ttcacagaaa	gcagaatcct	2460
taaaaataac	cctcttagtt	cacatctgtg	gtcagctctgg	gcttaatggc	accccatcct	2520
ccccatttgc	tcatttggtc	tcagcagtga	atggaaaaag	tgtctcgtec	tgacccccctg	2580
cttccctttc	ctacttctctg	gaaatecaca	ggatgctgca	tttgctcage	agatttaaca	2640
gcccacttat	cactcatgga	agatccctcc	tcctgcttga	ctccgcctc	tctccctctg	2700
cccgctttca	ataagaggca	gagacagcag	ccagaggaac	cgagaggctg	agactaaccc	2760
agaaacatcc	aattctcaaa	ctgaagctcg	cactctcgcc	tccagcatga	aagtctctgc	2820
cgcccttctg	tgcttctctg	tcatagcage	caccttcatt	ccccaggggc	tcgctcagcc	2880
aggtaaggcc	ccctcttctt	ctccttgaac	cacattgtct	tctctctgag	ttatcatgga	2940
ccatccaagc	agacgtggta	cccacagtct	tgctttaacg	ctacttttcc	aagataaggt	3000
gactcagaaa	aggacaaggg	gtgagcccaa	ccacacagct	gctgctcggc	agagcctgaa	3060
ctagaattcc	agctgtgaac	cccaaateca	gctccttcca	ggattccagc	tctgggaaca	3120
cactcagcgc	agttactccc	ccagctgctt	ccagcagagt	ttggggatca	gggtaataca	3180
agagaggggtg	ggtgtgtagg	ctgtttccag	acacgctgga	g		3221

## FIG. 7D

SEQ ID NO: 4 Human Ccl2 gene and enhancer region

Sequence 4:

```
ggtacctcct ccagccttgg ccacagtgtc atccttgggc cccttaggtt tcagcctctt      60
gagtttgcaac ttgcagggtt ggctgttgct ctcaaagcag gactattgca tcaacatggc      120
agggtgcagag gtcttcccgc ctcaategtc acccactgat ttctctgcca tggccttgaa      180
ctcaggcgac caatccagtt ggaacctccc cacactctcc gtggctaata attttggact      240
cagaagaaaa agcctcaatt tctctcctct caggaggctc cttggtcctt gagcaaatgt      300
atccatttct tctctatct ccagtccttg ggccccaaa ggttttttcc tccctttctc      360
caggacaatg agtgccctatt tacaagtgcc tgtttctact tgaataaggc ttctataaac      420
taagaagtgt tccttaggga cacaagtaac tggcactcct gttggaaaat gctaagatct      480
aggtcacgcy cacttcccc aacagacaca tacacacatt cacacacaca cacacacaca      540
cacacacaca cacacacaca cacacataca gcttgtctgc actctagcac tggcactgac      600
gctaacgcta taatcctggg caactttatt tccccatctt acattaagca gtggtgcagg      660
gattttcaac tctgggatct ctatcacacc tcccagetct gattgcttcc taatttacat      720
atltattgag catctgatgc taggtcctca tgctgggatg gcaggagtaa actagacaga      780
caaaagtccg tgccccacat tgtctgacac ctacacacct gctgttcgga ctccattaca      840
aacagctcca aggggaacag tgcacttgta aagtttctct cattaccatg gccacatccg      900
tgagcaataa ataagttgca tagttgaatt atttgataat gctttgtttt taactccttg      960
cacttaagtc agagatgtgt gtgctttgga aaactatttc tctgactca ttagacaaat     1020
actatttgca tttttattca gcttcttccc tcagaacteta atttacagta aaggcaagag     1080
gatttttgaa tggagccagt gctttgcaat gtggggctcc accagctagc cgactgaaat     1140
cattaataaa gaagcctttt taagtggctg aagtttcccc tttttggcat gcaacatttt     1200
gcaaccaagc ggaagaaaca tcatccgcaa agaagaatcc atgtggcccc tgaaaatcac     1260
tctctctgct acaggctccc cactccccag tgctcccctt agccctgcca ctatctctcc     1320
tccagatgga aaaagtgagg aactcaggga accaaaagtc ttgcttcttt actaatttcc     1380
```

FIG. 7D (continued)

SEQ ID NO: 4 Human Ccl2 gene and enhancer region

Sequence 4:

ctgtctgaca	ttaaatac	ctacagttca	gatatctggg	ggaagtgact	agagattctt	1440
gaactgttaa	taattaattt	aatgatatt	tgtaagaac	ctacgacatg	gaagatactg	1500
taccaggtgc	tggggtccag	catgggcaaa	ggcctcaagg	tggaatggag	ctatggtgtg	1560
ttctggaagc	agagagtggg	gctgaggggtg	acatgaggtg	aggagacagg	agagggcctg	1620
gcaggggtggg	accttctggt	gagagctggc	tgctgtgtga	ggagctgagg	ccctggcctg	1680
attctggggg	tacttctttg	accttcagct	ttttgtcatg	ggcagacaga	atggggatga	1740
aaaaaagctt	aggaaatgga	aacctcccta	tgcattatat	aataaaaatg	gccaacacat	1800
tttcatagca	agaaatcaca	gcagaagctt	gtactgggca	tcaggactgt	aggcatccaa	1860
tgcccagaaa	ctggcatgtg	ccctgggaca	tcccctgaga	aggcatgcca	cgagccctca	1920
gactgacaca	gctctttaca	agttgcttac	agagcaactct	tggtttatta	attcatacaa	1980
gtctcatgac	aatgtcagaa	gcagctgtct	tactaatccc	ctttgacaga	agaggcccag	2040
agaggccaag	ggacttgctc	aaggccacac	agctagaaag	aggcagagcc	aggcctttgg	2100
ccctgggtgtt	ctgacaccac	ctggggctcc	ttctgttatt	ccatgctacc	tcttctttct	2160
cttccgtatt	cccttctcgt	tcccttccct	cttgtgtctt	gcttcttata	tgctgtact	2220
tattcctggt	ggtgcctccc	agctcagcca	gcatagetct	gtcttcaaat	accccatgct	2280
tcattctggg	gtcccataca	cagtctgaca	atcatctgag	ggggctgtgg	gaggacatag	2340
aaaaaataca	gctttacata	gaaaaaatg	caaattgtag	ccaggcgcag	tggtctatgc	2400
ctgtaacccc	agcactttgg	gaggccgagg	caggtggatc	acctgaggte	aggagtctga	2460
gaccagcctg	gccaatgtag	taaaactcct	tctctactaa	aaatataaaa	attagccagg	2520
cgtgatgtca	tgtgctgtga	gtcccagcta	ctcgggaggc	tgaggcagga	gaacctcttg	2580
aatccaggag	gcgcaggttg	cagtgagcag	agatagtgcc	actgcactcc	agcctgggtg	2640
acagagtgag	actctgtctc	aaaaaaataa	aataaaataa	aaaatgcaga	ctgtgattca	2700
gcaggtctgg	gttgaagccc	agaactctct	gataaattca	atggcactta	actacttggg	2760
ggcatggat	gcctttgcta	atctaataga	agctactgac	cctctctcca	gaaaaatgca	2820

FIG. 7D (continued)

SEQ ID NO: 4 Human Ccl2 gene and enhancer region

Sequence 4:

caaaaacata	aatgtggaag	acaactcctg	atggatctgg	gagcctatcc	aagggccaca	2880
gacaagagtc	ctggctctga	caaaatgagc	tgctcagtat	tttcccacct	ggccagcatt	2940
tcctatccaa	agacaaatgt	taaagttggt	ctagcagagc	catgcaccag	cagcagtatc	3000
atcacctggg	aaccggttag	caatgcagaa	ccgcaggccc	accccaaacc	tacagtcaga	3060
atctctactt	tagcaagatc	ctaaggagat	gggtaagcac	attacaattt	gcaacctttg	3120
taagtttgcc	caaaatgtga	ccctccttc	accacccgat	cgccaagggt	caaaaatctg	3180
cccaaccctt	gagcccatct	taaagtacc	atcacgagcc	ttcctggggc	ccctcagctg	3240
ggactctcac	cgctctgtat	ctttctgggt	aatgcaatta	ttctgttccc	ttagatgacc	3300
ccagcacagg	tgctaaagga	gtcaacaaaa	ggctattgtc	aaaaaagtgt	ttctgtctcc	3360
actccatctg	atctctgttt	ccctaagacc	tgcccatccc	cctctcccag	ttcggcacct	3420
tgacccccctc	atcacactgc	tcaggccacc	ttgtacaatg	caagccccaa	atgaggaaag	3480
cattttctcc	cccaatgtgt	aacacgaaaag	tgctgtagag	tggtctacgc	tgcccttagc	3540
ctaagaattt	atttaactct	tacccccaac	ccacatcagt	ctcctccctc	tagggctcag	3600
gtgctaactc	gtgagggctg	gctcagaaga	caatctaaag	aacaagcctc	ttgcttcctc	3660
aggcatcact	actcctcacc	accatcacc	ccaccacca	actcaggcca	ctactcttcc	3720
tgttctcata	tgctatgccc	atcgccacc	ctattcccat	gctcaggagt	attcttggct	3780
actgcatgca	attagacctg	gggcagatcc	aatccagaaa	gcaagaaatc	ttagatgctg	3840
gaagcttggg	gtaagtactg	atcagattta	ttcctaaatt	cagtcctact	ttccatggat	3900
tcttacttta	gcatctcttc	tgaaaaggaa	gcatcatgtc	taattcactt	ctccctccct	3960
gtgcagtcct	ctacctgggtg	ctctgcacag	ggatgtgct	aattgtatga	atgttataat	4020
aaagagatag	tgcagtagat	gacaaaagggc	actacattga	gagcccagaa	ataagcaaac	4080
cagcaciaaat	gtagccattc	gtcttctatc	tcaccttgag	cctgtcacta	acctgttcat	4140
ggcctcagtc	tccccatcag	agaaacaggt	agatggctctc	taaggtctcg	ttcattttct	4200
gacattctgt	gaaaaattaa	ggaaaagattt	tcatccttga	caggaaaggg	attgcagagt	4260



FIG. 7D (continued)

SEQ ID NO: 4 Human Ccl2 gene and enhancer region

Sequence 4:

agcggccctg ggaaaatggg ctctattcta cctggagcta gcctggagga gaggccttga 4320  
gtggggggtt tctagaaagg acatggtgag tgcagageta cgggtgcatct ctcttgaagg 4380  
ctgagtgaag ggagcaccag caaggagacc tgcactaggt ggggagggac aagtgaaccg 4440  
cagaagttgg tgggagccca ggcagtggct tcagatcttt ccagagagct cacttttact 4500  
tcctcttttt ttcaccctg aactgagtg ggagtctgca gcgatgacca aggttcatgc 4560  
agaggatctt agtgggtggg tcagaccccg ggaggaatga agaaagcatt attcaccaag 4620  
aggagctttt ccattcttta tctatgagtt gatagagagg aggccccggg gtaactgagg 4680  
attctggaca gcatcagagc attgaccctc attttcccca tagccctctt gggggccttt 4740  
cccttgtgtg tccccaagcg agagtccaac caaggtttgt gccagagcct aaccaggt 4800  
tgtgccgaga tgttcccagc acagcccat gtgagagctc cctggctccg ggcccagtat 4860  
ctggaatgca ggctccagcc aatgcatte tcttctacgg gatctgggaa cttccaaagc 4920  
tgctctctca gagtgggaat ttccactcac ttctctcag ccagcactga cctcccagcg 4980  
ggggagggca tctttcttg acagagcaga agtgggaggc agacagctgt cactttccag 5040  
aagactttct tttctgattc atacccttca ccttccctgt gtttactgtc tgatatatgc 5100  
aaaggccaag tcactttcca gagatgacaa ctcttctctg aagtagagac atgcttccaa 5160  
cactcagaag cctatgtgaa cactcagcca gcaaagctgg gaagtttttc tctgtgacca 5220  
tgggctaatt ggtctcttc tctggattgt ggctttatca gataaaaaca agtggctcatg 5280  
ccacaggatg tctataagcc cattgattct gggattctat gagtgatgct gatatgacta 5340  
agccaggaga gacttattta aagatctcag catctttcag cttgttaacc tagagaaaac 5400  
ccgaagcatg actggattat aaagggaaat tgaatgcggg ccaccaagtt catggtaaag 5460  
gatgactaa cagattagag agaggtttcc cctgatatga ggaaaacttc ttggaagatg 5520  
aggtgagatg gcctaggaag aaattcctac acaaagttgc acagtctcta gtccctggaaa 5580  
cattttatc attggataag aatggattga ggcatgagca gaggactgag acaaacacag 5640  
agaagtttca aactgggtg gggagaaaag gagtaactag tgagattcag gcagaacaag 5700

## FIG. 7D (continued)

SEQ ID NO: 4 Human Ccl2 gene and enhancer region

Sequence 4:

aataaggctc	ctcaagaggc	acaagcaaag	cagggctcga	gttgatttgt	tctctcttca	5760
tcttgctttt	tgtaattcca	ccagagtctg	aaatggccac	tccatagagt	ctctgctctg	5820
ggattctcca	ggaaaccaat	atccatcatg	agacatcaag	tctagtccca	ggaagaagag	5880
attctggaat	ggaaacatcc	tgggtgggag	tctcagcaca	tctactatcc	tgtctgagtt	5940
actggacaaa	taacttcagt	tttaacctaa	cgaaagctgg	gttggttggg	ggactgggca	6000
ggcagcgctg	gaaagtatgt	cagcaccata	cctgactccc	tgaatgcact	caacaatgcc	6060
attactgacc	acttactaga	aataaaacag	tcatttgttg	aatacaacce	gtttcttttt	6120
acaagtgtag	tgaaaagtgt	tttctttcaa	gaaaccccat	gcatttatag	acattgcctc	6180
agtgaccctt	tatgaaagaa	gtcactagtc	tttgtatgcc	cattgggcaa	gggcaccgca	6240
aggctcagaa	ggaggaggca	gtgggctagg	agaatcgaga	gatcagaatt	ttaaactcag	6300
cccagccatt	aacatgcctc	aagtactcct	atcatatttg	taagagacaa	cagttcactg	6360
aaatgaatcc	taaggtcttt	gggtttttat	cagtgtgctt	ctgtagtttc	tgaggaaatc	6420
taaggcacia	ctgaggaatg	aagtcaggct	ttccaattcc	cgaaatactc	ctccactgct	6480
tactcatgtc	ccatggaaat	taagaaggaa	gccaggagaa	tagctgccat	aaccagggat	6540
gaactttctg	tccactgctg	cctgctatgc	tagcaacagc	ctcctaactc	ataatgactt	6600
agccatgagg	aatgtttcta	gattctcctt	tagctgtctg	cccatttgga	agatgctgag	6660
gacagagaga	ggaccecaagc	aggcaactag	ttggaggact	tgtacacggt	tccttcacgc	6720
agtatgtcag	agaggtggca	gcccactggg	gacagggctg	cctgggttct	gtgctcgagg	6780
ggaccttgag	caggctattt	aacccttctg	tgctcagtt	gcctgatcta	taacatgaaa	6840
attagcaatc	cctactagat	aaagttgggg	aatttacaga	gttaatat	gtaaaggctc	6900
gagaatatcc	ctggcagagt	aagcactctg	tgagtatgac	actggcattt	cttctgcagc	6960
actacatgct	gtctatgctt	ttgtccaagt	ctgaaaccct	agaactctta	gaattcagtt	7020
caatgtttac	acaatcctac	agttctgcta	ggcttctatg	atgctactat	tctgcatttg	7080
aatgagcaaa	tggatttaat	gcattgtcag	ggagccggcc	aaagcttgag	agctccttcc	7140

FIG. 7D (continued)

SEQ ID NO: 4 Human Ccl2 gene and enhancer region

Sequence 4:

tggctgggag	gccccttggg	atgtggcctg	aaggtaagct	ggcagcgagc	ctgacatgct	7200
ttcatctagt	ttcctcgctt	ccttcctttt	ctgcagtttt	cgcttcacag	aaagcagaat	7260
ccttaaaaat	aaccctctta	gttcacatct	gtggtcagtc	tgggcttaat	ggcaccccat	7320
cctccccatt	tgctcatttg	gtctcagcag	tgaatggaaa	aagtgtctcg	tcttgacccc	7380
ctgcttcctt	ttcctacttc	ctggaaatcc	acaggatgct	gcatttgctc	agcagattta	7440
acagcccact	tatcactcat	ggaagatccc	tctcctgctt	tgactccgcc	ctctctccct	7500
ctgcccgtct	tcaataagag	gcagagacag	cagccagagg	aaccgagagg	ctgagactaa	7560
cccagaaaca	tccaattctc	aaactgaagc	tcgcaactctc	gcctccagca	tgaaagtctc	7620
tgccgcctct	ctgtgcctgc	tgctcatagc	agccaccttc	attcccccaag	ggctcgtctc	7680
gccaggtaag	gccccctctt	cttctccttg	aaccacattg	tcttctctct	gagttatcat	7740
ggaccatcca	agcagacgtg	gtacccacag	tcttgcttta	acgctacttt	tccaagataa	7800
ggtgactcag	aaaaggacaa	ggggtgagcc	caaccacaca	gctgctgctc	ggcagagcct	7860
gaactagaat	tccagctgtg	aacccccaat	ccagctcctt	ccaggattcc	agctctggga	7920
acacactcag	cgcagttact	ccccagctg	cttcacagcag	agtttgggga	tcagggtaat	7980
caaagagagg	gtgggtgtgt	aggctgtttc	cagacacgct	ggagaccag	aatctggctc	8040
gtgcttcatt	caacttagct	tccagagacg	gtgactctgc	agaggtaatg	agtatcaggg	8100
aaactcatga	ccaggcatag	cctattcaga	gtctaaaagg	aggctcatag	tggggctccc	8160
cagctgatct	tccctgggtg	tgatcatctg	gattattggg	ccgtcttaat	gacacttgta	8220
ggcattatct	agctttaact	ctgtccatta	tcaatgttat	atacccattt	tacagcatag	8280
gaaactgagt	cattgggtca	aagatcacat	tctagctctg	aggtataggg	agaagcactg	8340
ggatttaatg	agctctttct	cttctcctgc	ctgccttttg	ctttttcctc	atgactcttt	8400
tctgctctta	agatcagaat	aatccagttc	atcctaaaat	gctttttctt	tgtggtttat	8460
tttccagatg	caatcaatgc	cccagtcacc	tgctgctata	acttcaccaa	taggaagatc	8520
tcagtgcaga	ggctcgcgag	ctatagaaga	atcaccagca	gcaagtgtcc	caaagaagct	8580

## FIG. 7D (continued)

SEQ ID NO: 4 Human Ccl2 gene and enhancer region

Sequence 4:

```
gtgatgtgag ttcagcacac caaccttccc tggcctgaag ttcttccttg tggagcaagg 8640
gacaagcctc ataaacctag agtcagagag tgcactatth aacttaatgt acaaaggttc 8700
ccaatgggaa aactgaggca ccaagggaaa aagtgaacce caacatcact ctccacctgg 8760
gtgcctatth agaacacccc aatttcttta gcttgaagtc aggatggctc cacctggaca 8820
cctataggag cagtttgccc tgggttccct ccttccacct gcgttccctc tctagctccc 8880
atggcagccc tttggtgcag aatgggctgc acttctagac caaaactgca aaggaacttc 8940
atctaactct gtctcctc cccacagctt caagaccatt gtggccaagg agatctgtgc 9000
tgacccaag cagaagtggg ttcaggattc catggaccac ctggacaagc aaacccaac 9060
tccgaagact tgaacactca ctccacaacc caagaatctg cagctaactt attttcccct 9120
agctttccc agacaccttg ttttatttta ttataatgaa ttttgtttg t gatgtgaaa 9180
cattatgect taagtaatgt taattcttat ttaagttatt gatgttttaa gtttatcttt 9240
catggtaacta gtgtttttta gatacagaga cttggggaaa ttgcttttcc tcttgaacca 9300
cagttctacc cctgggatgt tttgagggtc tttgcaagaa tcattaatac aaagaattht 9360
ttttaacatt ccaatgcatt gctaaaatat tattgtggaa atgaatatht tgtaactatt 9420
acaccaaata aatatatht tgtacaaaac ctgacttcca gtgttttctt gaaggaaatt 9480
acaaagctga gagtatgagc ttggtggtga caaaggaaca tgatttcaga gggtagggct 9540
tacathttga aggaatggga aagtggattg gccccggtct tctccactgg gtggtctect 9600
ctgagctctc gtagaagaat ctttatggca ggccagttag gcattaaagc accaccctc 9660
cagtcttcaa cataagcagc ccagagtcca atgaccctgg tcacccttht agcaagagcc 9720
caacccccat tcttttctc acagaccctg accctgcat gcaattctt ccttaacata 9780
ttgcaactgc cccctaactg ggctaccac ccccaatct gtacctctc aattaatacc 9840
ccaacctgga gtaatacaga cactgccagt attaggaaat aaggaaagag ttaatcacca 9900
tagataagat gattagattg aagttcata gagatgatga gacctgaact tattathat 9960
gaatgaagaa ggcttttcta ggaaaattat aggatcatta agaaaggaga aggaagagtg 10020
```

FIG. 7D (continued)

SEQ ID NO: 4 Human Ccl2 gene and enhancer region

Sequence 4:

```
ggagcaaata cctggaggta gaaatggtga tgatgtgtac atcaagcagg gagaaaacca 10080
atgaaccaga tgcaattcg ggcccacacc aatgtcaagg gatgacaatt agaaaggaag 10140
gttgagtcaa gggatttgaa tgttaggggtg aaaagttact actcaactct gtaggttaaa 10200
aggaaacgtt gagaatcttc agtccaatga ggagggatgt gccatgttta gagattcaga 10260
gataagtttc aggaaatgta acttatagat tttatacata cacagagaaa tacggactag 10320
tgagaagcta ttgccatggt ccaagcaaga gatgatgaag gcctaaatat ggagccaaag 10380
aggcagcaat gaagaatgag ccatgcaggg tgaaatgctg catgttgtaa atggaggaga 10440
aagacctgtg acttcagata tgaaaacctc atcttcaacc cacattttaa gggggcagct 10500
tcctgaaac cagaatgtgt ttccctccat tactataccc ccatcccaat ctcaggcacc 10560
tggaatcatc catttaaaca gatgagcctt ctattcctaa atagccacct gaagtgtgta 10620
ttcctttgca tgatatttgt cccacctaaa gcattcgacc tgccctgggca cccacaccac 10680
gccaacactc aggaaagcag atgtcttget ctgttgaata aactgcatgg ttcttaactt 10740
cccagtctgg tggggaaatg accactgtgt caacctagag caggcagtgc ttttggcagc 10800
atgaggtgct ggggacaact ttgactggca agaagcacac tcaggttctc accccgcac 10860
cagcgctgac tcgctttgtc agtcaagaca ggtcagatat tctgagccta catcgatcat 10920
acaggtatga taatgtgtta caaataggaa occagaggaa aggttccctt tcggatctgg 10980
gagcacatct gttgaaaaac ttccatttct actaactgga gttgcagagg gagagaaggg 11040
attctgcttc tacattctct agccagtcca gggtccctga atcagactac cgaatccctt 11100
caaagctcca agtaccctga tatatcagtc agcagacaat ttattgacag ctatttagaa 11160
aactcactga ccctcactcc aggtcaagca gcgteccctg cctctctct acccctacat 11220
tcctggcct tgatcaccag tcaggagtga aatctcaaat tgcagtagat gccaagaggc 11280
aaaaagagaa tagaatgcaa acaaatgaga cctcatcata tggcttccga gcagcaacct 11340
tttgaccca ggcagatttg aggcagacag tctgggagga gaggaggcag agaaaggggg 11400
gatccacatg ctcaaaccct aaattaatct gottacattc cccttgcagg ccacatctct 11460
```

FIG. 7D (continued)

SEQ ID NO: 4 Human Ccl2 gene and enhancer region

Sequence 4:

```
tcattttcag gaagtcttga ctccatactg ttttccaccc aagcatggaa ttccctttcat 11520
gatgaaactg aacacagggc attggcagtg gtgagactct gttttagaag aaagtgccaa 11580
gtgcaatgca ttcatttctt gttgctgcca acaatcagtt ccaggaaatc taggcttttt 11640
atgtcatgct caaaattctt ccagcctatg ctccattatc aaatocaaag ccacatccac 11700
atctgtaggt gttagttaca gaagcaccat atttccaggt accaaaatct gtattagttt 11760
cttattgtta ctgtaacaaa ttcccataag ctt 11793
```



## FIG. 8A

SEQ ID NO: 5 Human Ccr2 gene variant A

Sequence 5:

```
caggactgcc tgagacaagc cacaagctga acagagaaag tggattgaac aaggacgcat      60
ttccccagta catccacaac atgctgtcca catctcgttc tcggtttatac agaaatacca      120
acgagagcgg tgaagaagtc accacctttt ttgattatga ttacgggtgct cctgtcata      180
aatttgacgt gaagcaaatt ggggccaac tcctgcctcc gctctactcg ctggtgttca      240
tctttggttt tgtgggcaac atgctggtcg tcctcatctt aataaactgc aaaaagctga      300
agtgcttgac tgacatttac ctgctcaacc tggccatctc tgatctgett tttcttatta      360
ctctcccatt gtgggctcac tctgctgcaa atgagtgggt ctttggaat gcaatgtgca      420
aattattcac agggctgtat cacatcggtt attttgggg aatcttcttc atcatcctcc      480
tgacaatcga tagatacctg gctattgtcc atgctgtgtt tgcttataaaa gccaggacgg      540
tcacctttgg ggtggtgaca agtgtgatca cctggttggg ggctgtgttt gcttctgtcc      600
caggaatcat ctttactaaa tgccagaaag aagattctgt ttatgtctgt ggccttatt      660
ttccaagagg atggaataat ttccacacaa taatgaggaa cattttgggg ctggtcctgc      720
cgctgctcat catggctcctc tgctactcgg gaatcctgaa aacctgctt cgggtctgaa      780
acgagaagaa gaggcataag gcagtgagag tcactctcac catcatgatt gtttactttc      840
tcttctggac tcctataaac attgtcattc tcctgaacac ctccaggaa ttcttcggcc      900
tgagtaactg tgaagcacc agtcaactgg accaagccac gcaggtgaca gagactcttg      960
ggatgactca ctgctgcctc aatccatca tctatgcctt cgttggggag aagttcagaa     1020
gcctttttca catagctctt ggctgtagga ttgccccact ccaaaaacca gtgtgtggag     1080
gtccaggagt gagaccagga aagaatgtga aagtgactac acaaggactc ctcgatggtc     1140
gtggaaaagg aaagtcaatt ggcagagccc ctgaagccag tcttcaggac aaagaaggag     1200
cctagagaca gaaatgacag atctctgctt tggaaatcac acgtctggtt tcacagatgt     1260
gtgattcaca gtgtgaatct tgggtgtctac gttaccaggc aggaaggctg agaggagaga     1320
gactccagct gggttggaaa acagtatctt ccaaactacc ttccagttcc tcatttttga     1380
atacaggcat agagttcaga ctttttttaa atagtaaaaa taaaattaaa gctgaaaact     1440
```

FIG. 8A (continued)

SEQ ID NO: 5 Human Ccr2 gene variant A

Sequence 5:

```
gcaacttgta aatgtggtaa agagttagtt tgagttgcta tcatgtcaaa cgtgaaaatg 1500
ctgtattagt cacagagata attctagctt tgagcttaag aatTTtgagc aggtgggatg 1560
tttgggagac tgctgagtca acccaatagt tgttgattgg caggagttgg aagtgtgtga 1620
tctgtgggca cattagccta tgtgcatgca gcatctaagt aatgatgtcg tttgaatcac 1680
agtatacget ccategctgt catctcagct ggatctccat tctctcagge ttgetgceaa 1740
aagccttttg tgttttgttt tgtatcatta tgaagtcatg cgtttaatca cattcgagtg 1800
tttcagtget tgcagatgt ccttgatget catattgttc cctaatttgc cagtgggaac 1860
tctaaatca aattggcttc taatcaaage ttttaaacce tattggtaaa gaatggaagg 1920
tggagaagct cctgaagta agcaaagact ttctcttag tcgagccaag ttaagaatgt 1980
tcttatgttg ccagtggtg ttctgatctg atgcaagcaa gaaacactgg gcttctagaa 2040
ccaggcaact tgggaactag actcccaage tggactatgg ctctactttc aggccacatg 2100
gctaaagaag gtttcagaaa gaagtgggga cagagcagaa ctttcacett catatatttg 2160
tatgatccta atgaatgcat aaaatgttaa gttgatggtg atgaaatgta aatactgttt 2220
ttaacaacta tgatttggaa aataaatcaa tgctataact atgttgataa aag 2273
```

## FIG 8B

SEQ ID NO: 6 Human Ccr2 gene variant B

Sequence 6:

```
caggactgcc tgagacaagc cacaagctga acagagaaaag tggattgaac aaggacgcat      60
ttccccagta catccacaac atgctgtcca catctcgttc tcggtttatc agaaatacca      120
acgagagcgg tgaagaagtc accacctttt ttgattatga ttacgggtgct ccctgtcata      180
aatttgacgt gaagcaaatt ggggcccac tcttgcctcc gctctactcg ctgggtgttca      240
tctttggttt tgtgggcaac atgctggteg tcttcatctt aataaactgc aaaaagctga      300
agtgcttgac tgacatttac ctgctcaacc tggccatctc tgatctgctt tttcttatta      360
ctctcccatt gtgggctcac tctgctgcaa atgagtgggt ctttgggaat gcaatgtgca      420
aattattcac agggctgtat cacatcgggt attttggcgg aatcttcttc atcatcctcc      480
tgacaatcga tagatactg gctattgtcc atgctgtggt tgctttaaaa gccaggacgg      540
tcacctttgg ggtggtgaca agtgtgatca cctggttggt ggctgtggtt gcttctgtcc      600
caggaatcat ctttactaaa tgccagaaaag aagattctgt ttatgtctgt ggccttatt      660
ttccacgagg atggaataat ttccacacaa taatgaggaa cttttggggg ctggctctgc      720
cgctgctcat catggtcadc tgetactcgg gaatcctgaa aaccctgctt cgggtgctgaa      780
acgagaagaa gaggcatagg gcagtgagag tcatcttcac catcatgatt gtttactttc      840
tcttctggac tccctataac attgtcattc tctgaacac ctccaggaa ttctctggcc      900
tgagtaactg tgaaagcacc agtcaactgg accaagccac gcaggtgaca gagactcttg      960
ggatgactca ctgctgcadc aatcccatca tctatgcctt cgttggggag aagttcagaa     1020
ggtatctctc ggtgttcttc cgaaagcaca tcaccaagcg cttctgcaaa caatgtccag     1080
ttttctacag ggagacagtg gatggagtga cttcaacaaa cacgccttcc actggggagc     1140
aggaagtctc ggctggttta taaaacgagg agcagtttga ttggtgttta taaagggaga     1200
taacaatctg tatataacaa caaacttcaa gggtttgttg aacaatagaa acctgtaaag     1260
caggtgcca ggaacctcag ggctgtgtgt actaatagc actatgtcac ccaatgcata     1320
tccaacatgt gctcagggaa taatccagaa aaactgtggg tagagacttt gactctccag     1380
aaagctcadc tcagctcctg aaaaatgect cattaccttg tgctaatect cttttctag     1440
```

FIG 8B (continued)

SEQ ID NO: 6 Human Ccr2 gene variant B

Sequence 6:

```
tcttcataat ttcttcactc aatctctgat tctgtcaatg tcttgaaatc aagggccagc 1500
tggaggtgaa gaagagaatg tgacaggcac agatgaatgg gagtgagggg tagtggggtc 1560
agggctgaga ggagaaggag ggagacatga gcatggctga gcctggacaa agacaaaggt 1620
gagcaaaggg ctcacgcatt cagccaggag atgatactgg tccttagccc catctgccac 1680
gtgtatttaa ccttgaaggg ttcaccaggt cagggagagt ttgggaactg caataacctg 1740
ggagttttgg tggagtccga tgattctctt ttgcataagt gcatgacata tttttgcttt 1800
attacagttt atctatggca cccatgcacc ttacatttga aatctatgaa atatcatgct 1860
ccattgttca gatgcttctt aggccacatc cccctgtcta aaaattcaga aaatTTTTGT 1920
ttataaaaga tgcattatct atgatatgct aatatatgta tatgcaatat aaaatttag 1979
```

FIG. 8C

SEQ ID NO: 7 Human Ccr2 gene isoform A

Sequence 7:

```
gtttatgaaa ttacagggct ggagacaaaag atcacaatgt gaagacaaaa ttggagagcg      60
gtcctaataca gccagagcaa aatttctggc tcttgctctt ccccatcctg ggttgaatca     120
taggaacagg tggcaagatg ccagggtcag gagattccag aagtggcagc aagctcagtg     180
ttaccaggtc agggatgacc tgtcttatta ttgaaatctc agagatatgc tccaattccg     240
gcccagagac acattgagag acaactgggg aacttgctat gttcctgaac aggcaatgag     300
ctgtcttcca agaaaaaacc tgagaccctt caagtctcag gtcttactta gcacatatac     360
caggtcttac acaggacaca tggttacaac tgactgaaat ctgggctggg tgtaggagct     420
cacacctgta atcccagccc ttcaggaggc tgaggcaggc agattgcctg agcccaggag     480
ttcgagacca gcccgggcaa catgacaaaa ccccatctct acaaaaaata gtcaggcatg     540
gtggcatgca cctgtagtct cagctacttg ggaggctgag atgagaggat tgcttgaggt     600
tgagactgca gtgaagcatg atcatgccac cgcactccag cctaggcaac agagcaagat     660
cttgtcgcaa aagaaagcaa aaacacaaca taacacaaca acaacaacaa caacaacaac     720
agcaaaaaag ccaacttctt gaaatctgga aaggacacct ggactgccct gagcatttga     780
ttgttgttgg ctctagcagt ggatgcatcc ttcaacctct ggcactctgc agggctcaga     840
ctgttctgtt ctgtttgtta cctgtggagt gcctgccaga cctgctcta gctgctttag     900
gtccatttac cctcatagac cccagtcctt gttattcata tttcatattt gggaaatgga     960
aacttagaaa cttgccaagt ccacagcatg agatcctgcc tccggtgtct gctggattcc    1020
agaaaagtgc aggggccaac ttagatgaca ccatgttctc tgcacaatct taggaatgct    1080
cctagtctga tgtcccatt gcaaaattta cattatcttt taacaaaacg tctttccaag    1140
gaggggcatt taaaataact gaggttcttc ttgctaagga agttcctgac acaagagata    1200
athtagcatt tccttttcat taaaaagttt gaaatcctgt aatttgtgat aatgtggatg    1260
aacctagagg atgttaagtg aaataagcca cacacagata gacaaaatacc acgtgatctc    1320
actcttatgt ggaatTTTTT ttaaataaag ttgcttagcc gggcatgatg gcacacacct    1380
```

FIG. 8C (continued)

SEQ ID NO: 7 Human Ccr2 gene isoform A

Sequence 7:

gtaatcctag ctactcagga ggctgaggtg ggaggatggc ttgaactcag aaggtggagg	1440
ttgcagtgag ctgagactgt gccagtgcac tccggctctgg gtgacagaat gaaaccaat	1500
ttaaaaaaaa aaaaaaagtt gctatcttag aaaaagacag tagagcagtg gttaccagag	1560
actggggagg aaagagagga ggtgagaatg ggcagcagtt gatcaacggg tacaaagtta	1620
ccatgagata ggagaaacaa gtgctgggtgc tctgctccaa gtagggtgac ggtagttaat	1680
aatgaattct gtatatataa atagctagaa gagaggggtt tcaatatcat tattatttca	1740
aaagaaatga taaatgttcc agaggatgga tatgtaatta ccctgatttg atcattgcac	1800
aatgtataca tgtagcaaaa catcacattg tgtcccataa atatatacaa ttattatgtg	1860
aattaaataa aaaaaaattt taaagtctta tctaaatgaa atttctaacc agattctgaa	1920
tccatgatac cactgaaacc agcacacatg atcgcagtaa aacctcatta tacttctctc	1980
actatcacca atacccttta ttctctggaa catgaaacat tctgttgtgc tcatatcatg	2040
caaattatca ctagtaggag agcagagagt ggaaatgttc caggtataaa gaccacaag	2100
ataaagaagc tcagagtcgt tagaaacagg agcagatgta cagggtttgc ctgactcaca	2160
ctcaaggttg cataagcaag atttcaaaat taatcctatt ctggagacct caaccaatg	2220
tacaatgttc ctgactggaa aagaagaact atatcttctt gatttttttt tttcaaatct	2280
ttaccattag ttgccttgta tctccgcctt cactttctgc aggaaacttt atttctact	2340
tctgcatacc aagtttctac ctctagatct gtttggttca gttgctgaga agcctgacat	2400
accaggactg cctgagacaa gccacaagct ggtgagttgt aggcattttt tccattactt	2460
tctgattcat aggctcaacg cacctcaaag ctggaaatgc cgggtctggg tacaccctgg	2520
ggaaactgcaa agcctgcaca cttgggggga atgatcaaga tgagaggcag ggggtgggat	2580
ggcatgtgca ccaggagatg ttagagaaac cctgaggaag agcagcgtgc agcaggtgat	2640
gggggagagt gggcagcaag cgaggccagg acagccactc tgctcagtca ccagtccaca	2700
caccagggg ctcactctgc cctctgagc acccaaggac gttaaagagc tggaaactgtt	2760
agtctaaata taggaccatc caagctctga accaaaatgt gtcccttgcc tcaactcagg	2820



FIG. 8C (continued)

SEQ ID NO: 7 Human Ccr2 gene isoform A

Sequence 7:

```
agatccacag aggcagaagt aaggaattta ttttctgaaa gatagatttc tatcagttct 2880
gggtgacatg ttctgacact tgaaatgaca cctaggacag cacatttcag gcatcttgct 2940
cattgttcac tgtagtagaa gctacatgct agccagttgt aaaaatgaaa ttaagtaatg 3000
tgtgcacagc atttaacata gcatctgagc ttcaggagca ctcaattaat gaccacagtt 3060
gtgattcttt aggcagatgc atttttttcc aactttgatc agaggtctta tttagcttct 3120
ccagatttca agaactctggc tcagtgatat gaaatacaag acttgtgaaa agtgtcaatt 3180
gcaagagaaa tggaaggata aagtatacag gtgggtggaa aagaaattca cagtcactgc 3240
cagaaaaaaaa attcttgaga atcaagtccct gatgatgtta gggcttatag ttcttattat 3300
aaagagtttt atgtactcat tcagtgaaca tttattggtg cctcctttag ccaggtacta 3360
tcataagagc tgaaaataga agcataatcc agtccttgat cttgaggaac atgctgtgtg 3420
tagcagataa cataataagt gcttatctag atgcatgcag tgttatgtga taagagtaat 3480
atgacagagg atacagatta ggcttcacag agaaggggga tttgagcagg aggtattgaa 3540
gggtgaatag aagctcacca atcattttgg gcagaggggc aaggacctgc aaaaccactg 3600
aagcatgaag gaaatggtga gtttagggaa aatgaagaga agatggctgt gactgaagca 3660
caggatttgg gattggagaa gggactggag gtgaggctga aaagaggcaa actcagaaaa 3720
gatgttgtgc tgggcagtct ggacattatc tttgaagccc accacatata agtcataggg 3780
ctactggagg ttttaagcta agagtgacta ttcaatttca acttaagaga agatagggtg 3840
agagggaaaca tggcttgaga tgagccatga gcaaaggaaa gactacaaca aagccaggag 3900
tgaggagtgt gtgaagcaag aaagtgacag ttgaaagcag tgcagagggg atgaatctga 3960
gaggcatcta tgaggtggaa ctcaaagac atgataataa tacagggcat ttctctgtgt 4020
cagatgctgt cctaagtcct tactccattg atcttcacag caactcagca tagttaatat 4080
tttatgcata aagaaatcgg cacttgaagg agtaattggc cccagattac actgcctata 4140
aggattcaaa tccaggtttg tttggctcca aaaactggct cctaattttc agaaggagaa 4200
```

FIG. 8C (continued)

SEQ ID NO: 7 Human Ccr2 gene isoform A

Sequence 7:

```
gcgaccacag gcaatgcccc attttgcttc ttaggcaatg gaggaatcca caatcggaag 4260
gagttttcag cagtgcccc tttgggggtgg gttgaatttg aggtccctgc atgataccca 4320
ctttgctcac ttcagtgcct aaaactgagt atgggttcata gtaggtgttc aataagtgtt 4380
gatgcagtga atacatgcat ggggagatat gcatcaggca atgggaaatt caactctaag 4440
gcttagggga aagctggagc ttgaagacag agctttagaa aacagtagca tagaaggag 4500
taggaacat gagtttagac aatacaattc aggaagaact ttgtagcaag gataaagagg 4560
caaaaaatta aagaggtgag agctaagtgt ggtgcctggg gaatcttaag gtgtgggcac 4620
ggggaggaga tgccagcaaa gaacatgaat aaaaagcggg agcacagccc ctcccatctg 4680
gaagccaaaa agaattgtaa atggaggaag ttagcagaag gatcaaatac ttgaagaggg 4740
tggaattgga ataaaaccag ggcatttgaa aaattggggt gtcactgcaa tcttaacaag 4800
agaagttttg gcaggatgat ggaggcagaa agctgagaga atcatcagtt agaacgtttt 4860
tgacttcaga gaacagaaaa tgcagttcat aatggcttta aaacaggggc ttgtttttct 4920
cccagcaatt tgagaggcca aggcgggtgc atcaggaggt caagagaccg agaccatcct 4980
ggccaacatg gtgaatcccc atcttacta aaaatacaaa aattagcggg gcatggtggt 5040
gcacgcctat agtcccatct actcaggagg ctgaggcagg agaatcactt gaaccagga 5100
ggtggaggtt gcagtgagct gagatcatgg ccaactgcact atagcctgga gacacagcga 5160
gactccgtct ccaaaaaaaaa aaaaaaagaa ggcagaaggt gaatagttca aggggtgggt 5220
taggactcag tgataatagc attctgcctg gcttctcatg gttctctagg tcttccatto 5280
atggcaccat gccctcacta ggcattgctgc cagagcagga ggggcagggt gagggttctc 5340
ttgtgtctgt cttatcaggg aagaagagct ttctcagaag ccccagcag actccctttt 5400
catattatgg tccagcaatg agtcacagac ctatgcacca cctgcaaagg agccagagaa 5460
aacaacgcc cagcgttttt agcctgaaaa tgagaatctg gtttgctggg gaagataaag 5520
ggtgtcggaa aatggctgtt gggtaaatca ttgatgtctg ccaactaggaa tgaaaggcaa 5580
atcaggaact ggcacacatg ctttcaggga gatggctgca agggagaggg caaagactgg 5640
```

FIG. 8C (continued)

SEQ ID NO: 7 Human Ccr2 gene isoform A

Sequence 7:

```
gaagttgctt atgtgggtgcc agactatattg gaagatcatg gattgcggtg tttgtgttgt 5700
gtgggtcatca ttttgtttott tgtttacaga acagagaaaag tggattgaac aaggacgcat 5760
ttccccagta catccacaac atgctgtcca catctcgttc toggtttate agaaatacca 5820
acgagagcgg tgaagaagtc accacctttt ttgattatga ttacgggtgct ccoctgtcata 5880
aatttgacgt gaagcaaatt ggggcccaac tectgcctcc gctctactcg ctgggtgttca 5940
totttggttt tgtgggcaac atgctggteg tectcatott aataaaactgc aaaaagctga 6000
agtgttgac tgacatttac ctgctcaacc tggccatctc tgatctgctt tttcttatta 6060
ctctcccatt gtgggctcac tctgctgcaa atgagtgggt ctttgggaat gcaatgtgca 6120
aattattcac agggctgtat cacatcgggt attttggcgg aatcttcttc atcatctctc 6180
tgacaatcga tagatacctg gctattgtcc atgctgtggt tgctttaaaa gccaggacgg 6240
tcacctttgg ggtgggtgaca agtgtgatca cctggttggt ggetgtgttt gcttctgtcc 6300
caggaatcat ctttactaaa tgcagaaaag aagattctgt ttatgtctgt ggcocctatt 6360
ttccacgagg atggaataat ttccacacaa taatgaggaa cttttgggg ctggctctgc 6420
cgctgctcat catggctcctc tgctactcgg gaatcctgaa aacctgctt cgggtgtcgaa 6480
acgagaagaa gaggcatagg gcagtgcag tcactctcac catcatgatt gtttactttc 6540
tcttctggac tccctataat attgtcattc tectgaacac cttccaggaa ttcttcggcc 6600
tgagtaactg tgaaagcacc agtcaactgg accaagccac gcaggtgaca gagactcttg 6660
ggatgactca ctgctgcctc aatcccatca tctatgcctt cgttggggag aagttcagaa 6720
ggtatctctc ggtgttcttc cgaaagcaca tcaccaagcg cttctgcaaa caatgtccag 6780
ttttctacag ggagacagtg gatggagtga cttcaacaaa cacgccttcc actggggagc 6840
aggaagtctc ggctggttta taaaacgagg agcagtttga ttgttgttta taaagggaga 6900
taacaatctg tatataacaa caaacttcaa gggtttgttg aacaatagaa acctgtaaag 6960
cagggtccca ggaacctcag ggctgtgtgt actaatacag actatgtcac ccaatgcata 7020
```

FIG. 8C (continued)

SEQ ID NO: 7 Human Ccr2 gene isoform A

Sequence 7:

tccaacatgt	gctcagggaa	taatccagaa	aaactgtggg	tagagacttt	gactctccag	7080
aaagctcatc	tcagctcctg	aaaaatgcct	cattaccttg	tgctaactct	ctttttctag	7140
tcttcataat	ttcttcactc	aatctctgat	tctgtcaatg	tcttgaaatc	aagggccagc	7200
tggaggtgaa	gaagagaatg	tgacaggcac	agatgaatgg	gagtgagggg	tagtggggtc	7260
agggctgaga	ggagaaggag	ggagacatga	gcatggctga	gcctggacaa	agacaaaggt	7320
gagcaaaggg	ctcacgcatt	cagccaggag	atgatactgg	tccttagccc	catctgccac	7380
gtgtatttaa	ccttgaaggg	ttcaccaggt	cagggagagt	ttgggaactg	caataacctg	7440
ggagttttgg	tggagtccga	tgattctctt	ttgcataagt	gcatgacata	tttttgcttt	7500
attacagttt	atctatggca	cccatgcacc	ttacatttga	aatctatgaa	atatcatgct	7560
ccattgttca	gatgcttctt	aggccacatc	ccctgtctta	aaaattcaga	aaatttttgt	7620
ttataaaaga	tgcatatctt	atgatatgct	aatatatgta	tatgcaatat	atataggtct	7680
ttgcttgatc	tctccaggag	gtagtgatta	tgagaagggg	gtggagaatg	atgagttcct	7740
tcaccaggag	caaaggacgg	ggatcgtgtg	gaaccactgc	agaactatct	ccgaaatcaa	7800
ctaagtggag	agagccagga	aggctgcatc	agaaccctgt	aaagcttctt	gtctggatct	7860
gagctggttt	gtttttgtgt	tgcttttccc	tgctttgcca	ctccccctac	tcttctcttt	7920
tccccacagc	ctttttcaca	tagctcttgg	ctgtaggatt	gccccactcc	aaaaaccagt	7980
gtgtggaggt	ccaggagtga	gaccaggaaa	gaatgtgaaa	gtgactacac	aaggactcct	8040
cgatggtcgt	ggaaaaggaa	agtcaattgg	cagagcccct	gaagccagtc	ttcaggacaa	8100
agaaggagcc	tagagacaga	aatgacagat	ctctgctttg	gaaatcacac	gtctggcttc	8160
acagatgtgt	gattcacagt	gtgaatcttg	gtgtctacgt	taccaggcag	gaaggctgag	8220
aggagagaga	ctccagctgg	gttggaaaac	agtattttcc	aaactacctt	ccagttcctc	8280
atttttgaat	acaggcatag	agttcagact	ttttttaaat	agtaaaaata	aaattaaagc	8340
tgaaaactgc	aacttgtaaa	tgtggtaaag	agttagtttg	agttactatc	atgtcaaacg	8400
tgaaaatgct	gtattagtca	cagagataat	tctagctttg	agcttaagaa	ttttgagcag	8460

## FIG. 8C (continued)

SEQ ID NO: 7 Human Ccr2 gene isoform A

Sequence 7:

gtggtatggt	tgggagactg	ctgagtcaac	ccaatagttg	ttgattggca	ggagttggaa	8520
gtgtgtgatc	tgtgggcaca	ttagcctatg	tgcattgcagc	atctaagtaa	tgatgtcggt	8580
tgaatcacag	tatacgtctc	atcgctgtca	tctcagctgg	atctccattc	tctcaggtct	8640
gctgccaaaa	gccttttgtg	ttttgttttg	tatcattatg	aagtcattgcg	tttaatcaca	8700
ttcgagtgtt	tcagtgtctc	gcagatgtcc	ttgatgtctc	tattgttccc	tattttgcca	8760
gtgggaactc	ctaaatcaag	ttggcttcta	atcaaagctt	ttaaacccta	ttggtaaaga	8820
atggaagggtg	gagaagctcc	ctgaagtaag	caaagacttt	cctcttagtc	gagccaagtt	8880
aagaatgttc	ttatgttgc	cagtgtgttt	ctgatctgat	gcaagcaaga	aacctgtggc	8940
ttctagaacc	aggcaacttg	ggaactagac	tcccagctg	gactatggct	ctactttcag	9000
gccacatggc	taaagaaggt	ttcagaaaaga	agtggggaca	gagcagaact	ttcaccttca	9060
tatatttgta	tgatccta	gaatgcata	aatgttaagt	tgatgggtgat	gaaatgtaaa	9120
tactgttttt	aacaactatg	atgtggaaaa	taaatcaatg	ctataactat	gttgataaaa	9180
gatttaaaaa	caactggctg	tttttttaca	ctgtgggtgtg	gaagattgtg	ttgtgttcac	9240
aacttttcac	ttcttcccct	gtgtgattac	acacacctgc	ccttgtgggtg	tgacttgcag	9300
tgcgccctac	aggccacaca	accccatgcc	ctccaccact	ggctctgctg	ctggaatgtg	9360
agcagaagtg	acatctgctc	catccaagca	gagcctcttg	ctcagccaca	ggaaggccca	9420
ttccagatca	caccgctcag	cccgtgcgcc	ctgggtgaatg	agaagacaca	gggagctgca	9480
gccacatata	acatgagcaa	gaagtctgtg	tttgctgtga	taagccactg	agttttaggg	9540
gttgtttgtt	aagaagcaca	aaaaccgatt	aagacatgtg	gtatatagtg	acttcatata	9600
tagaatctgg	aaaactatcc	atatttttc	aatcatggaa	ttcaatatga	caagcatccc	9660
ggagggctca	cctatgccag	actgggttgg	aaacagaaaag	acagatgtta	atgccagtgt	9720
cctttacacc	tccaagtcca	gggccagctg	tggagtggga	ggggtagaga	aggtcctgtg	9780
cacagtcaca	gtgcgctgtg	cagagcagga	acagaggcat	ctgtgaaaag	tgctgagagc	9840

FIG. 8C (continued)

SEQ ID NO: 7 Human Ccr2 gene isoform A

Sequence 7:

```
ctggaggaca gagtgactaa tgcaatgaca gtcttgcatc ataggaataa cagccacagc 9900
aggattttat tgctgccaaa gaaactgcca tttaaaaatt gccagccatc cgggaggctg 9960
aggcaggaga atggcatgaa tccaggagge ggagcttgca gtgagccgag atcgggccac 10020
tgcactccag cctgggcaac agagccagac tccatctcaa aaaaaaaaaa aaa 10073
```



FIG. 8D

SEQ ID NO: 8 Human Ccr2 gene promoter

Sequence 8:

```
gcacacctgt aatcccagcc cttcaggagg ctgaggcagg cagattgcct gagcccagga      60
gttcgagacc agcccgggca acatgacaaa accccatctc tacaaaaaat agtcaggcat      120
ggtagcatgc acctgtagtc tcagctactt gggaggctga gatgagagga ttgcttgagg      180
ttgagactgc actgaagcat gatcatgcca cgcactcca gcctaggcaa cagagcaaga      240
tcttgctgca aaagaaagca aaaatacaac ataacacaac aacaacaaca acaacaacaa      300
cagcaaaaaa gccaaacttct tgaaatctgg aaaggacacc tccactgccc tcagcatttg      360
attgttgttg gctctagcag tggatgcac cttcaacctc tggcactctg caggggctca      420
gactgttctg ttctgtttgt tacctgtgga gtgcctgcca gacctgctc tagctgcttt      480
aggccattt accctcatag acccccagtc ttgttattca tatttcatat ttgggaaatg      540
gaaacttaga aacttgcaa gtccacagca tgagatcctg cctccgggtg ctgctggatt      600
ccagaaagtg ccagggggcca acttagatga caccatgttc tctgcacaat cttaggaatg      660
ctcctagtct gatgtcccca ttgcaaaatt tacattatct tttacaaaaa cgtctttcca      720
aggaggggca tttaaaataa ctgaggttct tcttgctaag gacgttctg acacaagaga      780
taatttagca tttccttttc attaaaaagt ttgaaatcct gtaatttgtg ataatgtgga      840
tgaacctaga ggatgttaag tgaaataagc cacacacaga tagacaaata ccacgtgatc      900
tcactcttat gtggaatttt tttttaaata agttgcttag ccgggcatga tggcacacac      960
ctgtaatcct agctactcag gaggetgagg tgggaggatg gcttgaactc agaagggtgga     1020
ggtagcagtg agctgagact gtgccagtgc actccggtct gggtgacaga atgaaaccca     1080
atttaaaaaa aaaaaaaaaag ttgctatctt agaaaaagac agtagagcag tggttaccag     1140
agactgggga ggaaagagag gaggtgagaa tgggcagcag ttgatcaacg ggtacaaagt     1200
taccatgaga taggagaaac aagtgctggt gctctgctcc aagtaggggtg acggtagtta     1260
ataatgaatt ctgtatatat aaatagctag aagagaggggt tttcaatatc attattattt     1320
caaaagaaat gataaatggt tcagaggatg gatatgtaat taccctgatt tgatcattgc     1380
acaatgtata catgtagcaa aacatcacat tgtgtcccat aaatatatac aattattatg     1440
```

FIG. 8D (continued)

SEQ ID NO: 8 Human Ccr2 gene promoter

Sequence 8:

```
tgaattaaat aaaaaaaaaat tttaaagtct tatctaaatg aaattttctaa ccagattctg 1500
aatccatgat accactgaaa ccagcacaca tgatcgcagt aaaacctcat tatacttctc 1560
ccactatcac caataccctt tattctctgg aacatgaaac attctgttgt gctcatatca 1620
tgcaaattat cactagtagg agagcagaga gtggaaatgt tccaggtata aagaccacaca 1680
agataaagaa gctcagagtc gttagaaaca ggagcagatg tacagggttt gcctgactca 1740
cactcaaggt tgcataagca agattttcaa ataatccta ttctggagac ctcaacccaa 1800
tgtacaatgt tcctgactgg aaaagaagaa ctatattttt ctgatttttt ttttcaaate 1860
tttaccatta gttgccctgt atctccgct tcactttctg caggaaactt tatttcctac 1920
ttctgcatgc caagtttcta cctctagatc tgtttggttc agttgctgag aagcctgaca 1980
taccaggact gcctgagaca agccacaagc tggtgagttg taggcatttt ttccattact 2040
ttctgattca taggetcaac gcacctcaaa gctggaaatg cc 2082
```

FIG. 9

SEQ ID NO: 9 Human C5 receptor gene

Sequence 9

```
ctacctccaa ccatgggcct tttgggaata ctttgTTTTT taatcttcoct ggggaaaacc      60
tggggacagg agcaaacata tgtcatttca gcaccaaaaa tattcoctgt tggagcatct      120
gaaaatattg tgattcaagt ttatggatac actgaagcat ttgatgcaac aatctctatt      180
aaaagttatc ctgataaaaa atttagttac tctcagggcc atgttcattt atoctcagag      240
aataaattcc aaaactctgc aatcttaaca atacaacca aacaattgcc tggaggacaa      300
aaccagttt cttatgtgta tttggaagtt gtatcaaagc atttttcaaa atcaaaaaga      360
atgccataa cctatgacaa tggatttctc tcattcata cagacaaaacc tgtttatact      420
ccagaccagt cagtaaaagt tagagtttat tCGTTgaatg acgacttgaa gccagccaaa      480
agagaaactg tcttaacctt catagatcct gaaggatcag aagttgacat ggtagaagaa      540
attgatcata ttggaattat ctcttttctc gacttcaaga tcoctctaa tcttagatat      600
ggtatgtgga cgatcaaggc taaatataaa gaggactttt caacaactgg aaccgcatat      660
tttgaagtta aagaatatgt cttgccacat ttttctgtct caatcgagcc agaatataat      720
ttcattgggtt acaagaactt taagaatttt gaaattacta taaaagcaag atatttttat      780
aataaagtag tcaactgaggc tgacgtttat atcacatttg gaataagaga agacttaaaa      840
gatgatcaaa aagaaatgat gcaaacagca atgcaaaaca caatgttgat aaatggaatt      900
gctcaagtca catttgatlc tgaaacagca gtcaaagaac tgtcactacta cagtttagaa      960
gatttaaaca acaagtacct ttatattgct gtaacagtca tagagtctac aggtggattt     1020
tctgaagagg cagaaatacc tggcatcaaa tatgtcctct ctccctaca actgaatttg     1080
gttgctactc ctcttttctc gaagcctggg attccatata ccatcaaggt gcaggttaaa     1140
gattcgcttg accagttggt aggaggagtc ccagtaatac tgaatgcaca aacaattgat     1200
gtaaaccaag agacatctga cttggatcca agcaaaagtg taacacgtgt tgatgatgga     1260
gtagcttctt ttgtgcttaa tctcccatct ggagtgacgg tgctggagtt taatgtcaaa     1320
actgatgctc cagatcttcc agaagaaaat caggccaggg aaggttaccg agcaatagca     1380
```

FIG. 9 (continued)

SEQ ID NO: 9 Human C5 receptor gene

Sequence 9

tactcatctc	tcagccaaag	ttacctttat	attgattgga	ctgataacca	taaggctttg	1440
ctagtgggag	aacatctgaa	tattattggt	acccccaaaa	gcccatatat	tgacaaaata	1500
actcactata	attacttgat	tttatccaag	ggcaaaatta	tccattttgg	cacgagggag	1560
aaattttcag	atgcatctta	tcaaagtata	aacattccag	taacacagaa	catggttcct	1620
tcateccgac	ttctggteta	ttatatcgtc	acaggagaac	agacagcaga	attagtgtct	1680
gattcagtct	ggttaaatat	tgaagaaaaa	tgtggcaacc	agctccaggt	tcactctgtct	1740
cctgatgcag	atgcatattc	tccaggccaa	actgtgtctc	ttaatatggc	aactggaatg	1800
gattcctggg	tggcattagc	agcagtggac	agtgtgtgt	atggagtcca	aagaggagcc	1860
aaaaagccct	tggaaagagt	atctcaatc	ttagagaaga	gtgatctggg	ctgtggggca	1920
ggtggtggcc	tcaacaatgc	caatgtgttc	cacctagctg	gacttacctt	cctcactaat	1980
gcaaatgcag	atgactccca	agaaaatgat	gaaccttgta	aagaaattct	caggccaaga	2040
agaacgctgc	aaaagaagat	agaagaaata	gctgctaaat	ataaacatc	agtagtgaag	2100
aatgtttgtt	acgatggagc	ctgctgtaat	aatgatgaaa	cctgtgagca	gcgagctgca	2160
cggattagtt	tagggccaag	atgcatcaaa	gctttcactg	aatgtttgt	cgctcgcaagc	2220
cagctccgtg	ctaatatctc	tcataaagac	atgcaattgg	gaaggctaca	catgaagacc	2280
ctgttaccag	taagcaagcc	agaaattcgg	agttatcttc	cagaaagctg	gttgtgggaa	2340
gttcatcttg	ttcccagaag	aaaacagttg	cagtttgccc	tacctgattc	tctaaccacc	2400
tgggaaatc	aaggcattgg	catttcaaac	actggtatat	gtggtgctga	tactgtcaag	2460
gcaaagggtg	tcaaagatgt	cttctgggaa	atgaatatac	catattctgt	tgtacgagga	2520
gaacagatcc	aattgaaagg	aactgtttac	aactatagga	cttctgggat	gcagttctgt	2580
gttaaaatgt	ctgctgtgga	gggaatctgc	acttcggaaa	gccagtcac	tgatcatcag	2640
ggcaciaaagt	cctccaaatg	tgtgcgccag	aaagtagagg	gctcctccag	tcacttggtg	2700
acattcactg	tgcttctct	ggaaattggc	cttcacaaca	tcaatttttc	actggagact	2760

FIG. 9 (continued)

SEQ ID NO: 9 Human C5 receptor gene

Sequence 9

tggtttgaa	aagaaatctt	agtaaaaaca	ttacgagtgg	tgccagaagg	tgtcaaaagg	2820
gaaagctatt	ctggtgttac	tttggatcct	aggggtatct	atggtacat	tagcagacga	2880
aaggagtcc	catacaggat	accottagat	ttggtcccca	aaacagaaat	caaaaggatt	2940
ttgagtgtaa	aaggactgct	tgtaggtgag	atcttgtctg	cagttctaag	tcaggaaggc	3000
atcaatatcc	taaccacact	ccccaaaggg	agtgcagagg	cggagctgat	gagcgttctc	3060
ccagtattct	atgtttttca	ctacctggaa	acaggaaatc	attggaacat	ttttcattct	3120
gaccattaa	ttgaaaagca	gaaactgaag	aaaaaattaa	aagaagggat	gttgagcatt	3180
atgtcctaca	gaaatgctga	ctactcttac	agtgtgtgga	aggggtggaag	tgctagcact	3240
tggttaacag	cttttgcttt	aagagtactt	ggacaagtaa	ataaatacgt	agagcagaac	3300
caaaattcaa	tttgtaattc	tttattgtgg	ctagttgaga	attatcaatt	agataatgga	3360
tctttcaagg	aaaattcaca	gtatcaacca	ataaaattac	agggtaacct	gcctgttgaa	3420
gcccagagaga	acagcttata	tcttacagcc	tttactgtga	ttggaattag	aaaggetttc	3480
gatatatgcc	ccctggtgaa	aatcgacaca	gctctaatta	aagctgacaa	ctttctgctt	3540
gaaaatacac	tgccagccca	gagcaacctt	acattggcca	tttctgcgta	tgctctttcc	3600
ctgggagata	aaactcacc	acagtttctg	tcaattgctt	cagctttgaa	gagagaagct	3660
ttggttaaag	gtaatccacc	catttatctg	ttttggaaag	acaatcttca	gcataaagac	3720
agctctgtac	ctaactctgg	taoggcacgt	atggtagaaa	caactgcta	tgctttactc	3780
accagtctga	acttgaaaga	tataaattat	gttaaccacg	tcatcaaatg	gctatcagaa	3840
gagcagaggt	atggaggtgg	cttttattca	accaggaca	ccatcaatgc	cattgagggc	3900
ctgacggaat	attcaactct	ggttaaacia	ctccgcttga	gtatggacat	cgatgtttct	3960
tacaagcata	aaggtgcctt	acataattat	aaaatgacag	acaagaattt	ccttgggagg	4020
ccagtagagg	tgcttctcaa	tgatgacctc	attgtcagta	caggatttgg	cagtggcttg	4080
gctacagtac	atgtaacaac	tgtagttcac	aaaaccagta	cctctgagga	agtttgcagc	4140
ttttatttga	aaatcgatac	tcaggatatt	gaagcatccc	actacagagg	ctaogggaaac	4200

FIG. 9 (continued)

SEQ ID NO: 9 Human C5 receptor gene

Sequence 9

tctgattaca aacgcatagt agcatgtgcc agctacaagc ccagcagggga agaatcatca	4260
tctggatcct ctcatgcggt gatggacatc tccttgcccta ctggaatcag tgcaaatgaa	4320
gaagacttaa aagcccttgt ggaaggggtg gatcaactat tcaactgatta ccaaatcaaa	4380
gatggacatg ttattctgca actgaattcg attccctcca gtgatttctt ttgtgtacga	4440
ttccggatat ttgaactctt tgaagttggg tttctcagtc ctgccacttt cacagtttac	4500
gaataccaca gaccagataa acagtgtacc atgttttata gcacttcca tatcaaaatt	4560
cagaaagtct gtgaaggagc cgcgtgcaag tgtgtagaag ctgattgtgg gcaaatgcag	4620
gaagaattgg atctgacaat ctctgcagag acaagaaaac aaacagcatg taaaccagag	4680
attgcatatg cttataaagt tagcatcaca tccatcactg tagaaaatgt ttttgtcaag	4740
tacaaggcaa cccttctgga tatctacaaa actggggaag ctgttgctga gaaagactct	4800
gagattacct tcattaaaaa ggtaacctgt actaacgctg agctggtaaa aggaagacag	4860
tacttaatta tgggtaaaga agccctccag ataaaaataca atttcagttt caggtacatc	4920
taccctttag attccttgac ctggattgaa tactggccta gagacacaac atgttcatcg	4980
tgtaagcat ttttagctaa tttagatgaa tttgccgaag atatcttttt aaatggatgc	5040
taaaattcct gaagttcagc tgcatacagt ttgcacttat ggactcctgt tgttgaagtt	5100
cgtttttttg ttttcttctt tttttaaaca ttcatagctg gtcttatttg taaagctcac	5160
tttacttaga attagtggca cttgctttta ttagagaatg atttcaaagtg ctgtaacttt	5220
ctgaaataac atggccttgg agggcatgaa gacagatact cctccaaggt tattggacac	5280
cggaaacaat aaattggaac acctcctcaa acctaccact caggaatggt tgctggggcc	5340
gaaagaacag tccattgaaa gggagtatta caaaaacatg gcctttgctt gaaagaaaat	5400
accaaggaac aggaaactga tcattaaagc ctgagtttgc tttc	5444

FIG. 10

SEQ ID NO: 10 Human C5a receptor gene fragment

Sequence 10:

```
ctacctccaa ccatgggcct tttgggaata ctttgTTTT taatcttctt ggggaaaacc      60
tggggacagg agcaaacata tgtcatttca gcaccaaaaa tattccgtgt tggagcatct    120
gaaaatattg tgattcaagt ttatggatac actgaagcat ttgatgcaac aatctctatt    180
aaaagttatc ctgataaaaa atttagttac tcctcaggcc at                          222
```

## METHODS AND ANIMAL MODEL FOR ANALYZING AGE-RELATED MACULAR DEGENERATION

This application is a Continuation of U.S. application Ser. No. 10/685,705, filed Oct. 16, 2003 now U.S. Pat. No. 7,595,430, claiming priority of U.S. Provisional Application No. 60/422,096, filed Oct. 30, 2002, the entire contents of each of which are hereby incorporated by reference.

### FIELD OF THE INVENTION

The invention relates to methods of determining the pathology of age-related macular degeneration and methods of testing treatment protocols and candidate drugs for age-related macular degeneration. More particularly, the invention relates to use of Ccl2-deficient, Ccr2-deficient, or both Ccl2 and Ccr2-deficient mice to analyze the pathology and treatment of age-related macular degeneration and test candidate drugs for treatment of age-related macular edema.

### BACKGROUND OF THE INVENTION

Age-related macular degeneration (AMD) is the principal cause of legal blindness in the United States and Western Europe. It affects over 11 million people in this country alone, and with the aging population will exact an even greater toll. The earliest visible abnormality in AMD is the accumulation of drusen (Gass, J. D. (1972) *Trans Am Ophthalmol Soc* 70, 409-36.), lipoproteinaceous deposits between the retinal pigment epithelium (RPE) and Bruch's membrane, the extracellular matrix between the RPE and the underlying choroid. Drusen are a significant risk factor for progression to choroidal neovascularization (CNV), the principal cause of vision loss in AMD (Macular Photocoagulation Study Group (1997) *Arch Ophthalmol* 115, 741-7). There is no animal model of drusen resembling that of patients with AMD. Drusen-like deposits in elderly primates (Hope, et al., (1992) *Br J Ophthalmol* 76, 11-6.) are dissimilar to human drusen both in ultrastructural morphology and biochemical composition (Hirata, A. & Feeney-Burns, L. (1992) *Invest Ophthalmol Vis Sci* 33, 2079-90; Mullins, R. F. & Hageman, G. S. (1997) in *Degenerative Retinal Diseases*, ed. LaVail, M. (Plenum Press, New York), pp. 1-10.). Attempts to create a murine model of drusen by high fat diet, disrupting the apolipoprotein E gene, inducing protoporphyria (Gottsch et al., (1993) *Arch Ophthalmol* 111, 126-9.), accelerating senescence (Majji, et al., (2000) *Invest Ophthalmol Vis Sci* 41, 3936-42), or combinations of the above (Dithmar et al., (2001) *Arch Ophthalmol* 119, 1643-9) have not succeeded in creating drusen.

The biogenesis of drusen involves RPE dysfunction, impaired digestion of photoreceptor outer segments, and subsequent debris accumulation (Hageman, et al., (2001) *Prog Retin Eye Res* 20, 705-32). The presence of complement C5, immunoglobulins, apolipoprotein E, vitronectin, and clusterin in human drusen (Loffler, et al., (1986) *Graefes Arch Clin Exp Ophthalmol* 224, 493-501; Hageman, G. S., et al., (1999) *FASEB J* 13, 477-84; Hageman, G. S. & Mullins, R. F. (1999) *Mol Vis* 5, 28 Johnson, et al., (2000) *Exp Eye Res* 70, 441-9; Mullins et al., (2000) *FASEB J* 14, 835-46; and Anderson, et al., (2001) *Am J Ophthalmol* 131, 767-81) suggests that focal concentration of these materials may produce a powerful chemotactic stimulus for leukocytes, possibly acting via a complement cascade (Killingsworth, et al., (2001) *Exp Eye Res* 73, 887-96). Consistent with this, macrophages appear to preferentially engulf the wide-banded collagen of basal deposits in patients with AMD, suggesting a role in

drusen clearance (Loffler, K. U. & Lee, W. R. (1986) *Graefes Arch Clin Exp Ophthalmol* 224, 493-501; Killingsworth, et al., (1990) *Eye* 4, 613-21; Penfold, P. L., et al., (1985) *Graefes Arch Clin Exp Ophthalmol* 223, 69-76; and van der Schaft, et al., (1993) *Br J Ophthalmol* 77, 657-61). Laser photocoagulation induced regression of drusen in humans (Ho, et al., (1999) *Ophthalmology* 106, 1367-73; and Olk, et al., (1999) *Ophthalmology* 106, 2082-90) is believed to result from recruitment of macrophages that resorb these deposits (Duvall, J. & Tso, M. O. (1985) *Arch Ophthalmol* 103, 694-703).

The lack of a faithful animal model of AMD has hampered both the study and treatment of age-related macular degeneration. Thus, there is a need for a faithful animal model of drusen development and accumulation to provide mechanistic insights into the development of AMD and assist in evaluating candidate drugs for the treatment of age-related macular degeneration.

### SUMMARY OF THE INVENTION

In one aspect of the invention there is provided a method for testing a candidate drug for treatment or prevention of age-related macular degeneration comprising administering the candidate drug to a Ccl2-deficient, Ccr2-deficient- or a Ccl2-deficient and -Ccr2-deficient mouse and analyzing the eye of the mouse for development or regression of drusen and/or lipofuscin accumulation therein, for affect of the candidate drug on Bruch's membrane and/or choroidal neovascularization of the eyes of the mouse.

There is also provided a method of screening a test compound for potential utility for treatment of age-related macular degeneration, comprising: (a) providing a mouse comprising a disrupted Ccl2 and/or CCR2 gene, wherein the mouse is homozygous for the disrupted gene or genes, and wherein the mouse exhibits drusen and/or lipofuscin deposits, retinal degeneration, and/or choroidal neovascularization in at least one eye at about nine to twenty-four months of age compared to a wild-type mouse that does not have the disrupted gene; (b) administering the test compound to the mouse; (c) determining the effect of the test compound on drusen, lipofuscin deposition, retinal degeneration, or choroidal neovascularization in at least one eye of the mouse; and (d) correlating the effect of the test compound on drusen, lipofuscin accumulation, retinal degeneration, and/or choroidal neovascularization with a potential utility to treat age-related macular degeneration.

In another aspect of the invention there is provided a method of monitoring the effects of expression of a Ccl2 gene in at least one eye of a Ccl2-/- mouse comprising (1) introducing a plurality of stem cells obtained from a wild type mouse into the Ccl2-/- mouse to obtain a transplanted mouse, wherein said stem cells express wild type Ccl2; and (2) observing at least one eye of the transplanted mouse for the effect of the wild type Ccr2 gene expression on drusen or lipofuscin deposition, retinal degeneration, or choroidal neovascularization in at least one eye of the transplanted mouse. There is also provided a method of monitoring the expression of a Ccr2 gene in at least one eye of a Ccr2-/- mouse comprising (1) introducing a plurality of stem cells obtained from a wild type mouse into the Ccr2-/- mouse to obtain a transplanted mouse, wherein said stem cells express wild type Ccr2; and (2) observing at least one eye of the transplanted mouse for the effect of the wild type Ccr2 gene expression on drusen or lipofuscin deposition, retinal degeneration, or choroidal neovascularization in at least one eye of the transplanted mouse. There is also provided a method of monitoring the effects of expression of a Ccl2



gene, Ccr2 gene or both in at least one eye of a Ccl2 deficient, Ccr2 deficient mouse comprising (1) introducing a plurality of stem cells obtained from a wild type mouse into the Ccl2 deficient, Ccr2 deficient mouse to obtain a transplanted mouse, wherein said stem cells express wild type Ccl2 and Ccr2; and (2) observing at least one eye of the transplanted mouse for the effect of the wild type Ccl2 and/or Ccr2 gene expression on drusen or lipofuscin deposition, retinal degeneration, or choroidal neovascularization in at least one eye of the transplanted mouse.

In a further aspect of the invention there is provided a Ccl2-deficient/CCR2-deficient dual knockout mouse.

The present invention also provides a method of identifying mutations in the Ccl2 gene, Ccr2 gene or both comprising (1) obtaining an AMD DNA library or genomic DNA from a blood sample of an AMD patient; (2) screening the AMD DNA library or genomic DNA for sequences that hybridize under high stringency conditions to a wild type Ccl2 gene, Ccr2 gene, or both; and (3) sequencing the sequences that hybridize to determine the identity of any mutations contained therein.

In a further aspect of the invention there are provided expression vectors comprising SEQ ID NO.9 and/or SEQ ID NO. 10.

In yet a further aspect of the invention there is provided a method of screening for mutations that potentially cause or affect the development of AMD in a human comprising (1) obtaining an AMD DNA library or genomic DNA from a blood sample of an AMD patient; (2) screening the AMD DNA library or genomic DNA for sequences that hybridize under high stringency conditions to a wild type C5 receptor gene or C5a receptor gene; (3) sequencing the sequences that hybridize to determine the identity of any mutations contained therein.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIG. 1. Ccl2<sup>-/-</sup> and Ccr2<sup>-/-</sup> mice develop early AMD. (a) Fundus photo of 15-month-old Ccl2<sup>-/-</sup> mouse. Inset shows higher magnification. (b) Drusen deposits in knockout mice increase with age (n=4). (c) Collagen and elastin fibers (asterisks) of thickened Bruch membrane (indicated by bracket) in 9-month-old Ccl2<sup>-/-</sup> mouse are disrupted, and choriocapillaries are highly fenestrated (arrowheads). (d) Bruch membrane is thickened in 10- to 12-month-old knockout mice (n=5). Asterisk P<0.05. (e) TIMP-3 (red) immunoreactivity in RPE and Bruch membrane (BM) of 14-month-old Ccl2<sup>-/-</sup> mouse. There was no staining in photoreceptors (PR) or choroid (CH). (f) Lipofuscin autofluorescence (red) in light micrograph of RPE (arrow) of 15-month-old Ccl2<sup>-/-</sup> mouse. (g) Lipofuscin granules (arrows) in electron micrograph of 15-month-old Carl mouse. (h) MALDI spectrum of RPE of 12-month-old Ccl2<sup>-/-</sup> mouse, showing A2E signal. NPP, N-perfluoroalkyl pyridine. Scale bar=0.5 μm (c), 50, μm (e), 10 μm (f), or 2 μm (g).

FIG. 2. Ccl2<sup>-/-</sup> and Ccr2<sup>-/-</sup> mice develop retinal degeneration. a, Fundus of an 18-month-old Ccr2<sup>-/-</sup> mouse shows geographic atrophy (arrows). b,c, Electron micrographs show healthy photoreceptor cell bodies in 14-month-old wild-type mouse (b) and attenuated photoreceptors with pyknotic nuclei (arrows) in 16-month-old Ccl2<sup>-/-</sup> mouse (c). d,e, Orderly arrays of photoreceptor outer segments in 14-month-old wild-type mouse (d) and marked degeneration and seg-

ments (asterisk) with pigment-laden RPE cells (arrows) amidst disorganized tissue in 16-month-old Ccl2<sup>-/-</sup> mouse (e). f, RPE of 16-month-old Ccl2<sup>-/-</sup> mouse shows marked vacuolization (black arrows), degenerated nucleus (black asterisk), and few pigment granules (white arrow). Choroid is filled with abundant melanocytes (white asterisks) but no choriocapillaris vessels. g,h, Retina in Ccl2<sup>-/-</sup> mouse outside these atrophic areas contains normal photoreceptor cell bodies (g) and outer segments (h). Scale bar 10 μm (b,c,f,g) and 5 μm (d,e,h).

FIG. 3. Ccl2<sup>-/-</sup> and Ccr2<sup>-/-</sup> mice develop neovascular AMD and overexpress VEGF in RPE. a-c, Electron micrograph in 20-month-old Ccl2<sup>-/-</sup> mouse shows dilated choriocapillaries (CC) inserting processes (blue arrows) into Bruch's membrane (BM), with fragmented collagen and elastin layers (asterisks) of BM in a 20-month-old Ccl2<sup>-/-</sup> mouse. Inner BM (white arrowheads) is intact whereas outer BM (black arrowheads) is breached by choriocapillary processes (blue arrows) and fractures (red arrows). Higher magnification of insets (white area-b and black area-c) shows breaks (red arrows) in outer BM and endothelial processes (blue arrows) inserted into BM, disrupting outer collagenous (black asterisk) and elastin and inner collagenous layers (white asterisks), and large fenestrae (arrowheads) (c). d-f, CNV/ in 24-month-old Ccr2<sup>-/-</sup> mouse where an endothelial cell (E) and fibrocytes (asterisks) invade sub-RPE space through a defect in BM (arrowheads), disrupting overlying photoreceptors (PR). Higher magnification of insets shows (e) fibrocytes (F) invading BM and disrupting overlying RPE (r) extracellular matrix, and (f) an endothelial cell (E) and fibrocyte processes (asterisks) that have broken through a discontinuity in BM (arrowheads) to displace an RPE cell (R) from its intact monolayer (r). VEGF staining (blue) is minimally present in RPE of 18-month-old wild-type (g) but markedly expressed in RPE and choroid of 18-month-old Ccl2<sup>-/-</sup> mouse (h). Scale bars 2 μm (a,e,f), 1 μm (b,c), 10 μm (d), and 100 μm (g,h). Intrachoroidal neovascularization leaks indocyanine green but not fluorescein. i, Late phase (12 min) fluorescein angiogram corresponding to area in a-c shows no leakage (arrow) in the region whereas j-l, indocyanine green angiography reveals a focal area (arrow) of hyperfluorescence that increases over time (j-3 min, k-6 min, l-10 min). m,n, Choroidal neovascularization leaks fluorescein. m, Fluorescein angiography shows focal early (2 min) hyperfluorescence (m) that increases both in intensity and area in the late (9 min) frame (n) corresponding to region in d-f.

FIG. 4. Complement proteins and IgG deposition in Ccl2<sup>-/-</sup> and Ccl2<sup>-/-</sup> mice, and C5a and IgG stimulate Ccl2 and VEGF secretion in RPE cells and CEC. a, Complement C5 (blue) staining in RPE and choroid (CH) of 18-month-old Ccr2<sup>-/-</sup> mouse. b, IgG staining (blue) in choroid and RPE in 14-month-old Ccl2<sup>-/-</sup> mouse. c, Colocalization of complement C3c (red) and IgG (green) around choroidal vessel (V) wall and in RPE of 14-month Ccl2<sup>-/-</sup> mouse. Merged picture shows yellow costaining. d, Vitronectin immunoreactivity in RPE and choroid of 18-month-old Ccr2<sup>-/-</sup> mouse. e, CD46 staining in RPE of 14-month-old Ccl2<sup>-/-</sup> mouse. f, Serum amyloid P component staining in RPE and choroid of 14-month Ccl2<sup>-/-</sup> mouse. RPE, asterisks. Choroid, CH. Scale bar 100 μm (a,b), 25 μm (c), 50 μm (d-f). g, Western blot. RPE and choroid lysates from 6-month-old wild-type (Young WT), 18-month-old wild-type (Old WT), 6-month old Ccl2<sup>-/-</sup> (Young CCL2), 16-month-old Ccl2<sup>-/-</sup> (Old CCL2), 6-month-old Ccr2<sup>-/-</sup> (Young CCR2), and 18-month-old Ccr2<sup>-/-</sup> (Old CCR2) mice were analyzed by antibody against mouse IgG. A 23 kD reactive fragment corresponding to IgG light chain was identified. h, Ccl2 release at 24 h from C5a-

stimulated RPE cells and IgG-stimulated choroidal endothelial cells (CEC). i, C5a and IgG upregulate RPE secretion of VEGF at 8 h. Asterisks  $P < 0.05$ .

FIG. 5. Ccl2 overexpression and macrophage infiltration in aged wild-type mice. Ccl2 fluorescence (blue) is not observed in 4-month-old wild-type (a) but marked immunoreactivity is present in RPE and choroid of 12-month-old wild-type mouse (b). Cluster of F4/80 positive (blue) macrophages in choroid of 12-month-old wild-type (c) but not in 16-month-old  $Ccl2^{-/-}$  mouse (d). Scale bar 150  $\mu\text{m}$  (a,b) and 15  $\mu\text{m}$  (c,d). e, Percentage of choroidal cells expressing F4/80 (macrophages) in young (3-month-old; white bars) and old (12-month-old; black bars) wild-type and knockout mice.  $n=4$ . Asterisk  $P < 0.01$ . f, Western blot. RPE and choroid lysates from 6-month-old wild-type (Young WT), 18-month-old wild-type (Old WT), 6-month-old  $Ccl2^{-/-}$  (Young CCL2), 16-month-old  $Ccl2^{-/-}$  (Old CCL2), 6-month-old  $Ccr2^{-/-}$  (Young CCR2), and 18-month-old  $Ccr2^{-/-}$  (Old CCR2) mice were analyzed by antibody against mouse C5aR. A 50 kD reactive fragment corresponding to a reduced C5a receptor fragment was identified.

FIG. 6. Macrophages are immobilized by, adhere to, and degrade C5 and IgG. a, Migration of wild-type peritoneal macrophages, toward Ccl2, across membranes coated with CIV and BSA, C5a, or IgG. \*  $P < 0.05$ , #  $P < 0.01$  compared with BSA.  $n=3$ . b, Adhesion of wild-type peritoneal macrophages to slides coated with CIV and C5a or IgG. \*  $P < 0.05$ , #  $P < 0.01$  compared to BSA.  $n=3$ . c,d, Choroidal macrophages of 12-month-old wild-type mice clear C5 and IgG in situ. Quantitation shows significantly less C5 (c) and IgG (d) immunoreactivity in sections from 12-14-month-old knockout mice incubated with macrophages (M $\phi$ ) compared with sections without macrophages. \*  $P < 0.05$ , #  $P < 0.01$ .  $n=4-7$ . e-g, Confocal images from 12-month-old  $Ccr2^{-/-}$  mouse eye section incubated with wild-type choroidal macrophages for 2 h. An F4/80 positive (blue) macrophage adheres to the section (e). IgG-immunoreactive material (red) (f) seems closely associated with and engulfed by macrophage in the merged image (g). Scale bar 15  $\mu\text{m}$ .

FIG. 7A-D) is the nucleotide sequence of the human Ccl2 gene (variants, promoter, and enhancer regions) (SEQ ID NO. 1-4).

FIG. 8A-D is the nucleotide sequence of the human Ccr2 gene (variants, isoforms, promoter regions) (SEQ ID NO. 5-8)

FIG. 9 is the nucleotide sequence of the human C5 receptor gene (SEQ ID NO. 9).

FIG. 10 is the nucleotide sequence of the human C5a receptor gene (SEQ ID NO. 10).

#### DETAILED DESCRIPTION OF THE INVENTION

The inventors have discovered two strains of genetically modified mice that develop many features of AMD as they age. Elderly mice (9-24 months) deficient in the gene for monocyte chemoattractant protein-1 (Ccl2, formerly referred to as MCP-1) (Lu, B et al., (1998) *J Exp Med* 187, 601-8) or its cognate receptor CC chemokine receptor-2 (Ccr2) (Kuziel, et al., (1997) *Proc Natl Acad Sci USA* 94, 12053-8.) develop drusen, lipofuscin, and thickened Bruch's membrane (the extracellular matrix between the RPE and choroid), the earliest manifestations of AMD in humans, as well as intrachoroidal neovascularization. They also develop degeneration of the outer neural retina, which is seen in many patients with AMD (Green, W. R. & Enger, C. (1993) *Ophthalmology* 100, 1519-35). These pathologies are absent in age-matched wild-type mice and several other knockout strains of mice.

The present inventors have discovered that the development of drusen is more pronounced in the Ccl2 mice in comparison to the Ccr2 mice. Also, the accumulation of drusen occurs earlier in the Ccl2 mice. However,  $Ccr2^{-/-}$  mice also display evidence of drusen on fundus examination (FIG. 1). Just as Ccl2 deficient mice, Ccr2-deficient mice also exhibit phenotypic variation: some have the discrete hard drusen, while others have confluent drusen.

The subretinal deposits observed in the Ccl2 and Ccr2 mice have ophthalmoscopic and angiographic (FIG. 1) characteristics similar to drusen in AMD. Some deposits are discrete while others are confluent like hard or soft drusen, respectively, in patients with AMD (FIG. 1). The deposits are histologically similar to the human counterpart and absent in wild-type mice (FIG. 1). Bruch's membrane is visibly thickened in the knockout mice as in AMD. The choroid is markedly hypervascular and thickened, resembling the histologic appearance of intrachoroidal neovascularization (FIG. 3a-c). The outer nuclear layer of the neural retina is markedly attenuated, and photoreceptor inner & outer segments are nearly absent in many regions of the retina (FIG. 2), as seen in human AMD in regions of RPE cells compromised by drusen.

RPE cells of the knockout mice are engorged with lipofuscin (FIG. 1g), autofluorescent lysosomal storage bodies abundant in patients with AMD. Basal membranogranular deposits, the earliest pathological changes in AMD (Green et al., (1993) *Ophthalmology* 100, 1519-35; and Green, et al., (1977) *Trans Am Ophthalmol Soc* 75, 180-254), are seen in  $Ccl2^{-/-}$  mice (FIG. 1). Bruch's membrane was markedly thickened and internally fragmented in these mice, with disruption of the collagen and elastin layers (FIG. 4d). The average thickness of Bruch's membrane in nine month-old knockout mice (1.8  $\mu\text{m}$ ) is significantly higher than in wild-type mice at the same age (0.45  $\mu\text{m}$ ). By comparison, in humans with AMD, the average thickness of Bruch's membrane is approximately 3  $\mu\text{m}$  (Ramrattan, et al., (1994) *Invest Ophthalmol Vis Sci* 35, 2857-64). Lipofuscin granules, autofluorescent lysosomal residual bodies that accumulate with age in RPE cells of human, have been implicated in AMD development (Delori et al., 2000) and are found in  $Ccl2^{-/-}$  mice in an age dependent fashion, as is A2E, the principal fluorophore of lipofuscin (FIG. 1h).

Choroidal neovascularization (CNV) is observed in Ccl2 mice. FIG. 3 shows leakage due to CNV as captured by indocyanine angiography. FIG. 3a-c are transmission electron micrographs of CNV that depicts breaks in Bruch's membrane with choroidal endothelium injecting processes through these breaks. This pathology, which is identical to the earliest event in the development of CNV in human patients with AMD, has not previously been described in a spontaneous model.

Examination of human drusen revealed the presence of C5a within the deposits. It was also found that recombinant complement 5a up-regulates the secretion of Ccl2 in human R1E cells (FIG. 4h). This may explain the presence of subretinal deposits in Ccl2 and Ccr2 deficient mice, which cannot recruit macrophages, which are thought to aid drusen clearance (Duvall and Tso, 1985). This provides a mechanistic link between drusen and macrophage recruitment, and suggests a causal link between the gene defects and the presence of drusen in these knockout mice.

The totality of the data suggests that macrophages play a critical role in drusen resorption, which is impaired in the absence of Ccl2 or its receptor Ccr2. The presence of both drusen and CNV (the respective key findings of both types (non-exudative and exudative) of macular degeneration) in these mice at an age similar to human (adjusted for species

longevity) makes this an attractive model for investigating AMD and the role of senescence. This model not only provides evidence for a macrophage role in drusen clearance, but also provides a powerful platform to study the molecular etiology of AMD and the effect of candidate drugs or treatments on the development or progression of AMD.

Current animal models of CNV (the neovascular form of AMD that accounts for over 80% of visual loss in patients with AMD) relying on laser injury to fracture Bruch's membrane or viral transfection of VEGF into RPE cells, although useful for experimental study, are poor facsimiles of the human condition. Thus, particularly remarkable was the identification of CNV with frank evidence of angiographic leakage in 4 of 15 Ccl2<sup>-/-</sup> and 3 of 13 Ccr2<sup>-/-</sup> mice older than 18 months, and in none of 16 age-matched wild-type mice. This frequency of conversion to the neovascular stage is comparable to the rate of progression from drusen to CNV in humans with AMD1. At earlier stages (15-19 months), CNV had breached the outer, but not inner, aspect of Bruch's membrane (intrachoroidal neovascularization), showing angiographic leakage of indocyanine green but not fluorescein (FIG. 3a-c, i-l). This nascent angiogenesis later (18-27 months) completely breached Bruch's membrane, causing RPE and photoreceptor disruption due to the accumulation of subretinal fluid leakage from these immature vessels, which was visible on fluorescein angiography (FIG. 3d-f, m, n). It is shown in FIG. 3 that VEGF was overexpressed in the RPE in senescent Ccl2 or Ccr2 deficient, but not age-matched wild-type, mice (FIG. 3g,h), consistent with its putative role as the angiogen driving CNV.

Recent evidence suggests that complement activation and immune complex deposition occur in eyes of humans with AMD. (Mullins, et al., FASEB J 14, 835-846 (2000); Johnson, et al., Exp Eye Res 70, 441-449 (2000); and Anderson et al., Am J Ophthalmol 134, 411-431 (2002). The deposition of many of these proteins in aging Ccl2<sup>-/-</sup> and Ccr2<sup>-/-</sup> mice was observed in the present studies. Complement component C5 (FIG. 4a), immunoglobulin G (IgG) (FIG. 4b,c,g), the complement regulatory proteins vitronectin (Vn) and CD46 (membrane cofactor protein) (FIG. 4d,e), serum amyloid P component (SAP), a potential activator of the complement cascade (FIG. 4f), and advanced glycation endproducts (AGE) (data not shown) were present in RPE or choroid of both strains of knockout mice, but not age-matched wild-types, similar to their distribution in eyes with AMD. Colocalization of IgG and C3c in choroidal vessel walls (FIG. 4c) not only suggests the presence of immune complexes, but also reflects ongoing immune deposit formation because C3c, a split-product of surface bound C3b, is cleared within hours. The joint presence of CD46, a membrane-bound regulator that facilitates inactivation of the activated complement components C3b/C4b, and vitronectin, a fluid-phase regulator that binds to the terminal complement complex to regulate complement-mediated lysis, along with localization of complement intermediates suggests that complement activation occurs to completion. These deposits were identified in 6 of 7 Ccl2<sup>-/-</sup> and 4 of 6 Ccr2<sup>-/-</sup> mice as young as 6 months of age, predating the changes visible on fundus examination, consistent with a potential causal role. Such deposits were not identified in wild-type mice.

In other immune complex deposition disorders, it has been postulated that these proteins serve as an inflammatory nidus by inciting macrophage recruitment through Fc and complement receptor binding, triggering humoral activation and phagocytosis. Consistent with this hypothesis, it is shown herein that Ccl2 secretion by human RPE and choroidal endothelial cells (CEC) was upregulated by C5a (the acti-

vated form of C5) and IgG, respectively (FIG. 4h). AGE also stimulates human RPE cell secretion of Ccl2 (ref. 27).

These data may explain the presence of subretinal deposits in Ccl2 and Ccr2 deficient mice which are impaired in recruiting macrophages requisite for clearance and degradation of drusen and other debris. Consistent with this hypothesis, there was an age-dependent increase in the expression of Ccl2 in the RPE (FIG. 5a,b), and in macrophage infiltration in the choroid of wild-type mice (FIG. 5c-e). Using flow cytometry, we found that aging was associated with a marked increase (15-fold) in the number of macrophages in the choroid of wild-types compared with only a modest (2-3 fold) increase in knockout mice (FIG. 5e). These data suggest that macrophage recruitment in aged wild-type mice is principally directed along the Ccl2-Ccr2 axis. Along with overexpression of C5 in the RPE and choroid of Ccl2<sup>-/-</sup> and Ccr2<sup>-/-</sup> mice, marked upregulation of the C5a receptor (C5aR) in both strains of knockout mice starting at an early age, and in wild-type mice at a later age was observed (FIG. 5f). These findings suggest that in the wild-type animal ongoing stimulation by C5a, which upregulates C5aR expression, leads to Ccl2 production and subsequent clearance of C5 and molecules tagged by this opsonin. The inability to summon sufficient numbers of or appropriately stimulated macrophages in knockout mice however, would lead to continued C5 deposition.

Both C5a and IgG stimulated human RPE cells to increase their secretion of the potent angiogenic cytokine vascular endothelial growth factor (VEGF) (FIG. 4i), which is consistent with RPEJ overexpression of VEGF in senescent Ccl2 or Ccr2 deficient mice (FIG. 3h). AGE also upregulates human RPE and CEC secretion of VEGF. Together these processes may underlie the development of CNV and highly fenestrated choroidal capillaries (FIG. 1c, 3c), both of which can be induced by VEGF in these mice.

Cell culture inserts were used to examine the migration of macrophages across a porous membrane coated with collagen IV (CIV, an abundant constituent of Bruch's membrane) in response to Ccl2. The migration of macrophages across this CIV-coated membrane when simultaneously coated with C5a or IgG was then tested to determine whether macrophages recruited to these protein-deposition sites by locally secreted Ccl2 are immobilized when they contact these proteins in the extracellular matrix. It was found that Ccl2-induced macrophage chemotaxis was inhibited both by C5a and IgG (FIG. 6a). Such immobilization indicates that macrophages adhere to C5a or IgG coated surfaces. Using CIV-coated multi-spot slides coated with C5a or IgG, it was shown that macrophages adhere to these proteins in a dose-dependent fashion (FIG. 6b). Collectively these data suggest that macrophages recruited by Ccl2 become immobilized when they contact C5a or IgG and associate with them in the extracellular matrix.

Because macrophages were immobilized by and adhered to C5 and IgG in vitro, and aging was associated with macrophage infiltration into the choroid of wild-type mice, it is possible that these cells scavenge immune complexes identified in the eyes of Ccl2<sup>-/-</sup> or Ccr2<sup>-/-</sup> mice. To test this hypothesis, macrophages were purified from aged wild-type choroids by magnetic cell sorting and plated on unfixed eye sections from Ccl2<sup>-/-</sup> or Ccr2<sup>-/-</sup> mice which were rich in C5 and IgG deposits in their RPE and choroids. Incubation with wild-type macrophages for 24 hours markedly reduced the total RPE/choroidal area occupied by C5 or IgG, compared with untreated sections (FIGS. 6c,d). Within 2 hours, macrophages were spread out over the tissue and intimately associated with protein deposits (FIG. 6e-g). These results indicate that macrophages clear C5 and IgG deposits in silu and

assign a pivotal role for macrophage deficiency in the accumulation of complement components and immunoglobulins in *Ccl2*<sup>-/-</sup> or *Ccr2*<sup>-/-</sup> mice.

This present invention provides the first animal model of AMD that recapitulates the key elements of the human condition in senescent mice lacking the macrophage chemoattractant *Ccl2* or its cognate receptor *Ccr2*. The presence of similar pathology in two ligand/receptor strains that are defective in induced macrophage trafficking strengthens the hypothesis that macrophage dysfunction plays a role in its pathogenesis. The accumulation of several complement components, complement regulatory proteins, and IgG in these mutant mice, as in humans with AMD, suggests that impaired macrophage recruitment allows accretion of proteins associated with complement activation and immune complex deposition. Inability to summon macrophages is thus associated with senescence-associated development of features strongly reminiscent of human AMD, corroborated by several lines of evidence. In particular the present inventors have shown that *Ccl2*-driven macrophages are immobilized by and adhere to C5a and IgG *in vitro*, and that macrophages degrade these proteins *in situ*. Combined with the observation of a marked deficiency of macrophages in the choroids of aged knockout mice, these data suggest that impaired macrophage mobilization *in vivo* leads to non-clearing of these proteins since these cells are known to scavenge immune complexes via complement opsonization *in vivo*.

Since deposition of complement-related proteins and IgG precedes the development of drusen and lipofuscin, it is likely that AMD-like pathology is due, at least in part, to complement activation and immune complex deposition rather than the converse. Because RPE cells in eyes with AMD that are immunoreactive for complement-related proteins and IgG exhibit anatomic prelethal signs it has been suggested that accumulation of these proteins compromises RPE function. The presence of IgG along with complement C3 and C5 intermediates is strongly suggestive of the presence of immune complexes, and is consistent with the presence of circulating retinal auto-antibodies in patients with AMD. Furthermore, patients with membranoproliferative glomerulonephritis, in which complement activation and immune complex deposition cause glomerular injury, develop drusen resembling AMD-associated drusen in ultrastructure and composition, including C5 and IgG deposition, as well as CNV. Collectively these findings support the concept that complement activation and immune complex deposition may injure the RPE in AMD. RPE injury, which may be manifested by secondary photoreceptor degradation, also can be triggered by excessive accumulation of lipofuscin. SAP and TIMP-3 also may impair drusen clearance by functioning as protease inhibitors. RPE overexpression of VEGF stimulated by complement components and IgG combined with fragmentation of Bruch's membrane provides an environment permissive for CNV.

The presence of both atrophic and neovascular pathologies in *Ccl2*<sup>-/-</sup> or *Ccr2*<sup>-/-</sup> mice at an age corresponding to human senescence makes these mice attractive models for investigating both early and late AMD. Because mouse retina does not contain a specialized macula, this model is not an exact replica of the human condition. However, the pathology in human AMD, while pronounced in the macular area, is not confined to this central region, and the findings observed in aged *Ccl2*<sup>-/-</sup> or *Ccr2*<sup>-/-</sup> mice closely resemble those of the clinical condition in anatomical appearance, biochemical composition, and functional disruption. More importantly, they define a system for molecular dissection of the determi-

nants of AMD pathogenesis, and provide a platform to develop and validate novel therapeutic strategies and test compounds

- Ccl2*<sup>-/-</sup> *Ccr2*<sup>-/-</sup> mice and dual knockout mice, *Ccl2*<sup>-/-</sup>/*Ccr2*<sup>-/-</sup> mice may be used to characterize the temporal development of AMD, preferably from ages of about 9 to about 24 months by ophthalmoscopy, angiography, and histopathology, for example, as compared to wild-type age-matched mice. In characterizing the development of AMD the eyes of these mice are systematically examined at various ages, such as for example, at 1, 3, 6, 9, 12, 18, and 24 months to characterize the temporal development of the retinal and subretinal pathology. For example, the eyes of the mice may be examined by:
1. Clinical Retinal Evaluation—examination & fundus photography through dilated pupil, e.g., 50 degree fundus photography to quantify yellow spots (drusen);
  2. Fluorescein angiography—Staining or leakage within the eye may be identified;
  3. Histology Paraffin embedded and frozen sections of affected eyes may be studied for morphology and biochemical composition (lipid, cholesterol, lipofuscin);
  4. Immunohistochemistry Drusen (C5a, C5b-9, ApoE, vitronectin, clusterin staining for human correlation); Proliferating cell nuclear antigen (PCNA)+CD31 (proliferating choroidal endothelium); and/or
  5. Electron Microscopy—Morphology and morphometry of various structures, e.g., photoreceptors, RPE, Bruch's membrane (integrity and thickness), choroidal vasculature may be examined.

In one aspect of the invention, the *Ccl2*, *Ccr2* and/or *Ccl2*/*Ccr2* (dual knockout) knockout mice may be used to test candidate drugs for treatment of AMD. Dual knockout mice are created by a series of genetic backcrosses using the cross-backcross-intercross scheme, which is well known in the art. *Ccr2*<sup>-/-</sup> mice are mated with *Ccl2*<sup>-/-</sup> mice to yield heterozygous F1 offspring. The F1 mice are intercrossed and the progeny screened by PCR, for example, for *Ccr2* and *Ccl2*. B1 progeny, heterozygous for *Ccr2* and *Ccl2* are intercrossed, and mice homozygous for both disrupted genes are selected for example, by PCR typing for continued backcrossing. Mice are genotyped by any method, such as by analyzing tail DNA samples using Southern blot strategies or by PCR analysis with multiprimer sets that amplify in the disrupted gene, tansgene insert or neomycin resistance gene insert.

Candidate drugs include pharmaceutical compounds, small molecules, peptides, antibodies, antibody fragments and nucleic acids, including oligonucleotides and polynucleotides in sense or antisense orientation and aptamers. In this aspect of the invention the candidate drug is administered to the mouse orally, systemically, e.g., intravenously, intraperitoneally, intravitreally (e.g., by injection or sustained delivery implant), transsclerally or topically, and preferably by topical application to at least one eye of a test group of *Ccl2* mice, *Ccr2* mice, dual knockout mice or all three types of mutant mice, and the eye(s) of the treated mice are periodically examined to determine the effect of the candidate drug on drusen accumulation, lipofuscin accumulation, Bruch's membrane or any other symptomatic marker of AMD. A decrease in drusen or lipofuscin accumulation or thinning of Bruch's membrane, an affect on retinal degeneration or choroidal neovascularization, for example, is an indication of the ability of the candidate drug to effectively treat AMD.

In one embodiment of the invention, the genetic defect is treated by introducing a wild-type gene *Ccl2* or *Ccr2* gene into the mouse. Chemotactic deficiency in *Ccl2*<sup>-/-</sup> mice may be reversed by delivering a recombinant vector, such as for

example an adeno-associated virus (rAAV) vector expressing the cDNA for Ccl2. Although Ccl2 can be delivered via an osmotic pump, rAAV vector administration is not only as effective as systemic administration, but also confines production and secretion of Ccl2, and is likely to restrict chemotactic activity to the eye. Reconstituting Ccl2 function via AAV transduction is also superior to systemic delivery as the former permits intra-animal inter-eye comparisons, thus providing greater statistical and biological fidelity to the hypothesis testing. Also rAAV vectors have demonstrated long-term, sustained high-level expression in the retina for two years, eliminating the need for pump replacement.

Similarly, the Ccr2 defect may be treated by administering a vector encoding wild-type Ccr2 gene to determine whether rescue of Ccr2 function prevents or causes regression of AMD in Ccr2 mice or dual knockout mice. Alternatively the Ccr2 defect may be corrected by stem cell transplantation of cells from Ccr2+/+ animals, either by adoptive transfer or following bone marrow ablation. Similarly, the Ccl2 defect may be corrected by stem cell transplantation of cells from Ccl2+/+ animals, either by adoptive transfer or following bone marrow ablation, for example.

The rAAV-vector cassette preferably includes a promoter, such as for example a chicken  $\beta$ -actin (CBA) promoter, which preferably is composed of an enhancer element or elements, such as a cytomegalovirus (CMV) immediate-early enhancer (381 bp) and a CBA promoter-exon1-intron1 element (1,352 bp) upstream of a simian virus 40 early splice donor/splice-acceptor site, the Ccl2, gene, or both and a polyadenylation sequence, preferably the simian virus 40 polyadenylation sequence. The entire expression cassette containing the Ccl2 cDNA or Ccr2 cDNA is preferably flanked by AAV2 terminal repeats required for viral packaging. Viral vectors are packaged and purified as described (Raisler, B. J., Berns, K. I., Grant, M. B., Beliaev, D. & Hauswirth, W. W. (2002) *Proc Natl Acad Sci USA* 99, 8909-14). The CBA promoter is preferably used as it supports expression well in both RPE cells and photoreceptors (Acland et al. (2001) *Nat Genet* 28, 92-5).

Efficacy of transduction by the rAAV-CBA-Ccl2, -Ccr2 or vector encoding both Ccl2 and Ccr2 may be confirmed by any method including any combination of the following:

1. In vitro expression: RPE cells harvested and cultured from eyes of wild-type and Ccl2 -/- mice may be probed by PCR amplification for the presence or absence of the wild-type Ccl2 transgene or Ccr2 transgene, respectively. Wild-type RPE cells and mutant RPE cells transfected with rAAV-CBP-Ccl2, -Ccr2 or vector encoding both Ccl2 and CCR2 may be subjected to PCR amplification, and optionally ELISA of the supernatant for expression of Ccl2, which is constitutively secreted (Elner, et al., (1997) *Exp Eye Res* 65, 781-9).
2. In vivo expression: The amount of ocular protein in mice expressed from the vector construct may be assayed after subretinal vector inoculation by ELISA about six weeks after injection. Approximately  $10^{10}$  particles ( $2 \times 10^8$  infectious units) in a volume of 1  $\mu$ l of therapeutic vector is injected into one eye and the same volume of null vector in the fellow eye.
3. AAV-CBA-Ccl2, -Ccr2 or both Ccl2 and Ccr2 is injected into eyes of Ccl2 deficient mice, preferably about eight-week-old Ccl2 deficient, Ccr2-deficient mice, or dual knockout mice, and the temporal development of retinal and subretinal lesions is compared to fellow eyes injected with null vector over 24 months with interval measurements. In addition a vector such as AAV-CBA-Ccl2, AAV-CBA-Ccr2 or both or a single vector encoding both Ccl2 and Ccr2 may be injected into eyes of one-year-old Ccl2

deficient mice, one year old Ccr2 deficient mice or dual knockout mice, and the stabilization or regression of ocular lesions evaluated in comparison to fellow eyes.

In addition Ccl2 and Ccr2 function can be reconstituted by bone marrow transplantation from Ccl2 +/+ or Ccr2 -/+ mice.

In another aspect of the invention, there is provided a double knockout mouse which has both the Ccl2 and Ccr2 deletions. The mouse may be generated as described above, or by any method known to the skilled practitioner. The mouse is useful for determining the pathology of age-related macular degeneration and testing candidate drugs for treatment of age-related macular degeneration.

It is also contemplated that the genes, vectors and expression vectors of the invention may be used for stem cell transplantation to restore Ccr2 function. For example, stem cells obtained from a normal mouse, i.e., containing a wild type Ccr2 gene, may be introduced either by adoptive transfer or following bone marrow ablation. For example, the normal stem cells may be introduced by intravenous injection into a Ccr2-/- mouse or other animal. The eyes of the animal receiving the stem cell transplant are then observed to determine the effect of the transplantation. Alternatively, a Ccr2-/- mouse or other animal can be subjected to bone marrow irradiation to deplete stem cells. Following ablation of the endogenous stem cells, stem cells obtained from a wild type mouse are administered to the irradiated Ccr2-/- mouse, preferably by intravenous injection. The eyes of the transplanted mouse are then observed to determine the effect of the transplantation. Similar procedures can be employed to restore Ccl2 function in a Ccl2-/- mouse or other animal.

It is also contemplated that AMD can be treated or prevented in mammals, including humans, by administering to a patient in need, a wild type Ccr2 gene, wild type Ccl2 gene or both to compensate for a defective Ccr2 gene or Ccl2 gene or both. The wild type gene can be administered by any method known in the art, such as by administering the gene(s) via an expression vector, such as a replication defective adenovirus vector, directly into the eye, via an implant or via intravenous injection. Alternatively, the wild type gene can be introduced into the eye via stem cell transplantation as described above.

It is further contemplated that wild type Ccl2 and/or Ccr2 genes or small molecules that promote the function of Ccl2 and/or Ccr2 are used for the manufacture of a medicament for the treatment or prevention of AMD in a mammal.

It is further contemplated that the genes, vectors and expression vectors, including the promoter/enhancer regions of the genes for Ccl2 and/or Ccr2 may be used in identifying mutations or polymorphisms that place people at increased or decreased risk for developing AMD. The human Ccl2 gene, its promoter and enhancer (SEQ ID NO. 1-4) and human Ccr2 gene and its promoter (SEQ ID NO. 5-8) are shown in FIGS. 7A-D and 8A-D, respectively. These sequences can be used to isolate the Ccr2 and/or Ccl2 gene from genomic DNA obtained from patients suspected of having or believed to be at risk of developing age-related macular degeneration. Also, the wild type Ccl2 and/or Ccr2 sequences or fragments thereof can be used directly or oligonucleotides based on these sequences can be generated and used to screen genomic or cDNA AMD libraries using any method known in the art. Generally, high stringency conditions are used in the screening process. Methods for screening genomic DNA and gene libraries and selection of stringency conditions are well known to those of skill in the art. See, e.g. Maniatis et al., *Molecular Cloning A laboratory Manual*. The isolated genes or gene fragments can then be sequenced to determine the presence of mutations in the isolated DNA. Once specific

AMD mutations or polymorphisms are identified, these mutations can be used to screen patients for the presence of the mutation:

Applicants' studies have shown that C5 and C5a accumulate in the eyes of the Ccl2<sup>-/-</sup> and Ccr2<sup>-/-</sup> mice with aging, and that the inability of macrophages to clear these deposits leads to macular degeneration-like changes in the mice. Thus, defects in the C5 receptor and C5a receptor genes may promote macular degeneration. Therefore, an analysis of the C5 receptor gene and C5a receptor genes in AMD patients for the presence or absence of mutations or polymorphisms will confirm the role of these genes in the development of AMD. The sequence of each of the human C5 receptor and C5a receptor genes is shown in SEQ ID NO.9 and 10, respectively. As discussed above for the Ccl2 and Ccr2 genes, the wild type C5 receptor and C5a receptor genes may be used to screen AMD libraries or genomic DNA obtained from AMD patients for the C5 receptor and C5a receptor genes therein and the genes so isolated can be characterized, by nucleotide sequencing to determine the presence or absence of mutations or polymorphisms, for example. Also, the C5 receptor and C5a receptor genes may be cloned into an appropriate expression vector or expression vector and further characterized.

### EXAMPLES

Animals: Wild-type C57BL/6 mice (Jackson Laboratories), and Ccl2<sup>-/-</sup> and Ccr2<sup>-/-</sup> strains, generated as described previously (Lu, et al., J Exp Med 187, 601-608 (1998); Kuziel, et al, Proc Natl Acad Sci USA 94, 12053-12058 (1997)) (incorporated herein by reference) and backcrossed 10 times to C57BL/6, were anesthetized by intramuscular injection of ketamine (50 mg/kg) and xylazine (10 mg/kg).

Fundus photography and angiography: Photographs and angiograms performed after intraperitoneal injection of fluorescein sodium (Akorn; 60 mg/kg) or indocyanine green (Sigma-Aldrich; 6 mg/kg) were captured with a TRC-501A camera (Topcon) and evaluated by two masked readers.

Immunohistochemistry and electron microscopy: Frozen sections fixed in Histochoice MB (Amresco) and blocked with 5% donkey serum (Jackson Immunoresearch) were stained with rabbit anti-mouse C3c (1:1000, gift of J. D. Lambris, University of Pennsylvania, Philadelphia, Pa.), mouse anti-mouse C5 (1:1000; gift of J. D. Lambris), rabbit anti-human CD46 (1:500; Santa Cruz Biotechnologies), goat anti-mouse MCP-1 (15 micro g/ml; R&D Systems), goat anti-human SAP (1:500; Santa Cruz), rabbit anti-mouse TIMP-3 (1:2500; gift of B. H. F. Weber, University of Wuerzburg, Wuerzburg, Germany), goat anti-mouse VEGF (15 micro g/ml; R&D Systems), rabbit polyclonal anti-AGE antibodies (1:1000, gift of A. Gugliucci, Touro University, Vallejo, Calif.), or goat anti-human vitronectin (1:500; Santa Cruz). Bound antibodies were detected with Cy3-conjugated goat secondaries or Cy5-conjugated donkey secondaries (1:100; Jackson Immunoresearch). Alternatively sections were stained directly with FITC-conjugated goat anti-mouse IgG (1:100; BD Pharmingen), Cy5-conjugated donkey anti-mouse IgG (1:00; Jackson Immunoresearch) or Cy5-conjugated F4/80 (5 micro g/ml; Serotec). A "mouse-on-mouse" kit (Vector Laboratories) was used for C5 staining. Lipofuscin autofluorescence was detected through the Cy3 channel: Transmission electron microscopic studies were performed on uranyl acetate/lead citrate-stained ultrathin sections. Bruch's membrane thicknesses were measured 150 micro m from the optic nerve by averaging thinnest and thickest parts. Western blotting: Equal amounts of total protein from RPE/choroid were resolved in SDS 4-20% polyacrylamide gradi-

ent gel and transferred to nitrocellulose membranes for western blotting with antibodies against mouse C5aR (gift of J. D. Lambris) or mouse IgG (Transduction Laboratories).

Flow cytometry: Single cell suspensions of RPE/choroids were incubated in Fc block (0.5 mg/ml BD Pharmingen) for 15 min on ice, stained with Cy5-F4/80 antibody (1:30), and live cells were detected by gating on forward versus side scatter, followed by analysis of F4/80 in the fluorescence channel (FACScalibur, BD Biosciences).

Migration: Wild-type peritoneal macrophage migration (10,000 cells/well) toward 30 nM of mouse Ccl2 (R&D Systems) was assayed using 24-well transwell chambers (Corning) separated by a 5 micrometer polycarbonate filter coated with 50 micro g/ml collagen IV (CIV; Fluka), with or without overlay of human C5a (50 nM; Calbiochem), mouse IgG (50 micro g/well; Jackson Immunoresearch), or bovine serum albumin (BSA; 50 micro g/well; Sigma-Aldrich), by counting numbers of migrated cells after 3 hours incubation at 37 degrees C.

Adherence: Adherence of wild-type peritoneal macrophages (105 cells/spot) plated on multispot glass slides (Shandon) coated with 50 micro g/ml CIV overlaid with human C5a, mouse IgG, or BSA (0-8 micro g/spot) was quantitated using CyQuantGR (Molecular Probes) after incubation at 37 degrees C. for 1 h.

Degradation: Frozen unfixed eye sections from knockout mice were transferred to 24-well culture plates and incubated with or without wild-type (12-month-old) choroidal macrophages (10,000 cells/well), purified via magnetic cell sorting using MicroBeads conjugated with CD 11b antibody (clone M1/70.15.11.5; Miltenyi Biotec), for up to 24 h at 37 degrees C. Sections were fixed with Histochoice MB, stained for C5, IgG, or F4/80, and imaged by scanning confocal microscopy. Relative areas of C5 or IgG immunoreactivity were measured for 4-7 sections using image-analysis software (Photoshop, ver. 6.0 Adobe Systems).

Cell stimulation: Serum starved human CEC (gift of D. R. Hinton, University of Southern California, Los Angeles, Calif.) and human RPE cells were stimulated with human C5a (50 ng/ml) or immobilized human IgG (50 micro g/well; Sigma-Aldrich) after attaining 80% confluence. Ccl2 and VEGF levels measured by ELISA (R&D Systems) at 8 and 24 h after stimulation were normalized to total protein.

MALDI-TOF mass spectrometry: RPE extracts and standards of synthetic N-retinylidene-N-retinylethanolamine (A2E; gifts of E. Rodriguez-Boulan, New York University, N.Y. New York and G. H. Travis, University of California, Los Angeles, Calif.) were dissolved in 50% methanol/50% water (Fisher Scientific), transferred to C18 PrepSep solid phase extraction columns (Fisher), and eluted with 1 ml methanol containing 0.1% trifluoroacetic acid (TFA; Fisher). N-perfluoroalkyl pyridine (NPP; gift of S. Rankin, University of Kentucky, Lexington, Ky.; 250 ng) was added to samples as an external standard. The MALDI target was prepared by adding 0.5 micro l sample to deposited 0.5 micro l matrix (alpha-cyano-4-hydroxycinnamic acid; Sigma-Aldrich). Positive ion spectra were acquired on a Bruker Autoflex MALDI-TOF mass spectrometer (Bruker Daltonic). The A2E response (m/z 592.5) was normalized to the NPP response (m/z 576.1).

Statistics: Data are represented as the mean  $\pm$  s.e.m. of at least 3 independent experiments and were compared using a two-tailed Student's t-test. The null hypothesis was rejected at P<0.05.

### Example 1

Eyes of greater than 60 Ccl2 and Ccr2<sup>-/-</sup> mice and 40 age-matched wild-type mice ranging from 3 to 27 months

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were subjected to fundus examination. Of these, eyes from 25 Ccl2<sup>-/-</sup>, 21 Ccr2<sup>-/-</sup> and 18 age-matched wild-type (<12 months: 6; 12-24 months: 7; >24 months: 5) mice were extensively examined histopathologically. Before 9 months of age, the fundi of Ccl2<sup>-/-</sup> and Ccr2<sup>-/-</sup> mice were indistinguishable from wild-type mice. Thereafter subretinal deposits with ophthalmoscopic and pathologic features of drusen in patients with AMD were observed in all mice of both knockout strains and increased in number with age as in humans (FIG. 1a, b). In contrast, no such changes were visible in wild-type mice even at 24 months of age (n=5). Bruch's membrane (the extracellular matrix between the RPE and choroid) was markedly thickened in senescent Ccl2 or Ccr2 deficient mice compared with age-matched wild-types and that its collagen and elastin layers were severely disrupted with internal fragmentation (FIG. 1e), features observed in AMD. As in patients with AMD, intense immunostaining of tissue inhibitor of metalloproteinases (TIMP)-3, produced by the RPE and thought to contribute to thickening of Bruch's membrane, was observed in aged knockout mice (FIG. 1e). As Ccl2<sup>-/-</sup> and Ccr2<sup>-/-</sup> mice aged, increasing amounts of lipofuscin granules (autofluorescent lysosomal residual bodies which accumulate with age in RPE cells of humans and have been implicated in AMD development) were observed in swollen and vacuolated RPE cells (FIG. 1f, g) at 9 months and thereafter. Ultrastructural analysis of these RPE cells showed significant intracellular accumulation of dense bodies (FIG. 1h) including large ellipsoid and spherical structures of high electron density, presumably representing melanosomes and melanolipofuscin fusion particles, respectively, and numerous smaller structures of variable density representing lipofuscin granules. RPE extracts were tested for the presence of N-retinylidene-N retinylethanolamine (A2E), the principal lipofuscin fluorophore by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry. RPE extracts from 12-month-old knockouts contained 25 pmol of A2E per eye (FIG. 1i). No A2E was detected in RPE of age-matched wild-type mice. Lipofuscin accumulation is thought to promote RPE dysfunction in AMD.

## Example 2

Retinal Degeneration and Choroidal Neovascularization in Ccl2<sup>-/-</sup> and Ccr2<sup>-/-</sup> mice

As Ccl2<sup>-/-</sup> and Ccr2<sup>-/-</sup> mice aged, they exhibited several of the late findings seen in human AMD, including progressive outer retinal degeneration and CNV, similar to that seen in patients with late AMD. Despite evidence of RPE and choroidal pathology, differences in neural retinal morphology between knockout strains and wild types were not observed before 16 months of age. At 16 months of age and thereafter,

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both knockout strains exhibited confluent areas of visible atrophy similar to "geographic atrophy" seen in advanced AMD (FIG. 2a). These areas were characterized by cell loss in the outer nuclear layer of the retina and atrophy of photoreceptor segments (FIG. 2b-e), as well as attenuation of the RPE and choriocapillaris (FIG. 2f) as in late AMD. In these regions the RPE was hypopigmented along with prominent vacuolization and degeneration of most intracellular organelles, and was devoid of basal infoldings. The choriocapillaris was nearly obliterated with few or no patent inner choroidal vessels observed in the areas corresponding to fundus atrophy. Regions outside these areas did not display such atrophy (FIG. 2g,h).

## Example 3

CCR2 rescue of the ocular abnormalities in Ccr2 deficient mice is accomplished by creating chimeric mice using bone marrow transplantation (BMT). In vitro AAV transduction results in loss of stem cell activity during infection, while in vivo transduction results in non-specific and low-level target expression (only 1 per 15,000 bone marrow cells are stem cells); neither approach will guarantee sustained expression in vivo. Ccr2<sup>-/-</sup> mice are irradiated and repopulated with bone marrow stem cells from wildtype Ccr2<sup>+/+</sup> mice. Ccr2<sup>-/-</sup> mice are maintained on antibiotic-containing water for one week before irradiation. These mice are irradiated with 900 cGy from a cesium source (delivered in two equal doses of 450 cGy 3-4 hours apart), and donor bone marrow cells (1×10<sup>7</sup>) are injected into a tail vein. Mice are maintained on antibiotic containing water for four weeks after transplantation. Engraftment is verified by PCR detection of the Ccr2 gene in the bone marrow of all irradiated mice. Eyes of eight-week-old chimeric mice are compared to ungrafted Ccr2<sup>-/-</sup> mice over 24 months with interval measurements. In addition, eyes of Ccr2<sup>-/-</sup> mice repopulated with bone marrow at one year of age are compared to ungrafted mice over the following year.

## Example 4

A candidate drug for the treatment of AMD is applied to one or both eyes of a Ccl2 mouse, which was previously confirmed to have developed AMD symptoms, e.g., drusen and/or lipofuscin deposits in the eye, thickening of Bruch's membrane. Treatment is repeated at least once daily for one to several weeks. Examination of the treated eye(s) by visual and/or fundus examination through dilated pupil is carried out periodically during treatment and the effect of treatment is compared to placebo treated wild-type eyes.

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&lt;210&gt; SEQ ID NO 8

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&lt;400&gt; SEQUENCE: 8

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&lt;400&gt; SEQUENCE: 9

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&lt;400&gt; SEQUENCE: 10

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What is claimed is:

1. A method for testing a candidate drug for treatment of age-related macular degeneration (AMD) comprising:

- (i) administering the candidate drug to at least one eye of a Ccl2-/-/Ccr2-/- dual knockout mouse, wherein the at least one eye exhibits at least one symptom comprising drusen accumulation, lipofuscin accumulation, thickening of Bruch's membrane, retinal degeneration, choroidal neovascularization, or a combination thereof;
- (ii) determining the effect of the candidate drug on the at least one symptom, and
- (iii) correlating the effect of the test compound on the at least one symptom with a potential utility to treat AMD.

2. The method of claim 1 wherein the candidate drug is nucleic acid.

3. The method of claim 2 wherein the candidate drug comprises a viral vector encoding wild-type Ccl2.

4. The method of claim 1 wherein the candidate drug comprises a viral vector encoding wild type Ccr2.

5. The method of claim 1 wherein step (ii) comprises analyzing the at least one eye to determine amount and type of drusen or lipofuscin accumulation, extent of retinal degeneration, or neovascularization developed therein or a combination thereof.

6. The method according to claim 1 wherein the at least one eye is analyzed by ophthalmoscopy, angiography, histopathology or a combination thereof.

7. The method of claim 1 wherein the candidate drug is administered to the mouse orally, intravenously, intraperitoneally, intravitreally, transsclerally or topically.

8. The method of claim 7 wherein the candidate drug is administered topically to at least one eye of the mouse.

9. The method of claim 1 wherein the candidate drug is a pharmaceutical compound, small molecule, peptide, antibody, antibody fragment, aptamer or nucleic acid.

10. The method of claim 9 wherein the nucleic acid is an oligonucleotide or polynucleotide in either the sense or antisense orientation or an aptamer.

11. A method of screening a candidate drug for potential utility for treatment of age-related macular degeneration, comprising:

- (a) providing a Ccl2-/-/Ccr2-/- dual knockout mouse which exhibits drusen accumulation, lipofuscin accumulation, thickening of Bruch's membrane, retinal degeneration, choroidal neovascularization, or a combination thereof in at least one eye;
- (b) administering the candidate drug to the knockout mouse;
- (c) determining the effect of the candidate drug on drusen, lipofuscin deposition, retinal degeneration, and/or choroidal neovascularization in at least one eye of the knockout mouse; and
- (d) correlating the effect of the candidate drug on drusen, lipofuscin accumulation, retinal degeneration, and/or

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choroidal neovascularization with a potential utility to treat age-related macular degeneration.

12. The method of claim 11 wherein the candidate drug is administered topically to at least one eye of the mouse.

13. The method of claim 11 wherein the candidate drug is nucleic acid.

14. The method of claim 11 wherein the candidate drug comprises a viral vector encoding wild-type Ccl2.

15. The method of claim 11 wherein the candidate drug comprises a viral vector encoding wild type Ccr2.

16. The method of claim 11 wherein step (c) comprises analyzing the at least one eye to determine amount and type of drusen or lipofuscin accumulation, extent of retinal degeneration, or neovascularization developed therein or a combination thereof.

17. The method according to claim 11 wherein the at least one eye is analyzed by ophthalmoscopy, angiography, histopathology, mass spectrometry or a combination thereof.

18. The method of claim 11 wherein the candidate drug is administered to the mouse orally, intravenously, intraperitoneally, intravitreally, transsclerally or topically.

19. The method of claim 11 wherein the candidate drug is a pharmaceutical compound, small molecule, peptide, antibody, antibody fragment, aptamer or nucleic acid.

20. The method of claim 19 wherein the nucleic acid is an oligonucleotide or polynucleotide in either the sense or antisense orientation or an aptamer.

21. A Ccl2-/-/Ccr2-/- dual knockout mouse which exhibits at least one symptom comprising drusen accumulation, lipofuscin accumulation, thickening of Bruch's membrane, retinal degeneration, choroidal neovascularization, or a combination thereof in at least one eye.

22. The method of claim 7 wherein the candidate drug is administered intravitreally by injection or by sustained delivery implant, to at least one eye of the mouse.

23. The method of claim 7 wherein the candidate drug is administered transsclerally to at least one eye of the mouse.

24. The method of claim 11 wherein candidate drug is administered intravitreally by injection or by sustained delivery implant, to at least one eye of the mouse.

25. The method of claim 11 wherein the candidate drug is administered transsclerally to at least one eye of the mouse.

26. The method of claim 18 wherein, the candidate drug is administered transsclerally to at least one eye of the mouse.

27. The method of claim 1 wherein the candidate drug comprises stem cells obtained from a wild-type mouse and intravitreally injected into the Ccl2-/-/Ccr2-/- dual knockout mouse.

28. The method of claim 11 wherein the candidate drug comprises stem cells obtained from a wild-type mouse and injected intravitreally into the Ccl2-/-/Ccr2-/- dual knockout mouse.

\* \* \* \* \*