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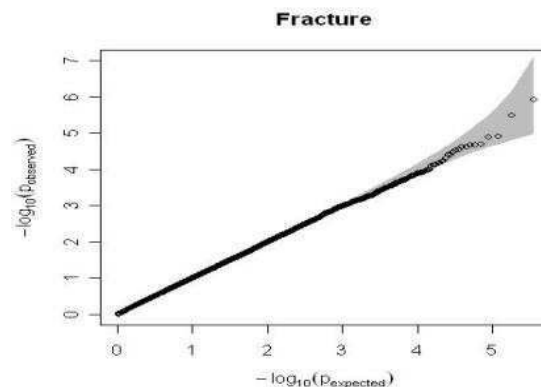
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(54) 발명의 명칭 골다공증 또는 골다공성 골절 발생 위험도 예측용 다형성 마커

(57) 요약

본 발명은 골다공증 및 골다공증으로 인한 골절 발생 위험을 효과적으로 진단 및 예측할 수 있는 신규한 다형성 마커, 예측용 조성물 및 이를 이용한 골다공성 골절 발생 위험도 예측 방법에 관한 것으로, 본 발명자들은 간편한 검체 시료 검사를 통해 골다공증 또는 골다공성 골절 발생의 위험성을 신속 정확하게 예측할 수 있는 새로운 바이오 마커를 개발하던 중, MECOM 유전자의 SNP가 골다공성 골절의 유전적 감수성을 진단할 수 있는 마커로 사용될 수 있음을 확인하고 본 발명을 완성하였다. 따라서 본 발명은 MECOM 유전자의 다형성 부위를 포함하는 폴리뉴클레오티드를 포함하는 골다공증 또는 골다공성 골절 감수성 예측용 마커 또는 예측용 조성물을 제공함으로써, 골다공성 골절 발생의 위험성을 조기에 신속하고 정확하게 진단 및 예측할 수 있는 효과가 있으며, 골다공증의 조기발견과 조기치료를 통해 환자의 치료율을 증가시킬 수 있다.

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연구과제명 골다공증 후보 유전자군의 한국인 특이 SNP 발견 및 골다공증 관련 유전자 규명(9)

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특허청구의 범위

청구항 1

골다공증 또는 골다공성 골절 발생 위험도 예측용 MECOM 유전자 다형성 마커.

청구항 2

제1항에 있어서,

상기 마커는 서열번호 1로 이루어진 폴리뉴클레오티드에서 서열번호 1의 410333번째 염기 G가 A로 치환되고, 상기 서열번호 1의 410333번째 염기를 포함하는 폴리뉴클레오티드인 것을 특징으로 하는 MECOM 유전자 다형성 마커.

청구항 3

서열번호 1로 이루어진 MECOM 유전자 내의 다형성 부위와 특이적으로 결합하는 폴리뉴클레오티드를 포함하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 조성물.

청구항 4

제3항에 있어서,

상기 폴리뉴클레오티드는 상기 다형성 부위를 증폭할 수 있는 프라이머 또는 프로브인 것을 특징으로 하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 조성물.

청구항 5

(a) 생물학적 시료로부터 게놈 DNA를 추출하는 단계;

(b) 상기 (a) 단계의 게놈 DNA 상에서 서열번호 1로 이루어진 MECOM 유전자 내의 다형성 부위의 염기서열을 검출하는 단계; 및

(c) 상기 (b) 단계에서 검출된 염기서열을 통해 골다공증 또는 골다공성 골절 발생의 고위험군 또는 저위험군을 판별하는 단계를 포함하는 골다공증 또는 골다공성 골절 발생 위험도를 예측하는 방법.

청구항 6

제5항에 있어서,

상기 (b) 단계는 시퀀싱 분석, 마이크로어레이(microarray)에 의한 혼성화, 대립유전자 특이적인 PCR(allele specific PCR), 다이내믹 대립 유전자 혼성화방법(dynamic allele-specific hybridization, DASH), PCR 연장 분석 및 TaqMan 기법으로 이루어진 군에서 선택되는 것에 의해 수행되는 것을 특징으로 하는 골다공증 또는 골다공성 골절 발생 위험도를 예측하는 방법.

청구항 7

제5항에 있어서,

상기 (b) 단계의 다형성 부위는 서열번호 1의 410333번째 염기인 것을 특징으로 하는 골다공증 또는 골다공성 골절 발생 위험도를 예측하는 방법.

청구항 8

서열번호 1로 이루어지는 MECOM 유전자 내의 다형성 부위를 증폭할 수 있는 폴리뉴클레오티드를 포함하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 키트.

청구항 9

제8항에 있어서,

상기 다형성 부위는 서열번호 1의 410333번째 염기인 것을 특징으로 하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 키트.

명세서

기술분야

[0001] 본 발명은 골다공증 및 골다공증으로 인한 골절 위험을 효과적으로 진단 및 예측할 수 있는 신규한 예측용 다형성 마커, 진단용 조성물 및 이를 이용한 골절위험 예측 방법에 관한 것이다.

배경기술

[0002] 골다공증은 낮은 골밀도(bone mineral density; 이하, BMD) 및 증가된 골절 위험성의 특징을 갖는 전신성 골질환으로, 골조성(骨組成)이 정상인 채로 골밀도가 감소하는 것을 특징으로 한다. 특히 40세 이후의 여성에게는 폐경에 따른 폐경후 골다공증이 높은 빈도로 나타났다. 또한 70세 이후에는 남녀를 불문하고 노인성 골다공증을 쉽게 볼 수 있다. 골다공증은 유전적 요소, 영양, 호르몬 및 약물 치료 등을 포함하는 다중 위험 인자의 영향을 받는데, 그 중 유전적 인자는 중요한 역할을 수행하는 것으로 알려져 있다.

[0003] 골밀도의 감소가 경미한 동안에는 눈에 띄는 임상 증상이 나타나지는 않으나, 골밀도 감소의 정도가 커지면, 골절이나 골변형(骨變形)을 일으키기 쉽다. 특히 척추는 골절이나 골변형을 일으키기 쉬워, 추체(椎體)의 압박골절이나 척추변형에 따른 요통 등의 증세가 나타난다. 더욱이 대퇴골경부(大腿骨頸部)나 골반(骨盤)의 골절은 때에 따라서 환자의 운동기능을 상실하게 하여, 이후의 생활에도 중대한 영향을 미치는 경우가 있다.

[0004] 상기와 같이 골다공증으로 인한 여러 병증이 있다 하더라도, 오늘날 골다공증으로 인한 가장 흔한 관련 병증은 바로 골절 현상이다. 골다공증에 걸린 환자들은 낮은 골밀도로 인해 약한 충격에도 쉽사리 뼈에 금이 가거나 부러지는 골절 현상을 쉽게 경험하게 되기 때문이다. 2007년 정형외과 추계학술대회에서 발표된 ‘골다공성 골절의 사회경제적 비용(백승훈, 양규현, 문성환, 공정희, 김진현)’ 논문에 의하면 골다공성 고관절 골절과 척추골절, 손목관절 골절로 인한 사회경제적 비용이 1조50억원에 이른다고 한다. 이처럼 수많은 사람들이 골다공증 및 이로 인한 골절 질환으로 고통을 겪고 있어 다각도의 해결책이 필요하다.

[0005] 현재, 골다공증의 진단은 X선 촬영 등의 물리학적 방법을 통하여 이루어지고 있지만, 이 방법은 대규모 진단장치를 필요로 하고, 또한 X선을 사용함에 따른, 안전성 등의 문제가 있을 뿐만 아니라, 골절 위험을 정확하게 예측하고 치료할 환자를 선정하는 데에 많은 한계점을 가지고 있다.

[0006] 따라서 폐경 후 골다공증이나 노인성 골다공증을 비롯하여 각종 골다공증의 발생을 조기에 예측할 뿐만 아니라 좀 더 정확하게 골다공증성 골절을 예측할 수 있는 마커의 개발이 절실하다.

발명의 내용

해결하려는 과제

[0007] 이에 본 발명자들은 간편한 검체 시료 검사를 통해 골다공증 또는 골다공성 골절 발생의 위험성을 신속 정확하게 예측할 수 있는 새로운 바이오 마커를 개발하던 중, MECOM 유전자가 골다공성 골절의 유전적 감수성을 진단할 수 있는 마커로 사용될 수 있음을 확인하고 본 발명을 완성하였다.

[0008] 따라서 본 발명의 목적은 MECOM 유전자의 다형성 부위를 포함하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 바이오 마커를 제공하는 것이다.

[0009] 또한, 본 발명의 다른 목적은 MECOM 유전자의 다형성 부위와 특이적으로 결합하는 폴리뉴클레오티드를 포함하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 조성물을 제공하는 것이다.

[0010] 또한, 본 발명의 다른 목적은 MECOM 유전자의 다형성 부위의 염기서열을 확인하는 단계를 포함하는 골다공증 또는 골다공성 골절 발생 위험도를 예측하는 방법을 제공하는 것이다.

[0011] 또한, 본 발명의 다른 목적은 상기 본 발명의 예측용 조성물을 포함하는 골절 또는 골다공성 골절 발생 위험도 예측용 키트 또는 마이크로어레이를 제공하는 것이다.

과제의 해결 수단

- [0012] 상기와 같은 본 발명의 목적을 달성하기 위해서, 본 발명은 골다공증 또는 골다공성 골절 발생 위험도 예측용 MECOM 유전자 다형성 마커를 제공한다.
- [0013] 본 발명의 일실시예에 있어서, 상기 마커는 서열번호 1로 이루어진 폴리뉴클레오티드에서 서열번호 1의 410333번째 염기 G가 A로 치환되고, 상기 서열번호 1의 410333번째 염기를 포함하는 폴리뉴클레오티드일 수 있다.
- [0014] 또한, 본 발명은 서열번호 1로 이루어진 MECOM 유전자 내의 다형성 부위와 특이적으로 결합하는 폴리뉴클레오티드를 포함하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 조성물을 제공한다.
- [0015] 본 발명의 일실시예에 있어서, 상기 폴리뉴클레오티드는 상기 다형성 부위를 증폭할 수 있는 프라이머 또는 프로브일 수 있다.
- [0016] 또한, 본 발명은 (a) 생물학적 시료로부터 게놈 DNA를 추출하는 단계; (b) 상기 (a) 단계의 게놈 DNA 상에서 서열번호 1로 이루어진 MECOM 유전자 내의 다형성 부위의 염기서열을 검출하는 단계; 및 (c) 상기 (b) 단계에서 검출된 염기서열을 통해 골다공증 또는 골다공성 골절 발생의 고위험군 또는 저위험군을 판별하는 단계를 포함하는 골다공증 또는 골다공성 골절 발생 위험도를 예측하는 방법을 제공한다.
- [0017] 본 발명의 일실시예에 있어서, 상기 생물학적 시료는 조직, 세포, 혈액, 혈청, 혈장, 타액 및 뇨로 이루어진 군 중에서 선택될 수 있다.
- [0018] 본 발명의 일실시예에 있어서, 상기 (b) 단계는 시퀀싱 분석, 마이크로어레이(microarray)에 의한 혼성화, 대립 유전자 특이적인 PCR(allele specific PCR), 다이내믹 대립 유전자 혼성화 방법(dynamic allele-specific hybridization, DASH), PCR 연장 분석 및 TaqMan 기법으로 이루어진 군에서 선택되는 것에 의해 수행될 수 있다.
- [0019] 본 발명의 일실시예에 있어서, 상기 (b) 단계의 다형성 부위는 서열번호 1의 410333번째 염기서열일 수 있다.
- [0020] 본 발명의 일실시예에 있어서, 상기 (c) 단계에서 상기 (b) 단계에서 검출된 서열번호 1의 뉴클레오티드의 410333번째 염기가 G인 경우 골다공증 또는 골다공성 골절 발생 위험도는 저위험군일 수 있으며, 염기가 A인 경우 골다공증 또는 골다공성 골절 발생 위험도는 고위험군일 수 있다.
- [0021] 또한, 본 발명은 서열번호 1로 이루어지는 MECOM 유전자 내의 다형성 부위를 증폭할 수 있는 폴리뉴클레오티드를 포함하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 키트를 제공한다.
- [0022] 본 발명의 일실시예에 있어서, 상기 다형성 부위는 서열번호 1의 410333번째 염기일 수 있다.
- [0023] 더불어, 본 발명은 서열번호 1로 이루어지는 MECOM 유전자 내의 다형성 부위를 포함하는 폴리뉴클레오티드를 포함하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 마이크로어레이를 제공한다.

발명의 효과

- [0024] 본 발명은 MECOM 유전자가 골다공증 또는 골다공성 골절과 일련의 상관관계가 있음을 발견하고, 이를 기초로 MECOM 유전자의 SNP(single nucleotide polymorphism)가 정상인과 골다공증 또는 골다공성 골절을 겪는 환자들과는 다른 양상을 보이는 것을 확인하였다. 따라서 본 발명은 MECOM 유전자의 다형성 부위를 포함하는 폴리뉴클레오티드를 골다공증 또는 골다공성 골절 발생 위험도 예측용 마커 또는 진단용 조성물을 제공하는 바, 본 발명은 골다공성 골절의 위험성을 조기에 신속하고 정확하게 진단 및 예측할 수 있는 효과가 있으며, 골다공증의 조기발견과 조기치료를 통해 환자의 치료율을 증가시킬 수 있다.

도면의 간단한 설명

- [0025] 도 1은 골다공성 골절의 Q-Q 플롯(quantile-quantile plots)을 나타낸 그래프이다.
- 도 2는 GWAS 단계에서 3번 염색체(chromosome 3)에서의 rs784288의 위치적 연관 시그널(regional association signal)을 나타낸 그래프이다.

발명을 실시하기 위한 구체적인 내용

- [0026] 본 발명은 MECOM 유전자의 다형성 부위를 포함하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 바이오 마커를 제공함에 그 특징이 있다.
- [0027] 본 발명자들은 MECOM 유전자가 골다공증 또는 골다공성 골절과 강한 상관관계가 있음을 발견하고, 이를 기초로

MECOM 유전자의 다형성을 통해 골다공증 또는 골다공성 골절이 발생할 위험성, 유전적 감수성을 예측할 수 있음을 확인하였다.

- [0028] 일반적으로 개체의 유전적 소인이 질환의 발생을 결정하는 주요 요인이라는 개념은 이미 보편화된 개념이다. 개체간의 유전적 차이는 개체 간의 DNA 염기서열의 차이에 의해 초래되고 이러한 염기서열의 차이는 변이(mutation)와 다형성(polymorphism)으로 구분되는데, 다형성은 미세한 표현형 차이(phenotypic change)를 초래하며 그 빈도가 인구의 1% 이상인 경우 다형성 유전형으로 정의된다.
- [0029] 인간 게놈에 존재하는 다형성 가운데 단일염기다형성(single nucleotide polymorphism, SNP)은 염기서열에서 한 개의 염기가 다른 경우로, 자연적으로 나타나는 다형성의 약 90%를 차지하기 때문에 이러한 단일염기다형성은 임상에서 개체의 질병에 대한 감수성(susceptibility)을 예측하는 지표로 사용될 수 있고, 약물에 대한 효과 및 부작용을 예측할 수 있는 지표로 사용될 수 있어, 개체의 유전적 특성에 적합한 진단 및 치료전략을 구사하는 맞춤형의학(personalized medicine)에 이용될 수 있을 것으로 생각된다.
- [0030] 개체의 유전적 특성은 개인간의 차이뿐 아니라 인종에 따라서도 상당한 차이를 보인다. 즉 특정 다형성의 존재 여부와 그 빈도 그리고 다형성 간의 관계는 인종에 따라 상당한 차이가 있기 때문에 한국인의 골다공증에 대한 유전적 감수성 진단용 다형성 마커는 한국인의 게놈 데이터를 바탕으로 고안된 다형성 마커이어야 할 것이다.
- [0031] 이에 본 발명자들은 골다공증(osteoporosis) 및 골다공성 골절(osteoporotic fracture)을 겪고 있는 환자와 정상인들의 유전적 특성의 차이를 조사한 결과, MECOM 유전자 내의 단일염기다형성(single nucleotide polymorphism, SNP)을 확인하고, 이를 골다공증 또는 골다공성 골절의 예측을 위한 마커로 사용하고자 본 발명을 완성하였다.
- [0032] MECOM은 호모 사피엔스(Homo sapiens)에서 나타나는 MDS1 및 EVI1의 복합 유전자 좌위(complex locus)를 가르는 말로, 구체적으로는 골수이형성증후군(myelodysplastic syndrome, MDS) 1 및 동종지향성 바이러스 인테그레이션 사이트(ecotropic viral integration site, EVI) 1을 인코딩한 유전자로서, 전사 인자(transcription factors)로 알려져 있으며, 골수성 백혈병(myelogenous leukemia) 및 조혈모세포의 분화에 연관되어 있다고 알려져 있다. 기능적으로, MECOM은 각종 질병(3q21q26 syndrome)과 연관되어 있음이 연구되어 왔고(Ogawa, S. et al. Structurally altered Evi-1 protein generated in the 3q21q26 syndrome. *Oncogene* **13**, 183-91(1996)), 전사 인자 활성화, 금속 이온 결합 및 주석 이온 결합과 같은 다세포 기관의 발생에 있어 요구되는 분자적 기능에 참여하고 있음이 밝혀졌다(Alliston, T. et al. Repression of bone morphogenetic protein and activin-inducible transcription by Evi-1. *JBiolChem*, 24227-37(2005), Kurokawa, M. et al. The oncoprotein Evi-1 represses TGF-beta signalling by inhibiting Smad3. *Nature*, 92-6(1998), Vinatzer, U., Taplick, J., Seiser, C., Fonatsch, C. & Wieser, R. The leukaemia-associated transcription factors EVI-1 and MDS1/EVI1 repress transcription and interact with histone deacetylase. *BrJHaematol*, 566-73(2001)). 또한 MECOM 유전자는 다양한 스플라이싱(splicing)을 통해 2개 이상의 서로 다른 전사체를 갖는 것으로 알려져 있다.
- [0033] 그러나 지금까지 뼈 대사에 MECOM 유전자가 연관되어 있음을 밝힌 연구는 없었다. 이에, 본 발명자들은 MECOM 유전자, 특히 MECOM 유전자 내에서도 염기서열의 다형성(polymorphism)이 골다공증 및 골다공성 골절 위험의 강한 유전적 결정인자임을 확인하고, 이를 기초로 MECOM 유전자가 뼈 대사에 매우 중요한 역할을 함을 밝혔다.
- [0034] 그동안 골절과 BMD 간의 미약한 상관관계에도 불구하고, 골다공증은 낮은 BMD를 특징으로 하고 있는데다, 고령화 사회에서 상당한 질병률과 사망률을 초래하고 있는 골다공증은 다양한 골격 위치에서(skeletal sites)에서 골다공성 골절을 야기하고 있어, 골다공성 골절의 발생 위험에 대한 임상적이며 효과적인 예측적 평가는 매우 시급한 것이었다.
- [0035] 이에 본 발명은 골다공성 골절-특히 변수를 동정하고, 이를 동 아시아인 개인의 골다공성 골절 위험을 예측하는데 적용할 수 있는 유전적 기초로 제공하였다는 특징이 있다.
- [0036] 따라서 본 발명은 MECOM 유전자에 존재하는 SNP 뉴클레오타이드를 포함하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 다형성 마커를 제공할 수 있다.
- [0037] 상기 “다형성(polymorphism)”이란 유전학적으로 결정된 집단 내에서 2 이상의 대체적 서열 또는 대립형질의 발생을 의미한다. 바람직한 마커는 선택된 집단에서 1% 이상, 더욱 바람직하기로는 10% 또는 20% 이상의 발생빈도를 나타내는 두개 이상의 대립형질을 가진다.

- [0038] 본 발명자들은 골다공증 또는 골다공성 골절 발생에 관여하는 여러 유전자들의 다형성 가운데 상기 질환의 위험도와 관련성이 있는 MECOM 유전자의 다형성을 발굴하였다. 본 발명자들은 MECOM 유전자가 골다공증 및 골다공성 골절의 발생과 연관이 있음을 확인하고, 일반적인 분자실험학적 비교 실험 및 본 발명의 일실시예에서 수행한 GWAS(genomic wide association study)를 통해 특별히 MECOM 유전자 내에 존재하는 여러 다형성 존재 여부와 상기 골다공증 또는 골다공성 골절의 발병 여부를 분석하였다.
- [0039] 그 결과, MECOM 유전자 내에 존재하는 여러 SNP 존재와 골다공증 또는 골다공성 골절과의 유의적인 상관관계를 확인할 수 있었다.
- [0040] 그리하여, 본 발명은 골다공증 또는 골다공성 골절 발생 위험도 예측용 MECOM 유전자 다형성 마커를 제공할 수 있으며, 상기 MECOM 유전자의 염기서열(full sequence)은 서열번호 1에 기재하였다.
- [0041] 본 발명의 일실시예에 따르면, 본 발명자들은 골다공증 및 골다공성 골절 발생과 유의미한 연관성을 지니는 MECOM 유전자의 여러 SNP 중에서, rs784288을 동정하고, 그 연관 정도를 분석하였다.
- [0042] 구체적으로, 본 발명자들은 GWAS 분석 이전에, 분자생물학적 실험 및 연구를 통한 MECOM 유전자의 염기서열 비교 분석을 통해, 골다공증 및 골다공성 골절 질환을 겪고 있는 환자들과 그렇지 않은 정상인 간에 MECOM 유전자의 특유 SNP의 차이, 즉 대조군(정상인)에서는 MECOM 유전자의 염기서열인 서열목록 1의 폴리뉴클레오티드 염기서열 중에서, 410333번째 염기서열이 대다수 G였던 반면에, 실험군(환자군)에서는 서열목록 1의 410333번째 염기서열이 대부분 A로 확인되어, 대조군과 실험군간의 유의미한 차이를 발견할 수 있었는 바, MECOM 유전자의 다형성이 골다공증 및 골다공성 골절과 연관되어 있음을 뒷받침하는 본 발명의 분자생물학적 근거를 확보한 바 있었다.
- [0043] 본 발명의 일실시예에 따른 GWAS 분석에서도, 본 발명의 서열번호 1의 410333번째 염기가 골다공증 및 골다공성 골절에 걸리지 않은 정상인에게서는 대다수 G를 보이나, GWAS 분석 결과, 골다공증 및 골다공성 골절을 겪고 있는 환자들에게서는 A로 치환되어 있음을 나타내는 유의미한 분석 결과를 통해 확인할 수 있었다. 상기 다형성 부위가 포함된 rs784288의 특성은 하기의 표 1과 같다.

표 1

[0044]	rs_num	rs784288
	allele	G/A
	reference allele	A
	chromosome	3
	position	168971231
	strand	+
	gene id	2122
	gene symbol	MECOM
	gene cytoband	3q26.2
	gene start (5')	168801287
	gene end (3')	169381563
	func	intron2
	sequence	서열목록2 (TGGTAATTAATATGTTTTCTATTGCTGTGTAT(G/A) CAAAACAACCTTTTAGGA)

- [0045] 상기의 rs784288은 MECOM 유전자 내에 포함된 일부분이며, 상기 서열목록2에 나타난 33번째 염기에 해당하는 “G/A” 부분이, 본 발명의 특징이 되는 SNP로서, “A” 염기서열을 가질 때 골다공증 또는 골다공성 골절 발생 고 위험군에 해당되게 된다.
- [0046] 따라서, 상기의 결과들을 토대로, 본 발명은 서열번호 1로 이루어지는 MECOM 유전자의 염기서열에서, 서열번호 1의 410333번째 염기 G가 A로 치환되고, 상기 서열번호 1의 410333번째 염기를 포함하는 폴리뉴클레오티드인 것을 특징으로 하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 MECOM 유전자 다형성 마커를 제공할 수 있다.
- [0047] 상기 폴리뉴클레오티드는 그 크기가 제한되지 않으나, 본 발명에 따른 SNP를 포함하는 10-200bp의 연속적인 DNA 일 수 있으며, 일반적으로 20-100bp의 연속적인 DNA 서열로 이루어질 수도 있다.

- [0048] 한편, 본 발명은 서열번호 1호 이루어진 MECOM 유전자 내의 다형성 부위와 특이적으로 결합하는 폴리뉴클레오티드를 포함하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 조성물을 제공할 수 있다.
- [0049] 또한, 상기 다형성 부위는 MECOM 유전자의 여러 SNP를 모두 포함할 수 있으며, 바람직하게는 서열번호 1의 410333번째 염기 일 수 있다.
- [0050] 따라서, 그러한 경우, 본 발명은 서열번호 1로 이루어진 폴리뉴클레오티드의 410333번째 염기 G가 A로 치환된 다형성 부위에 특이적으로 결합하는 폴리뉴클레오티드를 포함하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 조성물을 제공한다.
- [0051] 본 발명에 따른 골다공증 또는 골다공성 골절 발생 위험도 예측용 조성물에서, MECOM 유전자의 다형성 부위와 특이적으로 결합하는 폴리뉴클레오티드는, 상기 다형성 부위와 특이적인 결합을 통해 다형성 부위의 염기서열을 검출해 낼 수 있는 목적을 위해 사용 될 수 있다. 따라서, 상기 폴리뉴클레오티드에 MECOM 유전자의 SNP 염기서열을 검출해 내기 위하여 사용될 수 있는, MECOM 유전자의 다형성 부위와 특이적으로 결합할 수 있는 폴리뉴클레오티드는 모두 포함되며, 바람직하게는 다형성 부위를 증폭할 수 있는 프라이머 또는 프로브일 수 있다.
- [0052] 또한 본 발명은, 서열번호 1로 이루어지는 MECOM 유전자 내에 존재하는 다형성 부위를 증폭할 수 있는 폴리뉴클레오티드를 포함하는 골다공증 또는 골다공성 골절 발생 위험도 예측용 키트를 제공한다. 물론, 상기 다형성 부위는 서열번호 1의 410333번째 염기일 수 있다.
- [0053] 상기 골다공증 또는 골다공성 골절 발생 위험도 예측용 키트에는 다형성 부위를 증폭할 수 있는 폴리뉴클레오티드 뿐만 아니라, 중합 반응에 필요한 시약, 예를 들면 dNTP, 각 종의 중합효소 및 발색제 등을 포함할 수 있다.
- [0054] 본 발명에서 상기 다형성 부위를 증폭할 수 있는 폴리뉴클레오티드는, 이에 제한되지는 않으나, MECOM 유전자의 SNP를 포함하는 유전자의 특정 영역을 특이적으로 증폭할 수 있는 프라이머 또는 프로브일 수 있으며, 상기 프라이머 또는 프로브는 당업계에 알려진 방법을 통해 디자인할 수 있다.
- [0055] 상기 증폭 반응은 핵산 분자를 증폭하는 반응을 말하며, 이러한 유전자의 증폭 반응들에 대해서는 당업계에 잘 알려져 있고, 예컨대, 중합효소 연쇄반응(PCR), 역전사 중합효소 연쇄반응(RT-PCR), 리가아제 연쇄반응(LCR), 전자 중재 증폭(TMA), 핵산 염기서열 기반 증폭(NASBA) 등이 포함될 수 있다.
- [0056] 상기 "프라이머(primer)"란 적절한 버퍼 중의 적절한 조건(예를 들면, 4개의 다른 뉴클레오시드 트리포스페이트 및 DNA, RNA 폴리머라제 또는 역전사 효소와 같은 중합제) 및 적당한 온도 하에서 주형-지시 DNA 합성의 시작점으로서 작용할 수 있는 단일가닥 올리고뉴클레오티드를 말한다.
- [0057] 상기 프라이머의 적절한 길이는 사용 목적에 따라 달라질 수 있으나, 통상 15 내지 30 뉴클레오티드이다. 짧은 프라이머 분자는 일반적으로 주형과 안정한 혼성체를 형성하기 위해서는 더 낮은 온도를 필요로 한다. 프라이머 서열은 주형과 완전하게 상보적일 필요는 없으나, 주형과 혼성화 할 정도로 충분히 상보적이어야 한다. 상기 프라이머는 다형성 부위를 포함하는 표적 DNA에 혼성화하고, 상기 프라이머가 완전한 상동성을 보이는 대립형질 형태의 증폭을 개시한다. 이 프라이머는 반대편에 혼성화하는 제2 프라이머와 쌍을 이루어 사용된다. 증폭에 의하여 두 개의 프라이머로부터 산물이 증폭되고, 이는 특정 대립형질 형태가 존재한다는 것을 의미한다.
- [0058] 본 발명에서, 상기 프로브라는 용어는 자연의 또는 변형된 모노머 또는 연쇄(linkages)의 선형 올리고머를 의미하며, 디옥시리보뉴클레오타이드 및 리보뉴클레오타이드를 포함하고 타겟 뉴클레오타이드 서열에 특이적으로 혼성화할 수 있으며, 자연적으로 존재하거나 또는 인위적으로 합성된 것을 말한다. 본 발명에 따른 프로브는 단일쇄일 수 있으며, 바람직하게는 올리고디옥시리보뉴클레오타이드일 수 있다. 본 발명의 프로브는 자연 dNMP(즉, dAMP, dGMP, dCMP 및 dTMP), 뉴클레오타이드 유사체 또는 유도체를 포함할 수 있다. 또한, 본 발명의 프로브는 리보뉴클레오타이드도 포함할 수 있다. 예컨대, 본 발명의 프로브는 골격 변형된 뉴클레오타이드, 예컨대, 펩타이드 핵산 (PNA) (M. Egholm et al., Nature, 365:566-568(1993)), 포스포로티오에이트 DNA, 포스포로디티오에이트 DNA, 포스포로아미데이트 DNA, 아마이드-연결된 DNA, MMI-연결된 DNA, 2'-O-메틸 RNA, 알파-DNA 및 메틸 포스포네이트 DNA, 당 변형된 뉴클레오타이드 예컨대, 2'-O-메틸 RNA, 2'-플루오로 RNA, 2'-아미노 RNA, 2'-O-알킬 DNA, 2'-O-알릴 DNA, 2'-O-알카이닐 DNA, 핵소스 DNA, 피라노실 RNA 및 안히드로핵시톨 DNA, 및 염기 변형을 갖는 뉴클레오타이드 예컨대, C-5 치환된 피리미딘(치환기는 플루오로-, 브로모-, 클로로-, 아이오도-, 메틸-, 에틸-, 비닐-, 포르밀-, 에틸-, 프로피닐-, 알카이닐-, 티아조틸-, 이미다조틸-, 피리딜- 포함), C-7 치환기를 갖는 7-데아자퓨린 (치환기는 플루오로-, 브로모-, 클로로-, 아이오도-, 메틸-, 에틸-, 비닐-, 포르밀-, 알카이닐-, 알켄일-, 티아조틸-, 이미다조틸-, 피리딜-), 이노신 및 디아미노퓨린을 포함할 수 있다.

- [0059] 또한 본 발명은 서열번호 1로 이루어지는 MECOM 유전자 내의 다형성 부위를 포함하는 폴리뉴클레오티드로 구성된 골다공증 또는 골다공성 골절 발생 위험도 예측용 마이크로어레이를 제공한다. 물론, 상기 다형성 부위는 서열번호 1의 410333번째 염기일 수 있다.
- [0060] 상기 마이크로어레이는 DNA 또는 RNA 폴리뉴클레오티드를 포함하는 것일 수 있다. 상기 마이크로어레이는 본 발명의 폴리뉴클레오티드를 포함하는 것을 제외하고는 통상적인 마이크로어레이로 이루어진다.
- [0061] 프로브 폴리뉴클레오티드를 기관 상에 고정화하여 마이크로어레이를 제조하는 방법은 당업계에 잘 알려져 있다. 상기 "프로브 폴리뉴클레오티드"는 혼성화 할 수 있는 폴리뉴클레오티드를 의미하는 것으로, 핵산의 상보성 가닥에 서열 특이적으로 결합할 수 있는 올리고뉴클레오티드를 의미한다. 이러한 프로브에는 Nielsen 등, Science 254, 1497-1500(1991)에 기재된 펩티드 핵산을 포함한다.
- [0062] 본 발명의 프로브는 대립형질 특이적 프로브로서, 같은 종의 두 구성원으로부터 유래한 핵산 단편 중에 다형성 부위가 존재하여, 한 구성원으로부터 유래한 DNA 단편에는 혼성화하나, 다른 구성원으로부터 유래한 단편에는 혼성화하지 않는다. 이 경우 혼성화 조건은 대립형질간의 혼성화 강도에 있어서 유의한 차이를 보여, 대립형질 중 하나에만 혼성화하도록 충분히 엄격해야 한다. 이렇게 함으로써 다른 대립형질성 형태 간에 좋은 혼성화 차이를 유발할 수 있다. 여기서, 혼성화란 엄격한 조건, 예를 들면 1M 이하의 염 농도 및 25 °C 이상의 온도하에서 보통 수행될 수 있다. 예를 들면, 5x SSPE (750 mM NaCl, 50 mM Na Phosphate, 5 mM EDTA, pH 7.4) 및 25-30 °C의 조건이 대립형질 특이적 프로브 혼성화에 적합할 수 있다.
- [0063] 본 발명의 골다공증과 연관된 프로브 폴리뉴클레오티드의 기관 상에 고정화하는 과정도 또한 이러한 종래 기술을 사용하여 용이하게 제조할 수 있다. 또한, 마이크로어레이 상에서의 핵산의 혼성화 및 혼성화 결과의 검출은 당업계에 잘 알려져 있다. 상기 검출은 예를 들면, 핵산 시료를 형광 물질 예를 들면 Cy3 및 Cy5와 같은 물질을 포함하는 검출 가능한 신호를 발생시킬 수 있는 표지 물질로 표지한 다음, 마이크로어레이 상에 혼성화하고 상기 표지 물질로부터 발생하는 신호를 검출함으로써 혼성화 결과를 검출할 수 있다.
- [0064] 또한, 본 발명은 (a) 생물학적 시료로부터 게놈 DNA를 추출하는 단계; (b) 상기 (a) 단계의 게놈 DNA 상에 존재하는 서열번호 1로 이루어진 MECOM 유전자 내의 다형성 부위의 염기서열을 검출하는 단계; 및 (c) 상기 (b) 단계에서 검출된 염기서열을 통해 골다공증 또는 골다공성 골절 발생의 고위험군 또는 저위험군을 판별하는 단계를 포함하는 골다공증 또는 골다공성 골절 발생 위험도를 예측하는 방법을 제공한다.
- [0065] 상기 (a) 단계의 생물학적 시료로부터 핵산을 얻는 단계는 통상의 DNA 분리방법에 의하여 수행될 수 있으며, 상기 생물학적 시료로는 예를 들면, 이에 제한되지는 않으나, 조직, 세포, 혈액, 혈청, 혈장, 타액 및 뇨 등이 포함될 수 있다.
- [0066] 상기 (b) 단계의 다형성 부위의 염기서열을 검출하는 단계는 시퀀싱 분석, 마이크로어레이(microarray)에 의한 혼성화, 대립유전자 특이적인 PCR(allele specific PCR), 다이내믹 대립 유전자 혼성화 방법(dynamic allele-specific hybridization, DASH), PCR 연장 분석 및 TaqMan 기법으로 이루어진 군에서 선택된 것에 의하여 수행될 수 있다.
- [0067] 또한, 상기 (b) 단계의 다형성 부위는 서열번호 1로 표기되는 MECOM 유전자의 410333번째 염기 일 수 있다. 따라서, 이러한 경우 상기 (b) 단계의 401333번째 염기서열 검출 결과, 410333번째 염기가 G인 경우는 골다공증 또는 골다공성 골절 저위험군으로 판별할 수 있으며, 염기가 A인 경우에는 골다공증 또는 골다공성 골절 고위험군으로 판별할 수 있다.
- [0068] 상기 "고위험군"은 골다공증 또는 골다공성 골절에 걸릴 확률이 높은 개체군임을 의미하며, "저위험군"은 골다공증 또는 골다공성 골절에 걸릴 확률이 낮은 개체군임을 의미한다.
- [0069] 그리하여 본 발명에 따른 골다공증 또는 골다공성 골절 발생 위험성 예측용 마커 및 조성물은, 정상 대조군과의 MECOM 유전자 내의 다형성의 차이를 확인함으로써, 골다공증 또는 골다공성 골절의 발병 가능성을 예측 및 진단할 수 있다.
- [0070] 이하, 본 발명을 실시예에 의해 상세히 설명하기로 한다. 그러나 이들 실시예는 본 발명을 보다 구체적으로 설명하기 위한 것으로서, 본 발명의 범위가 이들 실시예에 한정되는 것은 아니다.

[0071] <실시예 1>

[0072] 실험 대상의 선정 및 통계학적 분석

[0073] <1-1> 실험 대상의 선정

[0074] 본 발명자들은 골절 표현형(fracture phenotype)의 유전적 원인을 찾기 위하여, 개체군-기반 코호트 연구(population-based cohort analysis)(KARE, n=1427)와 더불어 1, 2기의 한국인(Asan Medical Center, AMC, n=1,082) 및 일본인 여성(RIKEN, n=929)을 대상으로 병원-기반의 독립적 접근(hospital-based independent approaches)을 반복하였다.

[0075] 하기의 표 2에서는 코호트 개체군 내에서의 임상적 값의 분포 및 연구 샘플들의 일반적 특성을 기재하였다.

[0076] 우선 KARE 연구 대상들은 한국 지놈 역학적 연구 프로젝트(Korean Genome Epidemiologic Study project)에 기초해 모집되었고, KARE 코호트(Cho, Y.S. et al. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat Genet* **41**,527-34(2009))로부터, 본 발명자들은 실험군으로 골다공성 골절(osteoporotic fracture)에 걸린 40~69세 사이의 288명의 여성을 선별하였으며 악성 종양(malignancy)을 가졌던 전적이 있거나 혹은 뼈 대사에 영향을 줄 수 있는 약을 투여했었던 환자들은 모두 제외하였다. 대조군으로는 4-폴드 나이(4-fold age) 및 BMI-매칭 골절되지 않은 여성(BMI-matching non-fractured women) 1139명을 무작위적으로 선택하였다.

[0077] 한편, AMC 코호트는 Kim, B.J. et al. Association of SMAD2 polymorphisms with bone mineral density in postmenopausal Korean women. *OsteoporosInt.*에서 기재된 바와 유사한 방법으로 대상을 모집하였으며, 골다공증성 척추 골절(osteoporotic vertebral fracture)의 존재는 방사성 검사(radiological examination)를 통해 확인하였으며, 자가-설문지(self-questionnaire)를 표준화함으로서 분류하였다.

[0078] 또한, RIKEN 코호트에서는, 골다공증성 척추 골절을 겪고 있는 60세 또는 그 이상의 폐경기 후(post-menopausal females)의 369명의 여성들로 실험군을 구성하였으며, 이들은 바이오뱅크 일본 프로젝트(BioBank Japan project) (<http://biobankjp.org/>)로부터 모집하였다. 대조군은 일본에서 몇몇의 의료기관에서 골다공증성 척추 골절을 겪고 있지 않는 60세 또는 그 이상의 폐경기 후의 560명의 여성들로 구성하였다.

표 2

[0079] 실험군 및 대조군의 특성

코호트(Co hort)	실험군(case)				대조군(control)			
	인원수	나이 (year)	키(cm)	몸무게 (kg)	인원수	나이 (year)	키(cm)	몸무게 (kg)
KARE	288	59.93±6.77	152.11±5.94	57.53±8.60	1139	60.67±6.54	151.93±5.53	57.72 ±8.40
AMC	272	66.46±7.95	150.59±8.27	53.84±8.27	810	61.53±8.37	153.74±6.06	56.12 ±7.96
RIKEN	369	75.2±7.1	146.8±6.5	46.9±7.5	560	73.1±6.2	150.3±6.0	56.5±9.0

[0080] <1-2> GWA 분석

[0081] 유전형 알고리즘(Mahalanobis Distance Genotyping Algorithm, BRLMM)을 사용하여 베이시안 로버스트 평형 모델링(the Bayesian Robust Linear Modeling)의 과정 및 애피메트릭스 지놈-와이드 휴먼 SNP 어레이 5.0(Affymetrix Genome-Wide Human SNP array 5.0)을 사용하여 총 10,004 샘플들을 유전형(genotyping)화하였다.

[0082] 그러나 본 실험에서 하기의 SNP들은 배제되었다; genotyping calls rates < 96%(n=401), 이형접합성(heterozygosity) > 30%(n=11), 성별 불일치(sex inconsistency)(n=41), IBS 값(identity-by-state value) > 0.80(n=608), Markers with high missing call rate > 5%, MAF(minor allele frequency) < 1%, 하디-바인베르그 평형(HWE)에서의 P값이 1×10^{-6} 보다 작을 경우. 상기의 SNP들을 제외하고, 남은 359,918 SNPs는 추후의 GWA 분석에 사용되었다.

[0083] GWA(genomic wide association)는 부가 및 우성적 모델(additive and dominant model)의 로지스틱 분석

(logistic analysis)(자유도 1)을 통해 수행되었으며, 나이, 폐경여부, BMI는 공변량(covariates)으로서 PLINK(<http://pngu.mgh.harvard.edu/~purcell/plink/>) 및 SAS 프로그램(version 9.1; SAS institute Inc., Cary, NC, USA)을 통해 조절되었다.

[0084] 그 결과, 도 1에서와 같이, 트렌드 테스트(trend test)로부터 유래한 관찰된 P-값(observed P-value)의 Q-Q 플롯(quantile-quantile plots)은 오직 그래프의 꼬리 부분에서 나타나는 영분포(null distribution)로부터 유의미한 편차(deviation)를 보여주었다. 그리고 이는 진정한 관련 변수(associated variants)로 생각되었다. 또한, 추측된 지놈상의 대조군 일플레이션 요소(estimated genomic inflation factor)는 1.007이었으며, 이는 KARE 연구 샘플들의 개체군 계층화(population stratification)의 증거로서 판단되었다.

[0085] <실시예 2>

[0086] GWAS 발견 및 반복 연구

[0087] GWAS 발견 단계에서, 본 발명자들은 하기의 표 3에 나타난 바와 같이 P-값이 5×10^{-4} 보다 작은 골다공증적 골절(osteoporotic fracture)에 관련된 10개의 SNPs를 발견하였다. KARE 연구에서, SNP rs784288은 염색체 3q26.2b의 MECOM(MDS1 and EVI1) (OR=1.67, 95% CI=1.29-2.17, $p_{\text{dom}}=1.09 \times 10^{-4}$)에 위치해 있었다.

[0088] 우선, SNP 대치 분석(Imputation analysis)은 IMPUTE를 이용하여 총 1,573,409 SNPs의 HapMap 아시안 개체군(Asian(JTP/CHB) population)(release 22/NCBI build 36 and dbSNP bulid 126)을 대상으로 이뤄졌다. 이들 중, 각 코호트에서, 사후 확률 스코어(posterior probability score) < 0.90, high genotype information content(info < 0.5), HWE($P < 1 \times 10^{-7}$), SNP 결측율(missing rate) > 0.1 및 MAF < 0.01에 해당하는 SNPs를 탈락시켰다.

[0089] 또한, 연관 방법(Association methods)은 PLINK(<http://pngu.mgh.harvard.edu/~purcell/plink/>) 및 SAS programs(version 9.1; SAS institute Inc., Cary, NC, USA)을 통해 수행되었으며, 메타-분석(meta-analysis)은 각 연구 결과의 조합으로부터 수행되었다. 모든 코호트의 결과는 고정효과(fixed effects)를 가정한 inverse-variance meta-analysis method를 통해 결합되었으며, Cochran's Q-test는 세계의 연구 사이의 이형접합성(heterozygosity) 측정을 위해 사용되었고, 모든 메타-분석 계산들은 R 프로그램(version 2.7.1)을 사용하여 진행하였다.

[0090] 또한, 본 발명자들은 반복 코호트(replication cohorts)를 위하여, 9 SNPs(rs784288, rs17546738, rs7809764, rs11751618, rs1509957, rs12477614, rs4753784, rs13434517, rs2277730)에 대한 allelic discrimination assay를 골든게이트 어세이(Illumina Inc., San Diego, CA, USA)를 이용하여 수행하였으며, 하나의 SNP(rs10816453)을 위해서는 다른 플랫폼(platform)에서의 functioning assay를 고안할 수는 없었다.

[0091] 상기 실험들의 결과, 본 발명자들은 10개의 SNPs 중 rs784288가 AMC 코호트의 골다공증성 척추 골절(osteoporotic vertebral fracture)(OR = 1.38, 95% CI = 1.05-1.83, $p_{\text{dom}}=2.15 \times 10^{-2}$)과 관련하여 중요한 요인을 알 수 있었으며, 또한 일본인 RIKEN 코호트에서도 골다공증성 척추 골절(OR=1.45, 95%CI=1.11-1.89, $p_{\text{dom}}=5.81 \times 10^{-3}$)의 중요 관련인자임을 발견하였다. 더불어, GWAS 발견 및 메타-분석(meta-analysis)을 통한 반복 세트(replication sets)의 조합을 통해, rs784288가 같은 방향으로 지놈-와이드 유의도(genome-wide significance)(OR=1.5, 95%CI=1.29-1.75, $p_{\text{dom}}=2.23 \times 10^{-7}$)와 일관적으로 연관되어 있음을 알 수 있었다.

표 3

골다공증적 골질의 GWAS 및 반복 연구(replication study)의 결과

CHR	rs_num	BP	gene_symbol	M/m	freq		Mode	KARE		ACMC		RXEN		Combind			
					case	control		OR(CI)	P	OR(CI)	P	OR(CI)	P	OR(CI)	P	hetero_Q	hetero_P
3	rs784288	170453925	MECOM	G/A	0.34	0.25	DOM	1.67 (1.29-2.17)	1.09×10^{-4}	1.38 (1.05-1.83)	2.15×10^{-2}	1.45 (1.11-1.89)	5.81×10^{-3}	1.5 (1.29-1.75)	2.23×10^{-2}	1.05	0.590
10	rs17546738	36827724		T/C	0.07	0.03	DOM	2.35 (1.54-3.59)	7.95×10^{-5}	1.59 (1.01-2.51)	4.71×10^{-2}	-	-	1.96 (1.44-2.68)	2.19×10^{-5}	1.51	0.220
7	rs7809764	25912177	NFE2L3	T/G	0.53	0.42	ADD	1.58 (1.32-1.91)	1.21×10^{-6}	1.15 (0.95-1.4)	0.164	1.03 (0.86-1.24)	0.754	1.24 (1.11-1.38)	1.37×10^{-4}	11.05	0.004
6	rs11751618	87293047	HTF1E	T/C	0.33	0.25	ADD	1.49 (1.22-1.81)	9.10×10^{-5}	1.14 (0.91-1.43)	0.255	-	-	1.32 (1.14-1.54)	2.22×10^{-4}	3	0.083
10	rs1509957	64280724	EGR2	A/G	0.38	0.48	ADD	0.69 (0.57-0.83)	8.15×10^{-5}	0.84 (0.69-1.03)	0.926	0.93 (0.78-1.12)	0.440	0.81 (0.73-0.91)	2.26×10^{-4}	5.36	0.069
9	rs10816453	108716071	ZNF462	G/A	0.15	0.24	ADD	0.58 (0.45-0.74)	1.24×10^{-5}	-	-	0.96 (0.76-1.2)	0.718	0.76 (0.64-0.9)	1.30×10^{-3}	8.85	0.003
2	rs12477614	7009781	RNF144A	A/G	0.48	0.38	ADD	1.48 (1.23-1.79)	3.66×10^{-5}	1.12 (0.93-1.35)	0.222	1.01 (0.84-1.23)	0.888	1.19 (1.07-1.33)	1.40×10^{-3}	8.4	0.015
11	rs4753784	106493141	GUCY1A2	A/G	0.50	0.40	ADD	1.47 (1.23-1.77)	2.87×10^{-5}	0.99 (0.81-1.20)	0.911	1.12 (0.92-1.36)	0.276	0.19 (1.07-1.33)	2.02×10^{-3}	9.15	0.010
4	rs13434517	39716924	N4BP2	C/T	0.41	0.34	DOM	1.77 (1.34-2.33)	5.14×10^{-5}	1.04 (0.78-1.37)	0.802	-	-	1.36 (1.12-1.65)	2.30×10^{-3}	7.13	0.008
18	rs2277730	11874833	MPP1	G/C	0.37	0.47	ADD	0.67 (0.55-0.8)	2.10×10^{-5}	0.99 (0.81-1.21)	0.903	0.93 (0.77-1.12)	0.449	0.84 (0.76-0.94)	2.60×10^{-3}	9.6	0.008

Abbreviations: OR (CI) - odds ratio (confidence interval)

^a major allele/minor allele, ^b KARE frequency

<실시예 3>

MECOM 유전자의 인 실리코 분석(*In silico analysis*)

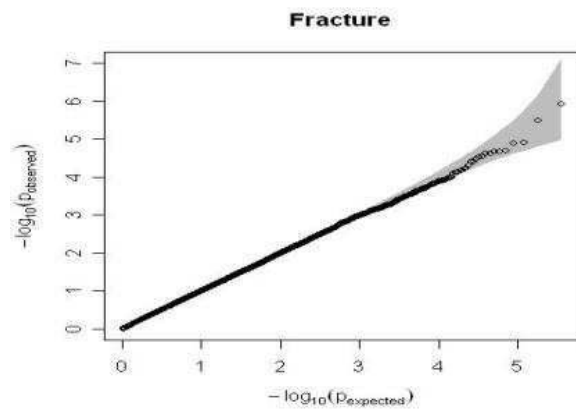
본 발명자들은 상기 실험에서 얻은 GWAS 데이터 및 아시아인 HapMap 데이터를 바탕으로 SNPs의 유전자형이 rs784288 인근에 위치하는 것임을 알 수 있었으며, 이는 도 2에 나타난 바와 같이 위치적 연관 시그널(regional association signals)을 의미하는 것임을 알 수 있었다. 도 2에서 파란색에서 빨간색으로의 농도변화의 컬러 스킴(color scheme)은 낮은 것에서부터 높은 LD 값(r²)를 반영하며, 스캐터 플롯(scatter plot)과 피크(peak)는 각 SNP와 재조합율(recombination rate)에 대한 P-값의 음성 로그(negative logarithm)을 의미하는 것이다.

3q에서의 rs784288에서의 연관성은 MECOM 유전자만을 포함하는 높은 연관불균형(linkage disequilibrium)의 200-kb 위치에 존재하는 것이었다. 대치 분석(imputation analysis)을 통해 추가적인 연관성이 드러났으나, 모두 동일한 높은 연관 불균형(high linkage equilibrium) 내에 존재하였다. 따라서 가장 유망한 변수(variant)는 아마도 스플라이싱 조절(splicing regulation)을 통하여 c-jun 및 NF-κB의 전사 인자(transcription factor)의 바인딩 사이트(binding sites)를 제거하는 작용을 유도할 것으로 판단된다.

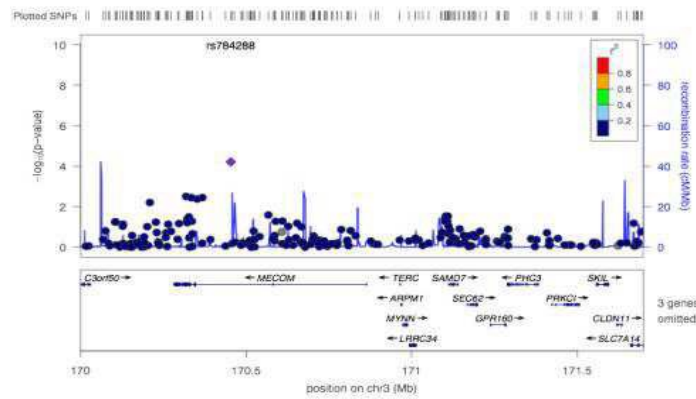
이제까지 본 발명에 대하여 그 바람직한 실시예들을 중심으로 살펴보았다. 본 발명이 속하는 기술 분야에서 통상의 지식을 가진 자는 본 발명이 본 발명의 본질적인 특성에서 벗어나지 않는 범위에서 변형된 형태로 구현될 수 있음을 이해할 수 있을 것이다. 그러므로 개시된 실시예들은 한정적인 관점이 아니라 설명적인 관점에서 고려되어야 한다. 본 발명의 범위는 전술한 설명이 아니라 특허청구범위에 나타나 있으며, 그와 동등한 범위 내에 있는 모든 차이점은 본 발명에 포함된 것으로 해석되어야 할 것이다

도면

도면1



도면2



서열 목록

SEQUENCE LISTING

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<160> 2

<170> PatentIn version 3.2

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<212> DNA

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