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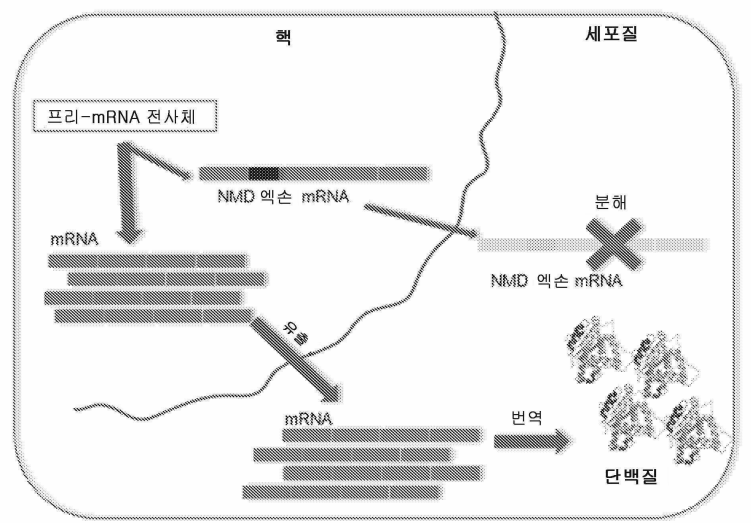
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(54) 발명의 명칭 **병태 및 질환 치료용 안티센스 올리고머**

(57) 요 약

유전자 *SCN1A*에서 선택적 스플라이싱 이벤트는 비생산적 mRNA 전사체를 초래할 수 있고, 이는 결국 비정상적 단백질 발현을 초래할 수 있고, *SCN1A* 유전자에서 선택적 스플라이싱 이벤트를 표적화할 수 있는 치료제는 드라벳 증후군 환자에서 기능성 단백질의 발현 수준을 조절하고/거나 비정상적 단백질 발현을 억제할 수 있다. 이러한 치료제는 SCN1A, SCN8A 또는 SCN5A 단백질 결핍에 의해 야기된 병태를 치료하는 데 사용될 수 있다.

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명 세 서

청구범위

청구항 1

SCN1A 단백질을 코딩하는, 넌센스 매개 RNA 붕괴 유도 엑손을 함유하는 mRNA(NMD 엑손 mRNA)를 갖는 세포에서 SCN1A 단백질의 발현을 조절하는 방법으로서, 치료제를 세포에 접촉시키는 단계를 포함하고, 그로 인하여 치료제가 SCN1A 단백질을 코딩하는 NMD 엑손 mRNA로부터 NMD 엑손의 스플라이싱을 조절하고, 이로써 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 조절하고, SCN1A 단백질의 발현을 조절하는 것인 방법.

청구항 2

치료를 필요로 하는 대상체의 세포에서 SCN1A 단백질의 발현을 조절함으로써 대상체에서 질환 또는 병태를 치료하는 방법으로서, 대상체의 세포를 넌센스 매개 mRNA 붕괴 유도 엑손(NMD 엑손)을 함유하고 SCN1A를 코딩하는 세포에서 mRNA로부터 NMD 엑손의 스플라이싱을 조절하는 치료제와 접촉시키는 단계를 포함하고, 그로 인하여 대상체의 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 조절하고, SCN1A 단백질의 발현을 조절하는 것인 방법.

청구항 3

제1항 또는 제2항에 있어서, 치료제는

(a) SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분에 결합하거나;

(b) NMD 엑손 mRNA의 스플라이싱에 관여하는 인자의 결합을 조절하거나;

(c) (a)와 (b)의 조합인 방법.

청구항 4

제3항에 있어서, 치료제는 표적화된 부분의 영역으로부터 NMD 엑손의 스플라이싱에 관여하는 인자의 결합을 방해하는 것인 방법.

청구항 5

제3항에 있어서, 표적화된 부분은 NMD 엑손에 근접한 것인 방법.

청구항 6

제5항에 있어서, 표적화된 부분은 NMD 엑손의 5' 말단의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 상류에 있는 것인 방법.

청구항 7

제5항에 있어서, 표적화된 부분은 NMD 엑손의 5' 말단의 적어도 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 상류에 있는 것인 방법.

청구항 8

제5항에 있어서, 표적화된 부분은 NMD 엑손의 3' 말단의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약

300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 하류에 있는 것인 방법.

청구항 9

제5항에 있어서, 표적화된 부분은 NMD 엑손의 3' 말단의 적어도 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 하류에 있는 것인 방법.

청구항 10

제3항에 있어서, 표적화된 부분은 SCN1A를 코딩하는 NMD 엑손 mRNA의 2개의 표준 엑손 영역 사이의 인트론 영역에 위치하며, 인트론 영역은 NMD 엑손을 함유하는 것인 방법.

청구항 11

제3항에 있어서, 표적화된 부분은 NMD 엑손과 적어도 부분적으로 중첩되는 것인 방법.

청구항 12

제3항에 있어서, 표적화된 부분은 NMD 엑손의 상류 인트론과 적어도 부분적으로 중첩되는 것인 방법.

청구항 13

제3항에 있어서, 표적화된 부분은 5' NMD 엑손-인트론 접합부 또는 3' NMD 엑손-인트론 접합부를 포함하는 것인 방법.

청구항 14

제3항에 있어서, 표적화된 부분은 NMD 엑손 내에 있는 것인 방법.

청구항 15

제3항에 있어서, 표적화된 부분은 NMD 엑손의 약 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30개 또는 그 이상의 연속적인 뉴클레오티드를 포함하는 것인 방법.

청구항 16

제1항 또는 제2항에 있어서, SCN1A를 코딩하는 NMD 엑손 mRNA는 서열 번호 2 또는 7 내지 10 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함하는 것인 방법.

청구항 17

제1항 또는 제2항에 있어서, SCN1A를 코딩하는 NMD 엑손 mRNA는 서열 번호 1 또는 3 내지 6과 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 유전자 서열에 의해 코딩되는 것인 방법.

청구항 18

제5항에 있어서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,803의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 상류에 있는 것인 방법.

청구항 19

제5항에 있어서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,803의 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300

뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 상류에 있는 것인 방법.

청구항 20

제5항에 있어서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,740의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 하류에 있는 것인 방법.

청구항 21

제5항에 있어서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,740의 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 하류에 있는 것인 방법.

청구항 22

제3항에 있어서, SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분은 서열 번호 2 또는 7 내지 10의 적어도 8개의 인접한 핵산을 포함하는 영역과 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함하는 것인 방법.

청구항 23

제1항 또는 제2항에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21 내지 67, 210 내지 256, 또는 304 내지 379 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

청구항 24

제3항에 있어서, SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 넌센스 매개 RNA 붕괴 유도 엑손 20x 내에 있는 것인 방법.

청구항 25

제24항에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 42 내지 50, 또는 231 내지 239 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

청구항 26

제3항에 있어서, SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 넌센스 매개 RNA 붕괴 유도 엑손 20x의 상류 또는 하류에 있는 것인 방법.

청구항 27

제26항에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21 내지 38, 53 내지 67, 210 내지 227, 또는 242 내지 256 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

청구항 28

제3항에 있어서, NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 엑손 20x의 엑손-인트론 접합부를 포함하는 것인 방법.

청구항 29

제28항에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 39 내지 41, 51, 52, 228 내지 230, 240, 또는 241 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

청구항 30

제1항 또는 제2항에 있어서, 치료제는 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 촉진하는 것인 방법.

청구항 31

제30항에 있어서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제는 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제와 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가하는 것인 방법.

청구항 32

제30항에 있어서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 증가시키는 것인 방법.

청구항 33

제30항에 있어서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 양은 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가하는 것인 방법.

청구항 34

제30항에 있어서, 치료제는 세포에서 SCN1A 단백질의 발현을 증가시키는 것인 방법.

청구항 35

제30항에 있어서, 치료제와 접촉된 세포에서 생성된 SCN1A의 양은 대조군 세포에서 생성된 SCN1A의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가하는 것인 방법.

청구항 36

제2항에 있어서, 질환 또는 병태는 $Na_v1.1$의 기능 상실 돌연변이에 의해 유발되는 것인 방법.

청구항 37

제36항에 있어서, 질환 또는 병태는 *SCN1A* 유전자의 반가불충분성과 관련되며, 대상체는 기능성 SCN1A를 코딩하

는 제1 대립 유전자, 및 SCN1A가 생성되지 않거나 감소한 수준으로 생성되는 제2 대립 유전자, 또는 비기능성 SCN1A 또는 부분 기능성 SCN1A를 코딩하는 제2 대립 유전자를 갖는 것인 방법.

청구항 38

제36항에 있어서, 질환 또는 병태는 뇌병증인 방법.

청구항 39

제38항에 있어서, 뇌병증은 간질성 뇌병증인 방법.

청구항 40

제36항에 있어서, 질환 또는 병태는 드라벳 증후군(DS); 유아기의 중증 근간대성 뇌전증(SMEI) 경계성(SMEB); 열성 발작(FS); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+); 조기 유아 간질성 뇌병증 13; 잠재성 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 뇌전증에서의 돌연사(SUDEP); 동기능 부전 증후군 1; 자폐증; 또는 유아기의 악성 이동 부분 발작인 방법.

청구항 41

제40항에 있어서, GEFS+는 열성 발작 플러스를 동반하는 전신 뇌전증 2형인 방법.

청구항 42

제40항에 있어서, 열성 발작은 가족성 열성 발작 3A인 방법.

청구항 43

제40항에 있어서, SMEB는 전신 극파가 없는 SMEB(SMEB-SW), 근간대성 발작이 없는 SMEB(SMEB-M), SMEI의 하나 초과의 특징이 결여된 SMEB(SMEB-O), 또는 전신 강직성 간대 발작을 동반한 난치성 소아기 뇌전증(ICEGTC)인 방법.

청구항 44

제36항에 있어서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 촉진하고 SCN1A의 발현을 증가시키는 것인 방법.

청구항 45

제36항에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 22 내지 24, 26, 27, 29 내지 35, 37 내지 62, 64 내지 67, 또는 304 내지 379 중 어느 하나에 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 서열을 포함하는 것인 방법.

청구항 46

제1항 또는 제2항에 있어서, 치료제는 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 억제하는 것인 방법.

청구항 47

제46항에 있어서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제는 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제와 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소하는 것인 방법.

청구항 48

제46항에 있어서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 감소시키는 것인 방법.

청구항 49

제46항에 있어서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 양은 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소하는 것인 방법.

청구항 50

제46항에 있어서, 치료제는 세포에서 SCN1A 단백질의 발현을 감소시키는 것인 방법.

청구항 51

제46항에 있어서, 치료제와 접촉된 세포에서 생성된 SCN1A의 양은 대조군 세포에서 생성된 SCN1A의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소하는 것인 방법.

청구항 52

제2항에 있어서, 질환 또는 병태는 $Na_v1.1$의 기능 획득 돌연변이에 의해 유발되는 것인 방법.

청구항 53

제52항에 있어서, 대상체는 세포에서 SCN1A가 증가한 수준으로 생성되는 대립 유전자, 또는 $Na_v1.1$의 증가한 활성을 유도하는 돌연변이 SCN1A를 코딩하는 대립 유전자를 갖는 것인 방법.

청구항 54

제52항에 있어서, 질환 또는 병태는 편두통인 방법.

청구항 55

제54항에 있어서, 편두통은 가족성 편마비 편두통 3인 방법.

청구항 56

제2항에 있어서, 질환 또는 병태는 $Na_v1.1$ 유전성 뇌전증인 방법.

청구항 57

제52항에 있어서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 억제하고 SCN1A의 발현을 감소시키는 것인 방법.

청구항 58

제52항에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21, 25, 28, 36, 또는 63 중 어느 하나에 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 서열을 포함하는 것인 방법.

청구항 59

제1항 또는 제2항에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 포스포로티오에이트 결합 또는 포스포로디아미데이트 결합을 포함하는 백본 변형을 포함하는 것인 방법.

청구항 60

제1항 또는 제2항에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 포스포로디아미데이트 모르폴리노, 잠금 핵산, 펩티드 핵산, 2'-O-메틸, 2'-플루오로, 또는 2'-O-메톡시에틸 모이어티를 포함하는 것인 방법.

청구항 61

제1항 또는 제2항에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 적어도 하나의 변형된 당 모이어티를 포함하는 것인 방법.

청구항 62

제61항에 있어서, 각각의 당 모이어티는 변형된 당 모이어티인 방법.

청구항 63

제1항 또는 제2항에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 8 내지 50개의 핵염기, 8 내지 40개의 핵염기, 8 내지 35개의 핵염기, 8 내지 30개의 핵염기, 8 내지 25개의 핵염기, 8 내지 20개의 핵염기, 8 내지 15개의 핵염기, 9 내지 50개의 핵염기, 9 내지 40개의 핵염기, 9 내지 35개의 핵염기, 9 내지 30개의 핵염기, 9 내지 25개의 핵염기, 9 내지 20개의 핵염기, 9 내지 15개의 핵염기, 10 내지 50개의 핵염기, 10 내지 40개의 핵염기, 10 내지 35개의 핵염기, 10 내지 30개의 핵염기, 10 내지 25개의 핵염기, 10 내지 20개의 핵염기, 10 내지 15개의 핵염기, 11 내지 50개의 핵염기, 11 내지 40개의 핵염기, 11 내지 35개의 핵염기, 11 내지 30개의 핵염기, 11 내지 25개의 핵염기, 11 내지 20개의 핵염기, 11 내지 15개의 핵염기, 12 내지 50개의 핵염기, 12 내지 40개의 핵염기, 12 내지 35개의 핵염기, 12 내지 30개의 핵염기, 12 내지 25개의 핵염기, 12 내지 20개의 핵염기, 또는 12 내지 15개의 핵염기로 이루어지는 것인 방법.

청구항 64

제3항에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 단백질을 코딩하는 NMD 엑손 mRNA의 표적화된 부분에 적어도 80%, 적어도 85%, 적어도 90%, 적어도 95%, 적어도 98%, 적어도 99%, 또는 100% 상보적인 것인 방법.

청구항 65

제1항에 있어서, SCN1A mRNA 또는 단백질 발현을 평가하는 단계를 추가로 포함하는 방법.

청구항 66

제2항에 있어서, 대상체는 인간인 방법.

청구항 67

제2항에 있어서, 대상체는 비인간 동물인 방법.

청구항 68

제2항에 있어서, 대상체는 태아, 배아, 또는 소아인 방법.

청구항 69

제1항 또는 제2항에 있어서, 세포는 생체 외 세포인 방법.

청구항 70

제2항에 있어서, 치료제는 대상체의 수막공간내 주사, 뇌실내 주사, 복강내 주사, 근육내 주사, 피하 주사, 안

내, 또는 정맥내 주사에 의해 투여되는 것인 방법.

청구항 71

제2항에 있어서, 대상체에게 제2 치료제를 투여하는 단계를 추가로 포함하는 방법.

청구항 72

제71항에 있어서, 제2 치료제는 소분자인 방법.

청구항 73

제71항에 있어서, 제2 치료제는 ASO인 방법.

청구항 74

제73항에 있어서, ASO는 서열 번호 115 내지 161 중 어느 하나에 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 서열을 포함하는 것인 방법.

청구항 75

제71항에 있어서, 제2 치료제는 인트론 유지를 교정하는 것인 방법.

청구항 76

제2항에 있어서, 질환 또는 병태는 알츠하이머병, SCN2A 뇌병증, SCN8A 뇌병증, 또는 SCN5A 부정맥인 방법.

청구항 77

제30항, 제32항 또는 제34항에 있어서, 질환 또는 병태는 알츠하이머병, SCN2A 뇌병증, SCN8A 뇌병증, 또는 SCN5A 부정맥인 방법.

발명의 설명

기 술 분 야

배 경 기 술

[0001] **상호 참조**

[0002] 본 출원은 2017년 8월 25일에 출원된 미국 가출원 제62/550,462호, 2017년 10월 23일에 출원된 미국 가출원 제62/575,901호, 2018년 5월 4일에 출원된 미국 가출원 제62/667,356호, 2018년 5월 15일에 출원된 미국 가출원 제62/671,745호의 이익을 주장하며, 이들 각각은 그 전문이 본원에 참고로 포함된다.

[0003] **발명의 배경**

[0004] 신경계 장애는 신경 흥분성, 뉴런 상호 작용, 및 전체적인 뇌 기능을 매개하는 이온 통로(채널)의 기능 장애를 특징으로 하는 이온통로병증과 종종 관련된다. 뉴런 전압 게이팅 나트륨 채널의 알파 세공 형성 서브유닛을 코딩하는 SCN1A-SCN2A-SCN3A 유전자 클러스터의 일부인 SCN1A 유전자의 돌연변이는 드라벳 증후군(DS: Dravet Syndrome)과 같은 질환 및 병태의 질환 수의 발달과 관련이 있다(Miller, et al., 1993-2015, GeneReviews, Eds. Pagon RA, et al. Seattle (WA): University of Washington, Seattle, Bookshelf ID: NBK1318, and Mulley, et al., 2005, Hum. Mutat. 25: 535-542).

발명의 내용

[0005] **발명의 요약**

[0006] 특정 실시양태에서, SCN1A 단백질을 코딩하는, 넌센스 매개 RNA 붕괴 유도 엑손(non-sense mediated RNA decay-inducing exon)을 함유하는 mRNA(NMD 엑손 mRNA)를 갖는 세포에서 SCN1A 단백질의 발현을 조절하는 방법으로서, 치료제를 세포에 접촉시키는 단계를 포함하고, 그로 인하여 치료제가 SCN1A 단백질을 코딩하는 NMD 엑

손 mRNA로부터 NMD 엑손의 스플라이싱을 조절하고, 이로써 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 조절하고, SCN1A 단백질의 발현을 조절하는 것인 방법이 본원에서 개시된다. 일부 실시양태에서, 치료제는 (a) SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분에 결합하거나; (b) NMD 엑손 mRNA의 스플라이싱에 관여하는 인자의 결합을 조절하거나; (c) (a)와 (b)의 조합이다. 일부 실시양태에서, 치료제는 표적화된 부분의 영역으로부터 NMD 엑손의 스플라이싱에 관여하는 인자의 결합을 방해한다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손에 근접해 있다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손의 5' 말단의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 상류에 있다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손의 5' 말단의 적어도 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 하류에 있다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손의 3' 말단의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 하류에 있다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손의 3' 말단의 적어도 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 하류에 있다. 일부 실시양태에서, 표적화된 부분은 SCN1A를 코딩하는 NMD 엑손 mRNA의 2개의 표준 엑손 영역 사이의 인트론 영역에 위치하며, 인트론 영역은 NMD 엑손을 함유한다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손과 적어도 부분적으로 중첩된다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손의 상류 인트론과 적어도 부분적으로 중첩된다. 일부 실시양태에서, 표적화된 부분은 5' NMD 엑손-인트론 접합부 또는 3' NMD 엑손-인트론 접합부를 포함한다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손 내에 있다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손의 약 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30개 또는 그 이상의 연속적인 뉴클레오티드를 포함한다. 일부 실시양태에서, SCN1A를 코딩하는 NMD 엑손 mRNA는 서열 번호 2 또는 7 내지 10 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함한다. 일부 실시양태에서, SCN1A를 코딩하는 NMD 엑손 mRNA는 서열 번호 1 또는 3 내지 6과 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 유전자 서열에 의해 코딩된다. 일부 실시양태에서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,803의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 상류에 있다. 일부 실시양태에서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,803의 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 상류에 있다. 일부 실시양태에서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,740의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 하류에 있다. 일부 실시양태에서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,740의 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 하류에 있다. 일부 실시양태에서, SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분은 서열 번호 2 또

는 7 내지 10의 적어도 8개의 인접한 핵산을 포함하는 영역과 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함한다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO: antisense oligomer)이며 ASO는 서열 번호 21 내지 67, 210 내지 256, 또는 304 내지 379 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함한다. 일부 실시양태에서, SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 넌센스 매개 RNA 붕괴 유도 엑손 20x 내에 있다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 42 내지 50, 또는 231 내지 239 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함한다. 일부 실시양태에서, SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 넌센스 매개 RNA 붕괴 유도 엑손 20x의 상류 또는 하류에 있다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21 내지 38, 53 내지 67, 210 내지 227, 또는 242 내지 256 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함한다. 일부 실시양태에서, NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 엑손 20x의 엑손-인트론 접합부를 포함한다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 39 내지 41, 51, 52, 228 내지 230, 240, 또는 241 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함한다. 일부 실시양태에서, 치료제는 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 촉진한다. 일부 실시양태에서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제는 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제와 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가한다. 일부 실시양태에서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 증가시킨다. 일부 실시양태에서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 양은 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가한다. 일부 실시양태에서, 치료제는 세포에서 SCN1A 단백질의 발현을 증가시킨다. 일부 실시양태에서, 치료제와 접촉된 세포에서 생성된 SCN1A의 양은 대조군 세포에서 생성된 SCN1A의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가한다. 일부 실시양태에서, 치료제는 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 억제한다. 일부 실시양태에서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제는 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제와 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소한다. 일부 실시양태에서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 감소시킨다. 일부 실시양태에서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 양은 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약

4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소한다. 일부 실시양태에서, 치료제는 세포에서 SCN1A 단백질의 발현을 감소시킨다. 일부 실시양태에서, 치료제와 접촉된 세포에서 생성된 SCN1A의 양은 대조군 세포에서 생성된 SCN1A의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소한다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 포스포로티오에이트 결합 또는 포스포로디아미데이트 결합을 포함하는 백본 변형을 포함한다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 포스포로디아미데이트 모르폴리노, 잠금 핵산, 펩티드 핵산, 2'-O-메틸, 2'-플루오로, 또는 2'-O-메톡시에틸 모이어티를 포함한다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 하나 이상의 변형된 당 모이어티를 포함한다. 일부 실시양태에서, 각각의 당 모이어티는 변형된 당 모이어티이다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 8 내지 50개의 핵염기, 8 내지 40개의 핵염기, 8 내지 35개의 핵염기, 8 내지 30개의 핵염기, 8 내지 25개의 핵염기, 8 내지 20개의 핵염기, 8 내지 15개의 핵염기, 9 내지 50개의 핵염기, 9 내지 40개의 핵염기, 9 내지 35개의 핵염기, 9 내지 30개의 핵염기, 9 내지 25개의 핵염기, 9 내지 20개의 핵염기, 9 내지 15개의 핵염기, 10 내지 50개의 핵염기, 10 내지 40개의 핵염기, 10 내지 35개의 핵염기, 10 내지 30개의 핵염기, 10 내지 25개의 핵염기, 10 내지 20개의 핵염기, 10 내지 15개의 핵염기, 11 내지 50개의 핵염기, 11 내지 40개의 핵염기, 11 내지 35개의 핵염기, 11 내지 30개의 핵염기, 11 내지 25개의 핵염기, 11 내지 20개의 핵염기, 11 내지 15개의 핵염기, 12 내지 50개의 핵염기, 12 내지 40개의 핵염기, 12 내지 35개의 핵염기, 12 내지 30개의 핵염기, 12 내지 25개의 핵염기, 12 내지 20개의 핵염기, 또는 12 내지 15개의 핵염기로 이루어진다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 단백질을 코딩하는 NMD 엑손 mRNA의 표적화된 부분에 적어도 80%, 적어도 85%, 적어도 90%, 적어도 95%, 적어도 98%, 적어도 99%, 또는 100% 상보적이다. 일부 실시양태에서, 방법은 SCN1A mRNA 또는 단백질 발현을 평가하는 단계를 추가로 포함한다. 일부 실시양태에서, 세포는 생체 외 세포이다.

[0007] 특정 실시양태에서, 치료를 필요로 하는 대상체의 세포에서 SCN1A 단백질의 발현을 조절함으로써 대상체에서 질환 또는 병태를 치료하는 방법으로서, 대상체의 세포를 NMD 엑손을 함유하고 SCN1A를 코딩하는 세포에서 mRNA로부터 넌센스 매개 mRNA 붕괴 유도 엑손(NMD 엑손)의 스플라이싱을 조절하는 치료제와 접촉시키는 단계를 포함하고, 그로 인하여 대상체의 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 조절하고, SCN1A 단백질의 발현을 조절하는 것인 방법이 본원에서 개시된다. 일부 실시양태에서, 치료제 (a)는 (a) SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분에 결합하거나; (b) NMD 엑손 mRNA의 스플라이싱에 관여하는 인자의 결합을 조절하거나; (c) (a)와 (b)의 조합이다. 일부 실시양태에서, 치료제는 표적 부분의 영역으로부터 NMD 엑손의 스플라이싱에 관여하는 인자의 결합을 방해한다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손에 근접해 있다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손의 5' 말단의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 상류에 있다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손의 5' 말단의 적어도 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 상류에 있다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손의 3' 말단의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 하류에 있다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손의 3' 말단의 적어도 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드,

약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 하류에 있다. 일부 실시양태에서, 표적화된 부분은 SCN1A를 코딩하는 NMD 엑손 mRNA의 2개의 표준 엑손 영역 사이의 인트론 영역에 위치하며, 인트론 영역은 NMD 엑손을 함유한다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손과 적어도 부분적으로 중첩된다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손의 상류 인트론과 적어도 부분적으로 중첩된다. 일부 실시양태에서, 표적화된 부분은 5' NMD 엑손-인트론 접합부 또는 3' NMD 엑손-인트론 접합부를 포함한다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손 내에 있다. 일부 실시양태에서, 표적화된 부분은 NMD 엑손의 약 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30개 또는 그 이상의 연속적인 뉴클레오티드를 포함한다. 일부 실시양태에서, SCN1A를 코딩하는 NMD 엑손 mRNA는 서열 번호 2 또는 7 내지 10 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함한다. 일부 실시양태에서, SCN1A를 코딩하는 NMD 엑손 mRNA는 서열 번호 1 또는 3 내지 6과 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 유전자 서열에 의해 코딩된다. 일부 실시양태에서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,803의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 상류에 있다. 일부 실시양태에서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,803의 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 상류에 있다. 일부 실시양태에서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,740의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 하류에 있다. 일부 실시양태에서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,740의 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 하류에 있다. 일부 실시양태에서, SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분은 서열 번호 2 또는 7 내지 10의 적어도 8개의 인접한 핵산을 포함하는 영역과 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함한다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 서열 번호 21 내지 67, 210 내지 256, 또는 304 내지 379 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함한다. 일부 실시양태에서, SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 넌센스 매개 RNA 붕괴 유도 엑손 20x 내에 있다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 42 내지 50, 또는 231 내지 239 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함한다. 일부 실시양태에서, SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 넌센스 매개 RNA 붕괴 유도 엑손 20x의 상류 또는 하류에 있다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21 내지 38, 53 내지 67, 210 내지 227, 또는 242 내지 256 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함한다. 일부 실시양태에서, NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 엑손 20x의 엑손-인트론 접합부를 포함한다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 39 내지 41, 51, 52, 228 내지 230, 240, 또는 241 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함한다. 일부 실시양태에서, 치료제는 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 촉진한다. 일부 실시양태에서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제는 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제와 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배

증가한다. 일부 실시양태에서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 증가시킨다. 일부 실시양태에서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 양은 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가한다. 일부 실시양태에서, 치료제는 세포에서 SCN1A 단백질의 발현을 증가시킨다. 일부 실시양태에서, 치료제와 접촉된 세포에서 생성된 SCN1A의 양은 대조군 세포에서 생성된 SCN1A의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가한다. 일부 실시양태에서, 치료제는 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 억제한다. 일부 실시양태에서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제는 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제와 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소한다. 일부 실시양태에서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 감소시킨다. 일부 실시양태에서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 양은 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소한다. 일부 실시양태에서, 치료제는 세포에서 SCN1A 단백질의 발현을 감소시킨다. 일부 실시양태에서, 치료제와 접촉된 세포에서 생성된 SCN1A의 양은 대조군 세포에서 생성된 SCN1A의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소한다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 포스포로티오에이트 결합 또는 포스포로디아미데이트 결합을 포함하는 백본 변형을 포함한다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 포스포로디아미데이트 모르폴리노, 잠금 핵산, 펩티드 핵산, 2'-O-메틸, 2'-플루오로, 또는 2'-O-메톡시에틸 모이어티를 포함한다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 하나 이상의 변형된 당 모이어티를 포함한다. 일부 실시양태에서, 각각의 당 모이어티는 변형된 당 모이어티이다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 8 내지 50개의 핵염기, 8 내지 40개의 핵염기, 8 내지 35개의 핵염기, 8 내지 30개의 핵염기, 8 내지 25개의 핵염기, 8 내지 20개의 핵염기, 8 내지 15개의 핵염기, 9 내지 50개의 핵염기, 9 내지 40개의 핵염기, 9 내지 35개의 핵염기, 9 내지 30개의 핵염기, 9 내지 25개의 핵염기, 9 내지 20개의 핵염기, 9 내지 15개의 핵염기, 10 내지 50개의 핵염기, 10 내지 40개의 핵염기, 10 내지 35개의 핵염기, 10 내지 30개의 핵염기, 10 내지 25개의 핵염기, 10 내지 20개의 핵염기, 10 내지 15개의 핵염기, 11 내지 50개의 핵염기, 11 내지 40개의 핵염기, 11 내지 35개의 핵염기, 11 내지 30개의 핵염기, 11 내지 25개의 핵염기, 11 내지 20개의 핵염기, 11 내지 15개

의 핵염기, 12 내지 50개의 핵염기, 12 내지 40개의 핵염기, 12 내지 35개의 핵염기, 12 내지 30개의 핵염기, 12 내지 25개의 핵염기, 12 내지 20개의 핵염기, 또는 12 내지 15개의 핵염기로 이루어진다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 단백질을 코딩하는 NMD 엑손 mRNA의 표적화된 부분에 적어도 80%, 적어도 85%, 적어도 90%, 적어도 95%, 적어도 98%, 적어도 99%, 또는 100% 상보적이다. 일부 실시양태에서, 방법은 SCN1A mRNA 또는 단백질 발현을 평가하는 단계를 추가로 포함한다. 일부 실시양태에서, 질환 또는 병태는 $Na_v1.1$의 기능 상실 돌연변이에 의해 유발된다. 일부 실시양태에서, 질환 또는 병태는 *SCN1A* 유전자의 반가불충분성과 관련되며, 대상체는 기능성 SCN1A를 코딩하는 제1 대립 유전자, 및 SCN1A가 생성되지 않거나 감소한 수준으로 생성되는 제2 대립 유전자, 또는 비기능성 SCN1A 또는 부분 기능성 SCN1A를 코딩하는 제2 대립 유전자를 갖는다. 일부 실시양태에서, 질환 또는 병태는 뇌병증이다. 일부 실시양태에서, 뇌병증은 간질성 뇌병증이다. 일부 실시양태에서, 질환 또는 병태는 드라벳 증후군(DS); 유아기의 중증 근간대성 뇌전증(SMEI: severe myoclonic epilepsy of infancy) 경계성(borderland)(SMEB); 열성 발작(FS: Febrile seizure); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+: epilepsy, generalized, with febrile seizures plus); 조기 유아 간질성 뇌병증 13(epileptic encephalopathy, early infantile, 13); 잠재성(cryptogenic) 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 뇌전증에서의 돌연사(SUDEP: sudden unexpected death in epilepsy); 동기능 부전 증후군 1(sick sinus syndrome 1); 자폐증; 또는 유아기의 악성 이동 부분 발작이다. 일부 실시양태에서, GEFS+는 열성 발작 플러스를 동반하는 전신 뇌전증 2형(epilepsy, generalized, with febrile seizures plus, type 2)이다. 일부 실시양태에서, 열성 발작은 가족성 열성 발작 3A(Febrile seizures, familial, 3A)이다. 일부 실시양태에서, SMEB는 전신 극파가 없는 SMEB(SMEB-SW: SMEB without generalized spike wave), 근간대성 발작이 없는 SMEB(SMEB-M: SMEB without myoclonic seizures), SMEI의 하나 초과의 특징이 결여된 SMEB(SMEB-O: SMEB lacking more than one feature of SMEI), 또는 전신 강직성 간대 발작을 동반한 난치성 소아기 뇌전증(ICEGTC: intractable childhood epilepsy with generalized tonic-clonic seizures)이다. 일부 실시양태에서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 촉진하고 SCN1A의 발현을 증가시킨다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 22 내지 24, 26, 27, 29 내지 35, 37 내지 62, 64 내지 67, 또는 304 내지 379 중 어느 하나에 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 서열을 포함한다. 일부 실시양태에서, 질환 또는 병태는 $Na_v1.1$의 기능 획득 돌연변이에 의해 유발된다. 일부 실시양태에서, 대상체는 세포에서 SCN1A가 증가한 수준으로 생성되는 대립 유전자, 또는 $Na_v1.1$의 증가한 활성을 유도하는 돌연변이 SCN1A를 코딩하는 대립 유전자를 갖는다. 일부 실시양태에서, 질환 또는 병태는 편두통이다. 일부 실시양태에서, 편두통은 가족성 편마비 편두통 3(migraine, familial hemiplegic, 3)이다. 일부 실시양태에서, 질환 또는 병태는 $Na_v1.1$ 유전성 뇌전증이다. 일부 실시양태에서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 억제하고 SCN1A의 발현을 감소시킨다. 일부 실시양태에서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21, 25, 28, 36 또는 63 중 어느 하나에 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 서열을 포함한다. 일부 실시양태에서, 대상체는 인간이다. 일부 실시양태에서, 대상은 비인간 동물이다. 일부 실시양태에서, 대상체는 태아, 배아, 또는 소아이다. 일부 실시양태에서, 치료제는 대상체의 수막공간내(intrathecal) 주사, 뇌실내(intracerebroventricular) 주사, 복강내 주사, 근육내 주사, 피하 주사, 안내(intravitreal) 주사, 또는 정맥내 주사에 의해 투여된다. 일부 실시양태에서, 방법은 대상체에게 제2 치료제를 투여하는 단계를 추가로 포함한다. 일부 실시양태에서, 제2 치료제는 소분자이다. 일부 실시양태에서, 제2 치료제는 ASO이다. 일부 실시양태에서, ASO는 서열 번호 115 내지 161 중 어느 하나에 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 서열을 포함한다. 일부 실시양태에서, 제2 치료제는 인트론 유지(intron retention)를 교정한다. 일부 실시양태에서, 질환 또는 병태는 알츠하이머병, SCN2A 뇌병증, SCN8A 뇌병증, 또는 SCN5A 부정맥이다. 일부 실시양태에서, 질환 또는 병태는 알츠하이머병, SCN2A 뇌병증, SCN8A 뇌병증, 또는 SCN5A 부정맥이다. 일부 실시양태에서, 세포는 생체 외 세포이다.

[0008] **참고의 포함**

[0009] 본 명세서에서 언급된 모든 간행물, 특허, 및 특허 출원은 각각의 개별 간행물, 특허, 또는 특허 출원이 참고로 포함된 것으로 구체적이고 개별적으로 명시된 것과 동일한 정도로 본원에 참고로 포함된다.

도면의 간단한 설명

[0010] 본 발명의 신규 특징은 첨부된 청구 범위에 구체적으로 기재되어 있다. 본 발명의 특징 및 장점은 본 발명의 원

리가 이용되는 예시적인 실시양태 및 하기의 첨부 도면을 설명하는 다음의 상세한 설명을 참조하여 더 잘 이해될 것이다:

[도 1]은 넌센스 매개 mRNA 붕괴 유도 엑손을 함유하는 표적 mRNA(NMD 엑손 mRNA), 및 전장의 표적 단백질 또는 기능성 RNA의 발현을 증가시키는 넌센스 매개 mRNA 붕괴 유도 엑손의 치료제 매개 배제의 개략도를 도시한다. [도 1a]는 핵 및 세포질 구획으로 분할된 세포를 나타낸다. 핵에서, 표적 유전자의 프리-mRNA(pre-mRNA) 전사체는 스플라이싱을 거쳐 mRNA를 생성하고, 이 mRNA는 세포질로 유출되고 표적 단백질로 번역된다. 이 표적 유전자에 대해, mRNA의 일부 분획은 세포질에서 분해되는 넌센스 매개 mRNA 붕괴 유도 엑손을 함유하여(NMD 엑손 mRNA) 표적 단백질 생산으로 이어지지 않는다. [도 1b]는 핵 및 세포질 구획으로 분할된 동일한 세포의 예를 보여준다. 안티센스 올리고머(ASO)와 같은 치료제에 의한 치료는 넌센스 매개 mRNA 붕괴 유도 엑손의 배제를 촉진하고 mRNA의 증가를 초래하며, 이는 결국 더 높은 수준의 표적 단백질로 번역된다. [도 1c]는 넌센스 매개 mRNA 붕괴 유도 엑손의 치료적 ASO 매개 배제를 개략적으로 나타내며, 이는 비생산적 mRNA를 생산적 mRNA로 바꾸고 생산적 mRNA로부터 전장의 표적 단백질의 발현을 증가시킨다.

[도 2]는 *SCN1A* 유전자에서 예시적인 넌센스 매개 mRNA 붕괴(NMD) 유도 엑손의 확인을 도시한다. 비교 게놈학을 사용하여 *SCN1A* 유전자에서 NMD 유도 엑손의 확인을 UCSC 게놈 브라우저에서 시각화하여 나타낸다. 상단 패널은 스케일링할 SCN1A 유전자를 도식적으로 표현하여 나타낸다. 100종의 척추동물 종에 대한 보존 수준을 피크로 나타낸다. 가장 높은 피크는 엑손(검은 박스)에 해당하는 한편, 대부분의 인트론(화살표가 있는 선)에는 피크가 관찰되지 않는다. 중간 패널에 나타낸 인트론 20(NM_006920)에서 보존 피크가 확인된다. 보존된 서열을 검사하여 3' 및 5' 스플라이스 부위(밑줄 친 서열)의 측면에 위치한 64 bp의 엑손 유사 서열(하부 패널, 회색으로 강조된 서열)을 확인하였다. 이 엑손의 포함은 프레임 시프트 및 엑손 21에 조기 종결 코돈의 도입을 초래하여 전사체를 표적으로 만든다.

[도 3a]는 시클로헥시미드 처리를 통한 NMD 유도 엑손의 확증을 도시한다. DMSO 처리(CHX-) 또는 시클로헥시미드 처리(CHX+) Neuro 2A(마우스 신경 전구 세포)의 세포질 RNA 및 엑손 21 및 하류 엑손 내 프라이머를 사용한 RT-PCR 분석으로 NMD 유도 엑손(21x)에 상응하는 밴드의 존재를 확증하였다. 생성물의 동일성은 서열분석에 의해 확증하였다. 밴드의 밀도 측정 분석을 수행하여 총 *SCN1A* 전사체의 퍼센트 엑손 21x 포함을 계산하였다. NMD를 억제하는 시클로헥시미드로 Neuro 2A의 처리(CHX+)는 세포질 분획에서 NMD 유도 엑손 21x에 상응하는 생성물의 2배 증가를 초래하였다(연회색 막대, CHX-와 진회색 막대, CHX+ 비교).

[도 3b]는 시클로헥시미드 처리를 통한 NMD 유도 엑손의 확증을 도시한다. DMSO 처리(CHX-) 또는 시클로헥시미드 처리(CHX+) RenCell VM(인간 신경 전구 세포)의 세포질 RNA 및 엑손 20 및 엑손 23 내 프라이머를 사용한 RT-PCR 분석으로 NMD 유도 엑손(20x)에 상응하는 밴드의 존재를 확증하였다. 생성물의 동일성은 서열분석에 의해 확증하였다. 밴드의 밀도 측정 분석을 수행하여 총 *SCN1A* 전사체의 퍼센트 엑손 20x 포함을 계산하였다. NMD를 억제하는 시클로헥시미드로 RenCell VM의 처리(CHX+)는 세포질 분획에서 NMD 유도 엑손 20x에 상응하는 생성물의 2배 증가를 초래하였다(참조: 연회색 막대, CHX-, 진회색 막대, CHX+).

[도 4]는 예시적인 *SCN1A* 엑손 20x 영역 ASO 워크(walk)를 도시한다. 2'-MOE ASO, PS 백본을 사용하여 3' 스플라이스 부위의 상류 서열, 3' 스플라이스 부위에 걸친 서열, 엑손 20x 서열, 5' 스플라이스 부위에 걸친 서열, 및 5' 스플라이스 부위의 하류 서열을 표적화하는 *SCN1A* 엑손 20x 영역에 수행된 ASO 워크를 도식으로 표현하여 나타낸다. ASO는 한 번에 5 뉴클레오티드를 이동시켜 이들 영역을 덮도록 설계하였다.

[도 5a]는 RT-PCR에 의해 평가된 *SCN1A* 엑손 20x 영역 ASO 워크를 도시한다. 대표적인 PAGE는 짐노틱(gymnotic) 흡수에 의해 RenCell VM 세포에서 모의 처리된(Sham), SMN-대조군 ASO 처리된(SMN), 또는 실시예 및 [도 4]의 설명에서 본원에 기재된 바와 같이 엑손 20x 영역을 표적화하는 20 μM 농도의 2'-MOE ASO로 처리된 SCN1A의 SYBR-세이프(SYBR-safe) 염색된 RT-PCR 생성물을 보여준다. 엑손 20x 포함(상부 밴드) 및 전장(엑손 20x 배제, 하부 밴드)에 해당하는 2개의 생성물을 정량화하였다.

[도 5b]는 [도 5a]의 데이터로부터 퍼센트 엑손 20x 포함을 플로팅한 그래프를 도시한다. 검은 선은 Sham에 대해 변화없음을 나타낸다.

[도 5c]는 *RPL32* 내부 대조군에 정규화된 전장 생성물의 그래프를 도시하고 Sham에 대한 배수 변화를 플로팅한다. 검은 선은 Sham에 대하여 1의 비율 및 변화없음을 나타낸다.

[도 6]은 RT-qPCR에 의해 평가된 예시적인 *SCN1A* 엑손 20x 영역 ASO 워크를 도시한다. [도 5]에 나타낸 바와 같이 SYBR-세이프 RT-PCR에 의해 평가된 동일한 ASO 흡수 실험을 사용하여 얻은, *RPL32*에 정규화된 SYBR-그린 RT-

qPCR *SCN1A* 증폭 결과는 Sham에 대한 배수 변화로서 플로팅하며, 이는 SYBR-세이프 RT-PCR 결과를 확증해준다. 검은 선은 1의 비율(Sham과 대하여 변화없음)을 나타낸다.

[도 7a]는 나트륨 전압 게이팅 채널 알파 서브유닛 구성원의 표를 도시한다. 화살표는 [도 7b]의 막대 색에 해당한다. X는 발현이 검출되지 않음을 나타낸다.

[도 7b]는 표적 선택도를 평가하기 위해 *SCN1A*, *SCN2A*, *SCN3A*, *SCN8A*, 및 *SCN9A*의 태크맨(Taqman) qPCR에 의해 평가된 선택된 ASO를 도시한다. Ex20x+1, IVS20x+18, 및 IVS20x+33 ASO를 사용하여 얻은, *RPL32*에 정규화된 태크맨-qPCR 증폭 결과는 Sham에 대한 배수 변화로서 플로팅한다. 검은 선은 1의 비율(Sham과 대하여 변화없음)을 나타낸다.

[도 8a]는 CXH 처리된 세포에서 선택된 ASO의 예시적인 용량 의존적 효과를 도시한다. RNAiMAX 형질 감염에 의해 Neuro 2A(마우스 신경 모세포종) 세포에서 모의 처리된(Sham, RNAiMAX 단독), 또는 엑손 21x(마우스 명명법, 인간 엑손 20x에 해당)를 표적화하는 30nM, 80nM, 및 200nM 농도의 Ex21x+1 2'-MOE ASO로 처리된 마우스 *Scn1a*의 SYBR-세이프 염색된 RT-PCR 생성물을 나타내는 대표적인 PAGE를 나타낸다. Ex21x+1(마우스 명명법)과 Ex20x+1(인간 명명법)은 동일하다. 엑손 20x 포함(상부 밴드) 및 전장(엑손 20x 배제, 하부 밴드)에 해당하는 2개의 생성물을 정량화하였다.

[도 8b]는 [도 7a]의 데이터로부터 퍼센트 엑손 20x 포함을 플로팅한 그래프를 도시한다. 검은 선은 Sham에 대하여 변화없음을 나타낸다.

[도 8c]는 *Hprt* 내부 대조군에 정규화된 전장 생성물의 예시적인 그래프를 도시하고, Sham에 대한 배수 변화를 플로팅한다. 검은 선은 Sham에 대하여 1의 비율 및 변화없음을 나타낸다.

[도 9a]는 C57BL6J 마우스(수컷, 3개월령)에서 선택된 ASO의 안내(IVT) 주사의 예시적인 결과를 도시한다. PBS 주사된(1μL) 좌안(-) 또는 IVS20x-21, Ex21x+1, IVS21x+18, IVS21x+33 또는 Cep290(음성 대조군 ASO; Gerard *et al, Mol. Ther. Nuc. Ac.*, 2015), 10mM 농도의 2'-MOE ASO 주사된(1μL) 우안(+)으로부터 마우스 *Scn1a*의 SYBR-세이프 염색된 RT-PCR 생성물의 PAGE 겔을 나타낸다. Ex21x+1, IVS21x+18, 및 IVS21x+33(마우스 명명법)과 Ex20x+1, IVS20x+18, 및 IVS20x+33(인간 명명법)은 동일하다. 엑손 21x 포함(상부 밴드) 및 전장(엑손 21x 배제, 하부 밴드)에 해당하는 2개의 생성물을 정량화하였다.

[도 9b]는 [도 9a]의 데이터로부터 퍼센트 엑손 21x 포함을 플로팅한 그래프를 도시한다. 흰색 막대는 ASO 주사된 눈에 해당하고 회색 막대는 PBS 주사된 눈에 해당한다(각 그룹에서 n = 5).

[도 9c]는 *Gapdh* 내부 대조군에 정규화된 전장 생성물의 그래프를 도시하며 PBS 주사된 눈에 대한 ASO 주사된 눈의 배수 변화를 플로팅한다. 검은 선은 PBS에 대하여 1의 비율 및 변화없음을 나타낸다(각 그룹에서 n = 5).

[도 10a]는 C57BL6J 마우스(수컷, 3개월령)에서 선택된 ASO의 뇌실내(ICV) 주사의 예시적인 결과를 도시한다. 주사되지 않은(-, ASO 없는 대조군) 또는 300 μg의 Cep290(음성 대조군 ASO; Gerard *et al, Mol. Ther. Nuc. Ac.*, 2015), Ex21x+1, IVS21x+18, IVS21x+33 2'-MOE ASO 주사된 뇌로부터 마우스 *Scn1a*의 SYBR-세이프 염색된 RT-PCR 생성물의 PAGE 겔을 나타낸다. Ex21x+1, IVS21x+18, 및 IVS21x+33(마우스 명명법)과 Ex20x+1, IVS20x+18, 및 IVS20x+33(인간 명명법)은 동일하다. 엑손 21x 포함(상부 밴드) 및 전장(엑손 21x 배제, 하부 밴드)에 해당하는 2개의 생성물을 정량화하였다.

[도 10b] 는 [도 10a]의 데이터로부터 퍼센트 엑손 21x 포함을 플로팅한 그래프를 도시한다(n = 6(각각의 표적화 ASO), n = 5(Cep290 ASO), n = 1(주사되지 않음, ASO 없는 대조군)).

[도 10c]는 엑손 21 및 22 접합부에 걸친 2개의 상이한 프로브를 사용하여 수행된 태크맨 qPCR 분석 결과의 그래프를 도시한다. 생성물을 *Gapdh* 내부 대조군에 정규화하였고 Cep290 주사된 뇌에 대한 ASO 주사된 뇌의 배수 변화를 플로팅한다. 검은 선은 Cep290에 대하여 1의 비율 및 변화없음을 나타낸다(n = 6(각각의 표적화 ASO), n = 5(Cep290 ASO), n = 1(주사되지 않음, ASO 없는 대조군)).

[도 11a]는 C57BL6J 마우스(수컷, 3 개월령)에서 선택된 ASO의 뇌실내(ICV) 주사의 예시적인 결과를 도시한다. 300ug의 Cep290(음성 대조군 ASO; Gerard *et al, Mol. Ther. Nuc. Ac.*, 2015), 또는 33ug, 100ug, 및 300ug의 Ex21x+1 2'-MOE ASO 주사된 뇌로부터 마우스 *Scn1a*의 SYBR-세이프 염색된 RT-PCR 생성물의 PAGE 겔을 나타낸다. Ex21x+1(마우스 명명법)과 Ex20x+1(인간 명명법)은 동일하다. 엑손 21x 포함(상부 밴드) 및 전장(엑손 21x 배제, 하부 밴드)에 해당하는 2개의 생성물을 정량화하였다.

[도 11b]는 [도 11a]의 데이터(각 그룹 n = 5)로부터 퍼센트 엑손 21x 포함을 플로팅한 그래프를 도시한다.

[도 11c]는 엑손 21 및 22 접합부에 걸친 2개의 상이한 프로브를 사용하여 수행된 태크맨 qPCR 분석 결과의 그래프를 도시한다. 생성물을 *Gapdh* 내부 대조군에 정규화하였고 Cep290 주사된 뇌에 대한 ASO 주사된 뇌의 배수 변화를 플로팅한다. 검은 선은 Cep290에 대하여 1의 비율 및 변화없음을 나타낸다(각 그룹 n = 5).

[도 12a]는 C57BL6J 마우스(생후 2일)에서 선택된 ASO의 뇌실내(ICV) 주사의 예시적인 결과를 도시한다. 주사되지 않은(-, ASO 없는 대조군), 또는 20ug의 Ex21x+1 2'-MOE ASO 주사된 뇌로부터 마우스 *Scn1a*의 SYBR-세이프 염색된 RT-PCR 생성물의 PAGE 겔을 나타낸다. 엑손 21x 포함(상부 밴드) 및 전장(엑손 21x 배제, 하부 밴드)에 해당하는 2개의 생성물을 정량화하였다. Ex21x+1(마우스 명명법)과 Ex20x+1(인간 명명법)은 동일하다.

[도 12b]는 [도 12a]의 데이터(각 그룹 n = 4)로부터 퍼센트 엑손 21x 포함을 플로팅한 그래프를 도시한다.

[도 12c]는 엑손 21 및 22 접합부에 걸친 2개의 상이한 프로브를 사용하여 수행된 태크맨 qPCR 분석 결과의 그래프를 도시한다. 생성물을 *Gapdh* 내부 대조군에 정규화하였고 ASO 없는 대조군 뇌에 대한 ASO 주사된 뇌의 배수 변화를 플로팅한다. 검은 선은 ASO 없는 대조군에 대하여 1의 비율 및 변화없음을 나타낸다(각 그룹 n = 4).

[도 13a]는 지시된 마우스 CNS 샘플에 퍼센트 엑손 21x 포함을 플로팅한 그래프를 도시한다.

[도 13b]는 지시된 인간 CNS 샘플에서 퍼센트 엑손 20x 포함을 플로팅한 그래프를 도시한다.

[도 14a]는 지시된 용량에서 엑손 21x 포함의 퍼센트 감소를 플로팅한 그래프를 도시한다.

[도 14b]는 지시된 용량에서 Scn1a mRNA의 퍼센트 증가를 플로팅한 그래프를 도시한다.

[도 14c]는 지시된 용량에서 Nav 1.1 단백질 수준의 퍼센트 증가를 플로팅한 그래프를 도시한다.

[도 15a]는 지시된 용량에서 엑손 21x 포함의 퍼센트 감소를 플로팅한 그래프를 도시한다.

[도 15b]는 지시된 용량에서 Scn1a mRNA의 퍼센트 증가를 플로팅한 그래프를 도시한다.

[도 16]은 표적 선택도를 평가하기 위해 생후 2일 마우스에서 ICV 주사를 통해 10ug 용량으로 투여된 선택된 *Scn1a* 표적화 ASO를 주사 후 5일에 *SCN1A, SCN2A, SCN3A, SCN4A, SCN5A, SCN7A, SCN8A, SCN9A, SCN10A,* 및 *SCN11A*의 태크맨 qPCR에 의해 평가하여 도시한다. Ex20x+1 ASO를 사용하여 얻은, *Gapdh*에 정규화된 태크맨-qPCR 증폭 결과는 PBS 주사된 마우스에 대한 배수 변화로서 플로팅한다.

[도 17]은 야생형(WT) 또는 129S-Scn1a^{tm1Kea} x C57BL/6J 교배의 이종 접합 드라벳 마우스(HET) F1 마우스에서 지시된 용량의 선택된 ASO의 출생 2일 후 뇌실내(ICV) 주사로부터 주사 3일 후의 예시적인 결과를 나타낸다.

[도 17a]는 엑손 21 및 22에 걸친 프로브를 사용하여 수행된 태크맨 qPCR 분석 결과의 그래프를 도시한다. 생성물을 *Gapdh* 내부 대조군에 정규화하였고 PBS 주사된 뇌에 대한 ASO 주사된 뇌의 배수 변화를 플로팅한다.

[도 17b]는 항-Nav1.1 항체를 사용하여 수행된 웨스턴 블롯의 결과의 그래프를 도시한다. 생성물을 폰소(Ponceau) 염색된 밴드에 정규화하였고 PBS 주사된 뇌에 대한 ASO 주사된 뇌의 배수 변화를 플로팅한다.

[도 18]은 자유 흡수를 통한 RenCell에서의 *SCN1A* 엑손 20x 영역 ASO 마이크로 워크의 예시적인 결과를 도시한다. ASO는 한 번에 1 뉴클레오티드를 이동 하거나(6 내지 41) ASO 17의 길이를 줄임으로써(1 내지 5) [도 6]에서 이전에 확인된 3개의 표적화 ASO(별표로 표시됨) 주위의 영역을 덮도록 설계하였다. 그래프는 SYBR-그린 qPCR에 의해 측정된 퍼센트 엑손 20x 포함을 도시한다. 검은 선은 ASO가 없는 것(-)에 대하여 변화없음을 나타낸다.

[도 19]는 *SCN1A* 표적화 ASO의 주사 후 시간 경과에 따른 마우스의 관상 뇌 슬라이스에서 *Scn1a* mRNA의 수준증가를 플로팅한 그래프이다. 도시된 바와 같이, *Scn1a* mRNA의 수준증가는 주사 후 적어도 80일 동안 유지되었다.

[도 20]은 드라벳 마우스 모델에서 *SCN1A* 표적화 ASO에 의해 제공되는 100% 생존 이점을 입증하는 예시적인 생존 곡선이다. +/+는 WT 유전자형을 나타내고, +/-는 129S-scn1a^{tm1Kea} 이형 접합 유전자형(드라벳 마우스 모델)을 나타내고; A는 PBS 처리를 나타내고, B는 ASO 처리를 나타낸다. 도시된 바와 같이, A +/- 그룹의 마우스(PBS 처리를 받은 드라벳 마우스)는 대략 생후 16일부터 죽기 시작했던 반면, B +/- 그룹(ASO 처리를 받은 드라벳 마우스)을 포함하는 다른 세 그룹의 모든 마우스는 적어도 생후 35일까지 생존하였다.

발명을 실시하기 위한 구체적인 내용

[0011] **스플라이싱 및 넌센스 매개 mRNA 붕괴**

[0012] 개재 서열 또는 인트론은 스플라이스좀이라 불리는 크고 매우 역동적인 RNA-단백질 복합체에 의해 제거되며, 이는 1차 전사체, 작은 핵 RNA(snRNA) 및 다수의 단백질 사이의 복잡한 상호 작용을 조정한다. 스플라이스좀은 U1 snRNA에 의한 5' 스플라이스 부위(5'ss) 또는 U2 경로에 의한 3' 스플라이스 부위(3'ss)의 인식으로 시작하여, 순차적인 방식으로 각 인트론에 특별하게 조립되며, 이는 3'ss의 영역에 U2 보조 인자(U2AF)의 결합을 수반하여 분지점 서열(BPS: branch point sequence)에 U2 결합을 용이하게 한다. U2AF는 폴리피리미딘 트랙(PPT: polypyrimidine tract)에 결합하는 U2AF2-코딩된 65 kD 서브유닛(U2AF65), 및 3'ss에서 고도로 보존된 AG 디뉴클레오티드와 상호 작용하고 U2AF65 결합을 안정화시키는 U2AF1-코딩된 35 kD 서브유닛(U2AF35)으로 구성된 안정한 이종 이량체이다. 정확한 스플라이싱에는 BPS/PPT 유닛 및 3'ss/5'ss 외에도 인트론 또는 엑손 스플라이싱 인핸서 또는 사일런서로 알려진, 스플라이스 부위 인식을 활성화 또는 억제하는 보조 서열 또는 구조가 필요하다. 이들 요소는 동일한 서열을 갖지만 진짜 부위가 수십 배나 더 많은 고등 진핵 세포의 게놈에서 광대한 과량의 잠재 또는 가성(pseudo) 부위 중에서 진짜 스플라이스 부위를 인식할 수 있게 한다. 이들은 종종 조절 기능을 갖지만, 이들의 활성화 또는 억제의 정확한 메커니즘은 잘 이해되지 않고 있다.

[0013] 스플라이스 여부에 대한 결정은 전형적으로 결정론적 과정보다는 확률론적 과정으로 모델링될 수 있어, 가장 결정적인 스플라이싱 신호조차 때때로 부정확하게 스플라이싱될 수 있다. 그러나 정상적인 조건하에서, 프리-mRNA 스플라이싱은 놀랍도록 높은 정확도로 진행된다. 이는 인접한 시스 작용 보조 엑손 및 인트론 스플라이싱 조절 요소(ESR 또는 ISR)의 활성에 부분적으로 기인한다. 전형적으로, 이들 기능적 요소는 스플라이싱을 자극하거나 억제하는 능력에 기초하여 각각 엑손 또는 인트론 스플라이싱 인핸서(ESE 또는 ISE) 또는 사일런서(ESS 또는 ISS)로 분류된다. 현재 일부 보조 시스 작용 요소가 U1 snRNP와 5'ss 사이의 복합체의 배열과 같은 스플라이스좀 어셈블리의 동역학에 영향을 미침으로써 작용할 수 있다는 증거가 있지만, 많은 요소는 트랜스 작용 RNA 결합 단백질(RBP)과 협력하여 기능을 할 가능성이 매우 크다. 예를 들어, 세린 및 아르기닌이 풍부한 RBP 패밀리(SR 단백질)는 엑손을 정의하는 데 중요한 역할을 하는 보존된 단백질 패밀리이다. SR 단백질은 프리-스플라이스좀의 성분을 인접한 스플라이스 부위로 동원하거나 근처에서 ESS의 효과를 길항함으로써 엑손 인식을 촉진한다. ESS의 억제 효과는 이종 핵 리보핵 단백질(hnRNP) 패밀리의 구성원에 의해 매개될 수 있으며, 인접 스플라이스 부위로의 코어 스플라이싱 인자의 동원을 변경할 수 있다. 스플라이싱 조절에서의 역할 외에도, 사일런서 요소는 전형적인 엑손 간격을 갖지만 기능적인 오픈 리딩 프레임이 없는 붕괴 인트론 스플라이스 부위 세트인 가성 엑손(pseudo exon)의 억제에 역할을 할 것으로 제안된다. ESE와 ESS는 동족 트랜스 작용 RBP와 협력하여 mRNA가 그들의 전구체로부터 언제, 어디서, 그리고 어떻게 조립되지를 지정하는 일련의 스플라이싱 제어에서 중요한 구성 요소를 나타낸다.

[0014] 엑손-인트론 경계를 표시하는 서열은 인간 유전자 내에서 높은 빈도로 발생할 수 있는 다양한 강도의 축퇴성 신호이다. 다중 엑손 유전자에서, 상이한 쌍의 스플라이스 부위가 많은 상이한 조합으로 서로 연결되어 단일 유전자로부터 다양한 전사체를 생성할 수 있다. 이것을 일반적으로 선택적 프리-mRNA 스플라이싱이라고 한다. 선택적 스플라이싱에 의해 생성된 대부분의 mRNA 아이소형이 핵으로부터 유출되어 기능성 폴리펩티드로 번역될 수 있지만, 단일 유전자로부터의 상이한 mRNA 아이소형은 그들의 번역 효율이 크게 달라질 수 있다. 엑손 접합부 복합체의 적어도 50 bp 상류에 조기 종결 코돈(PTC: premature termination codon)을 갖는 mRNA 아이소형은 넌센스 매개 mRNA 붕괴(NMD) 경로에 의한 분해에 대해 표적화될 가능성이 있다. 전통적(BPS/PPT/3'ss/5'ss) 및 보조적 스플라이싱 모티프에서의 돌연변이는 엑손 스키핑 또는 잠재성(또는 가성) 엑손 포함 또는 스플라이스 부위 활성화와 같은 비정상적인 스플라이싱을 유발할 수 있으며, 인간 이환율 및 사망률에 상당한 원인이 된다. 비정상적 및 선택적 스플라이싱 양상은 둘 다 엑손 및 인트론에서의 천연 DNA 변이에 의해 영향받을 수 있다.

[0015] 엑손-인트론 경계가 코돈의 3개 위치 중 어느 하나에서 발생할 수 있다고 가정하면, 선택적 스플라이싱 이벤트의 서브 세트만이 표준 오픈 리딩 프레임을 유지할 수 있다는 것은 명백하다. 예를 들어, 균등하게 3으로 나눌 수 있는 엑손만이 리딩 프레임을 변경하지 않고 스키핑되거나 mRNA에 포함될 수 있다. 적합한 상태가 없는 스플라이싱 이벤트는 프레임 시프트를 유발할 것이다. 하류 이벤트에 의해 역전되지 않는 한, 프레임 시프트는 확실히 하나 이상의 PTC로 이어질 수 있으며, 이는 아마도 NMD에 의한 후속 분해를 초래할 수 있다. NMD는 PTC를 함유하는 mRNA를 제거하는 번역 결합된 기전이다. NMD는 모든 진핵생물에 존재하는 감시 경로로서 기능을 할 수 있다. NMD는 조기 정지 코돈을 함유하는 mRNA 전사체를 제거함으로써 유전자 발현에서 오류를 감소시킬 수 있다. 이들 비정상적 mRNA의 번역은, 일부 경우에, 생성된 단백질의 유해한 기능 획득 또는 우성의 음성적 활성

으로 이어질 수 있다. NMD는 PTC가 있는 전사체뿐 아니라 많은 내인성 유전자로부터 발현된 광범위한 mRNA 아이소형을 표적화하며, 이는 NMD가 세포의 정상 상태(steady-state) RNA 수준에서 미세 조정과 대충 조정을 모두 유도하는 마스터 조절 인자임을 시사한다.

[0016] NMD 유도 엑손(NIE)은 인트론 내의 영역인 엑손 또는 가성 엑손이며, 성숙한 RNA 전사체에 포함되는 경우 NMD 경로를 활성화시킬 수 있다. 구성적 스플라이싱 이벤트에서, NIE를 함유하는 인트론은 일반적으로 스플라이싱되지만, 인트론 또는 그 일부(예를 들어, NIE)는 선택적 또는 비정상적 스플라이싱 이벤트 중에 유지될 수 있다. 이러한 NIE를 함유하는 성숙한 mRNA 전사체는 NMD 경로를 유도하는 프레임 시프트로 인해 비생산적일 수 있다. 성숙한 RNA 전사체에 NIE가 포함되면 유전자 발현을 하향 조절할 수 있다. NIE를 함유하는 mRNA 전사체는 본 개시 내용에서 "NIE 함유 mRNA" 또는 "NMD 엑손 mRNA"로 지칭될 수 있다.

[0017] 잠재성(또는 가성 스플라이스 부위)은 진짜 스플라이스 부위와 동일한 스플라이싱 인식 서열을 갖지만 스플라이싱 반응에는 사용되지 않는다. 이들은 인간 게놈에서 진짜 스플라이스 부위보다 수십 배 더 많으며 일반적으로 이렇게 잘 이해되지 않은 분자 기전에 의해 억제된다. 잠재성 5' 스플라이스 부위는 컨센서스 NNN/GUNNNN 또는 NNN/GCNNNN을 가지며, 여기서 N은 임의의 뉴클레오티드이고 엑손-인트론 경계이다. 잠재성 3' 스플라이스 부위는 컨센서스 NAG/N을 가진다. 이들의 활성화는 진짜 스플라이스 부위, 즉 각각 MAG/GURAGU 및 YAG/G(여기서 M은 C 또는 A, R은 G 또는 A, Y는 C 또는 U임)의 최적의 컨센서스와 더 유사하게 만드는 주변 뉴클레오티드에 의해 양성적으로 영향을 받는다.

[0018] 스플라이스 부위 및 그의 조절 서열은 예를 들어, 문헌[Kralovicova, J. and Vorechovsky, I. (2007) Global control of aberrant splice site activation by auxiliary splicing sequences: evidence for a gradient in exon and intron definition. *Nucleic Acids Res.*, 35, 6399-6413,(http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2095810/pdf/gkm680.pdf)]에 열거된 공개적으로 이용 가능한 적합한 알고리즘을 사용하여 당업자에 의해 용이하게 확인될 수 있다.

[0019] 잠재성 스플라이스 부위 또는 스플라이싱 조절 서열은 U2AF와 같은 RNA 결합 단백질에 대해 NIE의 스플라이스 부위와 경쟁할 수 있다. 일 실시양태에서, 작용제는 잠재성 스플라이스 부위 또는 스플라이싱 조절 서열에 결합하여 RNA 결합 단백질의 결합을 방지함으로써 NIE 스플라이스 부위의 이용을 유리하게 할 수 있다.

[0020] 일 실시양태에서, 잠재성 스플라이스 부위는 NIE의 5' 또는 3' 스플라이스 부위를 포함하지 않을 수 있다. 잠재성 스플라이스 부위는 NIE 5' 스플라이스 부위의 적어도 10 뉴클레오티드 상류에 있을 수 있다. 잠재성 스플라이스 부위는 NIE 5' 스플라이스 부위의 적어도 20 뉴클레오티드 상류에 있을 수 있다. 잠재성 스플라이스 부위는 NIE 5' 스플라이스 부위의 적어도 50 뉴클레오티드 상류에 있을 수 있다. 잠재성 스플라이스 부위는 NIE 5' 스플라이스 부위의 적어도 100 뉴클레오티드 상류에 있을 수 있다. 잠재성 스플라이스 부위는 NIE 5' 스플라이스 부위의 적어도 200 뉴클레오티드 상류에 있을 수 있다.

[0021] 잠재성 스플라이스 부위는 NIE 3' 스플라이스 부위의 적어도 10 뉴클레오티드 하류에 있을 수 있다. 잠재성 스플라이스 부위는 NIE 3' 스플라이스 부위의 적어도 20 뉴클레오티드 하류에 있을 수 있다. 잠재성 스플라이스 부위는 NIE 3' 스플라이스 부위의 적어도 50 뉴클레오티드 하류에 있을 수 있다. 잠재성 스플라이스 부위는 NIE 3' 스플라이스 부위의 적어도 100 뉴클레오티드 하류에 있을 수 있다. 잠재성 스플라이스 부위는 NIE 3' 스플라이스 부위의 적어도 200 뉴클레오티드 하류에 있을 수 있다.

[0022] **표적 전사체**

[0023] 일부 실시양태에서, 본 개시 내용의 방법은 *SCN1A* 유전자로부터 전사된 프리-mRNA에 NIE의 존재를 이용한다. 기능성 성숙 *SCN1A* mRNA를 생성하기 위한 확인된 *SCN1A* NIE 프리-mRNA 종의 스플라이싱은 NIE의 엑손 스키핑(exon skipping)을 자극하는 ASO와 같은 치료제를 사용하여 유도될 수 있다. 엑손 스키핑의 유도는 NMD 경로의 억제를 초래할 수 있다. 생성된 성숙한 *SCN1A* mRNA는 NMD 경로를 활성화시키지 않으면서 정상적으로 번역될 수 있으며, 이로써 환자 세포에서 SCN1A 단백질의 양을 증가시키고 SCN1A 결핍과 관련된 병태, 예를 들어 드라벳 증후군(DS); 열성 발작 플러스를 동반하는 전신 뇌전증 2형; 가족성 열성 발작 3A; 자폐증; 조기 유아 간질성 뇌병증 13; 동기능 부전 증후군 1; 알츠하이머병; 또는 SUDEP의 증상을 완화시킨다.

[0024] 다양한 실시양태에서, 본 개시 내용은 *SCN1A* mRNA 전사체를 표적화하여, 스플라이싱 또는 단백질 발현 수준을 조절, 예를 들어, 향상 또는 억제할 수 있는 치료제를 제공한다. 치료제는 소분자, 폴리뉴클레오티드, 또는 폴리펩티드일 수 있다. 일부 실시양태에서, 치료제는 ASO이다. *SCN1A* 프리-mRNA 상의 다양한 영역 또는 서열이 ASO와 같은 치료제에 의해 표적화될 수 있다. 일부 실시양태에서, ASO는 NIE를 함유하는 *SCN1A* 프리-mRNA 전사

체를 표적화한다. 일부 실시양태에서, ASO는 *SCN1A* 프리-mRNA 전사체의 NIE 내의 서열을 표적화한다. 일부 실시양태에서, ASO는 *SCN1A* 프리-mRNA 전사체의 NIE의 5' 말단으로부터 상류(또는 5') 서열(3'ss)을 표적화한다. 일부 실시양태에서, ASO는 *SCN1A* 프리-mRNA 전사체의 NIE의 3' 말단으로부터 하류(또는 3') 서열(5'ss)을 표적화한다. 일부 실시양태에서, ASO는 *SCN1A* 프리-mRNA 전사체의 NIE의 5' 말단의 측면에 위치한 인트론 내에 있는 서열을 표적화한다. 일부 실시양태에서, ASO는 *SCN1A* 프리-mRNA 전사체의 NIE의 3' 말단의 측면에 위치한 인트론 내에 있는 서열을 표적화한다. 일부 실시양태에서, ASO는 *SCN1A* 프리-mRNA 전사체의 NIE-인트론 경계를 포함하는 서열을 표적화한다. NIE-인트론 경계는 인트론 서열과 NIE 영역의 접합부를 지칭할 수 있다. 인트론 서열은 NIE의 5' 말단, 또는 NIE의 3' 말단의 측면에 위치할 수 있다. 일부 실시양태에서, ASO는 *SCN1A* 프리-mRNA 전사체의 엑손 내의 서열을 표적화한다. 일부 실시양태에서, ASO는 *SCN1A* 프리-mRNA 전사체의 인트론 내의 서열을 표적화한다. 일부 실시양태에서, ASO는 인트론의 일부 및 엑손의 일부를 둘 다 포함하는 서열을 표적화한다.

[0025] 일부 실시양태에서, 본원에 기재된 치료제는 NMD 엑손 mRNA의 스플라이싱에 관여하는 인자의 결합을 조절한다.

[0026] 일부 실시양태에서, 본원에 기재된 치료제는 NMD 엑손 mRNA의 스플라이싱에 관여하는 인자의 결합을 방해한다.

[0027] 일부 실시양태에서, 본원에 기재된 치료제는 NMD 엑손 mRNA의 스플라이싱에 관여하는 인자의 결합을 방지한다.

[0028] 일부 실시양태에서, 치료제는 SCN1A를 코딩하는 NMD 엑손 mRNA의 2개의 표준 엑손 영역 사이의 인트론 영역에 위치한 표적화된 부분을 표적화하며, 여기서 인트론 영역은 NMD 엑손을 함유한다.

[0029] 일부 실시양태에서, 치료제는 NMD 엑손과 적어도 부분적으로 중첩되는 표적화된 부분을 표적화한다.

[0030] 일부 실시양태에서, 치료제는 NMD 엑손의 상류 인트론과 적어도 부분적으로 중첩되는 표적화된 부분을 표적화한다.

[0031] 일부 실시양태에서, 치료제는 NMD 엑손 내의 표적화된 부분을 표적화한다.

[0032] 일부 실시양태에서, 치료제는 NMD 엑손의 적어도 약 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30개 또는 그 이상의 연속적인 뉴클레오티드를 포함하는 표적화된 부분을 표적화한다. 일부 실시양태에서, 치료제는 NMD 엑손의 최대 약 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30개 또는 그 이상의 연속적인 뉴클레오티드를 포함하는 표적화된 부분을 표적화한다. 일부 실시양태에서, 치료제는 NMD 엑손의 약 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30개 또는 그 이상의 연속적인 뉴클레오티드를 포함하는 표적화된 부분을 표적화한다.

[0033] 일부 실시양태에서, 치료제는 NMD 엑손에 근접한 표적화된 부분을 표적화한다.

[0034] 일부 실시양태에서, ASO는 NIE의 5' 말단으로부터 약 4 내지 약 300 뉴클레오티드 상류(또는 5')의 서열을 표적화한다. 일부 실시양태에서, ASO는 NIE 영역의 5' 말단으로부터 약 1 내지 약 20 뉴클레오티드, 약 20 내지 약 50 뉴클레오티드, 약 50 내지 약 100 뉴클레오티드, 약 100 내지 약 150 뉴클레오티드, 약 150 내지 약 200 뉴클레오티드, 약 200 내지 약 250 뉴클레오티드, 약 250 내지 약 300 뉴클레오티드, 약 350 내지 약 400 뉴클레오티드, 약 450 내지 약 500 뉴클레오티드, 약 550 내지 약 600 뉴클레오티드, 약 650 내지 약 700 뉴클레오티드, 약 750 내지 약 800 뉴클레오티드, 약 850 내지 약 900 뉴클레오티드, 약 950 내지 약 1000 뉴클레오티드, 약 1050 내지 약 1100 뉴클레오티드, 약 1150 내지 약 1200 뉴클레오티드, 약 1250 내지 약 1300 뉴클레오티드, 약 1350 내지 약 1400 뉴클레오티드, 또는 약 1450 내지 약 1500 뉴클레오티드 상류(또는 5')의 서열을 표적화한다. 일부 실시양태에서, ASO는 NIE의 5' 말단으로부터 300 뉴클레오티드 초과 상류의 서열을 표적화할 수 있다. 일부 실시양태에서, ASO는 NIE의 3' 말단으로부터 약 4 내지 약 300 뉴클레오티드 하류(또는 3')의 서열을 표적화한다. 일부 실시양태에서, ASO는 NIE의 3' 말단으로부터 약 1 내지 약 20 뉴클레오티드, 약 20 내지 약 50 뉴클레오티드, 약 50 내지 약 100 뉴클레오티드, 약 100 내지 약 150 뉴클레오티드, 약 150 내지 약 200 뉴클레오티드, 약 200 내지 약 250 뉴클레오티드, 약 250 내지 약 300 뉴클레오티드, 약 350 내지 약 400 뉴클레오티드, 약 450 내지 약 500 뉴클레오티드, 약 550 내지 약 600 뉴클레오티드, 약 650 내지 약 700 뉴클레오티드, 약 750 내지 약 800 뉴클레오티드, 약 850 내지 약 900 뉴클레오티드, 약 950 내지 약 1000 뉴클레오티드, 약 1050 내지 약 1100 뉴클레오티드, 약 1150 내지 약 1200 뉴클레오티드, 약 1250 내지 약 1300 뉴클레오티드, 약 1350 내지 약 1400 뉴클레오티드, 또는 약 1450 내지 약 1500 뉴클레오티드 하류의 서열을 표적화한다. 일부 실시양태에서, ASO는 NIE의 3' 말단으로부터 300 뉴클레오티드 초과 하류의 서열을 표적화한다.

[0035] 일부 실시양태에서, ASO는 NIE의 5' 말단으로부터 약 4 내지 약 300 뉴클레오티드 상류(또는 5')의 서열을 표적

화한다. 일부 실시양태에서, ASO은 NIE 영역의 5' 말단으로부터 적어도 약 1 뉴클레오티드, 적어도 약 10 뉴클레오티드, 적어도 약 20 뉴클레오티드, 적어도 약 50 뉴클레오티드, 적어도 약 80 뉴클레오티드, 적어도 약 85 뉴클레오티드, 적어도 약 90 뉴클레오티드, 적어도 약 95 뉴클레오티드, 적어도 약 96 뉴클레오티드, 적어도 약 97 뉴클레오티드, 적어도 약 98 뉴클레오티드, 적어도 약 99 뉴클레오티드, 적어도 약 100 뉴클레오티드, 적어도 약 101 뉴클레오티드, 적어도 약 102 뉴클레오티드, 적어도 약 103 뉴클레오티드, 적어도 약 104 뉴클레오티드, 적어도 약 105 뉴클레오티드, 적어도 약 110 뉴클레오티드, 적어도 약 120 뉴클레오티드, 적어도 약 150 뉴클레오티드, 적어도 약 200 뉴클레오티드, 적어도 약 300 뉴클레오티드, 적어도 약 400 뉴클레오티드, 적어도 약 500 뉴클레오티드, 적어도 약 600 뉴클레오티드, 적어도 약 700 뉴클레오티드, 적어도 약 800 뉴클레오티드, 적어도 약 900 뉴클레오티드, 또는 적어도 약 1000 뉴클레오티드 상류(또는 5')의 서열을 표적화한다. 일부 실시양태에서, ASO는 NIE의 3' 말단으로부터 약 4 내지 약 300 뉴클레오티드 하류(또는 3')의 서열을 표적화한다. 일부 실시양태에서, ASO는 NIE의 3' 말단으로부터 적어도 약 1 뉴클레오티드, 적어도 약 10 뉴클레오티드, 적어도 약 20 뉴클레오티드, 적어도 약 50 뉴클레오티드, 적어도 약 80 뉴클레오티드, 적어도 약 85 뉴클레오티드, 적어도 약 90 뉴클레오티드, 적어도 약 95 뉴클레오티드, 적어도 약 96 뉴클레오티드, 적어도 약 97 뉴클레오티드, 적어도 약 98 뉴클레오티드, 적어도 약 99 뉴클레오티드, 적어도 약 100 뉴클레오티드, 적어도 약 101 뉴클레오티드, 적어도 약 102 뉴클레오티드, 적어도 약 103 뉴클레오티드, 적어도 약 104 뉴클레오티드, 적어도 약 105 뉴클레오티드, 적어도 약 110 뉴클레오티드, 적어도 약 120 뉴클레오티드, 적어도 약 150 뉴클레오티드, 적어도 약 200 뉴클레오티드, 적어도 약 300 뉴클레오티드, 적어도 약 400 뉴클레오티드, 적어도 약 500 뉴클레오티드, 적어도 약 600 뉴클레오티드, 적어도 약 700 뉴클레오티드, 적어도 약 800 뉴클레오티드, 적어도 약 900 뉴클레오티드, 또는 적어도 약 1000 뉴클레오티드 하류의 서열을 표적화한다. 일부 실시양태에서, ASO는 NIE의 3' 말단으로부터 300 뉴클레오티드 초과 하류의 서열을 표적화한다.

[0036] 일부 실시양태에서, ASO는 NIE의 5' 말단으로부터 약 4 내지 약 300 뉴클레오티드 상류(또는 5')의 서열을 표적화한다. 일부 실시양태에서, ASO는 NIE 영역의 5' 말단으로부터 최대 약 10 뉴클레오티드, 최대 약 20 뉴클레오티드, 최대 약 50 뉴클레오티드, 최대 약 80 뉴클레오티드, 최대 약 85 뉴클레오티드, 최대 약 90 뉴클레오티드, 최대 약 95 뉴클레오티드, 최대 약 96 뉴클레오티드, 최대 약 97 뉴클레오티드, 최대 약 98 뉴클레오티드, 최대 약 99 뉴클레오티드, 최대 약 100 뉴클레오티드, 최대 약 101 뉴클레오티드, 최대 약 102 뉴클레오티드, 최대 약 103 뉴클레오티드, 최대 약 104 뉴클레오티드, 최대 약 105 뉴클레오티드, 최대 약 110 뉴클레오티드, 최대 약 120 뉴클레오티드, 최대 약 150 뉴클레오티드, 최대 약 200 뉴클레오티드, 최대 약 300 뉴클레오티드, 최대 약 400 뉴클레오티드, 최대 약 500 뉴클레오티드, 최대 약 600 뉴클레오티드, 최대 약 700 뉴클레오티드, 최대 약 800 뉴클레오티드, 최대 약 900 뉴클레오티드, 최대 약 1000 뉴클레오티드, 최대 약 1100 뉴클레오티드, 최대 약 1200 뉴클레오티드, 최대 약 1300 뉴클레오티드, 최대 약 1400 뉴클레오티드, 또는 최대 약 1500 뉴클레오티드 상류(또는 5')의 서열을 표적화한다. 일부 실시양태에서, ASO는 NIE의 3' 말단으로부터 약 4 내지 약 300 뉴클레오티드 하류(또는 3')의 서열을 표적화한다. 일부 실시양태에서, ASO는 NIE의 3' 말단으로부터 최대 약 10 뉴클레오티드, 최대 약 20 뉴클레오티드, 최대 약 50 뉴클레오티드, 최대 약 80 뉴클레오티드, 최대 약 85 뉴클레오티드, 최대 약 90 뉴클레오티드, 최대 약 95 뉴클레오티드, 최대 약 96 뉴클레오티드, 최대 약 97 뉴클레오티드, 최대 약 98 뉴클레오티드, 최대 약 99 뉴클레오티드, 최대 약 100 뉴클레오티드, 최대 약 101 뉴클레오티드, 최대 약 102 뉴클레오티드, 최대 약 103 뉴클레오티드, 최대 약 104 뉴클레오티드, 최대 약 105 뉴클레오티드, 최대 약 110 뉴클레오티드, 최대 약 120 뉴클레오티드, 최대 약 150 뉴클레오티드, 최대 약 200 뉴클레오티드, 최대 약 300 뉴클레오티드, 최대 약 400 뉴클레오티드, 최대 약 500 뉴클레오티드, 최대 약 600 뉴클레오티드, 최대 약 700 뉴클레오티드, 최대 약 800 뉴클레오티드, 최대 약 900 뉴클레오티드, 또는 최대 약 1000 뉴클레오티드, 최대 약 1100 뉴클레오티드, 최대 약 1200 뉴클레오티드, 최대 약 1300 뉴클레오티드, 최대 약 1400 뉴클레오티드, 또는 최대 약 1500 뉴클레오티드 하류의 서열을 표적화한다. 일부 실시양태에서, ASO는 NIE의 3' 말단으로부터 300 뉴클레오티드 초과 하류의 서열을 표적화한다.

[0037] 일부 실시양태에서, 본원에 기재된 바와 같은 NIE는 [도 2]에 도시된 바와 같이 GRCh37/hg19: chr2:166,863,740과 GRCh37/hg19: chr2:166,863,803 사이에 위치한다. 일부 실시양태에서, NIE의 5' 말단은 GRCh37/hg19: chr2:166,863,803에 위치한다. 일부 실시양태에서, NIE의 3' 말단은 GRCh37/hg19: chr2:166,863,740에 위치한다.

[0038] 일부 실시양태에서, ASO는 게놈 부위 GRCh37/hg19: chr2:166,863,803으로부터 약 4 내지 약 300 뉴클레오티드 상류(또는 5')의 서열을 표적화한다. 일부 실시양태에서, ASO는 게놈 부위 GRCh37/hg19: chr2:166,863,803으로부터 약 1 내지 약 20 뉴클레오티드, 약 20 내지 약 50 뉴클레오티드, 약 50 내지 약 100 뉴클레오티드, 약 100

내지 약 150 뉴클레오티드, 약 150 내지 약 200 뉴클레오티드, 약 200 내지 약 250 뉴클레오티드, 약 250 내지 약 300, 약 250 내지 약 300 뉴클레오티드, 약 350 내지 약 400 뉴클레오티드, 약 450 내지 약 500 뉴클레오티드, 약 550 내지 약 600 뉴클레오티드, 약 650 내지 약 700 뉴클레오티드, 약 750 내지 약 800 뉴클레오티드, 약 850 내지 약 900 뉴클레오티드, 약 950 내지 약 1000 뉴클레오티드, 약 1050 내지 약 1100 뉴클레오티드, 약 1150 내지 약 1200 뉴클레오티드, 약 1250 내지 약 1300 뉴클레오티드, 약 1350 내지 약 1400 뉴클레오티드, 또는 약 1450 내지 약 1500 뉴클레오티드 상류(또는 5')의 서열을 표적화한다. 일부 실시양태에서, ASO는 게놈 부위 GRCh37/hg19: chr2:166,863,803으로부터 300 뉴클레오티드 초과 상류의 서열을 표적화할 수 있다. 일부 실시양태에서, ASO는 GRCh37/hg19: chr2:166,863,740으로부터 약 4 내지 약 300 뉴클레오티드 하류(또는 3')의 서열을 표적화한다. 일부 실시양태에서, ASO는 GRCh37/hg19: chr2:166,863,740으로부터 약 1 내지 약 20 뉴클레오티드, 약 20 내지 약 50 뉴클레오티드, 약 50 내지 약 100 뉴클레오티드, 약 100 내지 약 150 뉴클레오티드, 약 150 내지 약 200 뉴클레오티드, 약 200 내지 약 250 뉴클레오티드, 약 250 내지 약 300 뉴클레오티드, 약 350 내지 약 400 뉴클레오티드, 약 450 내지 약 500 뉴클레오티드, 약 550 내지 약 600 뉴클레오티드, 약 650 내지 약 700 뉴클레오티드, 약 750 내지 약 800 뉴클레오티드, 약 850 내지 약 900 뉴클레오티드, 약 950 내지 약 1000 뉴클레오티드, 약 1050 내지 약 1100 뉴클레오티드, 약 1150 내지 약 1200 뉴클레오티드, 약 1250 내지 약 1300 뉴클레오티드, 약 1350 내지 약 1400 뉴클레오티드, 또는 약 1450 내지 약 1500 뉴클레오티드 하류의 서열을 표적화한다. 일부 실시양태에서, ASO는 GRCh37/hg19: chr2:166,863,740으로부터 300 뉴클레오티드 초과 하류의 서열을 표적화한다.

[0039] 일부 실시양태에서, ASO는 게놈 부위 GRCh37/hg19: chr2:166,863,803으로부터 약 4 내지 약 300 뉴클레오티드 상류(또는 5')의 서열을 표적화한다. 일부 실시양태에서, ASO는 게놈 부위 GRCh37/hg19: chr2:166,863,803으로부터 적어도 약 1 뉴클레오티드, 적어도 약 10 뉴클레오티드, 적어도 약 20 뉴클레오티드, 적어도 약 50 뉴클레오티드, 적어도 약 80 뉴클레오티드, 적어도 약 85 뉴클레오티드, 적어도 약 90 뉴클레오티드, 적어도 약 95 뉴클레오티드, 적어도 약 96 뉴클레오티드, 적어도 약 97 뉴클레오티드, 적어도 약 98 뉴클레오티드, 적어도 약 99 뉴클레오티드, 적어도 약 100 뉴클레오티드, 적어도 약 101 뉴클레오티드, 적어도 약 102 뉴클레오티드, 적어도 약 103 뉴클레오티드, 적어도 약 104 뉴클레오티드, 적어도 약 105 뉴클레오티드, 적어도 약 110 뉴클레오티드, 적어도 약 120 뉴클레오티드, 적어도 약 150 뉴클레오티드, 적어도 약 200 뉴클레오티드, 적어도 약 300 뉴클레오티드, 적어도 약 400 뉴클레오티드, 적어도 약 500 뉴클레오티드, 적어도 약 600 뉴클레오티드, 적어도 약 700 뉴클레오티드, 적어도 약 800 뉴클레오티드, 적어도 약 900 뉴클레오티드, 또는 적어도 약 1000 뉴클레오티드 상류(또는 5')의 서열을 표적화한다. 일부 실시양태에서, ASO는 GRCh37/hg19: chr2:166,863,740으로부터 약 4 내지 약 300 뉴클레오티드 하류(또는 3')의 서열을 표적화한다. 일부 실시양태에서, ASO는 GRCh37/hg19: chr2:166,863,740으로부터 적어도 약 1 뉴클레오티드, 적어도 약 10 뉴클레오티드, 적어도 약 20 뉴클레오티드, 적어도 약 50 뉴클레오티드, 적어도 약 80 뉴클레오티드, 적어도 약 85 뉴클레오티드, 적어도 약 90 뉴클레오티드, 적어도 약 95 뉴클레오티드, 적어도 약 96 뉴클레오티드, 적어도 약 97 뉴클레오티드, 적어도 약 98 뉴클레오티드, 적어도 약 99 뉴클레오티드, 적어도 약 100 뉴클레오티드, 적어도 약 101 뉴클레오티드, 적어도 약 102 뉴클레오티드, 적어도 약 103 뉴클레오티드, 적어도 약 104 뉴클레오티드, 적어도 약 105 뉴클레오티드, 적어도 약 110 뉴클레오티드, 적어도 약 120 뉴클레오티드, 적어도 약 150 뉴클레오티드, 적어도 약 200 뉴클레오티드, 적어도 약 300 뉴클레오티드, 적어도 약 400 뉴클레오티드, 적어도 약 500 뉴클레오티드, 적어도 약 600 뉴클레오티드, 적어도 약 700 뉴클레오티드, 적어도 약 800 뉴클레오티드, 적어도 약 900 뉴클레오티드, 또는 적어도 약 1000 뉴클레오티드 하류의 서열을 표적화한다. 일부 실시양태에서, ASO는 GRCh37/hg19: chr2:166,863,740으로부터 300 뉴클레오티드 초과 하류의 서열을 표적화한다.

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서, ASO는 GRCh37/hg19: chr2:166,863,740으로부터 약 4 내지 약 300 뉴클레오티드 하류(또는 3')의 서열을 표적화한다. 일부 실시양태에서, ASO는 GRCh37/hg19: chr2:166,863,740으로부터 최대 약 10 뉴클레오티드, 최대 약 20 뉴클레오티드, 최대 약 50 뉴클레오티드, 최대 약 80 뉴클레오티드, 최대 약 85 뉴클레오티드, 최대 약 90 뉴클레오티드, 최대 약 95 뉴클레오티드, 최대 약 96 뉴클레오티드, 최대 약 97 뉴클레오티드, 최대 약 98 뉴클레오티드, 최대 약 99 뉴클레오티드, 최대 약 100 뉴클레오티드, 최대 약 101 뉴클레오티드, 최대 약 102 뉴클레오티드, 최대 약 103 뉴클레오티드, 최대 약 104 뉴클레오티드, 최대 약 105 뉴클레오티드, 최대 약 110 뉴클레오티드, 최대 약 120 뉴클레오티드, 최대 약 150 뉴클레오티드, 최대 약 200 뉴클레오티드, 최대 약 300 뉴클레오티드, 최대 약 400 뉴클레오티드, 최대 약 500 뉴클레오티드, 최대 약 600 뉴클레오티드, 최대 약 700 뉴클레오티드, 최대 약 800 뉴클레오티드, 최대 약 900 뉴클레오티드, 또는 최대 약 1000 뉴클레오티드, 최대 약 1100 뉴클레오티드, 최대 약 1200 뉴클레오티드, 최대 약 1300 뉴클레오티드, 최대 약 1400 뉴클레오티드, 또는 최대 약 1500 뉴클레오티드 하류의 서열을 표적화한다. 일부 실시양태에서, ASO는 GRCh37/hg19: chr2:166,863,740으로부터 300 뉴클레오티드 초과 하류의 서열을 표적화한다.

[0041] 본원에서 실시예에 기재된 바와 같이, *SCN1A* 유전자(서열 번호 1)를 NIE에 대해 분석하고 인트론 20의 일부(서열 번호 4)의 포함이 관찰되었다(이 부분은 본 개시 내용 전체에 걸쳐 엑손 20x로 지칭됨). 일부 실시양태에서, 본원에 개시된 ASO는 *SCN1A* 게놈 서열로부터 전사된 NIE 함유 프리-mRNA(서열 번호 2)를 표적화한다. 일부 실시양태에서, ASO는 인트론 20의 일부를 포함하는 *SCN1A* 게놈 서열로부터의 NIE 함유 프리-mRNA 전사체를 표적화한다. 일부 실시양태에서, ASO는 엑손 20x(서열 번호 6)를 포함하는 *SCN1A* 게놈 서열로부터의 NIE 함유 프리-mRNA 전사체를 표적화한다. 일부 실시양태에서, ASO는 서열 번호 2 또는 12의 NIE 함유 프리-mRNA 전사체를 표적화한다. 일부 실시양태에서, ASO는 NIE를 포함하는 서열 번호 2 또는 12의 프리-mRNA 전사체를 표적화한다. 일부 실시양태에서, ASO는 엑손 20x을 포함하는 서열 번호 2의 NIE 함유 프리-mRNA 전사체(서열 번호 10)를 표적화한다. 일부 실시양태에서, 본원에 개시된 ASO는 *SCN1A* 프리-mRNA 서열(서열 번호 2 또는 12)을 표적화한다. 일부 실시양태에서, ASO는 NIE를 포함하는 *SCN1A* 프리-mRNA 서열(서열 번호 10 또는 20)을 표적화한다. 일부 실시양태에서, ASO는 서열 번호 7 내지 10 또는 17 내지 20 중 어느 하나에 따른 *SCN1A* 프리-mRNA 서열을 표적화한다. 일부 실시양태에서, ASO는 서열 번호 21 내지 67 중 어느 하나에 따른 서열을 갖는다. 일부 실시양태에서, ASO는 서열 번호 68 내지 114 중 어느 하나에 따른 서열을 갖는다. 일부 실시양태에서, ASO는 서열 번호 115 내지 209 중 어느 하나에 따른 서열을 갖는다. 일부 실시양태에서, ASO는 서열 번호 210 내지 256 중 어느 하나에 따른 서열을 갖는다. 일부 실시양태에서, ASO는 서열 번호 257 내지 303 중 어느 하나에 따른 서열을 갖는다. 일부 실시양태에서, ASO는 서열 번호 304 내지 341 중 어느 하나에 따른 서열을 갖는다. 일부 실시양태에서, ASO는 서열 번호 342 내지 379 중 어느 하나에 따른 서열을 갖는다.

[0042] 일부 실시양태에서, *SCN1A* NIE 함유 프리-mRNA 전사체는 서열 번호 1 또는 11과 적어도 약 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% 또는 100% 서열 동일성을 갖는 유전자 서열에 의해 코딩된다. 일부 실시양태에서, *SCN1A* NIE 프리-mRNA 전사체는 서열 번호 2 내지 10 및 12 내지 20 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% 또는 100% 서열 동일성을 갖는 서열을 포함한다.

[0043] 일부 실시양태에서, *SCN1A* NIE 함유 프리-mRNA 전사체(또는 NMD 엑손 mRNA)는 서열 번호 2, 7 내지 10, 12 및 17 내지 20 중 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함한다. 일부 실시양태에서, *SCN1A* NIE 함유 프리-mRNA 전사체(또는 NMD 엑손 mRNA)은 서열 번호 1, 3 내지 6, 11, 및 13 내지 16과 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열에 의해 코딩된다. 일부 실시양태에서, NMD 엑손 mRNA의 표적화된 부분은 서열 번호 2, 7 내지 10, 12, 및 17 내지 20의 적어도 8개의 인접한 핵산을 포함하는 영역과 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함한다.

[0044] 일부 실시양태에서, ASO는 NIE 엑손 20x를 포함하는 *SCN1A* NIE 함유 프리-mRNA의 엑손 20을 표적화한다. 일부 실시양태에서, ASO는 NIE 엑손 20x의 하류(또는 3')의 엑손 21 서열을 표적화한다. 일부 실시양태에서, ASO는 엑손 20x의 5' 말단으로부터 약 4 내지 약 300 뉴클레오티드 상류(또는 5')의 서열을 표적화한다. 일부 실시양태에서, ASO는 엑손 20x의 3' 말단으로부터 약 4 내지 약 300 뉴클레오티드 하류(또는 3')의 서열을 표적화한다. 일부 실시양태에서, ASO는 서열 번호 21 내지 67 중 어느 하나에 따른 서열을 갖는다. 일부 실시양태에서, ASO는 서열 번호 210 내지 256 중 어느 하나에 따른 서열을 갖는다.

[0045] 일부 실시양태에서, ASO는 NIE의 5' 말단으로부터 상류 서열을 표적화한다. 예를 들어, NIE(예를 들어, 인간 *SCN1A*에서 엑손 20x, 또는 마우스 *SCN1A*에서 엑손 21x)의 5' 말단으로부터 상류 서열을 표적화하는 ASO는 서열 번호 21 내지 38 중 어느 하나와 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함

할 수 있다. 다른 예에서, NIE(예를 들어, 인간 *SCN1A*에서 엑손 20x, 또는 마우스 *SCN1A*에서 엑손 21x)의 5' 말단으로부터 상류 서열을 표적화하는 ASO는 서열 번호 68 내지 85 중 어느 하나와 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함할 수 있다. 일부 실시양태에서, ASO는 엑손-인트론 경계(또는 접합부)를 함유하는 서열을 표적화한다. 예를 들어, 엑손-인트론 경계를 함유하는 서열을 표적화하는 ASO는 서열 번호 39 내지 41, 51, 52, 228 내지 230, 240, 또는 241 중 어느 하나와 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함할 수 있다. 다른 예에서, 엑손-인트론 경계를 함유하는 서열을 표적화하는 ASO는 서열 번호 86 내지 88 및 98 내지 99 중 어느 하나와 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함할 수 있다. 일부 실시양태에서, ASO는 NIE의 3' 말단으로부터 하류 서열을 표적화한다. 예를 들어, NIE(예를 들어, 인간 *SCN1A*에서 엑손 20x, 또는 마우스 *SCN1A*에서 엑손 21x)의 3' 말단으로부터 하류 서열을 표적화하는 ASO는 서열 번호 53 내지 67 중 어느 하나와 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함할 수 있다. 다른 예에서, NIE(예를 들어, 인간 *SCN1A*에서 엑손 20x, 또는 마우스 *SCN1A*에서 엑손 21x)의 3' 말단으로부터 하류 서열을 표적화하는 ASO는 서열 번호 100 내지 114 중 어느 하나와 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함할 수 있다. 일부 실시양태에서, ASO는 NIE 내의 서열을 표적화한다. 일부 실시양태에서, NIE(예를 들어, 인간 *SCN1A*에서 엑손 20x, 또는 마우스 *SCN1A*에서 엑손 21x) 내의 서열을 표적화하는 ASO는 서열 번호 42 내지 50, 또는 231 내지 239 중 어느 하나와 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함할 수 있다. 다른 예에서, NIE(예를 들어, 인간 *SCN1A*에서 엑손 20x, 또는 마우스 *SCN1A*에서 엑손 21x) 내의 서열을 표적화하는 ASO는 서열 번호 89 내지 97 중 어느 하나와 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함할 수 있다.

[0046] 일부 실시양태에서, ASO는 엑손 20x를 포함하는 *SCN1A* NIE 함유 프리-mRNA에서 엑손 20x를 표적화한다. 일부 실시양태에서, ASO는 *SCN1A* 프리-mRNA의 엑손 20x의 5' 말단으로부터 하류(또는 3')의 엑손 20x 서열을 표적화한다. 일부 실시양태에서, ASO는 *SCN1A* 프리-mRNA의 엑손 20x의 3' 말단으로부터 상류(또는 5')의 엑손 20x 서열을 표적화한다.

[0047] 일부 실시양태에서, *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분은 인트론 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 또는 25(NM_006920에서 mRNA 서열에 상응하는 인트론 넘버링)에 있다. 일부 실시양태에서, NIE 프리-mRNA의 표적화된 부분에 대한 ASO의 혼성화는 인트론 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 또는 25 내의 하나 이상의 NIE의 엑손 스키핑을 초래하고, 계속하여 SCN1A 단백질 생산을 증가시킨다. 일부 실시양태에서, NIE 프리-mRNA의 표적화된 부분에 대한 ASO의 혼성화는 인트론 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 또는 25 내의 하나 이상의 NIE의 엑손 스키핑을 억제 또는 차단하고, 계속하여 SCN1A 단백질 생산을 감소시킨다. 일부 실시양태에서, *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분은 인트론 20에 있다. 당업자는 본원에 제공된 인트론 서열에 기초하여 또는 NM_006920, NM_001202435, NM_001165964, 또는 NM_001165963에서 mRNA 서열과 관련하여 제공된 인트론 번호를 사용하여, 임의의 *SCN1A* 아이소형에서 상응하는 인트론 번호를 결정할 수 있다. 당업자는 또한 본원에 제공된 인트론 서열에 기초하여 또는 NM_006920, NM_001202435, NM_001165964, 또는 NM_001165963에서 mRNA 서열을 관련하여 제공된 인트론 번호를 사용하여, 본 발명의 방법을 사용하여 표적화하기 위한 임의의 *SCN1A* 아이소형에서 측면에 위치한 엑손의 서열을 결정할 수 있다.

[0048] 일부 실시양태에서, 본 개시 내용의 방법 및 조성물은 *SCN1A* NIE 함유 프리-mRNA의 가성 엑손의 엑손 스키핑을 유도하거나 억제함으로써 SCN1A의 발현을 조절, 예를 들어, 증가 또는 감소시키는 데 사용된다. 일부 실시양태에서, 가성 엑손은 인트론 1 내지 25 중 어느 하나 내의 서열이다. 일부 실시양태에서, 가성 엑손은 인트론 2, 4, 6, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 및 25 중 어느 하나 내의 서열이다. 가성 엑손은 인트론 15, 18, 및 19 중 어느 하나 내의 서열이다. 일부 실시양태에서, 가성 엑손은 임의의 *SCN1A* 인트론 또는 이의 일부일 수 있다. 일부 실시양태에서, 가성 엑손은 인트론 20 내에 있다. 본원에 사용된 SCN1A 인트론 넘버링은 NM_006920에서의 mRNA 서열에 상응한다. 인트론 넘버링은 상이한 *SCN1A* 아이소형 서열과 관련하여 변할 수 있는 것으로 이해된다.

[0049] **SCN1A 단백질**

[0050] *SCN1A* 유전자는 SCN1A(나트륨 채널, 전압-게이팅, I형, 알파 서브유닛) 단백질을 코딩할 수 있으며, 이는 또한 전압-게이팅 나트륨 채널 $Na_v1.1$의 알파-서브유닛으로 지칭될 수 있다. 또한, 전술한 바와 같이, DS에서 SCN1A

돌연변이는 전체 단백질에 걸쳐 퍼져있다. 데 노보(de novo) 발생이 더 약화된 100개 초과의 신규 돌연변이가 유전자 전체에 걸쳐 확인되었다. 이들은 절두(47%), 미스센스(43%), 결실(3%), 및 스플라이스 부위 돌연변이(7%)로 구성된다. SCN1A 돌연변이를 갖는 대상체의 백분율은 33 내지 100%로 다양하다. 대부분의 돌연변이는 신규 변화(88%)이다.

[0051] 일부 실시양태에서, 본원에 기재된 방법은 기능성 SCN1A 단백질의 생산을 조절, 예를 들어 증가 또는 감소시키기 위해 사용된다. 본원에 사용된 바와 같이, 용어 "기능성"은 치료되는 병태, 예를 들어 드라벳 증후군; 열성 발작 플러스를 동반하는 전신 뇌전증 2형; 가족성 열성 발작 3A; 자폐증; 조기 유아 간질성 뇌병증 13; 동기능 부전 증후군 1; 알츠하이머병; 또는 SUDEP의 임의의 하나 이상의 증상을 제거하는데 필요한 양의 SCN1A 단백질 활성 또는 기능을 지칭한다. 일부 실시양태에서, 방법은 부분 기능성 SCN1A 단백질의 생산을 증가시키는 데 사용된다. 본원에 사용된 바와 같이, 용어 "부분 기능성"은 질환 또는 병태의 임의의 하나 이상의 증상을 제거 또는 예방하는데 필요한 양의 활성 또는 기능보다 적은 임의의 양의 SCN1A 단백질 활성 또는 기능을 지칭한다. 일부 실시양태에서, 부분 기능성 단백질 또는 RNA는 완전 기능성 단백질 또는 RNA와 비교하여 10% 이상, 20% 이상, 30% 이상, 40% 이상, 50% 이상, 60% 이상, 70% 이상, 75% 이상, 80% 이상, 85% 이상, 90% 이상, 또는 95% 이상 적은 활성을 가질 것이다.

[0052] 일부 실시양태에서, 상기 방법은 SCN1A 단백질을 코딩하는 NIE 함유 프리-mRNA를 갖는 대상체의 세포에 의한 SCN1A 단백질의 발현을 증가시키는 방법으로서, 대상체는 결핍된 양의 SCN1A 단백질 활성에 의해 야기된 드라벳 증후군을 가지며, 결핍된 양의 SCN1A 단백질은 SCN1A 단백질의 반가불충분성에 의해 야기되는 것인 방법이다. 이러한 실시양태에서, 대상체는 기능성 SCN1A 단백질을 코딩하는 제1 대립 유전자, 및 SCN1A 단백질이 생성되지 않는 제2 대립 유전자를 갖는다. 이러한 또 다른 실시양태에서, 대상체는 기능성 SCN1A 단백질을 코딩하는 제1 대립 유전자, 및 비기능성 SCN1A 단백질을 코딩하는 제2 대립 유전자를 갖는다. 이러한 또 다른 실시양태에서, 대상체는 기능성 SCN1A 단백질을 코딩하는 제1 대립 유전자, 및 부분 기능성 SCN1A 단백질을 코딩하는 제2 대립 유전자를 갖는다. 이들 임의의 실시양태에서, 안티센스 올리고머는 제2 대립 유전자로부터 전사된 NIE 함유 프리-mRNA의 표적화된 부분에 결합함으로써 프리-mRNA로부터 가성 엑손의 엑손 스키핑을 유도하고, 대상체의 세포에서 기능성 SCN1A 단백질을 코딩하는 성숙한 mRNA의 수준 증가, 및 SCN1A 단백질의 발현 증가를 일으킨다.

[0053] 관련 실시양태에서, 방법은 단백질 또는 기능성 RNA의 발현을 증가시키기 위해 ASO를 사용하는 방법이다. 일부 실시양태에서, ASO는 SCN1A 단백질을 코딩하는 NIE 함유 프리-mRNA를 갖는 대상체의 세포에서 SCN1A 단백질의 발현을 증가시키는 데 사용되며, 여기서 대상체는 SCN1A 단백질의 양 또는 기능에 있어 결핍증, 예를 들어 드라벳 증후군(DS)(SMEI로도 알려짐); 유아기의 중증 근간대성 뇌전증(SMEI) 경계성(SMEB); 열성 발작(FS); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+); 조기 유아 간질성 뇌병증 13; 잠재성 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 뇌전증에서의 돌연사(SUDEP); 동기능 부전 증후군 1; 조기 유아 SCN1A 뇌병증; 조기 유아 간질성 뇌병증(EIEE); 또는 자폐증을 갖는다. 일부 실시양태에서, ASO는 대상체의 세포에서 SCN1A 단백질의 발현을 증가시키는 데 사용되며, 여기서 대상체는 SCN8A 단백질의 양 또는 기능에 있어 결핍증, 예를 들어 조기 유아 간질성 뇌병증 13을 갖는다. 일부 실시양태에서, ASO는 대상체의 세포에서 SCN1A 단백질의 발현을 증가시키는 데 사용되며, 여기서 대상체는 SCN5A 단백질의 양 또는 기능에 있어 결핍증, 예를 들어 동기능 부전 증후군 1을 갖는다.

[0054] 일부 실시양태에서, 질환 또는 병태를 유발하는 단백질을 코딩하는 NIE 함유 프리-mRNA 전사체는 본원에 기재된 ASO에 의해 표적화된다. 일부 실시양태에서, 질환을 유발하지 않는 단백질을 코딩하는 NIE 함유 프리-mRNA 전사체는 ASO에 의해 표적화된다. 예를 들어, 특정 경로에서 제1 단백질의 돌연변이 또는 결핍의 결과인 질환은 제2 단백질을 코딩하는 NIE 함유 프리-mRNA를 표적화함으로써 제2 단백질의 생성을 증가시켜 개선될 수 있다. 일부 실시양태에서, 제2 단백질의 기능은 (질환 또는 병태의 원인이 되는) 제1 단백질의 돌연변이 또는 결핍을 보상할 수 있다.

[0055] 일부 실시양태에서, 대상체는

[0056] (a) (i) SCN1A 단백질이 야생형 대립 유전자로부터의 생산과 비교하여 감소한 수준으로 생성되거나,

[0057] (ii) SCN1A 단백질이 동등한 야생형 단백질과 비교하여 기능이 감소한 형태로 생성되거나,

[0058] (iii) SCN1A 단백질 또는 기능성 RNA가 생성되지 않는

[0059] 제1 돌연변이 대립 유전자; 및

[0060] (b) (i) SCN1A 단백질이 야생형 대립 유전자로부터의 생산과 비교하여 감소한 수준으로 생성되거나,

[0061] (ii) SCN1A 단백질이 동등한 야생형 단백질과 비교하여 기능이 감소한 형태로 생성되거나,

[0062] (iii) SCN1A 단백질이 생성되지 않는

[0063] 제2 돌연변이 대립 유전자를 가지며,

[0064] 여기서 NIE 함유 프리-mRNA는 제1 대립 유전자 및/또는 제2 대립 유전자로부터 전사된다. 이들 실시양태에서, ASO는 제1 대립 유전자 또는 제2 대립 유전자로부터 전사된 NIE 함유 프리-mRNA의 표적화된 부분에 결합함으로써, NIE 함유 프리-mRNA로부터 가성 엑손의 엑손 스키핑을 유도하고, 대상체의 세포에서 SCN1A 단백질을 코딩하는 mRNA의 수준증가 및 표적 단백질 또는 기능성 RNA의 발현 증가를 일으킨다. 이들 실시양태에서, NIE 함유 프리-mRNA로부터의 가성 엑손의 엑손 스키핑으로 인한 발현 수준의 증가를 보이는 표적 단백질 또는 기능성 RNA는 동등한 야생형 단백질과 비교하여 기능이 감소한 형태(부분 기능성), 또는 동등한 야생형 단백질과 비교하여 완전한 기능을 보이는 형태(완전 기능성)이다.

[0065] 일부 실시양태에서, SCN1A 단백질을 코딩하는 mRNA의 수준은 대조군 세포, 예를 들어 안티센스 올리고머로 처리되지 않은 것 또는 *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분에 결합하지 않는 안티센스 올리고머로 처리된 것에서 생성된 SCN1A 단백질을 코딩하는 mRNA의 양에 비해 1.1 내지 10배 증가한다.

[0066] 일부 실시양태에서, 본 개시 내용의 방법을 사용하여 치료되는 대상체는 하나의 대립 유전자로부터 부분 기능성 SCN1A 단백질을 발현하며, 여기서 부분 기능성 SCN1A 단백질은 프레임 시프트 돌연변이, 넌센스 돌연변이, 미스센스 돌연변이, 또는 부분 유전자 결실에 의해 야기된다. 일부 실시양태에서, 본 발명의 방법을 사용하여 치료되는 대상체는 하나의 대립 유전자로부터 비기능성 SCN1A 단백질을 발현하며, 여기서 비기능성 SCN1A 단백질은 하나의 대립 유전자에서 프레임 시프트 돌연변이, 넌센스 돌연변이, 미스센스 돌연변이, 부분 유전자 결실에 의해 야기된다. 일부 실시양태에서, 본 발명의 방법을 사용하여 치료되는 대상체는 하나의 대립 유전자에서 *SCN1A* 전체 유전자 결실을 갖는다.

[0067] 일부 실시양태에서, 상기 방법은 SCN1A 단백질을 코딩하는 NIE 함유 프리-mRNA를 갖는 대상체의 세포에 의한 SCN1A 단백질의 발현을 감소시키는 방법으로서, 대상체는 $Na_v1.1$의 기능 획득 돌연변이를 갖는 것인 방법이다. 이러한 실시양태에서, 대상체는 SCN1A 단백질이 상승한 양으로 생성된 대립 유전자 또는 세포에서 $Na_v1.1$의 증가한 활성을 유도하는 돌연변이 SCN1A를 코딩하는 대립 유전자를 갖는다. 일부 실시양태에서, Nav1.1의 증가한 활성은 돌연변이 $Na_v1.1$ 통로에 의해 매개되는 연장된 또는 거의 지속성의 나트륨 전류, 빠른 불활성화의 둔화, 정상 상태 불활성화의 양(positive)의 이동, 반복적인 자극 중에 높은 통로 이용 가능성, 비-불활성화된 탈분극-유도된 지속성의 나트륨 전류의 증가, 불활성화로의 진입 지연, 빠른 불활성화로부터의 회복 가속화, 및/또는 저온에서의 인큐베이션 또는 상호 작용 단백질의 공동-발현에 의한 폴딩 결함의 구제를 특징으로 한다. 이들 임의의 실시양태에서, 안티센스 올리고머는 제2 대립 유전자로부터 전사된 NIE 함유 프리-mRNA의 표적화된 부분에 결합함으로써 프리-mRNA로부터 가성 엑손의 엑손 스키핑을 억제 또는 차단하고, 대상체의 세포에서 기능성 SCN1A 단백질을 코딩하는 성숙한 mRNA의 수준 감소, 및 SCN1A 단백질의 발현 감소를 일으킨다.

[0068] 관련 실시양태에서, 상기 방법은 단백질 또는 기능성 RNA의 발현을 감소시키기 위해 ASO를 사용하는 방법이다. 일부 실시양태에서, ASO는 SCN1A 단백질을 코딩하는 NIE 함유 프리-mRNA를 갖는 대상체의 세포에서 SCN1A 단백질의 발현을 감소시키는 데 사용된다. 일부 실시양태에서, 대상체는 $Na_v1.1$의 기능 획득 돌연변이를 가지며, 예를 들어 편두통을 갖는다. 일부 실시양태에서, ASO는 대상체의 세포에서 SCN1A 단백질의 발현을 감소시키는 데 사용되며, 대상체는 $Na_v1.1$의 기능 획득 돌연변이를 가지며, 예를 들어 가족성 편마비 편두통 3을 갖는다.

[0069] 일부 실시양태에서, SCN1A 단백질을 코딩하는 mRNA의 수준은 대조군 세포, 예를 들어 안티센스 올리고머로 처리되지 않은 것 또는 SCN1A NIE 함유 프리-mRNA의 표적화된 부분에 결합하지 않는 안티센스 올리고머로 처리된 것에서 생성된 *SCN1A* 단백질을 코딩하는 mRNA의 양과 비교할 때 1.1 내지 10배 감소한다.

[0070] 일부 실시양태에서, 본 개시 내용의 방법을 사용하여 치료되는 대상체는 하나의 대립 유전자로부터 돌연변이 SCN1A 단백질을 발현하며, 여기서 돌연변이 SCN1A 단백질은 프레임 시프트 돌연변이, 넌센스 돌연변이, 미스센스 돌연변이, 또는 부분 유전자 결실에 의해 야기되고, 돌연변이 SCN1A 단백질은 $Na_v1.1$의 상승한 활성 수준을 야기한다. 일부 실시양태에서, 본 개시 내용의 방법을 사용하여 치료되는 대상체는 프레임 시프트 돌연변이, 넌센스 돌연변이, 미스센스 돌연변이, 또는 부분 유전자 결실로 인해 하나의 대립 유전자로부터 상승한 양의

SCN1A 단백질을 발현한다.

[0071] 본 발명의 실시양태에서, 대상체는 *SCN1A*에서 돌연변이를 가질 수 있다. *SCN1A*에서 돌연변이는 상기 유전자 전체에 퍼져 있을 수 있다. SCN1A 단백질은 4개의 도메인으로 이루어질 수 있다. 상기 SCN1A 도메인은 막 관통 세그먼트를 가질 수 있다. 상기 SCN1A 단백질에서 돌연변이는 상기 단백질 전체에 걸쳐 발생할 수 있다. 상기 SCN1A 단백질은 둘 이상의 아이소형으로 이루어질 수 있다. SCN1A에서 돌연변이는 R931C, R946C, M934I, R1648C, 또는 R1648H를 포함할 수 있다. 일부 경우에는, SCN1A 단백질의 C-말단에서 돌연변이가 관찰될 수 있다. SCN1A 단백질에서 돌연변이는 또한 상기 SCN1A 단백질의 처음 3개 도메인의 세그먼트 5와 6 사이의 루프에서 발견될 수 있다. 일부 경우에, 돌연변이는 SCN1A 단백질의 N-말단에서 관찰될 수 있다. SCN1A 내의 예시적인 돌연변이는 R222X, R712X, I227S, R1892X, W952X, R1245X, R1407X, W1434R, c.4338+1G>A, S1516X, L1670fsX1678, 또는 K1846fsX1856을 포함하지만, 이에 제한되지는 않는다. 본 발명에 의해 표적화될 수 있는 돌연변이는 또한 이온 통로의 세공을 코딩할 수 있다.

[0072] 일부 실시양태에서, 본원에 기재된 방법 및 조성물은 DS를 치료하는 데 사용될 수 있다. 다른 실시양태에서, 본원에 기재된 방법 및 조성물은 유아기의 중증 근간대성 뇌전증(SMEI)을 치료하는 데 사용될 수 있다. 다른 실시양태에서, 본원에 기재된 방법 및 조성물은 경계성 드라벳 증후군; 열성 발작 플러스를 동반하는 전신 뇌전증 2형; 가족성 열성 발작 3A; 가족성 편마비 편두통 3; 자폐증; 조기 유아 간질성 뇌병증 13; 동기능 부전 증후군 1; 알츠하이머병 또는 SUDEP를 치료하는 데 사용될 수 있다. 본원에 기재된 방법 및 조성물은 또한 경계성 SMEI를 치료하는 데 사용될 수 있다. 또한, 본원에 기재된 방법 및 조성물은 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+)을 치료하는 데 사용될 수 있다. GEFS+는 SCN1B 또는 GABRG2와 같은 뇌전증 관련 이온통로 서브유닛에서의 돌연변이와 관련될 수 있다. 본원에 기재된 방법 및 조성물은 또한 나트륨 이온통로병증을 치료하는 데 사용될 수 있다. 나트륨 이온통로병증은 SCN1A의 돌연변이와 관련될 수 있다. 나트륨 이온통로병증은 또한 베타 서브유닛 SCN1B와 같은 SCN1A의 서브유닛과 관련될 수 있다. 일부 경우에, SCN1A 돌연변이와 관련된 추가 질환이 또한 본 개시 내용으로 치료될 수 있다. SCN1A 돌연변이와 관련된 연관 SCN1A 질환은 선천성 비정형 근긴장증, 고칼슘성 주기성 마비, 및 선천성 이상 근긴장증을 포함하지만 이에 제한되지는 않는다.

[0073] 일부 실시양태에서, 관련 기술 분야에 공지되어 있고 상기 언급된 문헌(예를 들어 문헌[Hamdan, *et al.*, 2009, Mulley, *et al.*, 2005])에 기재된 임의의 SCN1A 돌연변이를 갖는 대상체는 본원에 기재된 방법 및 조성물을 사용하여 치료될 수 있다. 일부 실시양태에서, 돌연변이는 임의의 *SCN1A* 인트론 또는 엑손 내에 있다.

[0074] **엑손 포함**

[0075] 본원에 사용된 바와 같이, "NIE 함유 프리-mRNA"는 하나 이상의 가성 엑손을 함유하는 프리-mRNA 전사체이다. 선택적 또는 비정상적 스플라이싱은 성숙한 mRNA 전사체에 하나 이상의 가성 엑손의 포함을 초래할 수 있다. 용어 "성숙한 mRNA" 및 "완전히 스플라이싱된 mRNA"는 완전히 가공된 mRNA를 기술하기 위해 본원에서 호환적으로 사용된다. 하나 이상의 가성 엑손의 포함으로 비생산적인 mRNA가 될 수 있고 성숙한 mRNA의 NMD를 유발할 수 있다. NIE를 함유하는 성숙한 mRNA는 때때로 비정상적 단백질 발현을 야기할 수 있다.

[0076] 일부 실시양태에서, 포함된 가성 엑손은 세포에서 표적 단백질을 코딩하는 유전자로부터 전사된 NIE 함유 프리-mRNA 집단에서 가장 풍부한 가성 엑손이다. 일부 실시양태에서, 포함된 가성 엑손은 세포에서 표적 단백질을 코딩하는 유전자로부터 전사된 NIE 함유 프리-mRNA 집단에서 가장 풍부한 가성 엑손이고, 여기서 NIE 함유 프리-mRNA의 집단은 2개 이상의 포함된 가성 엑손을 포함한다. 일부 실시양태에서, 표적 단백질을 코딩하는 NIE 함유 프리-mRNA 집단에서 가장 풍부한 가성 엑손에 표적화되는 안티센스 올리고머는 안티센스 올리고머가 표적화되거나 결합하는 가성 엑손을 포함하여, 집단에서 하나 또는 둘 이상의 가성 엑손의 엑손 스키핑을 유도한다. 실시양태에서, 표적화된 영역은 SCN1A 단백질을 코딩하는 NIE 함유 프리-mRNA에서 가장 풍부한 가성 엑손인 가성 엑손 내에 있다.

[0077] 엑손 포함의 정도는 퍼센트 엑손 포함, 예를 들어 주어진 가성 엑손이 포함된 전사체의 백분율로 표현될 수 있다. 간략하게, 퍼센트 엑손 포함은 엑손 포함을 갖는 RNA 전사체의 양의 평균 + 엑손 배제를 갖는 RNA 전사체의 양의 평균의 합에 대한 엑손 포함을 갖는 RNA 전사체의 양의 백분율로서 계산될 수 있다.

[0078] 일부 실시양태에서, 포함된 가성 엑손은 적어도 약 5%, 적어도 약 10%, 적어도 약 15%, 적어도 약 20%, 적어도 약 25%, 적어도 약 30%, 적어도 약 35%, 적어도 약 40%, 적어도 약 45%, 또는 적어도 약 50% 포함의 결정에 기초하여 포함된 가성 엑손으로서 확인된 엑손이다. 일부 실시양태에서, 포함된 가성 엑손은 5% 내지 약 100%, 약 5% 내지 약 95%, 약 5% 내지 약 90%, 약 5% 내지 약 85%, 약 5% 내지 약 80%, 약 5% 내지 약 75%, 약 5% 내지

약 70%, 약 5% 내지 약 65%, 약 5% 내지 약 60%, 약 5% 내지 약 55%, 약 5% 내지 약 50%, 약 5% 내지 약 45%, 약 5% 내지 약 40%, 약 5% 내지 약 35%, 약 5% 내지 약 30%, 약 5% 내지 약 25%, 약 5% 내지 약 20%, 약 5% 내지 약 15%, 약 10% 내지 약 100%, 약 10% 내지 약 95%, 약 10% 내지 약 90%, 약 10% 내지 약 85%, 약 10% 내지 약 80%, 약 10% 내지 약 75%, 약 10% 내지 약 70%, 약 10% 내지 약 65%, 약 10% 내지 약 60%, 약 10% 내지 약 55%, 약 10% 내지 약 50%, 약 10% 내지 약 45%, 약 10% 내지 약 40%, 약 10% 내지 약 35%, 약 10% 내지 약 30%, 약 10% 내지 약 25%, 약 10% 내지 약 20%, 약 15% 내지 약 100%, 약 15% 내지 약 95%, 약 15% 내지 약 90%, 약 15% 내지 약 85%, 약 15% 내지 약 80%, 약 15% 내지 약 75%, 약 15% 내지 약 70%, 약 15% 내지 약 65%, 약 15% 내지 약 60%, 약 15% 내지 약 55%, 약 15% 내지 약 50%, 약 15% 내지 약 45%, 약 15% 내지 약 40%, 약 15% 내지 약 35%, 약 15% 내지 약 30%, 약 15% 내지 약 25%, 약 20% 내지 약 100%, 약 20% 내지 약 95%, 약 20% 내지 약 90%, 약 20% 내지 약 85%, 약 20% 내지 약 80%, 약 20% 내지 약 75%, 약 20% 내지 약 70%, 약 20% 내지 약 65%, 약 20% 내지 약 60%, 약 20% 내지 약 55%, 약 20% 내지 약 50%, 약 20% 내지 약 45%, 약 20% 내지 약 40%, 약 20% 내지 약 35%, 약 20% 내지 약 30%, 약 25% 내지 약 100%, 약 25% 내지 약 95%, 약 25% 내지 약 90%, 약 25% 내지 약 85%, 약 25% 내지 약 80%, 약 25% 내지 약 75%, 약 25% 내지 약 70%, 약 25% 내지 약 65%, 약 25% 내지 약 60%, 약 25% 내지 약 55%, 약 25% 내지 약 50%, 약 25% 내지 약 45%, 약 25% 내지 약 40%, 또는 약 25% 내지 약 35% 포함의 결정에 기초하여 포함된 가성 엑손으로서 확인된 엑손이다. ENCODE 데이터(예를 들어, 문헌[Tilgner, *et al.*, 2012, "Deep sequencing of subcellular RNA fractions shows splicing to be predominantly co-transcriptional in the human genome but inefficient for lncRNAs," Genome Research 22(9):1616-25]에 기술됨)를 사용하여 엑손 포함을 확인하는데 도움을 줄 수 있다.

[0079] 일부 실시양태에서, *SCN1A* 프리-mRNA 전사체의 표적화된 부분에 상보적인 ASO와 세포를 접촉시킴으로써 ASO의 부재/치료의 부재하에 세포에 의해 생성된 SCN1A 단백질의 양과 비교하여 생성된 SCN1A 단백질의 양을 적어도 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, 또는 1000% 증가시킨다. 일부 실시양태에서, 안티센스 올리고머가 접촉되는 세포에 의해 생성된 SCN1A 단백질의 총량은 대조군 화합물에 의해 생성된 표적 단백질의 양과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가한다, 대조군 화합물은 예를 들어 프리-mRNA의 표적화된 부분에 상보적이지 않은 올리고뉴클레오티드일 수 있다.

[0080] 일부 실시양태에서, *SCN1A* 프리-mRNA 전사체의 표적화된 부분에 상보적인 ASO와 세포를 접촉시킴으로써 ASO의 부재/치료의 부재하에 세포에 의해 생성된 SCN1A 단백질의 양과 비교하여 생성된 SCN1A 단백질의 양을 적어도 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, 또는 1000% 감소시킨다. 일부 실시양태에서, 안티센스 올리고머가 접촉되는 세포에 의해 생성된 단백질의 총량은 대조군 화합물에 의해 생성된 표적 단백질의 양과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소한다. 대조군 화합물은 예를 들어 프리-mRNA의 표적 부분에 상보적이지 않은 올리고뉴클레오티드일 수 있다.

[0081] 일부 실시양태에서, *SCN1A* 프리-mRNA 전사체의 표적화된 부분에 상보적인 ASO와 세포를 접촉시킴으로써 표적 단백질을 코딩하는 성숙한 mRNA를 포함하여 SCN1A를 코딩하는 mRNA의 양을 증가시킨다. 일부 실시양태에서, SCN1A 단백질을 코딩하는 mRNA, 또는 SCN1A 단백질을 코딩하는 성숙한 mRNA의 양은 ASO의 부재/치료의 부재하에 세포에 의해 생성된 단백질의 양과 비교하여 적어도 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, 또는 1000% 증가한다. 일부 실시양태에서, 안티센스 올리고머가 접촉되는 세포에서 생성된 SCN1A 단백질을 코딩하는 mRNA, 또는 SCN1A 단백질을 코딩하는 성숙한 mRNA의 총량은 처리되지 않은 세포, 예를 들어, 처리되지 않은 세포 또는 대조군 화합물로 처리된 세포에서 생성된 성숙한 RNA의 양과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5

배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가한다. 대조군 화합물은 예를 들어 SCN1A NIE 함유 프리-mRNA의 표적화된 부분에 상보적이지 않은 올리고뉴클레오티드일 수 있다.

[0082] 일부 실시양태에서, *SCN1A* 프리-mRNA 전사체의 표적화된 부분에 상보적인 ASO와 세포를 접촉시킴으로써 표적 단백질을 코딩하는 성숙한 mRNA를 포함하여 SCN1A를 코딩하는 mRNA의 양을 감소시킨다. 일부 실시양태에서, SCN1A 단백질을 코딩하는 mRNA, 또는 SCN1A 단백질을 코딩하는 성숙한 mRNA의 양은 ASO의 부재/치료의 부재하에 세포에 의해 생성된 단백질의 양과 비교하여 적어도 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, 또는 1000% 감소한다. 일부 실시양태에서, 안티센스 올리고머가 접촉되는 세포에서 생성된 SCN1A 단백질을 코딩하는 mRNA, 또는 SCN1A 단백질을 코딩하는 성숙한 mRNA의 총량은 처리되지 않은 세포, 예를 들어, 처리되지 않은 세포 또는 대조군 화합물로 처리된 세포에서 생성된 성숙한 RNA의 양과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소한다. 대조군 화합물은 예를 들어 SCN1A NIE 함유 프리-mRNA의 표적화된 부분에 상보적이지 않은 올리고뉴클레오티드일 수 있다.

[0083] NIE는 임의의 길이일 수 있다. 일부 실시양태에서, NIE는 인트론의 전체 서열을 포함하고, 이 경우, 이는 인트론 유지로 지칭될 수 있다. 일부 실시양태에서, NIE는 인트론의 일부일 수 있다. 일부 실시양태에서, NIE는 5'ss 서열을 포함하는 인트론의 5' 말단 부분일 수 있다. 일부 실시양태에서, NIE는 3'ss의 서열을 포함하는 인트론의 3' 말단 부분일 수 있다. 일부 실시양태에서, NIE는 5'ss의 서열을 포함하지 않는 인트론 내의 일부일 수 있다. 일부 실시양태에서, NIE는 3'ss의 서열을 포함하지 않는 인트론 내의 일부일 수 있다. 일부 실시 예에서, NIE는 5'ss 또는 3'ss의 서열을 포함하지 않는 인트론 내의 일부일 수 있다. 일부 실시양태에서, NIE는 5 뉴클레오티드 내지 10 뉴클레오티드 길이, 10 뉴클레오티드 내지 15 뉴클레오티드 갈이, 15 뉴클레오티드 내지 20 뉴클레오티드 길이, 20 뉴클레오티드 내지 25 뉴클레오티드 길이, 25 뉴클레오티드 내지 30 뉴클레오티드 길이, 30 뉴클레오티드 내지 35 뉴클레오티드 길이, 35 뉴클레오티드 내지 40 뉴클레오티드 길이, 40 뉴클레오티드 내지 45 뉴클레오티드 길이, 45 뉴클레오티드 내지 50 뉴클레오티드 길이, 50 뉴클레오티드 내지 55 뉴클레오티드 길이, 55 뉴클레오티드 내지 60 뉴클레오티드 길이, 60 뉴클레오티드 내지 65 뉴클레오티드 길이, 65 뉴클레오티드 내지 70 뉴클레오티드 길이, 70 뉴클레오티드 내지 75 뉴클레오티드 길이, 75 뉴클레오티드 내지 80 뉴클레오티드 길이, 80 뉴클레오티드 내지 85 뉴클레오티드 길이, 85 뉴클레오티드 내지 90 뉴클레오티드 길이, 90 뉴클레오티드 내지 95 뉴클레오티드 길이, 또는 95 뉴클레오티드 내지 100 뉴클레오티드 길이일 수 있다. 일부 실시양태에서, NIE는 적어도 10 뉴클레오티드, 적어도 20 뉴클레오티드, 적어도 30 뉴클레오티드, 적어도 40 뉴클레오티드, 적어도 50 뉴클레오티드, 적어도 60 뉴클레오티드, 적어도 70 뉴클레오티드, 적어도 80 뉴클레오티드 길이, 적어도 90 뉴클레오티드, 또는 적어도 100 뉴클레오티드 길이일 수 있다. 일부 실시양태에서, NIE는 100 내지 200 뉴클레오티드 길이, 200 내지 300 뉴클레오티드 길이, 300 내지 400 뉴클레오티드 길이, 400 내지 500 뉴클레오티드 길이, 500 내지 600 뉴클레오티드 길이, 600 내지 700 뉴클레오티드 길이, 700 내지 800 뉴클레오티드 길이, 800 내지 900 뉴클레오티드 길이, 900 내지 1,000 뉴클레오티드 길이일 수 있다. 일부 실시양태에서, NIE는 1,000 뉴클레오티드 길이보다 더 길 수 있다.

[0084] 가성 엑손의 포함은 프레임 시프트 및 전사체를 NMD의 표적으로 만드는 성숙한 mRNA 전사체에 조기 종결 코돈(PIC)의 도입을 초래할 수 있다. NIE를 함유하는 성숙한 mRNA 전사체는 단백질 발현을 유발하지 않는 비생산적인 mRNA 전사체일 수 있다. PIC는 NIE의 하류 임의의 위치에 존재할 수 있다. 일부 실시양태에서, PIC는 NIE의 하류의 임의의 엑손에 존재할 수 있다. 일부 실시양태에서, PIC는 NIE 내에 존재할 수 있다. 예를 들어, *SCN1A* 유전자에 의해 코딩된 mRNA 전사체에 엑손 20x의 포함은 mRNA 전사체에 PIC, 예를 들어 mRNA 전사체의 엑손 21에 PIC가 유도될 수 있다.

[0085] **치료제**

[0086] 본 개시 내용의 다양한 실시양태에서, SCN1A의 단백질 발현 수준을 조절하기 위해 치료제를 포함하는 조성물 및 방법이 제공된다. 일부 실시양태에서, *SCNA1* 프리-mRNA의 선택적 스플라이싱을 조절하기 위한 조성물 및 방법이

본원에 제공된다. 일부 실시양태에서, 예를 들어 SCN1A 프리-mRNA의 스플라이싱 중에 가성 엑손의 스키핑을 유도하기 위해, SCN1A 프리-mRNA의 스플라이싱에서 엑손 스키핑을 유도하는 조성물 및 방법이 본원에 제공된다. 다른 실시양태에서, 단백질 발현 수준을 감소시키기 위해 치료제를 사용하여 엑손의 포함을 유도할 수 있다.

[0087] 일부 실시양태에서, 본원에 개시된 치료제는 소분자, 폴리펩티드, 또는 폴리핵산 중합체이다. 일부 경우에, 치료제는 소분자이다. 일부 경우에, 치료제는 폴리펩티드이다. 일부 경우에, 치료제는 폴리핵산 중합체이다. 일부 경우에, 치료제는 억제제 작용제이다. 추가의 경우에, 치료제는 인핸서 작용제이다.

[0088] 본원에 개시된 치료제는 NIE 억제제 작용제일 수 있다. 치료제는 폴리핵산 중합체를 포함할 수 있다.

[0089] 본 개시 내용의 한 측면에 따르면, 기능성 SCN1A 단백질의 수준을 증가시키기 위해 NIE 억제제 작용제를 대상체에게 투여하는 단계를 포함하는 기능성 SCN1A 단백질 결핍과 관련된 병태의 치료 또는 예방 방법으로서, 상기 작용제는 프리-mRNA 전사체의 영역에 결합하여 성숙한 전사체에 NIE의 포함을 감소시키는 것인 방법이 본원에 제공된다. 예를 들어, 기능성 SCN1A 단백질의 수준을 증가시키기 위해 NIE 억제제 작용제를 대상체에게 투여하는 단계를 포함하는 기능성 SCN1A 단백질 결핍과 관련된 병태의 치료 또는 예방 방법으로서, 상기 작용제는 프리-mRNA 전사체의 NIE를 함유하는 인트론의 영역(예를 들어, 인간 *SCN1A* 유전자에서 인트론 20) 또는 동일한 인트론 내 NIE 활성화 조절 서열에 결합하는 것인 방법이 본원에서 제공된다.

[0090] 성숙한 mRNA에 NIE 포함을 감소시키는 것이 언급되는 경우, 감소는 완전, 예를 들어 100%일 수 있거나, 부분적일 수 있다. 감소는 임상적으로 유의할 수 있다. 감소/교정은 치료 없는 대상체에서 NIE 포함 수준 또는 유사한 대상체 집단에서 NIE 포함 양에 상대적일 수 있다. 감소/교정은 평균 대상체, 또는 치료 전 대상체에 비해 NIE 포함이 10% 이상 적을 수 있다. 감소는 평균 대상체 또는 치료 전 대상체에 비해 NIE 포함이 20% 이상 적을 수 있다. 감소는 평균 대상체 또는 치료 전 대상체에 비해 NIE 포함이 40% 이상 적을 수 있다. 감소는 평균 대상체 또는 치료 전 대상체에 비해 NIE 포함이 50% 이상 적을 수 있다. 감소는 평균 대상체 또는 치료 전 대상체에 비해 NIE 포함이 60% 이상 적을 수 있다. 감소는 평균 대상체 또는 치료 전 대상체에 비해 NIE 포함이 80% 이상 적을 수 있다. 감소는 평균 대상체 또는 치료 전 대상체에 비해 NIE 포함이 90% 이상 적을 수 있다.

[0091] 활성 SCN1A 단백질 수준을 증가시키는 것이 언급되는 경우, 증가는 임상적으로 유의할 수 있다. 증가는 치료 없는 대상체에서 활성 SCN1A 단백질의 수준에 상대적일 수 있거나 유사한 대상체 집단에서 활성 SCN1A 단백질의 양에 상대적일 수 있다. 증가는 평균 대상체, 또는 치료 전 대상체에 비해 활성 SCN1A 단백질이 10% 이상 많을 수 있다. 증가는 평균 대상체, 또는 치료 전 대상체에 비해 20% 이상 많을 수 있다. 증가는 평균 대상체, 또는 치료 전 대상체에 비해 활성 SCN1A 단백질이 40% 이상 많을 수 있다. 증가는 평균 대상체, 또는 치료 전 대상체에 비해 활성 SCN1A 단백질이 50% 이상 많을 수 있다. 증가는 평균 대상체, 또는 치료 전 대상체에 비해 활성 SCN1A 단백질이 80% 이상 많을 수 있다. 증가는 평균 대상체, 또는 치료 전 대상체에 비해 활성 SCN1A 단백질이 100% 이상 많을 수 있다. 증가는 평균 대상체, 또는 치료 전 대상체에 비해 활성 SCN1A 단백질이 200% 이상 많을 수 있다. 증가는 평균 대상체, 또는 치료 전 대상체에 비해 활성 SCN1A 단백질이 500% 이상 많을 수 있다.

[0092] NIE 억제제 작용제가 폴리핵산 중합체를 포함하는 실시양태에서, 폴리핵산 중합체는 약 50 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 45 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 40 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 35 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 30 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 24 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 25 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 20 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 19 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 18 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 17 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 16 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 15 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 14 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 13 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 12 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 11 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 10 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 10 내지 약 50 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 10 내지 약 45 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 10 내지 약 40 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 10 내지 약 35 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 10 내지 약 30 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 10 내지 약 25 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 10 내지 약 20 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 15 내지 약 25 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 15 내지 약 30 뉴클레오티드 길이일 수 있다. 폴리핵산 중합체는 약 12 내지 약 30 뉴클레오티드 길이일 수 있다.

[0093] 폴리핵산 중합체의 서열은 mRNA 전사체, 예를 들어 부분적으로 가공된 mRNA 전사체의 표적 서열과 적어도 50%,

55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 또는 99.5% 상보적일 수 있다. 폴리핵산 중합체의 서열은 프리-mRNA 전사체의 표적 서열에 100% 상보적일 수 있다.

[0094] 폴리핵산 중합체의 서열은 프리-mRNA 전사체의 표적 서열과 4개 이하의 불일치를 가질 수 있다. 폴리핵산 중합체의 서열은 프리-mRNA 전사체의 표적 서열과 3개 이하의 불일치를 가질 수 있다. 폴리핵산 중합체의 서열은 프리-mRNA 전사체의 표적 서열과 2개 이하의 불일치를 가질 수 있다. 폴리핵산 중합체의 서열은 프리-mRNA 전사체의 표적 서열과 1개 이하의 불일치를 가질 수 있다. 폴리핵산 중합체의 서열은 프리-mRNA 전사체의 표적 서열과 불일치를 갖지 않을 수 있다.

[0095] 폴리핵산 중합체는 프리-mRNA 전사체의 표적 서열에 특이적으로 혼성화할 수 있다. 예를 들어, 폴리핵산 중합체는 프리-mRNA 전사체의 표적 서열에 대해 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% 또는 100% 서열 상보성을 가질 수 있다. 혼성화는 매우 엄격한 혼성화 조건하에 있을 수 있다.

[0096] 폴리핵산 중합체는 서열 번호 21 내지 67로 이루어진 군으로부터 선택된 서열과 적어도 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 또는 99.5% 서열 동일성을 갖는 서열을 가질 수 있다. 폴리핵산 중합체는 서열 번호 21 내지 67로 이루어진 군으로부터 선택된 서열과 100% 서열 동일성을 갖는 서열을 가질 수 있다. 일부 경우에, 폴리핵산 중합체는 서열 번호 68 내지 114로 이루어진 군으로부터 선택된 서열과 적어도 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 또는 99.5% 서열 동일성을 갖는 서열을 가질 수 있다. 일부 경우에, 폴리핵산 중합체는 서열 번호 68 내지 114로 이루어진 군으로부터 선택된 서열과 100% 서열 동일성을 갖는 서열을 가질 수 있다.

[0097] 폴리핵산 중합체 서열이 언급되는 경우, 당업자는 표적 서열에 혼성화하는 능력; 또는 표적 서열에 치환이 있는 경우, 표적 서열로 인식될 수 있는 능력이 유지되기 위해서 서열에서 하나 이상의 치환이 허용될 수 있고, 경우에 따라 2개의 치환이 허용될 수 있음을 이해할 것이다. 서열 동일성에 대한 기준은 표준/기본 파라미터를 사용한 BLAST 서열 정렬에 의해 결정될 수 있다. 예를 들어, 서열은 99% 동일성을 갖고 본 개시에 따라 여전히 기능을 할 수 있다. 다른 실시양태에서, 서열은 98% 동일성을 가질 수 있고 본 개시 내용에 따라 여전히 기능을 할 수 있다. 다른 실시 형태에서, 서열은 95% 동일성을 가질 수 있고 본 발명에 따라 여전히 기능을 할 수 있다. 다른 실시양태에서, 서열은 90% 동일성을 갖고 본 개시 내용에 따라 여전히 기능을 할 수 있다.

[0098] **안티센스 올리고머**

[0099] *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분에 결합함으로써 엑손 스키핑을 유도하는 안티센스 올리고머를 포함하는 조성물이 본원에 제공된다. 본원에 사용된 바와 같이, 용어 "ASO" 및 "안티센스 올리고머"는 호환적으로 사용되며, 왓슨-크릭(Watson-Crick) 염기 짝짓기 또는 워블(wobble) 염기 짝짓기(G-U)에 의해 표적 핵산(예를 들어, *SCN1A* NIE 함유 프리-mRNA) 서열에 혼성화하는 핵염기를 포함하는 폴리뉴클레오티드와 같은 올리고머를 지칭한다. ASO는 표적 서열에 상보적이거나 또는 상보성에 가까운(예를 들어, 표적 서열에 결합하고 스플라이스 부위에서 스플라이싱을 향상시키기에 충분한 상보성) 정확한 서열을 가질 수 있다. ASO는 이들이 표적 핵산(예를 들어, 프리-mRNA 전사체의 표적화된 부분)에 결합(혼성화)하고 생리 학적 조건하에서 혼성화된 상태를 유지하도록 설계된다. 전형적으로, 이들이 의도된(표적된) 핵산 서열 이외의 부위에 혼성화하는 경우, 이들은 표적 핵산이 아닌 제한된 수의 서열에(표적 핵산 이외의 몇몇 부위에) 혼성화된다. ASO가 다른 부위에 결합하고 "표적 외(off-target)" 효과를 야기할 가능성을 제한되도록 ASO의 설계는 프리-mRNA 전사체의 표적화된 부분의 핵산 서열의 발생 또는 게놈 또는 세포 프리-mRNA 또는 전사체의 다른 위치에서 충분히 유사한 핵산 서열의 발생을 고려할 수 있다. 당 업계에 알려진, 예를 들어 WO 2015/035091호로 공개된 PCT 출원 PCT/US2014/054151호(발명의 명칭 "Reducing Nonsense-Mediated mRNA Decay", 본원에서 참고로 포함됨)에서의 임의의 안티센스 올리고머는 본원에 기재된 방법을 실시하는 데 사용될 수 있다.

[0100] 일부 실시양태에서, ASO는 표적 핵산 또는 NIE 함유 프리-mRNA의 표적화된 부분에 "특이적으로 혼성화"하거나 "특이적"이다. 전형적으로 이러한 혼성화는 실질적으로 37℃ 초과, 바람직하게는 50℃ 이상, 전형적으로 60℃ 내지 대략 90℃의 T_m에서 발생한다. 이러한 혼성화는 바람직하게는 엄격한 혼성화 조건에 해당한다. 주어진 이온 강도 및 pH에서, T_m은 표적 서열의 50%가 상보적인 올리고뉴클레오티드에 혼성화하는 온도이다.

[0101] 올리고뉴클레오티드와 같은 올리고머는 2개의 단일 가닥 폴리뉴클레오티드 사이의 역평행 배열로 혼성화가 일어날 때 서로 "상보적"이다. 이중 가닥 폴리뉴클레오티드는 제1 폴리뉴클레오티드의 가닥 중 하나와 제2 폴리뉴클레오티드 사이에서 혼성화가 발생할 수 있는 경우 다른 폴리뉴클레오티드에 대해 "상보적"일 수 있다. 상보성(하나의 폴리뉴클레오티드가 다른 폴리뉴클레오티드와 상보적인 정도)은 일반적으로 허용되는 염기 짝짓기 규칙

에 따라 서로 수소 결합을 형성할 것으로 예상되는 반대 가닥의 염기의 비율(예를 들어, 백분율)에 대해 정량화할 수 있다. 안티센스 올리고머(ASO)의 서열은 혼성화하기 위해 그의 표적 핵산의 서열과 100% 상보적일 필요는 없다. 특정 실시양태에서, ASO는 그들이 표적화되는 표적 핵산 서열 내의 표적 영역과 적어도 70%, 적어도 75%, 적어도 80%, 적어도 85%, 적어도 90%, 적어도 95%, 적어도 96%, 적어도 97%, 적어도 98%, 또는 적어도 99% 서열 상보성을 포함할 수 있다. 예를 들어, 올리고머 화합물의 20개 핵염기 중 18개가 표적 영역에 상보적이고, 따라서 특이적으로 혼성화하는 ASO는 90% 상보성을 나타낸다. 이 예에서, 나머지 비-상보적인 핵염기는 함께 클러스터링되거나 상보적인 핵염기와 함께 산재될 수 있으며 서로 또는 상보적인 핵염기에 대해 인접할 필요는 없다. 표적 핵산의 영역과 ASO의 상보성 백분율은 당 업계에 공지된 BLAST 프로그램(기본 국소 정렬 검색 도구) 및 PowerBLAST 프로그램(Altschul, *et al.*, J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656)을 사용하여 통상적으로 결정될 수 있다.

[0102] ASO는 표적 서열에서 모든 핵염기에 혼성화할 필요는 없고, 그가 혼성화하는 핵염기는 인접하거나 비인접할 수 있다. ASO는 프리-mRNA 전사체의 하나 이상의 세그먼트에 걸쳐 혼성화할 수 있어서, 개재 또는 인접 세그먼트가 혼성화 이벤트에 관여하지 않는다(예를 들어, 루프 구조 또는 헤어핀 구조가 형성될 수 있음). 특정 실시양태에서, ASO는 표적 프리-mRNA 전사체에서 비인접한 핵염기에 혼성화한다. 예를 들어, ASO는 ASO가 혼성화하지 않는 하나 이상의 핵염기(들)에 의해 분리된 프리-mRNA 전사체에서 핵염기에 혼성화할 수 있다.

[0103] 본원에 기재된 ASO는 NIE 함유 프리-mRNA의 표적화된 부분에 존재하는 핵염기에 상보적인 핵염기를 포함한다. 용어 ASO는 올리고뉴클레오티드 및 표적 mRNA 상의 상보적 핵염기와 혼성화할 수 있는 핵염기는 포함하지만 펩티드 핵산(PNA)과 같은 당 모이어티를 포함하지 않는 임의의 다른 올리고머 분자를 포함한다. ASO는 자연 발생 뉴클레오티드, 뉴클레오티드 유사체, 변형된 뉴클레오티드, 또는 상기한 것 중 둘 또는 셋의 임의의 조합을 포함할 수 있다. 용어 "자연 발생 뉴클레오티드"는 데옥시리보뉴클레오티드 및 리보뉴클레오티드를 포함한다. 용어 "변형된 뉴클레오티드"는 변형되거나 치환된 당 기를 갖고/하거나 변형된 백본을 갖는 뉴클레오티드를 포함한다. 일부 실시양태에서, ASO의 모든 뉴클레오티드는 변형된 뉴클레오티드이다. 본원에 기재된 방법 및 조성물과 상용성인 ASO 또는 ASO의 성분의 화학적 변형은 당업자에게 명백할 것이며, 예를 들어 미국 특허 제8,258,109 B2호, 미국 특허 제5,656,612호, 미국 특허 공개 제2012/0190728호, 및 문헌[and Dias and Stein, Mol. Cancer Ther. 2002, 347-355]에서 찾아볼 수 있으며, 이들은 그 전문이 본원에 참고로 포함된다.

[0104] ASO의 하나 이상의 핵염기는 임의의 자연 발생, 변형되지 않은 핵염기, 예를 들어 아데닌, 구아닌, 시토신, 티민 및 우라실, 또는 변형되지 않은 핵염기와 충분히 유사하여 그것이 표적 프리-mRNA 상에 존재하는 핵염기와 수소 결합할 수 있는 임의의 합성 또는 변형된 핵염기일 수 있다. 변형된 핵염기의 예로는 히포크산틴, 크산틴, 7-메틸구아닌, 5,6-디히드로우라실, 5-메틸시토신, 및 5-히드록시메토일 시토신이 제한 없이 포함된다.

[0105] 본원에 기재된 ASO는 또한 올리고머의 성분을 연결하는 백본 구조를 포함한다. 용어 "백본 구조" 및 "올리고머 결합"은 호환적으로 사용될 수 있으며 ASO의 단량체 사이의 연결을 지칭한다. 자연 발생 올리고뉴클레오티드에서, 백본은 올리고머의 당 모이어티를 연결하는 3'-5' 포스포디에스테르 결합을 포함한다. 본원에 기재된 ASO의 백본 구조 또는 올리고머 결합은 포스포로티오에이트, 포스포로디티오에이트, 포스포로셀레노에이트, 포스포로디셀레노에이트, 포스포로아닐로티오에이트, 포스포라닐라데이트, 포스포라미데이트 등을 포함할 수 있다(그러나, 이에 이에 제한되지는 않음). 예를 들어, 문헌[LaPlanche, *et al.*, Nucleic Acids Res. 14:9081 (1986); Stec, *et al.*, J. Am. Chem. Soc. 106:6077 (1984), Stein, *et al.*, Nucleic Acids Res. 16:3209 (1988), Zon, *et al.*, Anti-Cancer Drug Design 6:539 (1991); Zon, et al., Oligonucleotides and Analogues: A Practical Approach, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec, *et al.*, 미국 특허 제5,151,510호; Uhlmann and Peyman, Chemical Reviews 90:543 (1990)]을 참조한다. 일부 실시양태에서, ASO의 백본 구조는 인을 함유하지 않고, 오히려 예를 들어 펩티드 핵산(PNA) 내, 펩티드 결합, 또는 카바메이트, 아미드, 및 선형 및 환식 탄화수소 기를 포함하는 연결 기를 함유한다. 일부 실시양태에서, 백본 변형은 포스포티오에이트 결합이다. 일부 실시양태에서, 백본 변형은 포스포라미데이트 결합이다.

[0106] 실시양태에서, ASO 백본의 인 뉴클레오티드 간 결합의 각각에서 입체 화학은 무작위적이다. 실시양태에서, ASO 백본의 인 뉴클레오티드 간 결합의 각각에서 입체 화학은 제어되고 무작위적이지 않다. 예를 들어, 본원에 참고로 포함된 미국 특허 출원 제2014/0194610호("Methods for the Synthesis of Functionalized Nucleic Acids,")는 핵산 올리고머에서 각각의 인 원자에서 키랄 선성(handedness of chirality)을 독립적으로 선택하는 방법을 기술한다. 실시양태에서, 표 5 및 6에 본원에 제시된 임의의 ASO를 포함하는, 그러나 이에 제한되지 않는 본 발명의 방법에 사용된 ASO는 무작위적이지 않은 인 뉴클레오티드 간 결합을 갖는 ASO를 포함한다. 실시양태에서, 본 발명의 방법에 사용된 조성물은 순수한 부분 입체 이성질체 ASO를 포함한다. 실시양태에서, 본 발명의

방법에 사용된 조성물은 약 90% 이상, 약 91% 이상, 약 92% 이상, 약 93% 이상, 약 94% 이상, 약 95% 이상, 약 96% 이상, 약 97% 이상, 약 98% 이상, 약 99% 이상, 약 100%, 약 90% 내지 약 100%, 약 91% 내지 약 100%, 약 92% 내지 약 100%, 약 93% 내지 약 100%, 약 94% 내지 약 100%, 약 95% 내지 약 100%, 약 96% 내지 약 100%, 약 97% 내지 약 100%, 약 98% 내지 약 100%, 또는 약 99% 내지 약 100%의 부분 입체 이성질체 순도를 갖는 ASO를 포함한다.

[0107] 실시양태에서, ASO는 그의 인 뉴클레오티드 간 결합에서 Rp 및 Sp 배열의 비무작위 혼합물을 갖는다. 예를 들어, 우수한 활성과 뉴클레아제 안정성 사이의 균형을 달성하기 위해 안티센스 올리고뉴클레오티드에서 Rp 및 Sp의 혼합이 필요하다는 것이 제안되었다(Wan, *et al.*, 2014, "Synthesis, biophysical properties and biological activity of second generation antisense oligonucleotides containing chiral phosphorothioate linkages," Nucleic Acids Research 42(22): 13456-13468, 본원에 참고로 포함됨). 실시양태에서, 서열 번호 21 내지 114에 본원에 제시된 임의의 ASO를 포함하는, 그러나 이에 제한되지 않는 본 발명의 방법에 사용되는 ASO는 약 5 내지 100% Rp, 적어도 약 5% Rp, 적어도 약 10% Rp, 적어도 약 15% Rp, 적어도 약 20% Rp, 적어도 약 25% Rp, 적어도 약 30% Rp, 적어도 약 35% Rp, 적어도 약 40% Rp, 적어도 약 45% Rp, 적어도 약 50% Rp, 적어도 약 55% Rp, 적어도 약 60% Rp, 적어도 약 65% Rp, 적어도 약 70% Rp, 적어도 약 75% Rp, 적어도 약 80% Rp, 적어도 약 85% Rp, 적어도 약 90% Rp, 또는 적어도 약 95% Rp, 그리고 나머지의 Sp, 또는 약 100% Rp를 포함한다. 실시양태에서, 서열 21 내지 114에 본원에 기재된 임의의 ASO를 포함하는, 그러나 이에 제한되지 않는 본 발명의 방법에 사용되는 ASO는 약 10% 내지 약 100% Rp, 약 15% 내지 약 100% Rp, 약 20% 내지 약 100% Rp, 약 25% 내지 약 100% Rp, 약 30% 내지 약 100% Rp, 약 35% 내지 약 100% Rp, 약 40% 내지 약 100% Rp, 약 45% 내지 약 100% Rp, 약 50% 내지 약 100% Rp, 약 55% 내지 약 100% Rp, 약 60% 내지 약 100% Rp, 약 65% 내지 약 100% Rp, 약 70% 내지 약 100% Rp, 약 75% 내지 약 100% Rp, 약 80% 내지 약 100% Rp, 약 85% 내지 약 100% Rp, 약 90% 내지 약 100% Rp, 또는 약 95% 내지 약 100% Rp, 약 20% 내지 약 80% Rp, 약 25% 내지 약 75% Rp, 약 30% 내지 약 70% Rp, 약 40% 내지 약 60% Rp, 또는 약 45% 내지 약 55% Rp, 그리고 나머지의 Sp를 포함한다.

[0108] 실시양태에서, 서열 21 내지 114에 본원에 기재된 임의의 ASO를 포함하는, 그러나 이에 제한되지 않는 본 발명의 방법에 사용되는 ASO는 약 5 내지 100% Sp, 적어도 약 5% Sp, 적어도 약 10% Sp, 적어도 약 15% Sp, 적어도 약 20% Sp, 적어도 약 25% Sp, 적어도 약 30% Sp, 적어도 약 35% Sp, 적어도 약 40% Sp, 적어도 약 45% Sp, 적어도 약 50% Sp, 적어도 약 55% Sp, 적어도 약 60% Sp, 적어도 약 65% Sp, 적어도 약 70% Sp, 적어도 약 75% Sp, 적어도 약 80% Sp, 적어도 약 85% Sp, 적어도 약 90% Sp, 또는 적어도 약 95% Sp, 그리고 나머지의 Rp, 또는 약 100% Sp를 포함한다. 실시양태에서, 서열 21 내지 114에 본원에 기재된 임의의 ASO를 포함하는, 그러나 이에 제한되지 않는 본 발명의 방법에 사용되는 ASO는 약 10% 내지 약 100% Sp, 약 15% 내지 약 100% Sp, 약 20% 내지 약 100% Sp, 약 25% 내지 약 100% Sp, 약 30% 내지 약 100% Sp, 약 35% 내지 약 100% Sp, 약 40% 내지 약 100% Sp, 약 45% 내지 약 100% Sp, 약 50% 내지 약 100% Sp, 약 55% 내지 약 100% Sp, 약 60% 내지 약 100% Sp, 약 65% 내지 약 100% Sp, 약 70% 내지 약 100% Sp, 약 75% 내지 약 100% Sp, 약 80% 내지 약 100% Sp, 약 85% 내지 약 100% Sp, 약 90% 내지 약 100% Sp, 또는 약 95% 내지 약 100% Sp, 약 20% 내지 약 80% Sp, 약 25% 내지 약 75% Sp, 약 30% 내지 약 70% Sp, 약 40% 내지 약 60% Sp, 또는 약 45% 내지 약 55% Sp, 그리고 나머지의 Rp를 포함한다.

[0109] 본원에 기재된 임의의 ASO는 자연 발생 뉴클레오티드에 존재하는 리보오스 또는 데옥시리보오스, 또는 변형된 당 모이어티, 또는 모르폴린 고리를 포함하는 당 유사체를 포함하는 당 모이어티를 함유할 수 있다. 변형된 당 모이어티의 비제한적 예는 2' 치환, 예를 들어 2'-O-메틸(2'-O-Me), 2'-O-메톡시에틸(2'MOE), 2'-O-아미노에틸, 2'F; N3'->P5' 포스포라미데이트, 2'디메틸아미노옥시에톡시, 2'디메틸아미노에톡시에톡시, 2'-구아니디니듐, 2'-O-구아니디늄 에틸, 카바메이트 변형된 당, 및 이환식 변형된 당을 포함한다. 일부 실시양태에서, 당 모이어티 변형은 2'-O-Me, 2'F, 및 2'MOE로부터 선택된다. 일부 실시양태에서, 당 모이어티 변형은 잠금 핵산(LNA)에서와 같은 추가의 가교 결합이다. 일부 실시양태에서, 당 유사체는 포스포로디아미데이트 모르폴리노(PMO)와 같은 모르폴린 고리를 함유한다. 일부 실시양태에서, 당 모이어티는 리보푸란실 또는 2'데옥시리보푸란실 변형을 포함한다. 일부 실시양태에서, 당 모이어티는 2'4'-구속된 2'O-메틸옥시에틸(cMOE) 변형을 포함한다. 일부 실시양태에서, 당 모이어티는 cEt 2', 4' 구속된 2'-O 에틸 BNA 변형을 포함한다. 일부 실시양태에서, 당 모이어티는 트리시클로 DNA(tcDNA) 변형을 포함한다. 일부 실시양태에서, 당 모이어티는 에틸렌 핵산(ENA) 변형을 포함한다. 일부 실시양태에서, 당 모이어티는 MCE 변형을 포함한다. 변형은 당 업계에 공지되어 있고 문헌, 예를 들어 본원에서 이러한 목적을 위해 참고로 포함된 문헌[Jarver, *et al.*, 2014, "A Chemical View of Oligonucleotides for Exon Skipping and Related Drug Applications," Nucleic Acid Therapeutics

24(1): 37-47]에 기술되어 있다.

[0110] 일부 실시양태에서, ASO의 각 단량체는 동일한 방식으로 변형되며, 예를 들어 ASO의 백본의 각각의 결합은 포스포로티오에이트 결합을 포함하거나 각각의 리보오스 당 모이어티는 2'O-메틸 변형을 포함한다. ASO의 각 단량체 성분에 존재하는 이러한 변형은 "균일한 변형"으로 지칭된다. 일부 예에서, 상이한 변형의 조합이 바람직할 수 있으며, 예를 들어, ASO는 포스포로디아미데이트 결합과 모르폴린 고리(모르폴리노)를 포함하는 당 모이어티의 조합을 포함할 수 있다. ASO에 대한 상이한 변형의 조합은 "혼합 변형" 또는 "혼합 화학"으로 지칭된다.

[0111] 일부 실시양태에서, ASO는 하나 이상의 백본 변형을 포함한다. 일부 실시양태에서, ASO는 하나 이상의 당 모이어티 변형을 포함한다. 일부 실시양태에서, ASO는 하나 이상의 백본 변형 및 하나 이상의 당 모이어티 변형을 포함한다. 일부 실시양태에서, ASO는 2'MOE 변형 및 포스포로티오에이트 백본을 포함한다. 일부 실시양태에서, ASO는 포스포로디아미데이트 모르폴리노(PMO)를 포함한다. 일부 실시양태에서, ASO는 펩티드 핵산(PNA)을 포함한다. 본원에 기재된 임의의 ASO 또는 ASO의 임의의 성분(예를 들어, 핵염기, 당 모이어티, 백본)은 ASO의 원하는 특성 또는 활성을 달성하거나 ASO의 원하지 않는 특성 또는 활성을 감소시키기 위해 변형될 수 있다. 예를 들어, ASO 또는 임의의 ASO의 하나 이상의 성분은 변형되어 프리-mRNA 전사체 상의 표적 서열에 대한 결합 친화성을 향상시키고/거나; 임의의 비-표적 서열에 대한 결합을 감소시키고/거나; 세포 뉴클레아제(즉, RNase H)에 의한 분해를 감소시키고/거나; 세포 및/또는 세포의 핵으로의 ASO의 흡수를 개선시키고/거나; ASO의 약동학 또는 약력학을 변경하고/거나; ASO의 반감기를 조절할 수 있다.

[0112] 일부 실시양태에서, ASO는 2'-O-(2-메톡시에틸)(MOE) 포스포로티오에이트-변형된 뉴클레오티드로 구성된다. 이러한 뉴클레오티드로 구성된 ASO는 본원에 개시된 방법에 특히 적합하며; 이러한 변형을 갖는 올리고머는 뉴클레아제 분해에 대한 내성이 상당히 향상되고, 생체 이용률이 증가하여, 예를 들어 본원에 기재된 일부 실시양태에서 경구 전달에 적합하게 만드는 것으로 나타났다. 예를 들어, 문헌[Geary, *et al.*, J Pharmacol Exp Ther. 2001; 296(3):890-7; Geary, *et al.*, J Pharmacol Exp Ther. 2001; 296(3):898-904] 참조.

[0113] ASO를 합성하는 방법은 당업자에게 공지될 것이다. 대안적으로 또는 추가로, ASO는 상업적 공급원으로부터 얻을 수 있다.

[0114] 달리 명시되지 않는 한, 단일 가닥 핵산(예를 들어, 프리-mRNA 전사체, 올리고뉴클레오티드, ASO 등) 서열의 좌측 말단은 5' 말단이고 단일 또는 이중 가닥 핵산 서열의 좌측 방향은 5' 방향으로 지칭된다. 유사하게, 핵산 서열(단일 또는 이중 가닥)의 우측 말단 또는 방향은 3' 말단 또는 방향이다. 일반적으로, 핵산에서 기준점에 대해 5'인 영역 또는 서열을 "상류"로 지칭하고, 핵산에서 기준점에 대해 3'인 영역 또는 서열을 "하류"로 지칭한다. 일반적으로 mRNA의 5' 방향 또는 말단은 개시 또는 시작 코돈이 위치하는 곳인 반면, 3' 말단 또는 방향은 종결 코돈이 위치하는 곳이다. 일부 측면에서, 핵산에서 기준점의 상류에 있는 뉴클레오티드는 음수로 지정될 수 있는 반면, 기준점의 하류에 있는 뉴클레오티드는 양수로 지정될 수 있다. 예를 들어, 기준점(예를 들어, mRNA에서의 엑손-엑손 접합부)은 "0(zero)" 부위로 지정될 수 있고, 기준점의 바로 인접하고 상류에 있는 뉴클레오티드는 "마이너스 1", 예를 들어 "-1"로 지정되는 반면, 기준점의 바로 인접하고 하류에 있는 뉴클레오티드는 "플러스 1", 예를 들어 "+1"로 지정된다.

[0115] 일부 실시양태에서, ASO는 *SCN1A* NIE 함유 프리-mRNA에 포함된 엑손의 5' 스플라이스 부위(또는 NIE의 3' 말단)의 하류(3' 방향)(예를 들어, 5' 스플라이스 부위에 대해 양수로 지정되는 방향)에 있는 *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분에 상보적이다(결합한다). 일부 실시양태에서, ASO는 포함된 엑손의 5' 스플라이스 부위(또는 3'말단)에 대해 약 +1 내지 약 +500의 영역 내에 있는 *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분에 상보적이다. 일부 실시양태에서, ASO는 포함된 엑손의 5' 스플라이스 부위(또는 3' 말단)에 대해 뉴클레오티드 +6 내지 +496의 영역 내에 있는 *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분에 상보적일 수 있다. 일부 측면에서, ASO는 포함된 엑손의 5' 스플라이스 부위(또는 3' 말단)에 대해 약 +1 내지 약 +500, 약 +1 내지 약 +490, 약 +1 내지 약 +480, 약 +1 내지 약 +470, 약 +1 내지 약 +460, 약 +1 내지 약 +450, 약 +1 내지 약 +440, 약 +1 내지 약 +430, 약 +1 내지 약 +420, 약 +1 내지 약 +410, 약 +1 내지 약 +400, 약 +1 내지 약 +390, 약 +1 내지 약 +380, 약 +1 내지 약 +370, 약 +1 내지 약 +360, 약 +1 내지 약 +350, 약 +1 내지 약 +340, 약 +1 내지 약 +330, 약 +1 내지 약 +320, 약 +1 내지 약 +310, 약 +1 내지 약 +300, 약 +1 내지 약 +290, 약 +1 내지 약 +280, 약 +1 내지 약 +270, 약 +1 내지 약 +260, 약 +1 내지 약 +250, 약 +1 내지 약 +240, 약 +1 내지 약 +230, 약 +1 내지 약 +220, 약 +1 내지 약 +210, 약 +1 내지 약 +200, 약 +1 내지 약 +190, 약 +1 내지 약 +180, 약 +1 내지 약 +170, 약 +1 내지 약 +160, 약 +1 내지 약 +150, 약 +1 내지 약 +140, 약 +1 내지 약 +130, 약 +1 내지 약 +120, 약 +1 내지 약 +110, 약 +1 내지 약 +100, 약 +1 내지 약 +90, 약 +1 내지 약 +80,

약 +1 내지 약 +70, 약 +1 내지 약 +60, 약 +1 내지 약 +50, 약 +1 내지 약 +40, 약 +1 내지 약 +30, 또는 약 +1 내지 약 +20의 영역 내에 있는 표적화된 부분에 상보적이다. 일부 측면에서, ASO는 포함된 엑손의 5' 스플라이스 부위(또는 3' 말단)에 대해 약 +1 내지 약 +100, 약 +100 내지 약 +200, 약 +200 내지 약 +300, 약 +300 내지 약 +400, 또는 약 +400 내지 약 +500의 영역 내에 있는 표적화된 부분에 상보적이다.

[0116] 일부 실시양태에서, ASO는 *SCN1A* NIE 함유 프리-mRNA에 포함된 엑손의 5' 스플라이스 부위(또는 3' 말단)의 상류(5' 방향)(예를 들어, 5' 스플라이스 부위에 대해 음수로 지정되는 방향)에 있는 *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분에 상보적이다(결합한다). 일부 실시양태에서, ASO는 포함된 엑손의 5' 스플라이스 부위(또는 3' 말단)에 대해 약 -4 내지 약 -270의 영역 내에 있는 *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분에 상보적이다. 일부 실시양태에서, ASO는 포함된 엑손의 5' 스플라이스 부위(또는 3' 말단)에 대해 뉴클레오티드 -1 내지 -264의 영역 내에 있는 *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분에 상보적일 수 있다. 일부 측면에서, ASO는 포함된 엑손의 5' 스플라이스 부위(또는 3' 말단)에 대해 약 -1 내지 약 -270, 약 -1 내지 약 -260, 약 -1 내지 약 -250, 약 -1 내지 약 -240, 약 -1 내지 약 -230, 약 -1 내지 약 -220, 약 -1 내지 약 -210, 약 -1 내지 약 -200, 약 -1 내지 약 -190, 약 -1 내지 약 -180, 약 -1 내지 약 -170, 약 -1 내지 약 -160, 약 -1 내지 약 -150, 약 -1 내지 약 -140, 약 -1 내지 약 -130, 약 -1 내지 약 -120, 약 -1 내지 약 -110, 약 -1 내지 약 -100, 약 -1 내지 약 -90, 약 -1 내지 약 -80, 약 -1 내지 약 -70, 약 -1 내지 약 -60, 약 -1 내지 약 -50, 약 -1 내지 약 -40, 약 -1 내지 약 -30, 또는 약 -1 내지 약 -20의 영역 내에 있는 표적화된 부분에 상보적이다. 일부 측면에서, ASO는 포함된 엑손의 5' 스플라이스 부위(또는 3' 말단)에 대해 약 -1 내지 약 -50, 약 -50 내지 약 -100, 약 -100 내지 약 -150, 약 -150 내지 약 -200, 또는 약 -200 내지 약 -250의 영역 내에 있는 표적화된 부분에 상보적이다.

[0117] 일부 실시양태에서, ASO는 *SCN1A* NIE 함유 프리-mRNA에 포함된 엑손의 3' 스플라이스 부위(또는 5' 말단)의 상류(5' 방향)(예를 들어, 음수로 지정되는 방향)에 있는 *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분에 상보적이다. 일부 실시양태에서, ASO는 포함된 엑손의 3' 스플라이스 부위(또는 5' 말단)에 대해 약 -1 내지 약 -500의 영역 내에 있는 *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분에 상보적이다. 일부 실시양태에서, ASO는 포함된 엑손의 3' 스플라이스 부위에 대해 -1 내지 -496의 영역 내에 있는 *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분에 상보적이다. 일부 측면에서, ASO는 포함된 엑손의 3' 스플라이스 부위에 대해 약 -1 내지 약 -500, 약 -1 내지 약 -490, 약 -1 내지 약 -480, 약 -1 내지 약 -470, 약 -1 내지 약 -460, 약 -1 내지 약 -450, 약 -1 내지 약 -440, 약 -1 내지 약 -430, 약 -1 내지 약 -420, 약 -1 내지 약 -410, 약 -1 내지 약 -400, 약 -1 내지 약 -390, 약 -1 내지 약 -380, 약 -1 내지 약 -370, 약 -1 내지 약 -360, 약 -1 내지 약 -350, 약 -1 내지 약 -340, 약 -1 내지 약 -330, 약 -1 내지 약 -320, 약 -1 내지 약 -310, 약 -1 내지 약 -300, 약 -1 내지 약 -290, 약 -1 내지 약 -280, 약 -1 내지 약 -270, 약 -1 내지 약 -260, 약 -1 내지 약 -250, 약 -1 내지 약 -240, 약 -1 내지 약 -230, 약 -1 내지 약 -220, 약 -1 내지 약 -210, 약 -1 내지 약 -200, 약 -1 내지 약 -190, 약 -1 내지 약 -180, 약 -1 내지 약 -170, 약 -1 내지 약 -160, 약 -1 내지 약 -150, 약 -1 내지 약 -140, 약 -1 내지 약 -130, 약 -1 내지 약 -120, 약 -1 내지 약 -110, 약 -1 내지 약 -100, 약 -1 내지 약 -90, 약 -1 내지 약 -80, 약 -1 내지 약 -70, 약 -1 내지 약 -60, 약 -1 내지 약 -50, 약 -1 내지 약 -40, 또는 약 -1 내지 약 -30의 영역 내에 있는 표적화된 부분에 상보적이다. 일부 측면에서, ASO는 포함된 엑손의 3' 스플라이스 부위에 대해 약 -1 내지 약 -100, 약 -100 내지 약 -200, 약 -200 내지 약 -300, 약 -300 내지 약 -400, 또는 약 -400 내지 약 -500의 영역 내에 있는 표적화된 부분에 상보적이다.

[0118] 일부 실시양태에서, ASO는 *SCN1A* NIE 함유 프리-mRNA에 포함된 엑손의 3' 스플라이스 부위(또는 5' 말단)의 하류(3' 방향)(예를 들어, 양수로 지정되는 방향)에 있는 i NIE 함유 프리-mRNA의 표적화된 부분에 상보적이다. 일부 실시양태에서, ASO는 포함된 엑손의 3' 스플라이스 부위에 대해 약 +1 내지 약 +100의 영역 내에 있는 *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분에 상보적이다. 일부 측면에서, ASO는 포함된 엑손의 3' 스플라이스 부위에 대해 약 +1 내지 약 +90, 약 +1 내지 약 +80, 약 +1 내지 약 +70, 약 +1 내지 약 +60, 약 +1 내지 약 +50, 약 +1 내지 약 +40, 약 +1 내지 약 +30, 약 +1 내지 약 +20, 또는 약 +1 내지 약 +10의 영역 내에 있는 표적화된 부분에 상보적이다.

[0119] 일부 실시양태에서, *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분은 포함된 엑손의 5' 스플라이스 부위(3' 말단)에 대해 +100 내지 포함된 엑손의 3' 스플라이스 부위(5' 말단)에 대해 -100의 영역 내에 있다. 일부 실시양태에서, *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분은 NIE 내에 있다. 일부 실시양태에서, *SCN1A* NIE 함유 프리-mRNA의 표적화된 부분은 가성 엑손과 인트론 경계를 포함한다.

[0120] ASO는 특이적 결합 및 스플라이싱의 효과적인 향상에 적합한 임의의 길이일 수 있다. 일부 실시양태에서, ASO는

8 내지 50개의 핵염기로 이루어진다. 예를 들어, ASO는 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, 또는 50개의 핵염기 길이일 수 있다. 일부 실시양태에서, ASO는 50개 초과의 핵염기로 이루어진다. 일부 실시양태에서, ASO는 8 내지 50개의 핵염기, 8 내지 40개의 핵염기, 8 내지 35개의 핵염기, 8 내지 30개의 핵염기, 8 내지 25개의 핵염기, 8 내지 20개의 핵염기, 8 내지 15개의 핵염기, 9 내지 50개의 핵염기, 9 내지 40개의 핵염기, 9 내지 35개의 핵염기, 9 내지 30개의 핵염기, 9 내지 25개의 핵염기, 9 내지 20개의 핵염기, 9 내지 15개의 핵염기, 10 내지 50개의 핵염기, 10 내지 40개의 핵염기, 10 내지 35개의 핵염기, 10 내지 30개의 핵염기, 10 내지 25개의 핵염기, 10 내지 20개의 핵염기, 10 내지 15개의 핵염기, 11 내지 50개의 핵염기, 11 내지 40개의 핵염기, 11 내지 35개의 핵염기, 11 내지 30개의 핵염기, 11 내지 25개의 핵염기, 11 내지 20개의 핵염기, 11 내지 15개의 핵염기, 12 내지 50개의 핵염기, 12 내지 40개의 핵염기, 12 내지 35개의 핵염기, 12 내지 30개의 핵염기, 12 내지 25개의 핵염기, 12 내지 20개의 핵염기, 12 내지 15개의 핵염기, 13 내지 50개의 핵염기, 13 내지 40개의 핵염기, 13 내지 35개의 핵염기, 13 내지 30개의 핵염기, 13 내지 25개의 핵염기, 13 내지 20개의 핵염기, 14 내지 50개의 핵염기, 14 내지 40개의 핵염기, 14 내지 35개의 핵염기, 14 내지 30개의 핵염기, 14 내지 25개의 핵염기, 14 내지 20개의 핵염기, 15 내지 50개의 핵염기, 15 내지 40개의 핵염기, 15 내지 35개의 핵염기, 15 내지 30개의 핵염기, 15 내지 25개의 핵염기, 15 내지 20개의 핵염기, 20 내지 50개의 핵염기, 20 내지 40개의 핵염기, 20 내지 35개의 핵염기, 20 내지 30개의 핵염기, 20 내지 25개의 핵염기, 25 내지 50개의 핵염기, 25 내지 40개의 핵염기, 25 내지 35개의 핵염기, 또는 25 내지 30개의 핵염기 길이이다. 일부 실시양태에서, ASO는 18 뉴클레오티드 길이이다. 일부 실시양태에서, ASO는 15 뉴클레오티드 길이이다. 일부 실시양태에서, ASO는 25 뉴클레오티드 길이이다.

[0121] 일부 실시양태에서, 상이한 화학을 가지지만 NIE 함유 프리-mRNA의 동일한 표적화된 부분에 상보적인 2개 이상의 ASO가 사용된다. 일부 실시양태에서, NIE 함유 프리-mRNA의 상이한 표적화된 부분에 상보적인 2개 이상의 ASO가 사용된다.

[0122] 실시양태에서, 본 발명의 안티센스 올리고뉴클레오티드는 하나 이상의 모이어티 또는 컨쥬게이트, 예를 들어, 올리고뉴클레오티드의 활성 또는 세포 흡수를 향상시키는 표적화 모이어티 또는 다른 컨쥬게이트에 화학적으로 연결된다. 이러한 모이어티는 지질 모이어티, 예를 들어 콜레스테롤 모이어티, 콜레스테릴 모이어티, 지방족 쇄, 예를 들어 도데칸디올 또는 운데실 잔기, 폴리아민 또는 폴리에틸렌 글리콜 쇄, 또는 아다만탄 아세트산을 포함하지만, 이에 제한되지는 않는다. 친유성 모이어티를 포함하는 올리고뉴클레오티드 및 제조 방법은 공개된 문헌에 기재되어 있다. 실시양태에서, 안티센스 올리고뉴클레오티드는 무염기 뉴클레오티드, 폴리에테르, 폴리아민, 폴리아미드, 펩티드, 탄수화물, 예를 들어 N-아세틸갈락토사민(GalNAc), N-Ac-글루코사민(GluNAc), 또는 만노스(예를 들어, 만노스-6-포스페이트), 지질, 또는 다가 탄화수소 화합물을 포함하는, 그러나 이에 제한되지 않는 모이어티로 컨쥬게이트된다. 컨쥬게이트는 예를 들어 링커를 사용하여 당 업계에 이해되고 문헌에 기재된 바와 같이 당, 염기 또는 포스페이트 기 상의 임의의 여러 위치에서 안티센스 올리고뉴클레오티드를 포함하는 하나 이상의 임의의 뉴클레오티드에 연결될 수 있다. 링커는 2가 또는 3가의 분지형 링커를 포함할 수 있다. 실시양태에서, 컨쥬게이트는 안티센스 올리고뉴클레오티드의 3' 말단에 부착된다. 올리고뉴클레오티드 컨쥬게이트를 제조하는 방법은 예를 들어, 본원에 참고로 포함된 미국 특허 제8,450,467호("Carbohydrate conjugates as delivery agents for oligonucleotides")에 기술되어 있다.

[0123] 일부 실시양태에서, ASO에 의해 표적화되는 핵산은 진핵 세포와 같은 세포에서 발현된 *SCN1A* NIE 함유 프리-mRNA이다. 일부 실시양태에서, 용어 "세포"는 세포 집단을 지칭할 수 있다. 일부 실시양태에서, 세포는 대상체에 있다. 일부 실시양태에서, 세포는 대상체로부터 단리된다. 일부 실시양태에서, 세포는 생체 외 세포이다. 일부 실시양태에서, 세포는 병태 또는 질환 관련 세포 또는 세포주이다. 일부 실시양태에서, 세포는 시험관 내 세포(예를 들어, 세포 배양물)이다.

[0124] **약학 조성물**

[0125] 기재된 조성물의 작용제, 예를 들어 안티센스 올리고뉴클레오티드를 포함하고 기재된 임의의 방법에 사용하기 위한 약학 조성물 또는 제제는 약학 산업에 널리 공지되어 있고 공개된 문헌에 기재된 통상적인 기술에 따라 제조될 수 있다. 실시양태에서, 대상체를 치료하기 위한 약학 조성물 또는 제제는 유효량의 본원에 기재된 바와 같은 임의의 안티센스 올리고머, 또는 그의 약학적으로 허용 가능한 염, 용매화물, 수화물 또는 에스테르를 포함한다. 안티센스 올리고머를 포함하는 약학 제제는 약학적으로 허용 가능한 부형제, 희석제 또는 담체를 추가로 포함할 수 있다.

[0126] 약학적으로 허용 가능한 염은 과도한 독성, 자극, 알레르기 반응 등 없이 인간 및 하등 동물의 조직과 접촉하여 사용하기에 적합하고, 합리적인 편익/위험 비에 상응한다(예를 들어, 이러한 목적을 위해 본원에 참고로 포함된 문헌[S. M. Berge, et al., J. Pharmaceutical Sciences, 66: 1-19 (1977)] 참조). 염은 화합물의 최종 단리 및 정제 중에 원위치에서 제조될 수 있거나, 유리 염기 작용기를 적합한 유기산과 반응시킴으로서 별도로 제조될 수 있다. 약학적으로 허용 가능한 비독성 산부가 염의 예는 염산, 브롬화수소산, 인산, 황산 및 과염소산과 같은 무기산, 또는 아세트산, 옥살산, 말레산, 타르타르산, 시트르산, 숙신산 또는 말론 산과 같은 유기산에 의해 또는 이온 교환과 같은 다른 문서화된 방법을 사용함으로써 형성된 아미노기의 염이다. 다른 약학적으로 허용 가능한 염은 아디페이트, 알기네이트, 아스코르베이트, 아스파테이트, 벤젠술포네이트, 벤조에이트, 비술페이트, 보레이트, 부티레이트, 캄포레이트, 캄포술포네이트, 시트레이트, 시클로펜탄프로피오네이트, 디글루코네이트, 도데실술페이트, 에탄술포네이트, 포르메이트, 푸마레이트, 글루코헵토 네이트, 글리세로포스페이트, 글루코네이트, 헤미술페이트, 헵타노에이트, 헥사노에이트, 히드로요오다이드, 2-히드록시-에탄술포네이트, 락토비오네이트, 락테이트, 라우레이트, 라우릴 술페이트, 말레이트, 말레에이트, 말로네이트, 메탄술포네이트, 2-나프탈렌술포네이트, 니코티네이트, 니트레이트, 올레에이트, 옥살레이트, 팔미테이트, 파모에이트, 펙티네이트, 퍼술페이트, 3-페닐프로피오네이트, 포스페이트, 피크레이트, 피발레이트, 프로피오네이트, 스테아레이트, 숙시네이트, 술페이트, 타르트레이트, 티오시아네이트, p-톨루엔술포네이트, 운데카노에이트, 발레레이트 염 등을 포함한다. 대표적인 알칼리 또는 알칼리 토금속 염은 나트륨, 리튬, 칼륨, 칼슘, 마그네슘 등을 포함한다. 추가의 약학적으로 허용 가능한 염에는, 적절한 경우, 할라이드, 히드록시드, 카르복실레이트, 술페이트, 포스페이트, 니레이트, 저급 알킬 술포네이트 및 아릴 술포네이트와 같은 반대 이온을 사용하여 형성된 비독성 암모늄, 4차 암모늄, 및 아민 양이온이 포함된다.

[0127] 실시양태에서, 조성물은 정제, 캡슐, 겔 캡슐, 액상 시럽, 연질 겔, 좌제, 및 관장제와 같은, 그러나 이에 제한되지 않는 가능한 많은 임의의 제형으로 제제화된다. 실시양태에서, 조성물은 수성, 비수성 또는 혼합 매질에서 현탁액으로 제제화된다. 수성 현탁액은 예를 들어 소듐 카르복시메틸셀룰로스, 소르비톨 및/또는 덱스트란을 포함하여 현탁액의 점도를 증가시키는 물질을 추가로 함유할 수 있다. 현탁액은 또한 안정화제를 함유할 수 있다. 실시양태에서, 본 발명의 약학 제제 또는 조성물은 용액, 에멀젼, 마이크로에멀젼, 폼 또는 리포솜 함유 제제(예를 들어, 양이온 또는 비양이온 리포솜)을 포함하지만, 이에 제한되지는 않는다.

[0128] 본원에 기재된 약학 조성물 또는 제제는 당업자에게 적절하고 널리 공지되거나 공개된 문헌에 기재된 하나 이상의 침투 증강제, 담체, 부형제 또는 다른 활성 또는 비활성 성분을 포함할 수 있다. 실시양태에서, 리포솜은 또한 입체적으로 안정화된 리포솜, 예를 들어 하나 이상의 특화된 지질을 포함하는 리포솜을 포함한다. 이러한 특화된 지질은 순환 수명이 향상된 리포솜을 생성한다. 실시양태에서, 입체적으로 안정화된 리포솜은 하나 이상의 당지질을 포함하거나 폴리에틸렌 글리콜 (PEG) 모이어티와 같은 하나 이상의 친수성 중합체로 유도체화된다. 실시양태에서, 계면활성제가 약학 제제 또는 조성물에 포함된다. 의약품, 제제 및 에멀젼에서 계면활성제의 사용은 당 업계에 잘 알려져있다. 실시양태에서, 본 발명은 안티센스 올리고뉴클레오티드의 효율적인 전달을 수행하기 위해 예를 들어 세포막을 통한 확산을 보조하고/거나 친유성 약물의 투과성을 향상시키기 위해 침투 증강제를 사용한다. 실시양태에서, 침투 증강제는 계면활성제, 지방산, 담즙 염, 킬레이트제 또는 비킬레이트 비-계면활성제이다.

[0129] 실시양태에서, 약학 제제는 다중 안티센스 올리고뉴클레오티드를 포함한다. 실시양태에서, 안티센스 올리고뉴클레오티드는 다른 약물 또는 치료제와 병용하여 투여된다.

[0130] **병용 요법**

[0131] 일부 실시양태에서, 본 개시 내용에 개시된 ASO는 하나 이상의 추가 치료제와 병용하여 사용될 수 있다. 일부 실시양태에서, 하나 이상의 추가 치료제는 소분자를 포함할 수 있다. 예를 들어, 하나 이상의 추가 치료제는 WO2016128343A1, WO2017053982A1, WO2016196386A1, WO2014284459A1, WO2015248876A2, WO2013119916A2, 및 WO2014209841A2에 기재된 소분자를 포함할 수 있으며, 이들은 그 전문으로 본원에 참고로 포함된다. 일부 실시양태에서, 하나 이상의 추가 치료제는 인트론 유지를 교정하는 데 사용될 수 있는 ASO를 포함한다. 일부 실시양태에서, 하나 이상의 다른 작용제는 표 1a 또는 표 1b에 열거된 ASO로부터 선택된다.

[0132] [표 1a]

인트론 유지를 교정하기 위한 예시적인 ASO

서열 번호	명칭	서열 (5'-3')	유지된 인트론
115	SCN1A-IVS21+6	CAGAGAAAAUAGUGUUCA	21
116	SCN1A-IVS21+11	AUAUUCAGAGAAAAUAGU	21
117	SCN1A-IVS21+16	UAAAAAUAUUCAGAGAAA	21
118	SCN1A-IVS21+21	AACAAUAAAAAUAUUCAG	21
119	SCN1A-IVS21+26	UUCCAAACAAUAAAAAUA	21
120	SCN1A-IVS21+31	UAUUAUUCCAAACAAUAA	21
121	SCN1A-IVS21+36	UUUGUUAUUAUUCCAAAC	21
122	SCN1A-IVS21+41	AUUAUUUUGUUAUUAUUC	21
123	SCN1A-IVS21+46	AUGUCAUUAUUUUGUUAU	21
124	SCN1A-IVS21+51	GAUGUAUGUCAUUAUUUU	21
125	SCN1A-IVS21+56	UAAUAGAUGUAUGUCAUU	21
126	SCN1A-IVS21+61	CUAAAUAAUAGAUGUAUG	21
127	SCN1A-IVS21+66	AGGAACUAAAUAAUAGAU	21
128	SCN1A-IVS21+71	UUCUUAGGAACUAAAUAA	21
129	SCN1A-IVS21+76	ACUUUUUCUUAGGAACUA	21
130	SCN1A-IVS21+81	UAUAUACUUUUUCUUAGG	21
131	SCN1A-IVS21-16	UGCAUGUUUUACUUUGGA	21
132	SCN1A-IVS21-21	GUUUUACUUUGGAGUAAA	21
133	SCN1A-IVS21-26	ACUUUGGAGUAAAAAUAA	21
134	SCN1A-IVS21-31	GGAGUAAAAAUAAUUUAG	21
135	SCN1A-IVS21-36	AAAAAUAAUUUAGACCUG	21
136	SCN1A-IVS21-41	UAAUUUAGACCUGAUGUU	21
137	SCN1A-IVS21-46	UAGACCUGAUGUUUAAUA	21
138	SCN1A-IVS21-51	CUGAUGUUUAAUAAAUAU	21
139	SCN1A-IVS21-56	GUUUAAUAAAUAUUCUUA	21
140	SCN1A-IVS21-61	AUAAAUAUUCUUACUGAU	21
141	SCN1A-IVS21-66	UAUUCUUACUGAUAUAAU	21
142	SCN1A-IVS21-71	UUACUGAUAUAAUUUUCA	21
143	SCN1A-IVS21-76	GAUAUAAUUUUCAAAAGG	21
144	SCN1A-IVS21-81	AAUUUUCAAAAGGGAAUA	21
145	SCN1A-IVS21-27	CUUUGGAGUAAAAAUAAU	21
146	SCN1A-IVS21-28	UUUGGAGUAAAAAUAAUU	21
148	SCN1A-IVS21-29	UUGGAGUAAAAAUAAUUU	21
149	SCN1A-IVS21-30	UGGAGUAAAAAUAAUUUA	21

[0133]

서열 번호	명칭	서열 (5'-3')	유지된 인트론
150	SCN1A-IVS21-32	GAGUAAAAAUAAUUUAGA	21
151	SCN1A-IVS21-33	AGUAAAAAUAAUUUAGAC	21
152	SCN1A-IVS21-34	GUAAAAAUAAUUUAGACC	21
153	SCN1A-IVS21-35	UAAAAAUAAUUUAGACCU	21
154	SCN1A-IVS21-72	UACUGAUAUAAUUUUCAA	21
155	SCN1A-IVS21-73	ACUGAUAUAAUUUUCAAA	21
156	SCN1A-IVS21-74	CUGAUAUAAUUUUCAAAA	21
157	SCN1A-IVS21-75	UGAUAUAAUUUUCAAAAG	21
158	SCN1A-IVS21-77	AUAUAAUUUUCAAAAGGG	21
159	SCN1A-IVS21-78	UAUAAUUUUCAAAAGGGA	21
160	SCN1A-IVS21-79	AUAAUUUUCAAAAGGGAA	21
161	SCN1A-IVS21-80	UAAUUUUCAAAAGGGAAU	21
162		CAAGGAUUAAAGGUAGCA	21

[0134]

[0135] [표 1b]

인트론 유지를 교정하기 위한 예시적인 ASO

서열 번호	명칭	서열 (5'-3')	유지된 인트론
163	SCN1A-IVS21+6	CAGAGAAAATAGTGTTCA	21
164	SCN1A-IVS21+11	ATATTCAGAGAAAATAGT	21
165	SCN1A-IVS21+16	TAAAAATATTCAGAGAAA	21
166	SCN1A-IVS21+21	AACAATAAAAATATTCAG	21
167	SCN1A-IVS21+26	TTCCAAACAATAAAAATA	21
168	SCN1A-IVS21+31	TATTATTCCAAACAATAA	21
169	SCN1A-IVS21+36	TTTGTTATTATTCCAAAC	21
170	SCN1A-IVS21+41	ATTATTTTGTTATTATTC	21
171	SCN1A-IVS21+46	ATGTCATTATTTTGTTAT	21
172	SCN1A-IVS21+51	GATGTATGTCATTATTTT	21
173	SCN1A-IVS21+56	TAATAGATGTATGTCATT	21
174	SCN1A-IVS21+61	CTAAATAATAGATGTATG	21
175	SCN1A-IVS21+66	AGGAACTAAATAATAGAT	21
176	SCN1A-IVS21+71	TTCTTAGGAACTAAATAA	21
177	SCN1A-IVS21+76	ACTTTTTCTTAGGAACTA	21
178	SCN1A-IVS21+81	TATATACTTTTTCTTAGG	21
179	SCN1A-IVS21-16	TGCATGTTTTACTTTGGA	21
180	SCN1A-IVS21-21	GTTTTACTTTGGAGTAAA	21
181	SCN1A-IVS21-26	ACTTTGGAGTAAAAATAA	21

[0136]

서열 번호	명칭	서열 (5'-3')	유지된 인트론
182	SCN1A-IVS21-31	GGAGTAAAAATAATTTAG	21
183	SCN1A-IVS21-36	AAAAATAATTTAGACCTG	21
184	SCN1A-IVS21-41	TAATTTAGACCTGATGTT	21
185	SCN1A-IVS21-46	TAGACCTGATGTTTAATA	21
186	SCN1A-IVS21-51	CTGATGTTTAATAAATAT	21
187	SCN1A-IVS21-56	GTTTAATAAATATTCTTA	21
188	SCN1A-IVS21-61	ATAAATATTCTTACTGAT	21
189	SCN1A-IVS21-66	TATTCTTACTGATATAAT	21
190	SCN1A-IVS21-71	TTACTGATATAATTTTCA	21
191	SCN1A-IVS21-76	GATATAATTTTCAAAAGG	21
192	SCN1A-IVS21-81	AATTTTCAAAAGGGAATA	21
193	SCN1A-IVS21-27	CTTTGGAGTAAAAATAAT	21
194	SCN1A-IVS21-28	TTTGGAGTAAAAATAATT	21
195	SCN1A-IVS21-29	TTGGAGTAAAAATAATTT	21
196	SCN1A-IVS21-30	TGGAGTAAAAATAATTTA	21
197	SCN1A-IVS21-32	GAGTAAAAATAATTTAGA	21
198	SCN1A-IVS21-33	AGTAAAAATAATTTAGAC	21
199	SCN1A-IVS21-34	GTAAAAATAATTTAGACC	21
200	SCN1A-IVS21-35	TAAAAATAATTTAGACCT	21
201	SCN1A-IVS21-72	TACTGATATAATTTTCAA	21
202	SCN1A-IVS21-73	ACTGATATAATTTTCAAA	21
203	SCN1A-IVS21-74	CTGATATAATTTTCAAAA	21
204	SCN1A-IVS21-75	TGATATAATTTTCAAAAG	21
205	SCN1A-IVS21-77	ATATAATTTTCAAAAGGG	21
206	SCN1A-IVS21-78	TATAATTTTCAAAAGGGA	21
207	SCN1A-IVS21-79	ATAATTTTCAAAAGGGAA	21
208	SCN1A-IVS21-80	TAATTTTCAAAAGGGAAT	21
209		CAAGGATTAAAGGTAGCA	21

[0137]

[0138] **대상체의 치료**

[0139] 본원에 제공된 임의의 조성물은 개체에게 투여될 수 있다. "개체"는 "대상체" 또는 "환자"와 호환적으로 사용될 수 있다. 개체는 포유동물, 예를 들어 인간 또는 비인간 영장류, 설치류, 토끼, 랫트, 마우스, 말, 당나귀, 염소, 고양이, 개, 소, 돼지, 또는 양과 같은 동물일 수 있다. 실시양태에서, 개체는 인간이다. 실시양태에서, 개체는 태아, 배아, 또는 소아이다. 다른 실시양태에서, 개체는 식물과 같은 다른 진핵생물일 수 있다. 일부 실시양태에서, 본원에 제공된 조성물은 생체 외 세포에 투여된다.

[0140] 일부 실시양태에서, 본원에 제공된 조성물은 질환 또는 장애의 치료 방법으로서 개체에게 투여된다. 일부 실시양태에서, 개체는 본원에 기재된 임의의 질환과 같은 유전 질환을 갖는다. 일부 실시양태에서, 개체는 본원에 기재된 임의의 질환과 같은 질환을 가질 위험에 있다. 일부 실시양태에서, 개체는 불충분한 단백질량 또는 단백질의 불충분한 활성에 의해 야기되는 질환 또는 장애를 가질 위험이 증가한다. 개체가 불충분한 단백질량 또는 단백질의 불충분한 활성에 의해 야기된 질환 또는 장애를 가질 "위험이 증가"한 경우, 방법은 방지 또는 예방 치료를 포함한다. 예를 들어, 개체는 질환의 가족력으로 인해 그러한 질환 또는 장애를 가질 위험이 증가할 수 있다. 전형적으로, 이러한 질환 또는 장애를 가질 위험이 증가한 개체는 예방 치료(예를 들어, 질환 또는 장애의 발병 또는 진행을 예방 또는 지연시킴으로써)가 유익하다. 실시양태에서, 태아는 예를 들어 ASO 조성물을 태아에게 직접 또는 간접적으로(예를 들어, 모체를 통해) 투여함으로써 자궁 내에서 치료된다.

[0141] 본 발명의 ASO의 투여에 적합한 경로는 ASO의 전달이 바람직한 세포 유형에 따라 달라질 수 있다. 다수의 조직 및 기관은 드라벳 증후군; 열성 발작 플러스를 동반하는 전신 뇌전증 2형; 가족성 열성 발작 3A; 가족성 편마비 편두통 3; 자폐증; 조기 유아 간질성 뇌병증 13; 동기능 부전 증후군 1; 뇌가 가장 크게 이환되는 조직인 알츠하이머병 또는 SUDEP에 의해 이환된다. 본 발명의 ASO는 비경구로, 예를 들어 수막공간내 주사, 뇌실내 주사, 복강내 주사, 근육내 주사, 피하 주사, 안내 주사, 또는 정맥내 주사에 의해 환자에게 투여될 수 있다.

[0142] 일부 실시양태에서, 질환 또는 병태는 $Na_v1.1$(*SCN1A* 유전자에 의해 코딩된 단백질)의 돌연변이에 의해 유발된다. 일부 경우에, 돌연변이는 Nav1.1의 기능 상실 돌연변이이다. 일부 경우에, $Na_v1.1$의 기능 상실 돌연변이는 야생형 $Na_v1.1$의 기능에 비해 Nav1.1의 기능을 (예를 들어, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% 또는 그 이상) 감소시키거나 손상시키는 하나 이상의 돌연변이를 포함한다. 일부 경우에, Nav1.1의 기능 상실 돌연변이는 질환 표현형을 초래하는 하나 이상의 돌연변이를 포함한다. 예시적인 기능 상실 돌연변이에는 R859C, T875M, V1353L, I1656M, R1657C, A1685V, M1841T, 및 R1916G가 포함되지만 이에 제한되지는 않는다.

[0143] 다른 경우에, 돌연변이는 $Na_v1.1$의 기능 획득 돌연변이이다. 이러한 경우, 기능 획득 돌연변이는 야생형 $Na_v1.1$의 기능에 비해 $Na_v1.1$의 활성화를 (예를 들어, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% 또는 그 이상) 연장시키는 하나 이상의 돌연변이를 포함한다. 이러한 경우, $Na_v1.1$의 기능 획득 돌연변이는 질환 표현형을 초래하는 하나 이상의 돌연변이를 포함한다. 예시적인 기능 획득 돌연변이에는 D188V, W1204R, R1648H, 및 D1866Y가 포함되지만 이에 제한되지는 않는다.

[0144] 일부 실시양태에서, 질환 또는 병태는 뇌병증이다. 일부 경우에, 뇌병증은 $Na_v1.1$의 기능 상실 돌연변이에 의해 유발된다.

[0145] 일부 실시양태에서, 뇌병증은 간질성 뇌병증이다. 예시적인 간질성 뇌병증에는 드라벳 증후군(DS)(유아기의 중증 근간대성 뇌전증 또는 SMEI로도 알려짐); 유아기의 중증 근간대성 뇌전증(SMEI) 경계성(SMEB); 열성 발작(FS); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+); 조기 유아 간질성 뇌병증 13; 잠재성 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 뇌전증에서의 돌연사(SUDEP); 조기 유아 SCN1A 뇌병증; 조기 유아 간질성 뇌병증(EIEE); 또는 동기능 부전 증후군 1이 포함되지만 이에 제한되지는 않는다. 일부 실시양태에서, 질환 또는 병태는 경우에 따라 드라벳 증후군(DS)(유아기의 중증 근간대성 뇌전증 또는 SMEI로도 알려짐); 유아기의 중증 근간대성 뇌전증(SMEI) 경계성(SMEB); 열성 발작(FS); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+); 조기 유아 간질성 뇌병증 13; 잠재성 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 뇌전증에서의 돌연사(SUDEP); 및 동기능 부전 증후군 1로부터 선택된 간질성 뇌병증이다.

[0146] 일부 경우에, GEFS+는 열성 발작 플러스를 동반하는 전신 뇌전증 2형이다.

[0147] 일부 경우에, 열성 발작은 가족성 열성 발작 3A이다.

[0148] 일부 경우에, SMEB는 전신 극파가 없는 SMEB(SMEB-SW), 근간대성 발작이 없는 SMEB(SMEB-M), SMEI의 하나 초과의 특징이 결여된 SMEB(SMEB-O), 또는 전신 강직성 간대 발작을 동반한 난치성 소아기 뇌전증(ICEGTC)이다.

[0149] 일부 실시양태에서, Nav1.1의 기능 상실 돌연변이에 의해 유발되는 질환 또는 병태는 드라벳 증후군(DS)(SMEI로도 알려짐); 유아기의 중증 근간대성 뇌전증(SMEI) 경계성(SMEB); 열성 발작(FS); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+); 조기 유아 간질성 뇌병증 13; 잠재성 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 뇌전증에서의 돌연사(SUDEP); 동기능 부전 증후군 1; 조기 유아 *SCN1A* 뇌병증; 조기 유아 간질성 뇌병증(EIEE); 자폐증; 또는 유아기의 악성 이동 부분 발작을 포함하지만 이에 제한되지는 않는다.

[0150] 일부 실시양태에서, 질환 또는 병태는 Nav1.1의 기능 획득 돌연변이에 의해 유발된다. $Na_v1.1$의 기능 획득 돌연변이와 관련된 예시적인 질환 또는 병태는 편두통을 포함하지만 이에 제한되지는 않는다. 일부 경우에, $Na_v1.1$의 기능 획득 돌연변이에 의해 유발되는 질환 또는 병태는 편두통이다.

[0151] 일부 경우에, 편두통은 가족성 편마비 편두통 3이다.

[0152] 일부 실시양태에서, 질환 또는 병태는 $Na_v1.1$ 유전성 뇌전증이다. Nav1.1 유전성 뇌전증은 $Na_v1.1$의 기능 손실 돌연변이 또는 Nav1.1의 기능 획득 돌연변이를 포함할 수 있다. 일부 경우에, $Na_v1.1$ 유전성 뇌전증은 하나 이상의 유전 돌연변이를 포함한다. 다른 경우에, $Na_v1.1$ 유전성 뇌전증은 하나 이상의 데 노보 돌연변이를 포함한다. 일부 경우에, $Na_v1.1$ 유전성 뇌전증은 드라벳 증후군(DS)(유아기의 중증 근간대성 뇌전증 또는 SMEI로도 알려짐); 유아기의 중증 근간대성 뇌전증(SMEI) 경계성(SMEB); 열성 발작(FS); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+); 조기 유아 간질성 뇌병증 13; 잠재성 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 조기 유아 *SCN1A* 뇌병증; 조기 유아 간질성 뇌병증(EIEE); 뇌전증에서의 돌연사(SUDEP); 또는 유아기의 악성 이동 부분 발작을 포함한다. 일부 경우에, 일부 경우에, Nav1.1의 기능 상실 돌연변이와 관련된 $Na_v1.1$ 유전성 뇌전증은 드라벳 증후군(DS)(유아기의 중증 근간대성 뇌전증 또는 SMEI로도 알려짐); 유아기의 중증 근간대성 뇌전증(SMEI) 경계성(SMEB); 열성 발작(FS); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+); 조기 유아 간질성 뇌병증 13; 잠재성 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 조기 유아 *SCN1A* 뇌병증; 조기 유아 간질성 뇌병증(EIEE); 뇌전증에서의 돌연사(SUDEP); 유아기의 악성 이동 부분 발작을 포함한다.

[0153] 일부 실시양태에서, 질환 또는 병태는 *SCN1A* 유전자의 반가불충분성과 관련된다. SCN1A 유전자의 반가불충분성과 관련된 예시적인 질환 또는 병태는 드라벳 증후군(DS)(SMEI로도 알려짐); 유아기의 중증 근간대성 뇌전증(SMEI) 경계성(SMEB); 열성 발작(FS); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+); 조기 유아 간질성 뇌병증 13; 잠재성 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 뇌전증에서의 돌연사(SUDEP); 동기능 부전 증후군 1; 조기 유아 *SCN1A* 뇌병증; 조기 유아 간질성 뇌병증(EIEE); 또는 유아기의 악성 이동 부분 발작을 포함하지만 이에 제한되지는 않는다. 일부 경우에, 질환 또는 병태는 드라벳 증후군(DS)(SMEI로도 알려짐); 유아기의 중증 근간대성 뇌전증(SMEI) 경계성(SMEB); 열성 발작(FS); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+); 조기 유아 간질성 뇌병증 13; 잠재성 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 뇌전증에서의 돌연사(SUDEP); 동기능 부전 증후군 1; 조기 유아 *SCN1A* 뇌병증; 조기 유아 간질성 뇌병증(EIEE); 또는 유아기의 악성 이동 부분 발작이다.

[0154] 일부 경우에, 질환 또는 병태는 드라벳 증후군(DS)이다.

[0155] 달리 중증의 유아기의 중증 근간대성 뇌전증(SMEI)으로 알려진 드라벳 증후군(DS)은 생후 첫 해에 나타나는 간

질성 뇌병증이다. 드라벳 증후군은 환자의 약 70 내지 80%에서 나트륨 채널 유전자 돌연변이의 발견에 의해 임상 진단이 뒷받침되는 간질성 뇌병증으로 점점 더 인식되고 있다. 이온 통로 유전자의 돌연변이는 다양한 뇌전증 증후군의 발병에 중요한 역할을 하며, 이온통로병증으로 간주되는 일부 뇌전증을 초래한다. 전압 게이팅 나트륨 채널(VGSC)은 신경 흥분성에 필수적인 역할을 한다: 따라서, DS와 관련된 많은 돌연변이가 VGSC 서브유닛을 코딩하는 유전자에서 확인되었다는 것은 놀라운 일이 아니다. 이 질환은 예를 들어, 문헌(Mulley, *et al*., 2005) 및 OMIM #607208(Online Mendelian Inheritance in Man, Johns Hopkins University, 1966-2015)의 질환 설명에 의해 설명되며, 둘 다 본원에 참고로 포함된다.

[0156] 환자의 70% 내지 80%는 나트륨 채널 α1 서브유닛 유전자(*SCN1A*) 이상을 보유하고, 절두 돌연변이가 약 40%를 차지하며, 이른 나이의 발작 시작과 유의한 상관관계를 갖는다. 시퀀싱 돌연변이는 약 70%의 사례에서 발견되며, 절두(40%) 및 미스센스 돌연변이(40%)를 포함하며, 나머지는 스플라이스 부위 변화이다. 대부분의 돌연변이는 드노 보이지만, 가족성 돌연변이가 5 내지 10%의 사례에서 발생하며 대개는 사실상 미스센스이다. 나머지 *SCN1A* 돌연변이는 스플라이스 부위 및 미스센스 돌연변이를 포함하며, 이들 대부분은 나트륨 채널의 세공 형성 영역에 속한다. 현재, 500개가 넘는 돌연변이가 DS와 관련되어 있고 유전자를 따라 무작위로 분포되어 있다(Mulley, et al., *Neurol*. 2006, 67, 1094-1095).

[0157] *SCN1A* 유전자는 인간 염색체 2q24 상의 나트륨 채널 유전자 클러스터에 위치하고 뉴런 전압 게이팅 나트륨 채널의 Nav1.1로 알려진 α-세공 형성 서브유닛을 코딩한다. *SCN1A* 유전자는 대략 100 kb의 게놈 DNA에 걸쳐 있으며 26개의 엑손을 포함한다. SCN1A 단백질은 각각 6개의 막 횡단 세그먼트를 갖는 4개의 도메인으로 이루어진다. 엑손 11에서 도메인 1과 2 사이의 세포질 루프에서 11개 아미노산의 존재 또는 부재에 있어 상이한 길고 짧은 아이소형을 생성하는 2개의 스플라이스 변이체가 확인되었다(Miller, et al., 1993-2015, and Mulley, et al., 2005, 25, 535-542, 본원에 참고로 포함됨).

[0158] *SCN1A* 유전자에서의 선택적 스플라이싱 이벤트는 비생산적 mRNA 전사체를 초래하여 결국 비정상적 단백질 발현으로 이어질 수 있으며, *SCN1A* 유전자에서 선택적 스플라이싱 이벤트를 표적화할 수 있는 치료제는 DS 환자에서 기능성 단백질의 발현 수준을 조절하고/거나 비정상적 단백질 발현을 억제할 수 있다. 이러한 치료제는 SCN1A 단백질 결핍에 의해 야기된 병태를 치료하는 데 사용될 수 있다.

[0159] 비생산적인 mRNA 전사체를 초래할 수 있는 선택적 스플라이싱 이벤트 중 하나는 넌센스 매개 mRNA 붕괴를 유도할 수 있는 mRNA 전사체에 추가의 엑손을 포함시키는 것이다. 본 개시 내용은 단백질을 코딩하는 성숙한 mRNA의 생성을 증가시켜 번역된 기능성 SCN1A 단백질을 증가시키기 위해 *SCN1A*의 선택적 스플라이싱을 조절하기 위한 조성물 및 방법을 제공한다. 이들 조성물 및 방법은 엑손 스키핑을 야기하고 *SCN1A* 프리-mRNA의 구성적 스플라이싱을 촉진할 수 있는 안티센스 올리고머(ASO)를 포함한다. 다양한 실시양태에서, SCN1A 단백질 결핍에 의해 야기된 병태를 치료하기 위해 본 개시 내용의 방법을 사용하여 기능성 SCN1A 단백질을 증가시킬 수 있다.

[0160] 일부 경우에, 질환 또는 병태는 SMEB이다.

[0161] 일부 경우에, 질환 또는 병태는 GEFS+이다.

[0162] 일부 경우에, 질환 또는 병태는 열성 발작(예를 들어, 가족성 열성 발작 3A)이다.

[0163] 일부 경우에, 질환 또는 병태는 자폐증(자폐 스펙트럼 장애 또는 ASD로도 알려짐)이다.

[0164] 일부 경우에, 질환 또는 병태는 편두통(예를 들어, 가족성 편마비 편두통 3)이다.

[0165] 일부 경우에, 질환 또는 병태는 알츠하이머병이다.

[0166] 일부 실시양태에서, 질환 또는 병태는 *SCN2A* 뇌병증이다.

[0167] 일부 실시양태에서, 질환 또는 병태는 *SCN8A* 뇌병증이다.

[0168] 일부 실시양태에서, 질환 또는 병태는 *SCN5A* 부정맥이다.

[0169] 실시양태에서, 안티센스 올리고뉴클레오티드는 당 업계에 공지된 임의의 방법에 의해 혈액-뇌 장벽을 통한 본 안티센스 올리고뉴클레오티드의 침투를 촉진할 수 있는 하나 이상의 작용제와 함께 투여된다. 예를 들어, 근육 조직에서 운동 뉴런으로 아데노 바이러스 벡터의 투여에 의한 작용제의 전달이 미국 특허 제6,632,427호("Adenoviral-vector-mediated gene transfer into medullary motor neurons", 본원에 참고로 포함됨)에 기재되어 있다. 뇌, 예를 들어 선조, 시상, 해마 또는 흑질로 직접적인 벡터의 전달이, 예를 들어 미국 특허 제6,756,523호("Adenovirus vectors for the transfer of foreign genes into cells of the central nervous

system particularly in brain", 본원에 참고로 포함됨)에 기재되어 있다.

[0170] 실시양태에서, 안티센스 올리고뉴클레오티드는 바람직한 약학적 또는 약력 학적 특성을 제공하는 작용제와 연결되거나 컨쥬게이트된다. 실시양태에서, 안티센스 올리고뉴클레오티드는 혈액-뇌 장벽를 통한 침투 또는 수송을 촉진하기 위해 당 업계에 공지된 물질, 예를 들어 트랜스페린 수용체에 대한 항체에 커플링된다. 실시양태에서, 안티센스 올리고뉴클레오티드는 바이러스 벡터와 연결되어, 예를 들어 안티센스 화합물을 더 효과적으로 만들거나 혈액-뇌 장벽을 통한 수송을 증가시킨다. 실시양태에서, 삼투성 혈액 뇌 장벽 파괴는 당, 예를 들어 메조 에리트리톨, 자일리톨, D(+) 갈락토오스, D(+) 락토오스, D(+) 자일로스, 둘시톨, 미오-이노시톨, L(-) 프룩토오스, D(-) 만니톨, D(+) 글루코오스, D(+) 아라비노스, D(-) 아라비노오스, 셀로비오스, D(+) 말토오스, D(+) 라피노스, L(+) 람노오스, D(+) 멜리비오스, D(-) 리보오스, 아도니톨, D(+) 아라비톨, L(-) 아라비톨, D(+) 푸코오스, L(-) 푸코오스, D(-) 릭소오스, L(+) 릭소오스, 및 L(-) 릭소오스, 또는 아미노산, 예를 들어 글루타민, 리신, 아르기닌, 아스파라긴, 아스파르트산, 시스테인, 글루탐산, 글리신, 히스티딘, 류신, 메티오닌, 페닐알라닌, 프롤린, 세린, 트레오닌, 티로신, 발린, 및 타우린의 주입에 의해 보조받는다. 혈액 뇌 장벽 침투를 향상시키기 위한 방법 및 물질은, 예를 들어 미국 특허 제9,193,969호("Compositions and methods for selective delivery of oligonucleotide molecules to specific neuron types"), 미국 특허 제4,866,042호("Method for the delivery of genetic material across the blood brain barrier"), 미국 특허 제6,294,520호("Material for passage through the blood-brain barrier"), 및 미국 특허 제6,936,589호("Parenteral delivery systems")에 기술되어 있으며, 이들 각각은 본원에 참고로 포함된다.

[0171] 실시양태에서, 본 발명의 ASO는 예를 들어 본원에 참고로 포함된 미국 특허 제9,193,969호에 기재된 방법을 사용하여 도파민 재흡수 억제제(DRI), 선택적 세로토닌 재흡수 억제제(SSRI), 노르아드레날린 재흡수 억제제(NRI), 노르에피네프린-도파민 재흡수 억제제(NDRI), 및 세로토닌-노르에피네프린-도파민 재흡수 억제제(SNDRI)에 커플링된다.

[0172] 실시양태에서, 방법 및 조성물을 사용하여 치료된 대상체는 당 업계에 공지되고 설명된 임의의 방법을 사용하여 병태의 개선에 대해 평가된다.

[0173] **엑손 스키핑을 유도하는 추가 ASO 확인 방법**

[0174] *SCN1A* NIE 함유 프리-mRNA의 엑손 스키핑을 유도하는 ASO를 확인 또는 결정하기 위한 방법이 또한 본 발명의 범위 내에 있다. 예를 들어, 방법은 SCN1A NIE 함유 프리-mRNA의 가성 엑손 스키핑을 유도하는 ASO를 확인 또는 결정하는 단계를 포함할 수 있다. 프리-mRNA의 표적 영역 내의 상이한 뉴클레오티드에 특이적으로 혼성화하는 ASO를 스크리닝하여 표적 인트론의 스플라이싱 속도 및/또는 정도를 개선시키는 ASO를 확인 또는 결정할 수 있다. 일부 실시 형태에서, ASO는 스플라이싱 억제제(들)/사일런서의 결합 부위(들)를 차단하거나 방해할 수 있다. 당해 기술 분야에 공지된 임의의 방법을 사용하여 엑손의 표적 영역에 혼성화될 때 원하는 효과(예를 들어, 가성 엑손 스키핑, 단백질 또는 기능성 RNA 생성)를 초래하는 ASO를 확인(결정)할 수 있다. 또한, 이들 방법을 사용하여 포함된 엑손 측면에 위치한 인트론 또는 포함되지 않은 엑손 내 표적화된 영역에 결합함으로써 포함된 엑손의 엑손 스키핑를 유도하는 ASO를 확인할 수 있다. 사용될 수 있는 방법의 예는 아래에 제공된다.

[0175] 프리-mRNA의 표적 영역에 혼성화하도록 설계된 ASO를 사용하여 ASO "워크"로 지칭되는 한 라운드의 스크리닝이 수행될 수 있다. 예를 들어, ASO 워크에 사용된 ASO는 포함된 엑손의 3' 스플라이스 부위의 대략 100 뉴클레오티드 상류(예를 들어, 표적/포함된 엑손의 상류에 위치한 엑손 서열의 일부)부터 표적/포함된 엑손의 3' 스플라이스 부위의 약 100 뉴클레오티드 하류까지 및/또는 포함된 엑손의 5' 스플라이스 부위의 약 100 뉴클레오티드 상류부터 표적/포함된 엑손의 5' 스플라이스 부위의 약 100 뉴클레오티드 하류(예를 들어, 표적/포함된 엑손의 하류에 위치한 엑손의 서열의 일부)까지 5 뉴클레오티드마다 타일링될 수 있다. 예를 들어, 15 뉴클레오티드 길이의 제1 ASO는 표적/포함된 엑손의 3' 스플라이스 부위에 대해 뉴클레오티드 +6 내지 +20에 특이적으로 혼성화하도록 설계될 수 있다. 제2 ASO는 표적/포함된 엑손의 3'스플라이스 부위에 대해 뉴클레오티드 +11 내지 +25에 특이적으로 혼성화하도록 설계될 수 있다. ASO는 프리-mRNA의 표적 영역에 이렇게 걸쳐있는 것으로 설계된다. 실시양태에서, ASO는 예를 들어 1, 2, 3 또는 4 뉴클레오티드마다 더 가까이 타일링될 수 있다. 또한, ASO는 5' 스플라이스 부위의 100 뉴클레오티드 하류부터 3' 스플라이스 부위의 100 뉴클레오티드 상류에 타일링될 수 있다. 일부 실시양태에서, ASO는 3' 스플라이스 부위의 약 1,160 뉴클레오티드 상류부터 5'스플라이스 부위의 약 500 뉴클레오티드 하류에 타일링될 수 있다. 일부 실시양태에서, ASO는 3' 스플라이스 부위의 약 500 뉴클레오티드 상류부터 3' 스플라이스 부위의 약 1,920 뉴클레오티드 하류에 타일링될 수 있다.

[0176] 하나 이상의 ASO, 또는 대조군 ASO(표적 영역에 혼성화하지 않을 것으로 예상되는 서열인 스크램블링된 서열을

갖는 ASO)는, 예를 들어 형질 감염에 의해, 표적 프리-mRNA(예를 들어, 본원에 기재된 NIE 함유 프리-mRNA)을 발현하는 질환 관련 세포주 내로 전달된다. 각각의 ASO의 엑손 스키핑 효과는 당 업계에 공지된 임의의 방법, 예를 들어 실시예 4에 기재된 바와 같이 스플라이스 접합부에 걸쳐있는 프라이머를 사용하는 역전사 효소(RT)-PCR에 의해 평가될 수 있다. 대조군 ASO 처리된 세포와 비교하여 ASO 처리된 세포에서 포함된 엑손(예를 들어, NIE의 측면 엑손 포함)을 함유하는 영역에 걸친 프라이머를 사용하여 생성된 더 긴 RT-PCR 생성물의 감소 또는 부재는 표적 NIE의 스플라이싱이 향상되었음을 나타낸다. 일부 실시양태에서, 엑손 스키핑 효율(또는 NIE를 함유하는 인트론을 스플라이싱하는 스플라이싱 효율), 스플라이싱되지 않은 프리-mRNA에 대한 스플라이싱된 프리-mRNA의 비율, 스플라이싱 속도 또는 스플라이싱 정도는 본원에 기재된 ASO를 사용하여 개선될 수 있다. 또한, 표적 프리-mRNA에 의해 코딩되는 단백질 또는 기능성 RNA의 양을 평가하여 각각의 ASO가 원하는 효과(예를 들어, 기능성 단백질 생산의 향상)를 달성했는지를 결정할 수 있다. 웨스턴 블롯팅, 유세포 분석, 면역 형광 현미경법, 및 ELISA와 같은 단백질 생산을 평가 및/또는 정량하기 위해 당 업계에 공지된 임의의 방법이 사용될 수 있다.

[0177] 프리-mRNA의 표적 영역에 혼성화하도록 설계된 ASO를 사용하여 ASO "마이크로 워크"라고 불리는 제2 라운드의 스크리닝을 수행할 수 있다. ASO 마이크로 워크에 사용된 ASO를 1 뉴클레오티드마다 타일링하여 ASO와 혼성화될 때 엑손 스키핑(또는 NIE의 향상된 스플라이싱)을 초래하는 프리-mRNA의 뉴클레오티드 서열을 추가로 정제(refine)한다.

[0178] 표적 인트론의 스플라이싱을 촉진하는 ASO에 의해 정의된 영역은 1-nt 스텝 간격의 ASO뿐만 아니라 더 긴 ASO, 전형적으로 18 내지 25 nt를 포함하는 ASO "마이크로 워크"에 의해 더 상세하게 탐색된다.

[0179] 상기 ASO 워크에 대해 설명된 바와 같이, 하나 이상의 ASO, 또는 대조군 ASO(표적 영역에 혼성화할 것으로 예상되지 않는 서열인 스크램블링된 서열을 갖는 ASO)를, 예를 들어 형질 감염에 의해, 표적 프리-mRNA를 발현하는 질환 관련 세포주 내로 전달하여 ASO 마이크로 워크를 수행한다. 각각의 ASO의 스플라이싱 유도 효과는 당 업계에 공지된 임의의 방법, 예를 들어 본원에 기재된 바와 같이 NIE에 걸친 프라이머를 사용하는 역전사 효소(RT)-PCR에 의해 평가될 수 있다(예를 들어, 실시예 4 참조). 대조군 ASO 처리된 세포와 비교하여 ASO 처리된 세포에서 NIE에 걸친 프라이머를 사용하여 생성된 더 긴 RT-PCR 생성물의 감소 또는 부재는 엑손 스키핑(또는 NIE를 함유하는 표적 인트론의 스플라이싱)이 향상되었음을 나타낸다. 일부 실시양태에서, 엑손 스키핑 효율(또는 NIE를 함유하는 인트론을 스플라이싱하는 스플라이싱 효율), 스플라이싱되지 않은 것에 대한 스플라이싱된 프리-mRNA의 비율, 스플라이싱 속도 또는 스플라이싱 정도는 본원에 기재된 ASO를 사용하여 개선될 수 있다. 또한, 표적 프리-mRNA에 의해 코딩되는 단백질 또는 기능성 RNA의 양을 평가하여 각각의 ASO가 원하는 효과(예를 들어, 기능성 단백질 생산의 향상)를 달성했는지를 결정할 수 있다. 웨스턴 블롯팅, 유세포 분석, 면역 형광 현미경법 및 ELISA와 같은 단백질 생산을 평가 및/또는 정량하기 위해 당 업계에 공지된 임의의 방법이 사용될 수 있다.

[0180] 프리-mRNA의 영역에 혼성화될 때 엑손 스키핑(또는 NIE를 함유하는 인트론의 스플라이싱 향상) 및 단백질 생산 증가를 초래하는 ASO는 동물 모델, 예를 들어 전장 인간 유전자가 녹인된 트랜스제닉 마우스 모델을 사용하여 또는 질환의 인간화된 마우스 모델에서 생체 내에서 시험될 수 있다. ASO의 투여를 위한 적절한 경로는 질환 및/또는 ASO의 전달이 요구되는 세포 유형에 따라 달라질 수 있다. ASO는 예를 들어 수막공간내 주사, 뇌실내 주사, 복강내 주사, 근육내 주사, 피하 주사, 안내 주사, 또는 정맥내 주사에 의해 투여될 수 있다. 투여 후, 모델 동물의 세포, 조직, 및/또는 기관은 예를 들어 당 업계에 공지되고 본원에 기재된 방법에 의해 스플라이싱(효율, 속도, 정도) 및 단백질 생산을 평가함으로써 ASO 처리의 효과를 결정하기 위해 평가될 수 있다. 동물 모델은 또한 질환 또는 질환 중증도의 표현형 또는 행동을 나타낼 수 있다.

[0181] 다양한 예에서 본원에 기술된 바와 같이, 인간 *SCN1A* 유전자의 엑손 20x는 마우스 *SCN1A* 유전자의 엑손 21x와 동등하다.

[0182] 또한, NMD 억제제, 예를 들어 시클로헥시미드의 존재 하에 NMD 유도 엑손을 식별 또는 확인하는 방법이 본 발명의 범위 내에 있다. 예시적인 방법이 [도 3] 및 실시예 2에 제공된다.

[0183] **구체적인 실시양태**

[0184] 실시양태 1. SCN1A 단백질을 코딩하는, 넌센스 매개 RNA 붕괴 유도 엑손을 함유하는 mRNA(NMD 엑손 mRNA)를 갖는 세포에서 SCN1A 단백질의 발현을 조절하는 방법으로서, 치료제를 세포에 접촉시키는 단계를 포함하고, 그로 인하여 치료제가 SCN1A 단백질을 코딩하는 NMD 엑손 mRNA로부터 NMD 엑손의 스플라이싱을 조절하고, 이로써 세

포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 조절하고, SCN1A 단백질의 발현을 조절하는 것인 방법.

[0185] 실시양태 2. 치료를 필요로 하는 대상체의 세포에서 SCN1A 단백질의 발현을 조절함으로써 대상체에서 질환 또는 병태를 치료하는 방법으로서, 대상체의 세포를 NMD 엑손을 함유하고 SCN1A를 코딩하는 세포에서 mRNA로부터 넌센스 매개 mRNA 붕괴 유도 엑손(NMD 엑손)의 스플라이싱을 조절하는 치료제와 접촉시키는 단계를 포함하고, 그로 인하여 대상체의 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 조절하고, SCN1A 단백질의 발현을 조절하는 것인 방법.

[0186] 실시양태 3. 실시양태 1 또는 2에 있어서, 치료제는

[0187] (a) SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분에 결합하거나;

[0188] (b) NMD 엑손 mRNA의 스플라이싱에 관여하는 인자의 결합을 조절하거나;

[0189] (c) (a)와 (b)의 조합인 방법.

[0190] 실시양태 4. 실시양태 3에 있어서, 치료제는 표적화된 부분의 영역으로부터 NMD 엑손의 스플라이싱에 관여하는 인자의 결합을 방해하는 것인 방법.

[0191] 실시양태 5. 실시양태 3 또는 4에 있어서, 표적화된 부분은 NMD 엑손에 근접한 것인 방법.

[0192] 실시양태 6. 실시양태 3 내지 5 중 어느 하나에 있어서, 표적화된 부분은 NMD 엑손의 5' 말단의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 상류에 있는 것인 방법.

[0193] 실시양태 7. 실시양태 3 내지 6 중 어느 하나에 있어서, 표적화된 부분은 NMD 엑손의 5' 말단의 적어도 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 상류에 있는 것인 방법.

[0194] 실시양태 8. 실시양태 3 내지 5 중 어느 하나에 있어서, 표적화된 부분은 NMD 엑손의 3' 말단의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 하류에 있는 것인 방법.

[0195] 실시양태 9. 실시양태 3 내지 5 또는 8 중 어느 하나에 있어서, 표적화된 부분은 NMD 엑손의 3' 말단의 적어도 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 하류에 있는 것인 방법.

[0196] 실시양태 10. 실시양태 3 내지 9 중 어느 하나에 있어서, 표적화된 부분은 SCN1A를 코딩하는 NMD 엑손 mRNA의 2개의 표준 엑손 영역 사이의 인트론 영역에 위치하며, 인트론 영역은 NMD 엑손을 함유하는 것인 방법.

[0197] 실시양태 11. 실시양태 3 내지 10 중 어느 하나에 있어서, 표적화된 부분은 NMD 엑손과 적어도 부분적으로 중첩되는 것인 방법.

[0198] 실시양태 12. 실시양태 3 내지 11 중 어느 하나에 있어서, 표적화된 부분은 NMD 엑손의 상류 인트론과 적어도 부분적으로 중첩되는 것인 방법.

[0199] 실시양태 13. 실시양태 3 내지 12 중 어느 하나에 있어서, 표적화된 부분은 5' NMD 엑손-인트론 접합부 또는 3' NMD 엑손-인트론 접합부를 포함하는 것인 방법.

[0200] 실시양태 14. 실시양태 3 내지 13 중 어느 하나에 있어서, 표적화된 부분은 NMD 엑손 내에 있는 것인 방법.

[0201] 실시양태 15. 실시양태 3 내지 14 중 어느 하나에 있어서, 표적화된 부분은 NMD 엑손의 약 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30개 또는 그 이상의 연속적

인 뉴클레오티드를 포함하는 것인 방법.

[0202] 실시양태 16. 실시양태 1 내지 15 중 어느 하나에 있어서, SCN1A를 코딩하는 NMD 엑손 mRNA는 서열 번호 2 또는 7 내지 10 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함하는 것인 방법.

[0203] 실시양태 17. 실시양태 1 내지 16 중 어느 하나에 있어서, SCN1A를 코딩하는 NMD 엑손 mRNA는 서열 번호 1 또는 3 내지 6과 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 유전자 서열에 의해 코딩되는 것인 방법.

[0204] 실시양태 18. 실시양태 3 내지 17 중 어느 하나에 있어서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,803의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 상류에 있는 것인 방법.

[0205] 실시양태 19. 실시양태 3 내지 18 중 어느 하나에 있어서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,803의 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 상류에 있는 것인 방법.

[0206] 실시양태 20. 실시양태 3 내지 17 중 어느 하나에 있어서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,740의 최대 약 1500 뉴클레오티드, 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드 하류에 있는 것인 방법.

[0207] 실시양태 21. 실시양태 3 내지 17 또는 20 중 어느 하나에 있어서, 표적화된 부분은 게놈 부위 GRCh37/hg19: chr2:166,863,740의 약 1000 뉴클레오티드, 약 800 뉴클레오티드, 약 700 뉴클레오티드, 약 600 뉴클레오티드, 약 500 뉴클레오티드, 약 400 뉴클레오티드, 약 300 뉴클레오티드, 약 200 뉴클레오티드, 약 100 뉴클레오티드, 약 80 뉴클레오티드, 약 70 뉴클레오티드, 약 60 뉴클레오티드, 약 50 뉴클레오티드, 약 40 뉴클레오티드, 약 30 뉴클레오티드, 약 20 뉴클레오티드, 약 10 뉴클레오티드, 약 5 뉴클레오티드, 약 4 뉴클레오티드, 약 2 뉴클레오티드, 약 1 뉴클레오티드 하류에 있는 것인 방법.

[0208] 실시양태 22. 실시양태 3 내지 21 중 어느 하나에 있어서, SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분은 서열 번호 2 또는 7 내지 10의 적어도 8개의 인접한 핵산을 포함하는 영역과 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함하는 것인 방법.

[0209] 실시양태 23. 실시양태 22에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21 내지 67, 210 내지 256, 또는 304 내지 379 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

[0210] 실시양태 24. 실시양태 3 내지 21 중 어느 하나에 있어서, SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 넌센스 매개 RNA 붕괴 유도 엑손 20x 내에 있는 것인 방법.

[0211] 실시양태 25. 실시양태 24에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 42 내지 50, 또는 231 내지 239 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

[0212] 실시양태 26. 실시양태 3 내지 21 중 어느 하나에 있어서, SCN1A를 코딩하는 NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 넌센스 매개 RNA 붕괴 유도 엑손 20x의 상류 또는 하류에 있는 것인 방법.

[0213] 실시양태 27. 실시양태 26에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21 내지 38, 53 내지 67, 210 내지 227, 또는 242 내지 256 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

[0214] 실시양태 28. 실시양태 3 내지 21 중 어느 하나에 있어서, NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 엑손 20x의 엑손-인트론 접합부를 포함하는 것인 방법.

[0215] 실시양태 29. 실시양태 28에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 39 내지 41, 51, 52, 228 내지 230, 240, 또는 241 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

[0216] 실시양태 30. 실시양태 1 내지 29 중 어느 하나에 있어서, 치료제는 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 촉진하는 것인 방법.

[0217] 실시양태 31. 실시양태 30에 있어서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제는 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제와 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가하는 것인 방법.

[0218] 실시양태 32. 실시양태 30 또는 31에 있어서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 증가시키는 것인 방법.

[0219] 실시양태 33. 실시양태 30 내지 32 중 어느 하나에 있어서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 양은 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가하는 것인 방법.

[0220] 실시양태 34. 실시양태 30 내지 33 중 어느 하나에 있어서, 치료제는 세포에서 SCN1A 단백질의 발현을 증가시키는 것인 방법.

[0221] 실시양태 35. 실시양태 30 내지 34 중 어느 하나에 있어서, 치료제와 접촉된 세포에서 생성된 SCN1A의 양은 대조군 세포에서 생성된 SCN1A의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가하는 것인 방법.

[0222] 실시양태 36. 실시양태 2 내지 35 중 어느 하나에 있어서, 질환 또는 병태는 Nav1.1의 기능 상실 돌연변이에 의해 유발되는 것인 방법.

[0223] 실시양태 37. 실시양태 2 내지 36 중 어느 하나에 있어서, 질환 또는 병태는 SCN1A 유전자의 반가불충분성과 관련되며, 대상체는 기능성 SCN1A를 코딩하는 제1 대립 유전자, 및 SCN1A가 생성되지 않거나 감소한 수준으로 생성되는 제2 대립 유전자, 또는 비기능성 SCN1A 또는 부분 기능성 SCN1A를 코딩하는 제2 대립 유전자를 갖는 것인 방법.

[0224] 실시양태 38. 실시양태 2 내지 37 중 어느 하나에 있어서, 질환 또는 병태는 뇌병증인 방법.

[0225] 실시양태 39. 실시양태 38에 있어서, 뇌병증은 간질성 뇌병증인 방법.

[0226] 실시양태 40. 실시양태 2 내지 37 중 어느 하나에 있어서, 질환 또는 병태는 드라벳 증후군(DS); 유아기의 중증 근간대성 뇌전증(SMEI) 경계성(SMEB); 열성 발작(FS); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+); 조기 유아 간질성 뇌병증 13; 잠재성 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증

후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 뇌전증에서의 돌연사(SUDEP); 동기능 부전 증후군 1; 자폐증; 또는 유아기의 악성 이동 부분 발작인 방법.

[0227] 실시양태 41. 실시양태 40에 있어서, GEFS+는 열성 발작 플러스를 동반하는 전신 뇌전증 2형인 방법.

[0228] 실시양태 42. 실시양태 40에 있어서, 열성 발작은 가족성 열성 발작 3A인 방법.

[0229] 실시양태 43. 실시양태 40에 있어서, SMEB는 전신 극파가 없는 SMEB(SMEB-SW), 근간대성 발작이 없는 SMEB(SMEB-M), SMEI의 하나 초과의 특징이 결여된 SMEB(SMEB-O), 또는 전신 강직성 간대 발작을 동반한 난치성 소아기 뇌전증(ICEGTC)인 방법.

[0230] 실시양태 44. 실시양태 1 내지 43 중 어느 하나에 있어서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 촉진하고 SCN1A의 발현을 증가시키는 것인 방법.

[0231] 실시양태 45. 실시양태 1 내지 44 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 22 내지 24, 26, 27, 29 내지 35, 37 내지 62, 64 내지 67, 또는 304 내지 379 중 어느 하나에 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 서열을 포함하는 것인 방법.

[0232] 실시양태 46. 실시양태 1 내지 29 중 어느 하나에 있어서, 치료제는 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 억제하는 것인 방법.

[0233] 실시양태 47. 실시양태 46에 있어서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제는 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제와 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소하는 것인 방법.

[0234] 실시양태 48. 실시양태 46 또는 47에 있어서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 감소시키는 것인 방법.

[0235] 실시양태 49. 실시양태 46 내지 48 중 어느 하나에 있어서, 치료제와 접촉된 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 양은 대조군 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소하는 것인 방법.

[0236] 실시양태 50. 실시양태 46 내지 49 중 어느 하나에 있어서, 치료제는 세포에서 SCN1A 단백질의 발현을 감소시키는 것인 방법.

[0237] 실시양태 51. 실시양태 46 내지 50 중 어느 하나에 있어서, 치료제와 접촉된 세포에서 생성된 SCN1A의 양은 대조군 세포에서 생성된 SCN1A의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 감소하는 것인 방법.

[0238] 실시양태 52. 실시양태 2 내지 29 또는 46 내지 49 중 어느 하나에 있어서, 질환 또는 병태는 Nav1.1의 기능 획득 돌연변이에 의해 유발되는 것인 방법.

[0239] 실시양태 53. 실시양태 52에 있어서, 대상체는 세포에서 SCN1A가 증가한 수준으로 생성되는 대립 유전자, 또는 Nav1.1의 증가한 활성을 유도하는 돌연변이 SCN1A를 코딩하는 대립 유전자를 갖는 것인 방법.

[0240] 실시양태 54. 실시양태 52 또는 53에 있어서, 질환 또는 병태는 편두통인 방법.

[0241] 실시양태 55. 실시양태 54에 있어서, 편두통은 가족성 편마비 편두통 3인 방법.

[0242] 실시양태 56. 실시양태 2 내지 49 중 어느 하나에 있어서, 질환 또는 병태는 Nav1.1 유전성 뇌전증인 방법.

[0243] 실시양태 57. 실시양태 46 내지 56 중 어느 하나에 있어서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 억제하고 SCN1A의 발현을 감소시키는 것인 방법.

[0244] 실시양태 58. 실시양태 46 내지 57 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21, 25, 28, 36, 또는 63 중 어느 하나에 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 서열을 포함하는 것인 방법.

[0245] 실시양태 59. 실시양태 1 내지 58 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 포스포로티오에이트 결합 또는 포스포로디아미데이트 결합을 포함하는 백본 변형을 포함하는 것인 방법.

[0246] 실시양태 60. 실시양태 1 내지 59 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 포스포로디아미데이트 모르폴리노, 잠금 핵산, 펩티드 핵산, 2'-O-메틸, 2'-플루오로, 또는 2'-O-메톡시에틸 모이어티를 포함하는 것인 방법.

[0247] 실시양태 61. 실시양태 1 내지 60 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 하나 이상의 변형된 당 모이어티를 포함하는 것인 방법.

[0248] 실시양태 62. 실시양태 61에 있어서, 각각의 당 모이어티는 변형된 당 모이어티인 방법.

[0249] 실시양태 63. 실시양태 1 내지 62 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 8 내지 50개의 핵염기, 8 내지 40개의 핵염기, 8 내지 35개의 핵염기, 8 내지 30개의 핵염기, 8 내지 25개의 핵염기, 8 내지 20개의 핵염기, 8 내지 15개의 핵염기, 9 내지 50개의 핵염기, 9 내지 40개의 핵염기, 9 내지 35개의 핵염기, 9 내지 30개의 핵염기, 9 내지 25개의 핵염기, 9 내지 20개의 핵염기, 9 내지 15개의 핵염기, 10 내지 50개의 핵염기, 10 내지 40개의 핵염기, 10 내지 35개의 핵염기, 10 내지 30개의 핵염기, 10 내지 25개의 핵염기, 10 내지 20개의 핵염기, 10 내지 15개의 핵염기, 11 내지 50개의 핵염기, 11 내지 40개의 핵염기, 11 내지 35개의 핵염기, 11 내지 30개의 핵염기, 11 내지 25개의 핵염기, 11 내지 20개의 핵염기, 11 내지 15개의 핵염기, 12 내지 50개의 핵염기, 12 내지 40개의 핵염기, 12 내지 35개의 핵염기, 12 내지 30개의 핵염기, 12 내지 25개의 핵염기, 12 내지 20개의 핵염기, 또는 12 내지 15개의 핵염기로 이루어지는 것인 방법.

[0250] 실시양태 64. 실시양태 3 내지 63 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 단백질을 코딩하는 NMD 엑손 mRNA의 표적화된 부분에 적어도 80%, 적어도 85%, 적어도 90%, 적어도 95%, 적어도 98%, 적어도 99%, 또는 100% 상보적인 것인 방법.

[0251] 실시양태 65. 실시양태 1 내지 64 중 어느 하나에 있어서, SCN1A mRNA 또는 단백질 발현을 평가하는 단계를 추가로 포함하는 방법.

[0252] 실시양태 66. 실시양태 2 내지 65 중 어느 하나에 있어서, 대상체는 인간인 방법.

[0253] 실시양태 67. 실시양태 2 내지 65 중 어느 하나에 있어서, 대상체는 비인간 동물인 방법.

[0254] 실시양태 68. 실시양태 2 내지 65 중 어느 하나에 있어서, 대상체는 태아, 배아, 또는 소아인 방법.

[0255] 실시양태 69. 실시양태 1 내지 68 중 어느 하나에 있어서, 세포는 생체 외 세포인 방법.

[0256] 실시양태 70. 실시양태 2 내지 69 중 어느 하나에 있어서, 치료제는 대상체의 수막공간내 주사, 뇌실내 주사, 복강내 주사, 근육내 주사, 피하 주사, 안내, 또는 정맥내 주사에 의해 투여되는 것인 방법.

[0257] 실시양태 71. 실시양태 2 내지 65 중 어느 하나에 있어서, 대상체에게 제2 치료제를 투여하는 단계를 추가로 포함하는 방법.

[0258] 실시양태 72. 실시양태 71에 있어서, 제2 치료제는 소분자인 방법.

[0259] 실시양태 73. 실시양태 71에 있어서, 제2 치료제는 ASO인 방법.

[0260] 실시양태 74. 실시양태 73에 있어서, ASO는 서열 번호 115 내지 161 중 어느 하나에 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 서열을 포함하는 것인 방법.

[0261] 실시양태 75. 실시양태 71에 있어서, 제2 치료제는 인트론 유지를 교정하는 것인 방법.

[0262] 실시양태 76. 실시양태 2 내지 65 중 어느 하나에 있어서, 질환 또는 병태는 알츠하이머병, SCN2A 뇌병증, SCN8A 뇌병증, 또는 SCN5A 부정맥인 방법.

[0263] 실시양태 77. 실시양태 30, 32 또는 34 중 어느 하나에 있어서, 질환 또는 병태는 알츠하이머병, SCN2A 뇌병증, SCN8A 뇌병증, 또는 SCN5A 부정맥인 방법.

[0264] 실시양태 78. 드라벳 증후군(DS); 열성 발작 플러스를 동반하는 전신 뇌전증 2형; 가족성 열성 발작 3A; 가족성 편마비 편두통 3; 자폐증; 조기 유아 간질성 뇌병증 13; 동기능 부전 증후군 1; 알츠하이머병 또는 뇌전증에서의 돌연사(SUDEP)의 치료를 필요로 하는 대상체의 세포에 의한 표적 단백질 또는 기능성 RNA의 발현을 증가시킴으로써 대상체에서 이를 치료하는 방법으로서, 세포는 넌센스 매개 RNA 붕괴 유도 엑손를 함유하는 mRNA(NMD 엑손 mRNA)를 가지며, NMD 엑손 mRNA는 표적 단백질 또는 기능성 RNA를 코딩하며, 대상체의 세포를 표적 단백질 또는 기능성 RNA를 코딩하는 NMD 엑손 mRNA의 표적화된 부분에 결합하는 치료제와 접촉시킴으로써, 넌센스 매개 RNA 붕괴 유도 엑손이 표적 단백질 또는 기능성 RNA를 코딩하는 NMD 엑손 mRNA로부터 배제되어, 이로써 대상체의 세포에서 표적 단백질 또는 기능성 RNA를 코딩하는 가공된 mRNA의 수준을 증가시키고, 표적 단백질 또는 기능성 RNA의 발현을 증가시키는 단계를 포함하는 방법.

[0265] 실시양태 79. 실시양태 78에 있어서, 표적 단백질은 SCN1A인 방법.

[0266] 실시양태 80. 넌센스 매개 NA 붕괴 유도 엑손을 함유하고(NMD 엑손 mRNA) SCN1A 단백질을 코딩하는 mRNA를 가진 세포에 의한 SCN1A 단백질의 발현을 증가시키는 방법으로서, 세포를 SCN1A 단백질을 코딩하는 NMD 엑손 mRNA의 표적화된 부분에 결합하는 작용제와 접촉시키는 단계를 포함하고, 그로 인하여 넌센스 매개 RNA 붕괴 유도 엑손이 SCN1A 단백질을 코딩하는 NMD 엑손 mRNA로부터 배제되고, 이로써 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 증가시키고, SCN1A 단백질의 발현을 증가시키는 것인 방법.

[0267] 실시양태 81. 질환 또는 병태의 치료를 필요로 하는 대상체의 세포에서 SCN1A 단백질의 발현을 증가시킴으로써 대상체에서 질환 또는 병태를 치료하는 방법으로서, 대상체의 세포를 SCN1A 단백질 또는 기능성 SCN1A RNA를 코딩하는 넌센스 매개 RNA 붕괴 유도 엑손 mRNA의 표적화된 부분에 결합하는 치료제와 접촉시키는 단계를 포함하고, 그로 인하여 넌센스 매개 RNA 붕괴 유도 엑손이 SCN1A 단백질 또는 기능성 SCN1A RNA를 코딩하는 NMD 엑손 mRNA로부터 배제되고, 이로써 대상체의 세포에서 SCN1A 단백질 또는 기능성 SCN1A RNA를 코딩하는 가공된 mRNA의 수준을 증가시키고, SCN1A 단백질 또는 기능성 SCN1A RNA의 발현을 증가시키며; 질환 또는 병태는 SCN1A 유전자 이외의 유전자의 돌연변이, SCN1A 유전자 이외의 유전자에 의해 코딩된 단백질의 비정상적 발현 또는 SCN1A 유전자 이외의 유전자에 의해 코딩된 RNA의 비정상적 발현과 관련되는 것인 방법.

[0268] 실시양태 82. 실시양태 81에 있어서, 질환 또는 병태의 증상은 약 2배, 3배, 4배, 5배, 6배, 7배, 8배, 9배, 10배 또는 그 이상 감소하는 것인 방법.

[0269] 실시양태 83. 실시양태 81 또는 82에 있어서, 질환 또는 병태의 증상은 SCN1A 단백질의 발현 증가에 의해 약 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 또는 100% 감소하는 것인 방법.

[0270] 실시양태 84. 실시양태 81 내지 83 중 어느 하나에 있어서, 질환 또는 병태의 진행은 SCN1A 단백질의 발현 증가에 의해 약 2배, 3배, 4배, 5배, 6배, 7배, 8배, 9배, 10배 또는 그 이상 감소하는 것인 방법.

[0271] 실시양태 85. 실시양태 81 내지 84 중 어느 하나에 있어서, 질환 또는 병태의 진행은 SCN1A 단백질의 발현 증가에 의해 약 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 또는 100% 감소하는 것인 방법.

[0272] 실시양태 86. 실시양태 81 내지 85 중 어느 하나에 있어서, SCN1A 단백질 또는 기능성 SCN1A RNA의 발현을 증가시키는 단계는 SCN1A 유전자 이외의 유전자의 돌연변이, SCN1A 유전자 이외의 유전자에 의해 코딩된 단백질의 비정상적 발현 또는 SCN1A 유전자 이외의 유전자에 의해 코딩된 RNA의 비정상적 발현을 보상하는 것인 방법.

[0273] 실시양태 87. 실시양태 81 내지 86 중 어느 하나에 있어서, 질환 또는 병태는 조기 유아 간질성 뇌병증 13인 방법.

[0274] 실시양태 88. 실시양태 81 내지 87 중 어느 하나에 있어서, 대상체는 SCN8A 유전자에 돌연변이를 갖는 것인 방

법.

[0275] 실시양태 89. 실시양태 81 내지 86 중 어느 하나에 있어서, 질환 또는 병태는 동기능 부전 증후군 1인 방법.

[0276] 실시양태 90. 실시양태 81 내지 86 또는 88 중 어느 하나에 있어서, 대상체는 SCN5A 유전자에 돌연변이를 갖는 것인 방법.

[0277] 실시양태 91. 실시양태 81 내지 86 중 어느 하나에 있어서, 질환 또는 병태는 알츠하이머병인 방법.

[0278] 실시양태 92. 질환 또는 병태의 치료를 필요로 하는 대상체에서 이를 치료하는 방법으로서, 안티센스 올리고머를 포함하는 조성물을 대상체에게 투여하는 단계를 포함하고, 안티센스 올리고머는 SCN1A의 인트론 20에 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 적어도 8개의 인접한 뉴클레오티드의 서열을 포함하는 것인 방법.

[0279] 실시양태 93. 질환 또는 병태의 치료를 필요로 하는 대상체에서 이를 치료하는 방법으로서, 안티센스 올리고머를 포함하는 조성물을 대상체에게 투여하는 단계를 포함하고, 안티센스 올리고머는 서열 번호 7 내지 10 중 어느 하나에 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 적어도 8개의 인접한 뉴클레오티드의 서열을 포함하는 것인 방법.

[0280] 실시양태 94. 실시양태 78 내지 93 중 어느 하나에 있어서, 넌센스 매개 RNA 붕괴 유도 엑손은 표적 단백질 또는 기능성 RNA를 코딩하는 NMD 엑손 mRNA로부터 스플라이싱되는 것인 방법.

[0281] 실시양태 95. 실시양태 78 내지 94 중 어느 하나에 있어서, 표적 단백질은 넌센스 매개 RNA 붕괴 유도 엑손에 의해 코딩되는 아미노산 서열을 포함하지 않는 것인 방법.

[0282] 실시양태 96. 실시양태 78 내지 95 중 어느 하나에 있어서, 표적 단백질은 전장 표적 단백질인 방법.

[0283] 실시양태 97. 실시양태 78 내지 96 중 어느 하나에 있어서, 작용제는 NMD 엑손 mRNA의 표적화된 부분에 상보적인 안티센스 올리고머(ASO)인 방법.

[0284] 실시양태 98. 실시양태 78 내지 97 중 어느 하나에 있어서, mRNA는 프리-mRNA인 방법.

[0285] 실시양태 99. 실시양태 78 내지 98 중 어느 하나에 있어서, 접촉 단계는 치료제를 세포의 핵에 있는 mRNA에 접촉시키는 것을 포함하는 것인 방법.

[0286] 실시양태 100. 실시양태 78 내지 99 중 어느 하나에 있어서, 표적 단백질 또는 기능성 RNA는 대상체에서 표적 단백질 또는 기능성 RNA의 결핍을 교정하는 것인 방법.

[0287] 실시양태 101. 실시양태 78 내지 100 중 어느 하나에 있어서, 세포는 SCN1A 단백질의 결핍된 양 또는 활성에 의해 야기되는 병태를 갖는 대상체에 있거나 그로부터 유래하는 것인 방법.

[0288] 실시양태 102. 실시양태 78 내지 101 중 어느 하나에 있어서, 표적 단백질의 결핍된 양은 표적 단백질의 반가불충분성에 의해 야기되고, 대상체는 기능성 표적 단백질을 코딩하는 제1 대립 유전자, 및 표적 단백질이 생성되지 않거나 감소한 수준으로 생성되는 제2 대립 유전자, 또는 비기능성 또는 부분 기능성 표적 단백질을 코딩하는 제2 대립 유전자를 가지며, 안티센스 올리고머는 제1 대립 유전자로부터 전사된 NMD 엑손 mRNA의 표적화된 부분에 결합하는 것인 방법.

[0289] 실시양태 103. 실시양태 78 내지 101 중 어느 하나에 있어서, 대상체는 표적 단백질의 양 또는 기능의 결핍으로 인한 장애에 의해 야기되는 병태를 가지며, 대상체는

[0290] (a) (i) 표적 단백질이 야생형 대립 유전자로부터의 생산과 비교하여 감소한 수준으로 생성되거나,

[0291] (ii) 표적 단백질이 동등한 야생형 단백질과 비교하여 기능이 감소한 형태로 생성되거나,

[0292] (iii) 표적 단백질이 생성되지 않는

[0293] 제1 돌연변이 대립 유전자; 및

[0294] (b) (i) 표적 단백질이 야생형 대립 유전자로부터의 생산과 비교하여 감소한 수준으로 생성되거나,

[0295] (ii) 표적 단백질이 동등한 야생형 단백질과 비교하여 기능이 감소한 형태로 생성되거나,

[0296] (iii) 표적 단백질이 생성되지 않는

[0297] 제2 돌연변이 대립 유전자를 가지며,

[0298] 대상체가 제1 돌연변이 대립 유전자 (a)(iii)을 가질 때, 제2 돌연변이 대립 유전자는 (b)(i) 또는 (b)(ii)이고, 대상체가 제2 돌연변이 대립 유전자 (b)(iii)을 가질 때, 제1 돌연변이 대립 유전자는 (a)(i) 또는 (a)(ii)이며, NMD 엑손 mRNA는 (a)(i) 또는 (a)(ii)인 제1 돌연변이 대립 유전자 및/또는 (b)(i) 또는 (b)(ii) 인 제2 대립 유전자로부터 전사되는 것인 방법.

[0299] 실시양태 104. 실시양태 103에 있어서, 표적 단백질은 동등한 야생형 단백질과 비교하여 기능이 감소한 형태로 생성되는 것인 방법.

[0300] 실시양태 105. 실시양태 103에 있어서, 표적 단백질은 동등한 야생형 단백질과 비교하여 완전히 기능적인 형태로 생성되는 것인 방법.

[0301] 실시양태 106. 실시양태 78 내지 105 중 어느 하나에 있어서, NMD 엑손 mRNA의 표적화된 부분은 넌센스 매개 RNA 붕괴 유도 엑손 내에 있는 것인 방법.

[0302] 실시양태 107. 실시양태 78 내지 105 중 어느 하나에 있어서, NMD 엑손 mRNA의 표적화된 부분은 넌센스 매개 RNA 붕괴 유도 엑손의 상류 또는 하류에 있는 것인 방법.

[0303] 실시양태 108. 실시양태 78 내지 107 중 어느 하나에 있어서, NMD 엑손 mRNA는 서열 번호 2, 7 내지 10, 12, 및 17 내지 20 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함하는 것인 방법.

[0304] 실시양태 109. 실시양태 78 내지 107 중 어느 하나에 있어서, NMD 엑손 mRNA는 서열 번호 1, 3 내지 6, 11, 및 13 내지 16과 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 유전자 서열에 의해 코딩되는 것인 방법.

[0305] 실시양태 110. 실시양태 78 내지 107 중 어느 하나에 있어서, NMD 엑손 mRNA의 표적화된 부분은 서열 번호 2, 7 내지 10, 12, 및 17 내지 20의 적어도 8개의 인접한 핵산을 포함하는 영역과 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함하는 것인 방법.

[0306] 실시양태 111. 실시양태 78 내지 110 중 어느 하나에 있어서, 작용제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21 내지 114 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

[0307] 실시양태 112. 실시양태 78 내지 105 중 어느 하나에 있어서, NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 넌센스 매개 RNA 붕괴 유도 엑손 20x 내에 있는 것인 방법.

[0308] 실시양태 113. 실시양태 112에 있어서, 작용제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 42 내지 50, 또는 231 내지 239 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

[0309] 실시양태 114. 실시양태 78 내지 105 중 어느 하나에 있어서, NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 넌센스 매개 RNA 붕괴 유도 엑손 20x의 상류 또는 하류에 있는 것인 방법.

[0310] 실시양태 115. 실시양태 114에 있어서, 작용제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21 내지 38, 53 내지 67, 210 내지 227, 또는 242 내지 256 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

[0311] 실시양태 116. 실시양태 78 내지 105 중 어느 하나에 있어서, NMD 엑손 mRNA의 표적화된 부분은 SCN1A의 엑손 20x의 엑손-인트론 접합부를 포함하는 것인 방법.

[0312] 실시양태 117. 실시양태 116에 있어서, 작용제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 39 내지 41, 51, 52, 228 내지 230, 240, 또는 241 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

[0313] 실시양태 118. 실시양태 78 내지 105 중 어느 하나에 있어서, NMD 엑손 mRNA의 표적화된 부분은 Scn1a의 넌센스 매개 RNA 붕괴 유도 엑손 21x 내에 있는 것인 방법.

[0314] 실시양태 119. 실시양태 118에 있어서, 작용제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 89 내지 97 중

어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

[0315] 실시양태 120. 실시양태 78 내지 105 중 어느 하나에 있어서, NMD 엑손 mRNA의 표적화된 부분은 Scn1a의 넌센스 매개 RNA 붕괴 유도 엑손 21x의 상류 또는 하류에 있는 것인 방법.

[0316] 실시양태 121. 실시양태 120에 있어서, 작용제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 68 내지 85 및 100 내지 114 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

[0317] 실시양태 122. 실시양태 78 내지 105 중 어느 하나에 있어서, NMD 엑손 mRNA의 표적화된 부분은 Scn1a의 엑손 21x의 엑손-인트론 접합부를 포함하는 것인 방법.

[0318] 실시양태 123. 실시양태 122에 있어서, 작용제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 86 내지 88 및 98 내지 99 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

[0319] 실시양태 124. 실시양태 78 내지 123 중 어느 하나에 있어서, 생성된 표적 단백질은 전장 단백질, 또는 야생형 단백질인 방법.

[0320] 실시양태 125. 실시양태 78 내지 124 중 어느 하나에 있어서, 안티센스 올리고머와 접촉된 세포에서 생성된 표적 단백질 또는 기능성 RNA를 코딩하는 가공된 mRNA의 총량은 대조군 세포에서 생성된 표적 단백질 또는 기능성 RNA를 코딩하는 가공된 mRNA의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가하는 것인 방법.

[0321] 실시양태 126. 실시양태 78 내지 124 중 어느 하나에 있어서, 안티센스 올리고머와 접촉된 세포에 의해 생성된 표적 단백질의 총량은 대조군 세포에 의해 생성된 표적 단백질의 총량과 비교하여 약 1.1 내지 약 10배, 약 1.5 내지 약 10배, 약 2 내지 약 10배, 약 3 내지 약 10배, 약 4 내지 약 10배, 약 1.1 내지 약 5배, 약 1.1 내지 약 6배, 약 1.1 내지 약 7배, 약 1.1 내지 약 8배, 약 1.1 내지 약 9배, 약 2 내지 약 5배, 약 2 내지 약 6배, 약 2 내지 약 7배, 약 2 내지 약 8배, 약 2 내지 약 9배, 약 3 내지 약 6배, 약 3 내지 약 7배, 약 3 내지 약 8배, 약 3 내지 약 9배, 약 4 내지 약 7배, 약 4 내지 약 8배, 약 4 내지 약 9배, 적어도 약 1.1배, 적어도 약 1.5배, 적어도 약 2배, 적어도 약 2.5배, 적어도 약 3배, 적어도 약 3.5배, 적어도 약 4배, 적어도 약 5배, 또는 적어도 약 10배 증가하는 것인 방법.

[0322] 실시양태 127. 실시양태 78 내지 126 중 어느 하나에 있어서, 작용제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 포스포로티오에이트 결합 또는 포스포로디아미데이트 결합을 포함하는 백본 변형을 포함하는 것인 방법.

[0323] 실시양태 128. 실시양태 78 내지 127 중 어느 하나에 있어서, 작용제는 안티센스 올리고머(ASO)이고, 안티센스 올리고머는 포스포로디아미데이트 모르폴리노, 잠금 핵산, 펩티드 핵산, 2'-O-메틸, 2'-플루오로, 또는 2'-O-메톡시에틸 모이어티를 포함하는 것인 방법.

[0324] 실시양태 129. 실시양태 78 내지 128 중 어느 하나에 있어서, 작용제는 안티센스 올리고머(ASO)이고, 안티센스 올리고머는 하나 이상의 변형된 당 모이어티를 포함하는 것인 방법.

[0325] 실시양태 130. 실시양태 129에 있어서, 각각의 당 모이어티는 변형된 당 모이어티인 방법.

[0326] 실시양태 131. 실시양태 78 내지 130 중 어느 하나에 있어서, 작용제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 8 내지 50개의 핵염기, 8 내지 40개의 핵염기, 8 내지 35개의 핵염기, 8 내지 30개의 핵염기, 8 내지 25개의 핵염기, 8 내지 20개의 핵염기, 8 내지 15개의 핵염기, 9 내지 50개의 핵염기, 9 내지 40개의 핵염기, 9 내지 35개의 핵염기, 9 내지 30개의 핵염기, 9 내지 25개의 핵염기, 9 내지 20개의 핵염기, 9 내지 15개의 핵염기, 10 내지 50개의 핵염기, 10 내지 40개의 핵염기, 10 내지 35개의 핵염기, 10 내지 30개의 핵염기, 10 내지 25개의 핵염기, 10 내지 20개의 핵염기, 10 내지 15개의 핵염기, 11 내지 50개의 핵염기, 11 내지 40개의 핵염기, 11 내지 35개의 핵염기, 11 내지 30개의 핵염기, 11 내지 25개의 핵염기, 11 내지 20개의 핵염

기, 11 내지 15개의 핵염기, 12 내지 50개의 핵염기, 12 내지 40개의 핵염기, 12 내지 35개의 핵염기, 12 내지 30개의 핵염기, 12 내지 25개의 핵염기, 12 내지 20개의 핵염기, 또는 12 내지 15개의 핵염기로 이루어지는 것인 방법.

[0327] 실시양태 132. 실시양태 78 내지 131 중 어느 하나에 있어서, 작용제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 단백질을 코딩하는 NMD 엑손 mRNA의 표적화된 부분에 적어도 80%, 적어도 85%, 적어도 90%, 적어도 95%, 적어도 98%, 적어도 99%, 또는 100% 상보적인 것인 방법.

[0328] 실시양태 133. 실시양태 78 내지 132 중 어느 하나에 있어서, SCN1A mRNA 또는 단백질 발현을 평가하는 단계를 추가로 포함하는 방법.

[0329] 실시양태 134. 실시양태 1 내지 133 중 어느 하나에 있어서, 드라벳 증후군; 열성 발작 플러스를 동반하는 전신 뇌전증 2형; 가족성 열성 발작 3A; 가족성 편마비 편두통 3; 자폐증; 조기 유아 간질성 뇌병증 13; 동기능 부전 증후군 1; 알츠하이머병 또는 뇌전증에서의 돌연사(SUDEP)가 치료되고 안티센스 올리고머는 SCN1A NMD 엑손 mRNA의 표적화된 부분에 결합하며, 표적화된 부분은 서열 번호 7 내지 10 및 17 내지 20으로부터 선택된 서열 내에 있는 것인 방법.

[0330] 실시양태 135. 실시양태 78 내지 134 중 어느 하나에 있어서, 대상체는 인간인 방법.

[0331] 실시양태 136. 실시양태 78 내지 135 중 어느 하나에 있어서, 대상체는 비인간 동물인 방법.

[0332] 실시양태 137. 실시양태 78 내지 136 중 어느 하나에 있어서, 대상체는 태아, 배아, 또는 소아인 방법.

[0333] 실시양태 138. 실시양태 78 내지 137 중 어느 하나에 있어서, 세포는 생체 외 세포인 방법.

[0334] 실시양태 139. 실시양태 78 내지 138 중 어느 하나에 있어서, 치료제는 대상체의 수막공간내 주사, 뇌실내 주사, 복강내 주사, 근육내 주사, 피하 주사, 안내 주사, 또는 정맥내 주사에 의해 투여되는 것인 방법.

[0335] 실시양태 140. 실시양태 78 내지 139 중 어느 하나에 있어서, 제2 치료제를 대상체에게 투여하는 단계를 추가로 포함하는 방법.

[0336] 실시양태 141. 실시양태 140에 있어서, 제2 치료제는 소분자인 방법.

[0337] 실시양태 142. 실시양태 140에 있어서, 제2 치료제는 ASO인 방법.

[0338] 실시양태 143. 실시양태 142에 있어서, ASO는 서열 번호 115 내지 161 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하는 것인 방법.

[0339] 실시양태 144. 실시양태 140 내지 142 중 어느 하나에 있어서, 제2 치료제는 인트론 유지를 교정하는 것인 방법.

[0340] 실시양태 145. 실시양태 78 내지 144 중 어느 하나의 방법에 사용되는 안티센스 올리고머.

[0341] 실시양태 146. 서열 번호 21 내지 114 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함하는 안티센스 올리고머.

[0342] 실시양태 147. 실시양태 145 또는 146의 안티센스 올리고머 및 부형제를 포함하는 약학 조성물.

[0343] 실시양태 148. 치료를 필요로 하는 대상체를 치료하는 방법으로서, 실시양태 147의 약학 조성물을 대상체에게 투여하는 단계를 포함하며, 투여 단계는 수막공간내 주사, 뇌실내 주사, 복강내 주사, 근육내 주사, 피하 주사, 안내 주사, 또는 정맥내 주사에 의한 것인 방법.

[0344] 실시 형태 149. 결핍된 단백질 또는 결핍된 기능성 RNA와 관련된 질환 또는 병태의 치료를 필요로 하는 대상체에서 이를 치료하기 위해 세포에 의한 표적 단백질 또는 기능성 RNA의 발현을 증가시키는 방법에 사용하기 위한 치료제를 포함하는 조성물로서, 결핍된 단백질 또는 결핍된 기능성 RNA는 대상체에서 양 또는 활성이 결핍되고, 표적 단백질은

[0345] (a) 결핍된 단백질; 또는

[0346] (b) 대상체에서 결핍된 단백질을 기능적으로 증대시키거나 대체하는 보상 단백질이고;

[0347] 기능성 RNA는

[0348] (c) 결핍된 RNA; 또는

[0349] (d) 대상체에서 결핍된 기능성 RNA를 기능적으로 증대시키거나 대체하는 보상 기능성 RNA이고;

[0350] 치료제는 표적 단백질 또는 기능성 RNA를 코딩하는 NMD 엑손 mRNA로부터의 넌센스 매개 RNA 붕괴 유도 엑손의 배제를 향상시킴으로써, 대상체에서 표적 단백질 또는 기능성 RNA의 생산 또는 활성을 증가시키는 것인 조성물.

[0351] 실시양태 150. 질환 또는 병태의 치료를 필요로 하는 대상체에서 이를 치료하는 방법에 사용하기 위한 치료제를 포함하는 조성물로서, 방법은 대상체의 세포에 의한 SCN1A 단백질의 발현을 조절하는 단계를 포함하고, 세포는 넌센스 매개 RNA 붕괴 유도 엑손을 함유하고(NMD 엑손 mRNA) SCN1A 단백질을 코딩하는 mRNA를 가지며, 방법은 세포를 치료제와 접촉시키는 단계를 포함하고, 그로 인하여 SCN1A 단백질을 코딩하는 NMD 엑손 mRNA로부터 넌센스 매개 RNA 붕괴 유도 엑손의 배제가 조절되고, 이로써 대상체의 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 조절하고, SCN1A 단백질의 발현을 조절하는 것인 조성물.

[0352] 실시양태 151. 실시양태 150에 있어서, 질환 또는 병태는 드라벳 증후군(DS); 유아기의 중증 근간대성 뇌전증(SMEI) 경계성(SMEB); 열성 발작(FS); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+); 조기 유아 간질성 뇌병증 13; 잠재성 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 뇌전증에서의 돌연사(SUDEP); 동기능 부전 증후군 1; 자폐증; 또는 가족성 편마비 편두통 3; 및 알츠하이머병으로 이루어진 군으로부터 선택되는 것인 조성물.

[0353] 실시양태 152. 실시양태 150 또는 151에 있어서, SCN1A 단백질 및 NMD 엑손 mRNA는 SCN1A 유전자에 의해 코딩되는 것인 조성물.

[0354] 실시양태 153. 실시양태 149 내지 152 중 어느 하나에 있어서, 넌센스 매개 RNA 붕괴 유도 엑손은 SCN1A 단백질을 코딩하는 NMD 엑손 mRNA로부터 스플라이싱되는 것인 조성물.

[0355] 실시양태 154. 실시양태 149 내지 153 중 어느 하나에 있어서, SCN1A 단백질은 넌센스 매개 RNA 붕괴 유도 엑손에 의해 코딩되는 아미노산 서열을 포함하지 않는 것인 조성물.

[0356] 실시양태 155. 실시양태 149 내지 154 중 어느 하나에 있어서, SCN1A 단백질은 전장 SCN1A 단백질인 조성물.

[0357] 실시양태 156. 실시양태 149 내지 155 중 어느 하나에 있어서, 치료제는 NMD 엑손 mRNA의 표적화된 부분에 상보적인 안티센스 올리고머(ASO)인 조성물.

[0358] 실시양태 157. 실시양태 149 내지 156 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 넌센스 매개 RNA 붕괴 유도 엑손 내에 있는 NMD 엑손 mRNA의 일부를 표적화하는 것인 조성물.

[0359] 실시양태 158. 실시양태 149 내지 156 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 넌센스 매개 RNA 붕괴 유도 엑손의 상류 또는 하류에 있는 NMD 엑손 mRNA의 일부를 표적화하는 것인 조성물.

[0360] 실시양태 159. 실시양태 149 내지 158 중 어느 하나에 있어서, 표적 단백질은 SCN1A인 조성물.

[0361] 실시양태 160. 실시양태 159에 있어서, NMD 엑손 mRNA는 서열 번호 2, 7 내지 10, 12, 및 17 내지 20 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함하는 것인 조성물.

[0362] 실시양태 161. 실시양태 159에 있어서, NMD 엑손 mRNA는 서열 번호 1, 3 내지 6, 11, 및 13 내지 16과 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 유전자 서열에 의해 코딩되는 것인 조성물.

[0363] 실시양태 162. 실시양태 159에 있어서, NMD 엑손 mRNA의 표적화된 부분은 서열 번호 2, 7 내지 10, 12, 및 17 내지 20의 적어도 8개의 인접한 핵산을 포함하는 영역과 적어도 80%, 85%, 90%, 95%, 97%, 또는 100% 서열 동일성을 갖는 서열을 포함하는 것인 조성물.

[0364] 실시양태 163. 실시양태 159 내지 162 중 어느 하나에 있어서, NMD 엑손 mRNA의 표적화된 부분은 (i) 넌센스 매개 RNA 붕괴 유도 엑손 20x 내에 있거나, (ii) 넌센스 매개 RNA 붕괴 유도 엑손 20x의 상류 또는 하류에 있거나, (iii) 넌센스 매개 RNA 붕괴 유도 엑손 20x의 엑손-인트론 접합부를 포함하는 것인 조성물.

[0365] 실시양태 164. 실시양태 159 내지 163 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21 내지 114 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 동일성인 서열을 포함하

는 것인 조성물

[0366] 실시양태 165. 실시양태 149 내지 164 중 어느 하나에 있어서, 질환 또는 병태는 Nav1.1의 기능 상실 돌연변이에 의해 유발되는 것인 조성물.

[0367] 실시양태 166. 실시양태 149 내지 165 중 어느 하나에 있어서, 질환 또는 병태는 SCN1A 유전자의 반가불충분성과 관련되며, 대상체는 기능성 SCN1A를 코딩하는 제1 대립 유전자, 및 SCN1A가 생성되지 않거나 감소한 수준으로 생성되는 제2 대립 유전자, 또는 비기능성 SCN1A 또는 부분 기능성 SCN1A를 코딩하는 제2 대립 유전자를 갖는 것인 조성물.

[0368] 실시양태 167. 실시양태 149 내지 166 중 어느 하나에 있어서, 질환 또는 병태는, 경우에 따라 Nav1.1의 기능 상실 돌연변이에 의해 유발되는, 뇌병증인 조성물.

[0369] 실시양태 168. 실시양태 167에 있어서, 뇌병증은 간질성 뇌병증인 조성물.

[0370] 실시양태 169. 실시양태 165 또는 166에 있어서, 질환 또는 병태는 드라벳 증후군(DS); 유아기의 중증 근간대성 뇌전증(SMEI) 경계성(SMEB); 열성 발작(FS); 열성 발작 플러스를 동반하는 전신 뇌전증(GEFS+); 조기 유아 간질성 뇌병증 13; 잠재성 전신 뇌전증; 잠재성 초점 뇌전증; 근간대성 무정위 뇌전증; 레녹스-가스토 증후군; 웨스트 증후군; 특발성 경련; 조기 근간대성 뇌병증; 진행성 근간대성 뇌전증; 소아기 교대성 편마비; 미분류 간질성 뇌병증; 뇌전증에서의 돌연사(SUDEP); 동기능 부전 증후군 1; 자폐증; 또는 유아기의 악성 이동 부분 발작인 조성물.

[0371] 실시양태 170. 실시양태 168에 있어서, GEFS+는 열성 발작 플러스를 동반하는 전신 뇌전증 2형인 조성물.

[0372] 실시양태 171. 실시양태 168에 있어서, 열성 발작은 가족성 열성 발작 3A인 조성물.

[0373] 실시양태 172. 실시양태 168에 있어서, SMEB는 전신 극파가 없는 SMEB(SMEB-SW), 근간대성 발작이 없는 SMEB(SMEB-M), SMEI의 하나 초과의 특징이 결여된 SMEB(SMEB-O), 또는 전신 강직성 간대 발작을 동반한 난치성 소아기 뇌전증(ICEGTC)인 조성물.

[0374] 실시양태 173. 실시양태 165 내지 172 중 어느 하나에 있어서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 촉진하고 SCN1A의 발현을 증가시키는 것인 조성물.

[0375] 실시양태 174. 실시양태 165 내지 173 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 22 내지 24, 26, 27, 29 내지 35, 37 내지 62, 또는 64 내지 67 중 어느 하나에 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 서열을 포함하는 것인 조성물.

[0376] 실시양태 175. 실시양태 149 내지 164 중 어느 하나에 있어서, 질환 또는 병태는 Nav1.1의 기능 획득 돌연변이에 의해 유발되는 것인 조성물.

[0377] 실시 형태 176. 실시 형태 149 내지 164 또는 175 중 어느 하나에 있어서, 대상체는 세포에서 SCN1A가 증가한 수준으로 생성되는 대립 유전자, 또는 Nav1.1의 증가한 활성을 유도하는 돌연변이 SCN1A를 코딩하는 대립 유전자를 갖는 것인 조성물.

[0378] 실시양태 177. 실시양태 149 내지 164, 175, 또는 176 중 어느 하나에 있어서, 질환 또는 병태는 편두통인 조성물.

[0379] 실시양태 178. 실시양태 177에 있어서, 편두통은 가족성 편마비 편두통 3인 조성물.

[0380] 실시양태 179. 실시양태 149 내지 164, 175, 또는 176 중 어느 하나에 있어서, 질환 또는 병태는 Nav1.1 유전성 뇌전증인 조성물.

[0381] 실시양태 180. 실시양태 149 내지 164, 또는 175 내지 179 중 어느 하나에 있어서, 치료제는 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA로부터 NMD 엑손의 배제를 억제하고 SCN1A의 발현을 감소시키는 것인 조성물.

[0382] 실시양태 181. 실시양태 149 내지 164, 또는 175 내지 180 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 ASO는 서열 번호 21, 25, 28, 36, 또는 63 중 어느 하나에 적어도 약 80%, 85%, 90%, 95%, 97%, 또는 100% 상보적인 서열을 포함하는 것인 조성물.

[0383] 실시양태 182. 실시양태 149 내지 181 중 어느 하나에 있어서, 표적 단백질 또는 기능성 RNA를 코딩하는 가공된 mRNA는 전장의 성숙한 mRNA, 또는 야생형의 성숙한 mRNA인 조성물.

[0384] 실시양태 183. 실시양태 149 내지 182 중 어느 하나에 있어서, 생성된 표적 단백질은 전장 단백질, 또는 야생형 단백질인 조성물.

[0385] 실시양태 184. 실시양태 149 내지 183 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 포스포로티오에이트 결합 또는 포스포로디아미데이트 결합을 포함하는 백본 변형을 포함하는 것인 조성물.

[0386] 실시양태 185. 실시양태 149 내지 184 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 안티센스 올리고뉴클레오티드인 조성물.

[0387] 실시양태 186. 실시양태 149 내지 185 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 포스포로디아미데이트 모르폴리노, 잠금 핵산, 펩티드 핵산, 2'-O-메틸, 2'-플루오로, 또는 2'-O-메톡시에틸 모이어티를 포함하는 것인 조성물.

[0388] 실시양태 187. 실시양태 149 내지 186 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 하나 이상의 변형된 당 모이어티를 포함하는 것인 조성물.

[0389] 실시양태 188. 실시양태 187에 있어서, 각각의 당 모이어티는 변형된 당 모이어티인 조성물.

[0390] 실시양태 189. 실시양태 149 내지 188 중 어느 하나에 있어서, 치료제는 안티센스 올리고머(ASO)이며 안티센스 올리고머는 8 내지 50개의 핵염기, 8 내지 40개의 핵염기, 8 내지 35개의 핵염기, 8 내지 30개의 핵염기, 8 내지 25개의 핵염기, 8 내지 20개의 핵염기, 8 내지 15개의 핵염기, 9 내지 50개의 핵염기, 9 내지 40개의 핵염기, 9 내지 35개의 핵염기, 9 내지 30개의 핵염기, 9 내지 25개의 핵염기, 9 내지 20개의 핵염기, 9 내지 15개의 핵염기, 10 내지 50개의 핵염기, 10 내지 40개의 핵염기, 10 내지 35개의 핵염기, 10 내지 30개의 핵염기, 10 내지 25개의 핵염기, 10 내지 20개의 핵염기, 10 내지 15개의 핵염기, 11 내지 50개의 핵염기, 11 내지 40개의 핵염기, 11 내지 35개의 핵염기, 11 내지 30개의 핵염기, 11 내지 25개의 핵염기, 11 내지 20개의 핵염기, 11 내지 15개의 핵염기, 12 내지 50개의 핵염기, 12 내지 40개의 핵염기, 12 내지 35개의 핵염기, 12 내지 30개의 핵염기, 12 내지 25개의 핵염기, 12 내지 20개의 핵염기, 또는 12 내지 15개의 핵염기로 이루어지는 것인 조성물.

[0391] 실시양태 190. 안티센스 올리고머를 포함하는 조성물로서, 안티센스 올리고머는 SCN1A의 인트론 20에 상보적인 적어도 80%, 85%, 90%, 95%, 97% 또는 100% 상보적인 적어도 8개의 인접한 뉴클레오티드의 서열을 포함하는 것인 조성물.

[0392] 실시 형태 191. 안티센스 올리고머를 포함하는 조성물로서, 안티센스 올리고머는 서열 번호 7 내지 10 중 어느 하나와 적어도 80%, 85%, 90%, 95%, 97% 또는 100% 상보적인 적어도 8개의 인접한 뉴클레오티드의 서열을 포함하는 것인 조성물.

[0393] 실시양태 192. 실시양태 149 내지 191 중 어느 하나의 조성물의 치료제 및 부형제를 포함하는 약학 조성물.

[0394] 실시양태 193. 치료를 필요로 하는 대상체를 치료하는 방법으로서, 실시양태 192의 약학 조성물을 대상체에게 투여하는 단계를 포함하며, 투여 단계는 수막공간내 주사, 뇌실내 주사, 복강내 주사, 근육내 주사, 피하 주사, 안내 주사, 또는 정맥내 주사에 의한 것인 약학 조성물.

[0395] 실시양태 194. SCN1A mRNA 전사체의 표적 서열에 혼성화하는 안티센스 올리고머 및 약학적으로 허용 가능한 부형제를 포함하는 약학 조성물로서, SCN1A mRNA 전사체는 넌센스 매개 RNA 붕괴 유도 엑손을 포함하며, 안티센스 올리고머는 SCN1A mRNA 전사체로부터의 넌센스 매개 RNA 붕괴 유도 엑손의 배제를 유도하는 것인 약학 조성물.

[0396] 실시양태 195. 실시양태 194에 있어서, SCN1A mRNA 전사체는 SCN1A NMD 엑손 mRNA 전사체인 약학 조성물.

[0397] 실시양태 196. 실시양태 194 또는 195에 있어서, SCN1A NMD 엑손 mRNA 전사체의 표적화된 부분은 (i) 넌센스 매개 RNA 붕괴 유도 엑손 20x 내에 있거나, (ii) 넌센스 매개 RNA 붕괴 유도 엑손 20x의 상류 또는 하류에 있거나, (iii) 넌센스 매개 RNA 붕괴 유도 엑손 20x의 엑손-인트론 접합부를 포함하는 것인 약학 조성물.

[0398] 실시양태 197. 실시양태 194 또는 196에 있어서, SCN1A NMD 엑손 mRNA 전사체는 서열 번호 1, 3 내지 6, 11, 및 13 내지 16 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% 또는 100% 서열 동일성을 갖는 유전자 서열에 의해 코딩되는 것인 약학 조성물.

[0399] 실시양태 198. 실시양태 194 또는 196에 있어서, SCN1A NMD 엑손 mRNA 전사체는 서열 번호 2, 7 내지 10, 12,

및 17 내지 20 중 어느 하나와 적어도 약 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% 또는 100% 서열 동일성을 갖는 서열을 포함하는 것인 약학 조성물.

[0400] 실시양태 199. 실시양태 194에 있어서, 안티센스 올리고머는 포스포로티오에이트 결합 또는 포스포로디아미데이트 결합을 포함하는 백본 변형을 포함하는 것인 약학 조성물.

[0401] 실시양태 200. 실시양태 194에 있어서, 안티센스 올리고머는 안티센스 올리고뉴클레오티드인 약학 조성물.

[0402] 실시양태 201. 실시양태 194에 있어서, 안티센스 올리고머는 포스포로디아미데이트 모르폴리노, 잠금 핵산, 펩티드 핵산, 2'-O-메틸, 2'-플루오로, 또는 2'-O-메톡시에틸 모이어티를 포함하는 것인 약학 조성물.

[0403] 실시양태 202. 실시양태 194에 있어서, 안티센스 올리고머는 하나 이상의 변형된 당 모이어티를 포함하는 것인 약학 조성물.

[0404] 실시양태 203. 실시양태 194에 있어서, 안티센스 올리고머는 8 내지 50개의 핵염기, 8 내지 40개의 핵염기, 8 내지 35개의 핵염기, 8 내지 30개의 핵염기, 8 내지 25개의 핵염기, 8 내지 20개의 핵염기, 8 내지 15개의 핵염기, 9 내지 50개의 핵염기, 9 내지 40개의 핵염기, 9 내지 35개의 핵염기, 9 내지 30개의 핵염기, 9 내지 25개의 핵염기, 9 내지 20개의 핵염기, 9 내지 15개의 핵염기, 10 내지 50개의 핵염기, 10 내지 40개의 핵염기, 10 내지 35개의 핵염기, 10 내지 30개의 핵염기, 10 내지 25개의 핵염기, 10 내지 20개의 핵염기, 10 내지 15개의 핵염기, 11 내지 50개의 핵염기, 11 내지 40개의 핵염기, 11 내지 35개의 핵염기, 11 내지 30개의 핵염기, 11 내지 25개의 핵염기, 11 내지 20개의 핵염기, 11 내지 15개의 핵염기, 12 내지 50개의 핵염기, 12 내지 40개의 핵염기, 12 내지 35개의 핵염기, 12 내지 30개의 핵염기, 12 내지 25개의 핵염기, 12 내지 20개의 핵염기, 또는 12 내지 15개의 핵염기를 포함하는 것인 약학 조성물.

[0405] 실시양태 204. 실시양태 194 또는 195에 있어서, 안티센스 올리고머는 SCN1A NMD 엑손 mRNA 전사체의 표적화된 부분에 적어도 80%, 적어도 85%, 적어도 90%, 적어도 95%, 적어도 98%, 적어도 99%, 또는 100% 상보적인 것인 약학 조성물.

[0406] 실시양태 205. 실시양태 194 또는 195에 있어서, SCN1A NMD 엑손 mRNA 전사체의 표적화된 부분은 서열 번호 2, 7 내지 10, 12, 및 17 내지 20으로부터 선택된 서열 내에 있는 것인 약학 조성물.

[0407] 실시양태 206. 실시양태 194에 있어서, 안티센스 올리고머는 서열 번호 21 내지 114 중 어느 하나와 적어도 약 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 또는 99% 서열 동일성인 뉴클레오티드 서열을 포함하는 것인 약학 조성물.

[0408] 실시양태 207. 실시양태 194에 있어서, 안티센스 올리고머는 서열 번호 21 내지 114로부터 선택된 뉴클레오티드 서열을 포함하는 것인 약학 조성물.

[0409] 실시양태 208. 실시양태 194 내지 실시양태 207 중 어느 하나에 있어서, 수막공간내 주사, 뇌실내 주사, 복강내 주사, 근육내 주사, 피하 주사, 안내 주사, 또는 정맥내 주사를 위해 제제화되는 약학 조성물.

[0410] 실시양태 209. 넌센스 매개 RNA 붕괴 유도 엑손의 제거를 촉진하게 하여 기능적 형태의 SCN1A 단백질을 코딩하는 완전히 가공된 SCN1A mRNA 전사체를 생성하기 위해 결핍된 SCN1A mRNA 전사체의 가공을 유도하는 방법으로서,

[0411] (a) 안티센스 올리고머를 대상체의 표적 세포에 접촉시키는 단계;

[0412] (b) 안티센스 올리고머를 결핍된 SCN1A mRNA 전사체에 혼성화시키는 단계로서, 결핍된 SCN1A mRNA 전사체는 기능적 형태의 SCN1A 단백질을 코딩할 수 있고 하나 이상의 넌센스 매개 RNA 붕괴 유도 엑손을 포함하는 것인 단계;

[0413] (c) 기능적 형태의 SCN1A 단백질을 코딩하는 완전히 가공된 SCN1A mRNA 전사체를 생성하기 위해 결핍된 SCN1A mRNA 전사체로부터 하나 이상의 넌센스 매개 RNA 붕괴 유도 엑손을 제거하는 단계; 및

[0414] (d) 완전히 가공된 SCN1A mRNA 전사체로부터 기능적 형태의 SCN1A 단백질을 번역하는 단계

[0415] 를 포함하는 방법.

[0416] 실시양태 210. SCN1A 단백질의 결핍된 양 또는 활성에 의해 야기되는 병태를 갖는 대상체를 치료하는 방법으로서, 서열 번호 24 내지 114 중 어느 하나와 적어도 약 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 또는 99% 서열 동일성을 갖는 뉴클레오티드 서열을 포함하는 안티센스 올리고머를 대상체에게 투여하는 단

계를 포함하는 방법.

[0417] 실시양태 211. 세포에 의한 표적 단백질 또는 기능성 RNA의 유전자 발현을 증가시키는 작용제를 스크리닝하는 방법으로서, 세포는 넌센스 매개 RNA 붕괴 유도 엑손을 함유하는 mRNA(NMD 엑손 mRNA)를 가지며, NMD 엑손 mRNA는 표적 단백질 또는 기능성 RNA를 코딩하고,

[0418] (a) NMD 엑손 mRNA를 표적화하는 시험 작용제를 제1 세포에 접촉시키는 단계;

[0419] (b) 대조 작용제를 제2 세포에 접촉시키는 단계;

[0420] (c) 제1 세포에서 제1 수준을 결정하는 단계로서, 제1 수준은 (i) RNA 붕괴유도 엑손을 포함하지 않는 NMD 엑손 mRNA에 의해 코딩된 RNA 전사체, 또는 (ii) RNA 붕괴 유도 엑손에 의해 코딩되는 아미노산 서열을 포함하지 않는 NMD 엑손 mRNA에 의해 코딩된 단백질의 수준인 단계;

[0421] (d) 제2 세포에서 제2 수준을 결정하는 단계로서, 제2 수준은 (i) RNA 붕괴 유도 엑손을 포함하지 않는 NMD 엑손 mRNA에 의해 코딩된 RNA 전사체, 또는 (ii) RNA 붕괴 유도 엑손에 의해 코딩되는 아미노산 서열을 포함하지 않는 NMD 엑손 mRNA에 의해 코딩된 단백질의 수준인 단계;

[0422] 제1 수준이 제2 수준보다 높은 경우;

[0423] (e) 시험 작용제를 선택하는 단계

[0424] 를 포함하는 방법.

[0425] 실시양태 212. 세포에 의한 표적 단백질 또는 기능성 RNA의 유전자 발현을 증가시키는 작용제를 스크리닝하는 방법으로서, 세포는 넌센스 매개 RNA 붕괴 유도 엑손을 함유하는 mRNA(NMD 엑손 mRNA)를 가지며, NMD 엑손 mRNA는 표적 단백질 또는 기능성 RNA를 코딩하고,

[0426] (a) NMD 엑손 mRNA를 표적화하는 시험 작용제를 제1 세포에 접촉시키는 단계;

[0427] (b) 대조 작용제를 제2 세포에 접촉시키는 단계;

[0428] (c) 제1 세포에서 제1 수준을 결정하는 단계로서, 제1 수준은 (i) RNA 붕괴유도 엑손을 포함하는 NMD 엑손 mRNA에 의해 코딩된 RNA 전사체, 또는 (ii) RNA 붕괴 유도 엑손에 의해 코딩되는 아미노산 서열을 포함하는 NMD 엑손 mRNA에 의해 코딩된 단백질의 수준인 단계;

[0429] (d) 제2 세포에서 제2 수준을 결정하는 단계로서, 제2 수준은 (i) RNA 붕괴 유도 엑손을 포함하는 NMD 엑손 mRNA에 의해 코딩된 RNA 전사체, 또는 (ii) RNA 붕괴 유도 엑손에 의해 코딩되는 아미노산 서열을 포함하는 NMD 엑손 mRNA에 의해 코딩된 단백질의 수준인 단계;

[0430] 제1 수준이 제2 수준보다 낮은 경우;

[0431] (e) 시험 작용제를 선택하는 단계

[0432] 를 포함하는 방법.

[0433] 실시양태 213. 실시양태 211 또는 212에 있어서, 단백질 합성 억제제를 제1 세포 및 제2 세포에 접촉시키는 단계를 포함하고; 제1 수준은 RNA 붕괴 유도 엑손을 포함하는 NMD 엑손 mRNA에 의해 코딩된 RNA 전사체의 수준이고; 제2 수준은 RNA 붕괴 유도 엑손을 포함하는 NMD 엑손 mRNA에 의해 코딩된 RNA 전사체의 수준인 방법.

[0434] 실시양태 214. 드라벳 증후군(DS), 열성 발작 플러스를 동반하는 전신 뇌전증 2형; 가족성 열성 발작 3A; 가족성 편마비 편두통 3; 자폐증; 조기 유아 간질성 뇌병증 13; 동기능 부전 증후군 1; 알츠하이머병 또는 SUDEP(뇌전증에서의 돌연사)의 치료를 필요로 하는 대상체의 세포에 의한 표적 단백질 또는 기능성 RNA의 발현을 증가시킴으로써 대상체에서 이를 치료하는 방법으로서, 세포는 넌센스 매개 RNA 붕괴 유도 엑손를 함유하는 mRNA(NMD 엑손 mRNA)를 가지며, NMD 엑손 mRNA는 표적 단백질 또는 기능성 RNA를 코딩하고, 표적 단백질 또는 기능성 RNA를 코딩하는 NMD 엑손 mRNA의 스플라이싱을 조절하는 치료제와 대상체의 세포를 접촉시킴으로써, 넌센스 매개 RNA 붕괴 유도 엑손이 표적 단백질 또는 기능성 RNA를 코딩하는 NMD 엑손 mRNA로부터 배제되어, 이로써 대상체의 세포에서 표적 단백질 또는 기능성 RNA를 코딩하는 가공된 mRNA의 수준을 증가시키고, 표적 단백질 또는 기능성 RNA의 발현을 증가시키는 단계

[0435] 를 포함하는 방법.

[0436] 실시양태 215. 넌센스 매개 RNA 붕괴 유도 엑손을 함유하고(NMD 엑손 mRNA) SCN1A 단백질을 코딩하는 mRNA를 갖는 세포에 의한 SCN1A 단백질의 발현을 증가시키는 방법으로서, 세포를 SCN1A 단백질을 코딩하는 NMD 엑손 mRNA의 스플라이싱을 조절하는 작용제에 접촉시킴으로써, 넌센스 매개 RNA 붕괴 유도 엑손이 SCN1A 단백질을 코딩하는 NMD 엑손 mRNA로부터 배제되어, 이로써 세포에서 SCN1A 단백질을 코딩하는 가공된 mRNA의 수준을 증가시키고, SCN1A 단백질의 발현을 증가시키는 단계를 포함하는 방법.

[0437] 실시양태 216. 실시양태 214 또는 215에 있어서, 작용제는

[0438] (a) 표적 단백질 또는 기능성 RNA를 코딩하는 NMD 엑손 mRNA의 표적화된 부분에 결합하거나;

[0439] (b) 스플라이스좀의 하나 이상의 성분에 결합하거나;

[0440] (c) (a)와 (b)의 조합인 방법.

[0441] 본 발명의 바람직한 실시양태가 여기에 제시되고 설명되었지만, 이러한 실시양태는 단지 예로서 제공된다는 것은 당업자에게 명백할 것이다. 당업자는 본 발명을 벗어나지 않으면서 다수의 변형, 변경 및 치환을 생각해 낼 것이다. 본원에 기술된 본 발명의 실시양태에 대한 다양한 대안이 본 발명을 실시하는 데 사용될 수 있음을 이해해야 한다. 다음의 청구 범위는 본 발명의 범위를 정의하고 이들 청구 범위의 범위 내의 방법 및 구조 및 그 등가물이 이에 의해 포함되는 것으로 의도된다.

[0442] **실시예**

[0443] 본 발명은 하기 실시예에 의해 더 구체적으로 설명될 것이다. 그러나 본 발명은 이들 실시예에 의해 어떠한 방식으로도 제한되지 않음을 이해해야 한다.

[0444] **실시예 1: 차세대 서열분석을 사용한 RNAseq에 의한 *SCN1A* 전사체에서 NMD 유도 엑손 포함 이벤트의 확인**

[0445] *SCN1A* 유전자에 의해 생성된 전사체의 스냅 샷을 밝혀 NIE 포함 이벤트를 확인하기 위해 차세대 서열 분석을 사용하여 전체 트랜스크립톰(transcriptome) 샷건 서열분석을 수행하였다. 이를 위해, HCN(인간 피질 뉴런)의 핵 및 세포질 분획으로부터 폴리A+ RNA를 단리하고 일루미나의 TruSeq 스트랜디드 mRNA 라이브러리 프렙 키트(Illumina's TruSeq Stranded mRNA library Prep Kit)를 사용하여 cDNA 라이브러리를 구축하였다. 라이브러리를 페어-엔드(pair-end) 서열분석하여 인간 게놈에 맵핑되는 100 뉴클레오티드 리드(read)를 생성하였다(Feb. 2009, GRCh37/hg19 assembly). *SCN1A*의 서열분석 결과는 [도 2]에 나타낸다. 간략하게, [도 2]는 UCSC 게놈 브라우저(UCSC 인포매틱스 그룹(UCSC Genome Informatics Group, 캘리포니아 대학교, 산타 크루즈(미국 캘리포니아주 95064 산타 크루즈 하이 스트리트 1156 소재) 바이오 분자 과학 및 공학 센터(Center for Biomolecular Science & Engineering))에 의해 운영되고 예를 들어, 문헌[Rosenbloom, *et al.*, 2015, "The UCSC Genome Browser database: 2015 update," Nucleic Acids Research 43, Database Issue, doi: 10.1093/nar/gku1177]에 의해 기술됨)를 사용하여 맵핑된 리드를 시각화하여 보여주며, 및 리드의 커버리지 및 수는 피크 신호에 의해 추론될 수 있다. 피크의 높이는 특정 영역에서 리드의 밀도로 주어진 발현 수준을 나타낸다. 상단 패널은 스케일링할 *SCN1A* 유전자를 도식으로 표현하여 나타낸다. 100종의 척추동물 종에 걸친 보존 수준을 피크로 나타낸다. 가장 높은 피크는 엑손(검은 박스)에 해당하는 한편, 대부분의 인트론(화살표가 있는 선)에는 피크가 관찰되지 않는다. 중간 패널에 나타낸 인트론 20(NM_006920)에서 보존 피크가 확인되었다. 보존된 서열을 검사하여 3' 및 5' 스플라이스 부위(밑줄 친 서열)의 측면에 위치한 64 bp의 엑손 유사 서열(하부 패널, 회색으로 강조된 서열)을 확인하였다. 이 엑손의 포함은 프레임 시프트 및 엑손 21에 조기 종결 코돈의 도입을 초래하여 전사체를 NMD의 표적으로 만든다.

[0446] 예시적인 *SCN1A* 유전자, 프리-mRNA, 엑손, 및 인트론 서열은 표 2에 요약한다. 각 엑손 또는 인트론에 대한 서열은 표 3에 요약한다.

[0447] [표 2]

표적 SCN1A 유전자 및 프리-mRNA 서열의 목록.

종	서열 번호	서열 유형
인간	서열 번호 1	SCN1A 유전자 (NC_000002.12)
	서열 번호 2	SCN1A 프리-mRNA (예를 들어, SCN1A mRNA NM_006920.5 코딩)
	서열 번호 3	엑손 20 유전자
	서열 번호 4	인트론 20 유전자
	서열 번호 5	엑손 21 유전자
	서열 번호 6	엑손 20x 유전자
	서열 번호 7	엑손 20 프리-mRNA
	서열 번호 8	인트론 20 프리-mRNA
	서열 번호 9	엑손 21 프리-mRNA
	서열 번호 10	엑손 20x 프리-mRNA
마우스	서열 번호 11	SCN1A 유전자 (NC_000068.7)
	서열 번호 12	SCN1A 프리-mRNA (예를 들어, SCN1A mRNA NM_001313997.1 코딩)
	서열 번호 13	엑손 21 유전자
	서열 번호 14	인트론 21 유전자
	서열 번호 15	엑손 22 유전자
	서열 번호 16	엑손 21x 유전자
	서열 번호 17	엑손 21 프리-mRNA
	서열 번호 18	인트론 21 프리-mRNA
	서열 번호 19	엑손 22 프리-mRNA
	서열 번호 20	엑손 21x 프리-mRNA

[0448]

[0449] [표 3]

SCN1A 프리-mRNA 전사체 에서 표적 엑손 또는 인트론의 서열.

서열 번호	서열 유형	서열
서열 번호 7	엑손 20 프리-mRNA	GUUUCAUUGGUCAGUUUAACAGCAAAUGCCUUGGGUUACUC AGAACUUGGAGCCAUCAAAUCUCUCAGGACACUAAGAGCUC UGAGACCUCUAAGAGCCUUAUCUCGAUUUGAAGGGAUGAGG
서열 번호 8	인트론 20 프리-mRNA	guaagaaaaaugaaagaaccugaaguauuguauauagccaaaauuaaacuaaauuaaauuuag aaaaaggaaaaucuaugcaugcaaaaggaauggcaaauucuugcaaaauugcuacuuuauugu uuuaucuguugcauauuuacuucuaggugauaugcaagagaaauaggccucucuugaaauga uauaauaucauuuaucugcugugcuuauuuaaaugacuuuauuuccuaauccaucuugggag

[0450]

		uuuccuuacaaaucuauauacaaaaaaaagcugaugcauuauuaaaguacuauguguaaugau auaaugguaaucuaaaguaaaauucuauaucagguacuuauucuuugugaugauauacuguac uuaacgaguuuuccugaaaauaaugugaaucacacaugugccuaaguaugaguguuaagaaa aaaaugaaaggaguuguuaaaacuuuugucuguauaaaugccaaaguuugcauuauuugaaua uauucaagauuagaugguuagauauuaaguguugacugaauuuauaaaacuaguaauacuaa cuuaaagauuacauacaaauccacaucauuuuuauaacaauaaaguaaaacacuuauaaugaac agaaaauauaauuuugacucauuacuauagguaauuuauacauuaaccuuaacuugcaucuua uuggucagagucacacaaaauguuauuuuauccuuuucaaagaugcaauaaucauuuuccau caugcauaacagauuagaaauuuugccauuauugacuuauuuuccaugccuuuuuuuacggc augaagcauuaguuuauagauauauaauauaaaaauuaguucugcuuuuuuuuaaaaaaaa auauuaucaaaacaaaacacugaauugugugauuccaauagaaaaacacugcucuuucaccuc cuaagguguaguuacuuuuauggaaacuaagcuguauuguagacuuccauuugcacuuugua gauuguuuauagccuuauguucucuucucaagucuuauuauaaaugucacuuuguaagaacg uaggacuugucuucgauuucccuaacauauaugaaaacuuugucccucauuaucgacaacucag aacaauauaauacaaguagucccuuuuauuucucacagagagccucaaauuuucaccaaaau guuaacagaaauuaucucugggguguauaagaauuaagucuguuuuccaauuaaaugucacu uuguuuuguuucagacuggcaguuucaguucuggagaaaaaaaaugucauuuguguacauuc uacuugaaaacauguugccugaaucaaaauaauauauuuuauauggcuugugaaaucugaac aaugcuaaacauuugaaaauauuauaaaccuuuuacauuugaccauuugaaaguuuauuaaau ucauuggucaagugcucagauauuuccauacauuacacuucauuucuauaaaaaagcugaucu uaucgguauacuuuuaauuuucucagaaauaaccauaucuauaauuauuaaucaauaaugccu uuuauauuaaaagagguuaguuuuugaaacuuggaguuuuagacauaaaaucccuuauaaaug cugauagugauauaacuaauaguuuaaauggucagauuuaugaauauggcucuauuccucau aaugacaacauacacacagcacuaaaaugacuaaucucuucaauacguguuuggcauuguaga gucaaaauaacguuauaauugauucuauuuuuuauacuucuaguguuuggauauuuuauuu uguaaaaauauaaucaugaaugauggugagguuggauauaagaaugaugauuaugauuggga agugagauuugaacaugcucagaaacucucauuuaauucuuugcccuagcagcauaaaaucac aauagcugcgucaaagcguaacucaggcacucauuuuauuuuuguuguucuguuauuuuuuc aaagcaugugcuuuuaugcaacauuacugaauaaagcauguuguacagugcuugauaagaag uuagaaaguaacaaauaaauuaucaucacguugcacuuuguguuuugcauguuuuaugcaca uuucuggcugacagcuuuuaaacauuuauuguauuucaaauuuccaguccaaauuuuucaac uuguaaaauuaaacugagugaauugaugucgugaauaucuaggguaaaauaaaauuuguguu uaaauuugauuuuuuaauuuccuaaccuaggaaaucuuaaauaccuucuuuuucaaaagaac ucaagucuuaauggauagggaaacagacggagagcaucaugaacaaaaaguaacaccaaaugu ucugucauaucagauuucuaacuaauaacaaacuauauauuucuauuuuguauaggauaauc uugcuccaacuuggaugggguggagcgcugguuccucccccugagcccuuuauuauggguacu guauuacccccuuuugcuaccuuuaaauccuugcacugugacuuauguguaguggggugaggg

[0451]

		agggauugggaaggguacuauuauugcaccacaguagggaaaauacauuauuuacauccuaa ucccucuuuucaauugucuuaaauuucauuugaaaaaaaaaaaaaccuuuaugaauuuaccc ucuguggauuuuaaccccaaugguugauaucuuuauuaaguuucauugaauaugauuuagu uauguguauauggaguuauccaucuuuggggagauuacuggauuggugagggcgggggacc cugguguagaaugauuauugugaaaaaacaauuuaacuuguuaagcucaugauacuguuugag gcauacagccccugcuguuuaguacauuggucuggguccugaaaauuaccaguuagauacca ucaguugauuauugauauguaugagcagauacuagggugcaauauuucagguuucauaagac ugguauugauugugaccacucucauuuuuuauuguguaaguucauaugggguuauuuucaa aauguuaacaaggcaaaaauauauuaagaaauaguugaauaagcacaugugaauugguguu aaacaaaaaguuagaauaaaaaaauccacuuauuugaauuaugcagaauagaauacauaccuag aaauaaaacaaaaacgucuuaucaugaguauuaagauaaaauuuaaggcauaaacucacuucu uagaauaaguaacucccaacuaacuuucuaggauuuuaaaacauaacacagugaaaacauacau aaacauaacucuacauuuuauuuauucuuaaaguuuaaguguauuauacaagaagaagaguu uauauucgagagacagaaaaagucagaauuuuuguuuggaucaccaauauaucauagcuuaca aaaaaacugucuuaauuaaaacccacaacauaauuuuuuuuagauuuuuaagaaagauucuauu auucuucuuuauacuuaaaaauggauguuccuacuuugcccacuuuuauuuuuauucacau agauuuucuuuauuucuauuagagaagcacuagaauucaugaauaguguugauuugaaguuc aaaguaauuaauucagauaaaaagacauuucugcauguaugaaaauuucuaaugugaauuug cauauuuaauuaucaauccuucauuuaguguagacuuauuuuuaaaaaugcagguaaugaac cagaaauagaauugguugugcuagaguagagaaacuuuauuugauguuguuuugaaaaaaa agcuucugagaagaaacaaccucuaguacaguauuaauucauuaagauagcuccuuucucaga cauuuccuuucauguagccugaaaguucaauuugaaauuuguucuuuccaauuuauucagac uaauucugccuacuuucuucccccccauaagaaccaauuacugcagcuuuauugagacugaaaa aaguuaauacaccuccuucuuugcugaaccaaggaauggcuuggaacucuugggaaaagacaa ucuuuucuaugaucuuucauugucuaauuuaauacaucauaaauaugacuauagcuuuguau aauaaacucccccaauacugugccagauguuuucuaagauaaaguuauuuuauguucacaaaaa aaauaaaacuuuucucugggccaaauguaugccaacuuugcaaaucauauccugaagugcacu gcugcagaguacaugcuugcgucauaaauuccauagaguucgcuuuaacucuaaaucaauccc caguuucaaaguaaaccucucaaacauauuaccuaagcacaaacuucucccugugcucaguuc cuuaauuauucucaucccauauucagaaauaacauuuaaaaauuaugcuuugaucaauaaaua cuaaucuaaacuuugcuucauuaacccauucauuuuugucaaccauuauuuuauuccuauau ucaaagcucucugguauguucuuauauucaagacacucaaggcccuggaagauucacgaacau auguuugcaucuuaaauuuuuagaaaaucuuacaaucugucaggauuacacugaacucuagu acagaguaauauggguaccagauaagugggagcaacucuuccacguagacuggaaacagcacu aaaugcuauuuauaggcuacuuucugaacuuaacuuguuuuaaccucauuuuucucauaugc caaaugagaacgcaauacugaauuaucuguacaguucuguucaguacuagaauucugauucu ugaauucaaaggggaaaacauucccucuuuauuuuggaggcuaaacuggggacaaaguuagg

[0452]

		cuccaugaaagaaguguauuugaacuaaagccuuuaagaggggagaguauuucagaagagg agcuauuagacaaggaauuucaauguaaauggcaucucaaucaccuggcaauuauauuagcac acgguuauuauauuaauugaaguggcaugaaguauagaugaccagggaaguuaaaacuggaa auauagauuguggagugaugugaauaccaagguaagaaaaauauuuguuaguuaccagagag ccaauaaauaacuuucaagugggacuuggggaagauuaauucaucuuacauagauuaaaugaa ggagaaggguuaggagacagaugacagugcaaguaugaaauaacagagggcaguucuaggugg ugacugugagaauggaaaagaggugcaaagcugagaaacguuucaaagaaaaaaugugagac agguaaugugaaaagaaaaucgagaaauagguauagauaaucaguguucugcucauacucuaa auugggguguugaaggcaaaauacguauuuuaauuaguacucuguguauacacacuagaaaca gcauuguaaucuggauaguggacaaaauauucagaaaagaagggaaauaguaacuugauuuc aauuuccaaaucucuaaucugaaagaaaucuaaauucuauucauccauuuaaaauaaauuauau aacgagaauuuaugaaguccauuguauuaaugcagacagucagaugagauaaggcaaagugu cacgugucagcuugguaguugcaucggccacaucauuuggguucugccuggauaacucaacca aauuaauuuuucauacucaucccccuccaccuuugucauuacugguauucuuauuuucuuugg cccacuuaucacacuguuuuauguucccagaaggccuagaguucuuuacaggcuuuaaacag ggaucagaaguauaagaaauuggcucauguauuuuuuuuucagacaggcaguuaaaaaaaau uguucuaaaaauacacuggcaucaaauggcaaauagaagauguuuugacgacuacuuccauug gaucagacugacaagaauaauacaagcacauagguggaauuaaacuuagcuauuaauguccaa guuugaggcagcugccccuuauaagcauuuuagggucuguuuuuagcuucccucuuagccac uccugugcagcuccagugggagguauggaggaaaaagcaaggaagccaucccuauguuguuu ccaaacaugaacacucaagauuuuuaacuagugguccagaaguaaagagggggaaaacauccu ucuauagaaaaaaaaaaaaguagauaauaauuugaacacagaacuucauguugaucacaucagau uugagaacuauguauggcaucccucuuuuucuuauuuuccuaagaaaugauuucuauuaugu uucauuugaaauaaguuuuuugaauuaaacucaguaaaugaaacaacugacaugacuggagc uugaaauaaacgaugugaugaucuaaugaaauacauaaugcaaauugucuugcuucuuaugc aaaaauuauuagucauagcaaugcaugaauaauuaaagacaauuauauuagguauuuaauaau auuuuuuauauuuaucaucugaauuuuuaaguuauuuuaaaaauauauuggucaaaucaacu cagguccaaauguuuuaguuuuguucuuuaauauauugccuuuuuaaaaugaguuaaacuuc uguauaggcuuuuuaacuuuucuuuauucugauaacacaauucugacuucaucuggcagcaa guuccucugauuuuccuuuuccuuuaaccuuuuaaugcuucucccucccuuuuuuuuaaaaa cauuuuuguuucauuucuuggguuauauugccuauaguuguuuuccuaaguguauugcuuaa gaaaaaaaaaugaauuuuaagauuuuuuugaaccuugcuuuuacauauccuagaauaaauagc auugauagaaaaaaagaauggaaagaccagagauuacuaggggaauuuuuuuucuuuauuaa cagauaagaauucugacuuuucuuuuuuuccauuuguguauuag
서열 번호 9	엑손 21 프리-mRNA	gauaaucuugcuccaacuuggaugggguggagcgcugguuccucccccugagcccuuuauuau gg
서열 번호	엑손 20x 프리-	GUGGUUGUGAAUGCCCUUUUAGGAGCAAUUCCAUCCAUCAU

[0453]

10	mRNA	GAAUGUGCUUCUGGUUUGUCUUAUAUUCUGGCUAAUUUUCA GCAUCAUGGGCGUAAAUUUGUUUGCUGGCAAAUUCUACCAC UGUAUUAACACCACAACUGGUGACAGGUUUGACAUCGAAGA CGUGAAUAAUCAUACUGAUUGCCUAAAACUAAUAGAAAGAA AUGAGACUGCUCGAUGGAAAAAUGUGAAAGUAAACUUUGAU AAUGUAGGAUUUGGGUAUCUCUCUUUGCUUCAAGUU
서열 번호 17	엑손 21 프리- mRNA	GUUUCAUUGGUCAGUUUAACAGCAAAUGCCUUGGGUUACUC UGAACUCGGGGCCAUCAAAUCCCUAAGGACACUAAGAGCUCU GAGACCCCUAAGAGCCUUAUCACGAUUUGAAGGGAUGAGG
서열 번호 18	인트론 21 프리- mRNA	guaagaaaaaggaaaacucugcagcguuguauauuugucaaagcuaggcugaguucaacuuaac uaacgaaaaacacgugcaugcaaaaggaauggcaacccuuugcaaacuugcuacuuuacccuu uucucuguugcauauuuacuucuuggugauaugcaagagaaaaucggccucuuugaaaauga uuuaauaucauuuaucugcuuugcuaauuaaaaugaccuuaguucauaaucgaucuugggag uuuccuuauaauucuaauacaaaggggagggcagauacucucuuaaagaacuaaguuga gucauguauauaauuaccuagagauaauuuuguuucauaucguucuccucuaugacagcccau caguacuuaagggauccuauggaaaguaaugugaaucacaaauguguaugaauacaaaggaaa aaaugaagaauuguuaaaauguuuugucuuuacaaugccaaaauucucauuauuugaauauau ccaagggcagauauuaaccauugacuggaguauaauaauacugccucaacuguaacuaaauua augacauugaauaaguaagacacuaauuuaauuacuauaaauacauacacaucuuaugacaau acagcugauaaggaaaaagaacauguauuuuuauucauugccauacauggcucgucaaccuua acuuaaaccucgguucucaguuacacagaguuuuauguugcucuuuugagcaaagcauuauu ccccucuccauaauucaacacguaucagauuuuugcauuuauuggcucauugguuaugaugau uauuucaaagcauuauacaaucauuuauagaagaugugccgugugaaaauuauuuuuuuauu aagauccaaaauuuacgcucuuuaaaccaaucagaugaaauguauaaggcaaagagugcucau uguccugacacuuacaaaccaaggcuccaacaaacaggcuccucucugcaccacauagagggcu uucagccugugucccccauaaaaaccuauuauaaauuuuauuauacuauacuguaagaaacc uguccaauuuuuauauucucuagcacauaugaaaacuucucuucaguagauccaaguaagcac aaaggagcuuugauucucacacaagaaaucacauuuguauuaaaaaauguaucauaaauuuucu cccaauuaugcaaaacuuaaaugcuuuuccaauuaaaagagcacuuucguuucagaauagcaa uuucaguugucaaggaaaacauuguuuuuauacauuuuauauaaaaauacaugagcuaaauu uaaauucacauuuuucaacuuuuuauggguuuuuuuauguuucuuuuucuuucgcauuuuuu aacaauccagccauuaccucuccucccuguccccuccuauaguuucucaucucauuccuccu ccccuugccucugagaggaugcucccucccucacuaggccucccucuucccagggcuucaa guuccucaaggauuauacacaucuucucccacugagaccaggccaggcaguccucugcuucug cccagccuguguauguuccugcuugguagcucagucucuggaagcucccuggggucuggguu uaguugggacugcuggucuuccuauggaguuacccucccccuucaacuucuucaauccuuccc cuaauucaaccacagguauccagacuucaguccaaugguugaguguaaauauuggcaucugu

[0454]

		cucugucagcuguuguuaggggcucagaggacagccaugcuaggcuccugccuacaagcagc acaccauaacaucaguaauaguucaggccuuuaaugaauaaugcuacguauauaagguugu uagauuauacuucaacuuugaucuuuuagaauauuauuaaauccagucguuuauuuuuuaua uguaauauuugacuuuccauaacaaaugagucuauuuuccuuuuguguagaaauaacuuuauc aauuauuguuaauaaugcuuuugucaauuauuguuugauaugcuucucuuuuuuaaaacugg agucaucaaaacaaaaauucuggguagauauuacgaagcugacuccuuggucagcuuggcaca uagugagaccacaaauaucucaaggucacggcaauuccucaccaccaguuuggcauugugaag ucaaaaccaaccuucuguugauucuggguuuuuguauuucuaguaugagacauuuucuacuuu guaagaguauauaacuguggauggcggcgagguugggcaugaugaugauuguaagcgggaa gugagcuagaguaucuucagaaacucucacuuuauccuccuuggcagcauagaaccgcaaucg cuguguccgagugucaaccaggcagucauuuuguuuugggguuuuuugucacucuuucaaag caugugcuucuacgcaacacuaccaaacacagcaugcugcauagugcuugagaaggaguuaga aaguaacaaacgaguuaucaucacguugcccuuuguguuuugcaugucuuaugcacacuuuu ggcugacagcuuuugaacauuuauuguauuucaaauuuccaguccaaauuuuuuuuucaacu ugugaaauugaacggaaugaaccgaugucgugaauaccuagggucaaauaaaacuuguauuu aaauucguaguuuuaauuucccaagcugggaaaaucguaaaaaccuuuuccaaagaacucaag ucuuaguugcuagggaaacaggcagugagcaucauauacaaaaaguaacaccaaauguucugu cauaucagcuuucuaacuaauaauaaacuauauauuucuauuuuauauaggauaaucuugcu ccaacuuggaugggguggagcgguggguuccucccccucagcccuuuauuauggguacuguauu accccuuuugcuaccuuuaauccuugcacugugacuuauguguagugggauugagggagggga gugggaaggguacaauugcaccacaguagggacaauacaggauuuauuuccaaauccacuacu uuuaaugagcuuaaaacuucuuuugggaaaaaaaaaguuaucucugacuuaccaucuguggau uauaaccccagaaguacauaucuuuauuacguuucacugaauaugauuuagcuauuuauacu ucauuguccauuuauggggaaauuacucaauuggugagggugggggacccugguguaggau gcugaugaaaacguuuucauuugucaagcucaugguagugacagagcauauaguccuuauuu uuucaacacacugcucuggucccucaaugggccagucacauuccaucaguugaucguugaug ugugcgagcaguggcuaaagguacaacaggccagguaucucaggguugccaauggguuaugau cauucucaucuuuauugcauaaaaaugugguuauuugcagaaaguagcaaggcaagaucccug ugaaacaagggaauacaaaaaaaaaaaaagaugugcuuuaaguuauaaaaccaaaacaugugaa aagucaacuucauugaaguauaaagaauaggauaugcaugaaaaacaaaaaaaucaugagcac uaagaaauuggguguauaagccaacuccuuguaagcuaccccaauuaacuucccagaaucuuag aaggcaucacagugcaccccaaaauaaaaagccaaacugacacuucugcuuccucuuaaaaugu aggagucuuggauaagaaagauaauuuuuauuguuuggaagaaaaaaaauuguuuggauaa uugaggcauuuaucuaucaaaaauauuuaucuuaauaaaauuucacaacacugauuuaguug uuggcuuuucuaaaaauuuuuuauauuucauauuaagaacucaugauuuuuacuuuccauuu uuuaaauucuuauucacauaugguuuuucucuauuucuuagaaaagcuauagaacccauggu uuccggugacuuaaaaaacuaaucuaaaugucuucacuuagaugauacuuucaaaugcacuga

[0455]

		aauuucuaauaucaacaagaauauuugccugguccuaauuuuucacugauuuaauaaaaaguaugaacccuaaagaagaaauagacuugaagaacugguugugugacaauacagaaauucugcugggagauguccuuuuaaaacauuguuagaagagacaaccucuacaauccacccauuaagcauacuucucucuuagacaucuccuuuuauguaccuuauaaccucaauguguucuuuccaauugacuuagaccaacacuucccagcgcaucccacaugggagccaauuacugacucucccuagagacugcaaagaauuaauauuguagaaccaagggauggguugggcucuugggagaggcaauccguuugugaucuuuugcucuggauguuaaugaaaucgcaacuuuaaguggauuuucaguggcaaauccucugauccuaugccaaauguucucuaagacaaacauccuuguaaaauaaaugucucacugggccaaaucuauggcaaauuugcacguuuuccugaacugcauuccuauauaguauuugccauccugaauucacuaauggggcauuacuuuuaauucaaaaccaguccccuucuucaaaggaaaucucucccauuuauuacauuaugcaaacugcuuucuuaugcaguggguuaaauccuuagccaggcaaguaugaggacuggaauuuggauauccagaacccucagaaaugucggauggcauaguagcuuacauguaauuccagagcuagaaagaugaaacuagcccuguccucaagcucugaauucaguuggugcaauaaauaagaaagaauccccccccccgaccccuacaucucuuuccucuacaucuauacauguauuuccccauacagcucuaugcuuccacauauaugcucacauaugugcaugcacacuugcacacauauguacacuugacacauugaagcaucauaaucuaacaauuggaauauaagaaaauauuuaacuuucacacagagcagucagagaaaaccauaagaggguugaaacucuugaaaaaacuugcaggauaaacagaaaagaguaugagaugccaauucuggucuacuuucugaaccuaacuuguuuuaccuucauuagucuaaauuuuccaaaugaaccccaagcaccaaauuguccuuauugcucuuuccaguacuaaauuauaauccucaauucgaaggcaaacacucucaucuuuguuaaggauguguagaguuagacucaauaaacgacauguauauuugagcuaaagccuggaggagggagauuauuucaagggcaaguacuuggcagggaguuucaaagaaagaaggcucucaauucucagacuaacaccuuagcgugagugacugucacagaggagggugaaguguaugucaccaguuagggaagcugaaaggggaaauguaucaggcuugugagaauccuucagcagccaagucuagcaccugagcacaaucccccagaacugaccccacauggugaaaggagaggaauaauuccugcaaauuuuccugugaccuccacccaaggcuauagaaaaugcaugcaugcccacaugcacacacaaauaaacauaguugcaaacuguuuugaggaaaauaaccucacaaacugucgagugauguaaaaugccaaagaaagagaaauguuuucuaauggcuagagaaccauuaaggaauuuuucaaaauggggacaugggauagauaaauuuaguauccauacugaaagaaggcaagcaaauaaaaucugauaagaauguaauucuuaguaaccgaggacagagcgaagauagagaacagggccaauggccaaggugguaaaggauuugaaggaagcagcaugaaaccuacagacuuguaccaaaauguucagugucaugaguguuaaaagugaaaagcuugcauguuaguauggauuucauauccucggcagacagagcaccucacugugaguggggagaugaaguauucagaaaugagaaacaacuacucaauuucaguguucauaucucaaauccaauaaacaacuuuaggguacaauuuuuuuaaaaaauuacauuaaaaauguuuuuaaaucucuucuuaauaauuuaaaaauuaaauugaaaauaacuuuaaaaaguaauauauacaggaaagccugugugcuaauuuuuuagggaggccauaaagggagauaguugcucauuaauuucuacacaucagccuaucuuuggcuucugccuugauagcgcacucugaauuaucuucuucauguucaucccucaucuuuauuguuacuggu

[0456]

		uucauuucccuuggccacauagcccacuauuuuguauuccccaauggauauuguuccuuaca aaguucagccagggcucagaaguacaaggaauuuggcucuuauacuucugucagacaggcaaaa acuucuaaaauuauacuauaauaaaaaucaaagagaugauauucauaauuaaacuaacaaaagu ggcaggccccccuccccccaacaugaguagaauuaaucugacguccauguucaagucugaaac acacuugccaauuaagagcacauuagggccagccuuuaucucccucuuaguuacuaaugugca guucaauggugagcuauagagaaggaagccaagacuaccauaugucaaauauaaaaaaaaaaa aucccauuuuaaaucuguagucccgaauuaaggacaagagagagggaaauaucuuugacauua gaaaauggagaaaauauuuuagcacaggacuuuacucagucacaucagaguugauaaguacgu augacaucccucuuuuuccuguuuuccugagaaaaugaucucucuaguguuucauuuaagau aaguuuauugaauuaaacucaguaaaugaaacaacugacaugacuggagcuugaaauaaacga ugugaugaucuacugaaauacaugaugcuaaauugucuugcuucuuaugcaaaaacuacuau uaguuauagcaaugcauggauaauuaaggccaaaaauauauuagauguuaaaaauaguuuua uauuuauacaucugaauuuuaauuuauauuuaaaguauauugguccaaucaauucaugccca aauguuuuaguucuauucuuugagauacuguuuuguuuugggauuuuuuuuuuaugagcuaa ucucuugccuaggaguuccuacuucucucuccccuccuuuuauuuuuucuaauaaacuacacau gugcuucauccaggagcuaacuucucccauuuugcuuuuccuuuagcaccuuuuuuauauu agauuucuuucuuuucuccaucucuuugcauauugccuauauuucuuuuccuaagcauaaua uuuaaaaaagacugaguuuuauguuaagauuauuucugcuuugcucuuacacagauaggaua aguagucuugauagaaaauaaaucaaugauuccuagggggaugucuuuuugcuuuuaaucaa uaaggauucugacuucucuuucucuccauuuguguauuag
서열 번호 19	엑손 22 프리-mRNA	gauaaucuugcuccaacuuggaugggguggagcggugguuccuccccucagcccuuuauuau gg
서열 번호 20	엑손 21x 프리-mRNA	GUGGUUGUGAAUGCCCUGUUAGGAGCAAUUCCAUCCAUCAU GAAUGUGCUUCUGGUUUGCCUUAUAUUCUGGCUAAUUUUCA GCAUCAUGGGCGUAAAUUUGUUUGCUGGCAAAUUCUACCAC UGUGUUAACACCACAACUGGUGACAUAUUUGAGAUCAGCGA AGUCAAUAAUCAUUCUGAUUGCCUAAAACUAAUAGAAAGAA AUGAGACCGCCCGGUGGAAAAAUGUGAAAGUAAACUUUGAU AAUGUAGGAUUUGGGUAUCUUUCUUUGCUUCAAGUU

[0457]

[0458] **실시예 2: 시클로헥시미드 처리를 통한 NIE의 확증**

[0459] DMSO 처리(CHX-) 또는 시클로헥시미드 처리(CHX+) 마우스 Neuro 2A 세포(도 3a) 및 RenCell VM(인간 신경 전구 세포)(도 3b)의 세포질 RNA 및 엑손 20 및 엑손 23 내 프라이머를 사용한 RT-PCR 분석은 NMD 유도 엑손(20x)에 상응하는 밴드의 존재를 확증하였다. 생성물의 동일성은 서열분석에 의해 확증하였다. 밴드의 밀도 측정 분석을 수행하여 총 *SCN1A* 전사체의 퍼센트 엑손 20x 포함을 계산하였다. NMD를 억제하는 시클로헥시미드(CHX+)로 RenCell VM의 처리는 세포질 분획에서 NMD 유도 엑손 20x에 상응하는 생성물의 2배 증가를 초래하였다(연회색 막대, CHX-와 진회색 막대, CHX+ 비교).

[0460] **실시예 3: SCN1A 엑손 20x 영역 ASO 워크**

[0461] 2'-MOE ASO, PS 백본을 사용하여 3' 스플라이스 부위 바로 상류 서열, 3' 스플라이스 부위에 걸친 서열, 엑손 20x 서열, 5' 스플라이스 부위에 걸친 서열, 및 5' 스플라이스 부위의 하류 서열을 표적화하는 *SCN1A* 엑손 20x 영역에 수행된 ASO 워크를 도식으로 표현하여 [도 4]에 나타낸다. ASO는 한 번에 5 뉴클레오티드를 이동시켜 이들 영역을 덮도록 설계하였다. *SCN1A*를 표적화하는 ASO의 목록은 표 4에 요약한다. ASO의 서열은 표 5a 및 표 5b 및 표 6a 및 표 6b에 요약한다.

[0462] [표 4]

SCN1A 를 표적화하는 ASO 의 목록

유전자 서열 번호	프리-mRNA 서열 번호	ASO 서열 번호	NIE
서열 번호 1	서열 번호 2	서열 번호 21-67, 210-256	엑손 20x
서열 번호 11	서열 번호 12	서열 번호 68-114, 257-303	엑손 21x

[0463]

[0464] [표 5a]

인간 *SCN1A* 를 표적화하는 ASO 의 서열

서열 번호	서열 명칭	ASO 서열
21	SCN1A-IVS19X-81	GATGCTCTCCGTCTGTTT
22	SCN1A-IVS19X-76	TTCATGATGCTCTCCGTC
23	SCN1A-IVS19X-71	TTTTGTTCATGATGCTCT
24	SCN1A-IVS19X-66	TTACTTTTTGTTCATGAT
25	SCN1A-IVS19X-61	TGGTGTTACTTTTTGTTC
26	SCN1A-IVS19X-56	ACATTTGGTGTTACTTTT
27	SCN1A-IVS19X-51	ACAGAACATTTGGTGTTA
28	SCN1A-IVS19X-46	ATATGACAGAACATTTGG
29	SCN1A-IVS19X-41	ATCTGATATGACAGAACA
30	SCN1A-IVS19X-36	TAGAAATCTGATATGACA
31	SCN1A-IVS19X-31	TTAGTTAGAAATCTGATA
32	SCN1A-IVS19X-26	TGTTATTAGTTAGAAATC
33	SCN1A-IVS19X-21	TAGTTTGTTATTAGTTAG
34	SCN1A-IVS19X-16	ATATATAGTTTGTTATTA
35	SCN1A-IVS19X-11	TAGAAATATATAGTTTGT
36	SCN1A-IVS19X-6	CAAAATAGAAATATATAG
37	SCN1A-IVS19X-3	ATACAAAATAGAAATATA
38	SCN1A-IVS19X-1	CTATACAAAATAGAAATA
39	SCN1A-I19X/E20X+2	TCCTATACAAAATAGAAA

[0465]

서열 번호	서열 명칭	ASO 서열
40	SCN1A-I19X/E20X+4	TATCCTATACAAAATAGA
41	SCN1A-I19X/E20X+6	ATTATCCTATACAAAATA
42	SCN1A-Ex20X+1	AGTTGGAGCAAGATTATC
43	SCN1A-Ex20X+6	ATCCAAGTTGGAGCAAGA
44	SCN1A-Ex20X+11	ACCCCATCCAAGTTGGAG
45	SCN1A-Ex20X+16	GCTCCACCCCATCCAAGT
46	SCN1A-Ex20X+21	CCAGCGCTCCACCCCATC
47	SCN1A-Ex20X-24	GAACCAGCGCTCCACCCC
48	SCN1A-Ex20X-19	GGGAGGAACCAGCGCTCC
49	SCN1A-Ex20X-3	ATAATAAAGGGCTCAGGG
50	SCN1A-Ex20X-1	CCATAATAAAGGGCTCAG
51	SCN1A-E20X/I20X-6	GTAATACAGTACCCATAA
52	SCN1A-E20X/I20X-4	GGGTAATACAGTACCCAT
53	SCN1A-IVS20X+13	TTAAAGGTAGCAAAAGGG
54	SCN1A-IVS20X+18	AAGGATTAAAGGTAGCAA
55	SCN1A-IVS20X+23	AGTGCAAGGATTAAAGGT
56	SCN1A-IVS20X+28	GTCACAGTGCAAGGATTA
57	SCN1A-IVS20X+33	CATAAGTCACAGTGCAAG
58	SCN1A-IVS20X+38	CTACACATAAGTCACAGT
59	SCN1A-IVS20X+43	CCCCACTACACATAAGTC
60	SCN1A-IVS20X+48	CCTCACCCCACTACACAT
61	SCN1A-IVS20X+53	CCCTCCCTCACCCCACTA
62	SCN1A-IVS20X+58	CCAATCCCTCCCTCACCC
63	SCN1A-IVS20X+63	CCTTCCCAATCCCTCCCT
64	SCN1A-IVS20X+68	AGTACCCTTCCCAATCCC
65	SCN1A-IVS20X+73	ATAATAGTACCCTTCCCA
66	SCN1A-IVS20X+78	GTGCAATAATAGTACCCT
67	SCN1A-IVS20X+83	CTGTGGTGCAATAATAGT

[0466]

[0467] [표 5b]

인간 *SCN1A* 를 표적화하는 ASO 의 서열

서열 번호	서열 명칭	ASO 서열
210	SCN1A-IVS19X-81	GAUGCUCUCCGUCUGUUU
211	SCN1A-IVS19X-76	UUCAUGAUGCUCUCCGUC
212	SCN1A-IVS19X-71	UUUUGUUCAUGAUGCUCU
213	SCN1A-IVS19X-66	UUACUUUUUGUUCAUGAU
214	SCN1A-IVS19X-61	UGGUGUUACUUUUUGUUC
215	SCN1A-IVS19X-56	ACAUUUGGUGUUACUUUU
216	SCN1A-IVS19X-51	ACAGAACAUUUGGUGUUA
217	SCN1A-IVS19X-46	AUAUGACAGAACAUUUGG
218	SCN1A-IVS19X-41	AUCUGAUAUGACAGAACA

[0468]

서열 번호	서열 명칭	ASO 서열
219	SCN1A-IVS19X-36	UAGAAAUCUGAUAUGACA
220	SCN1A-IVS19X-31	UUAGUUAGAAAUCUGAUA
221	SCN1A-IVS19X-26	UGUUAUUAGUUAGAAAUC
222	SCN1A-IVS19X-21	UAGUUUGUUAUUAGUUAG
223	SCN1A-IVS19X-16	AUAUAUAGUUUGUUAUUA
224	SCN1A-IVS19X-11	UAGAAAUAUAUAGUUUGU
225	SCN1A-IVS19X-6	CAAAAUAGAAAUAUAUAG
226	SCN1A-IVS19X-3	AUACAAAAUAGAAAUAUA
227	SCN1A-IVS19X-1	CUAUACAAAAUAGAAAUA
228	SCN1A-I19X/E20X+2	UCCUAUACAAAAUAGAAA
229	SCN1A-I19X/E20X+4	UAUCCUAUACAAAAUAGA
230	SCN1A-I19X/E20X+6	AUUAUCCUAUACAAAAUA
231	SCN1A-Ex20X+1	AGUUGGAGCAAGAUUAUC
232	SCN1A-Ex20X+6	AUCCAAGUUGGAGCAAGA
233	SCN1A-Ex20X+11	ACCCCAUCCAAGUUGGAG
234	SCN1A-Ex20X+16	GCUCCACCCCAUCCAAGU
235	SCN1A-Ex20X+21	CCAGCGCUCCACCCCAUC
236	SCN1A-Ex20X-24	GAACCAGCGCUCCACCCC
237	SCN1A-Ex20X-19	GGGAGGAACCAGCGCUCC
238	SCN1A-Ex20X-3	AUAAUAAAGGGCUCAGGG
239	SCN1A-Ex20X-1	CCAUAAUAAAGGGCUCAG
240	SCN1A-E20X/I20X-6	GUAAUACAGUACCCAUAA
241	SCN1A-E20X/I20X-4	GGGUAAUACAGUACCCAU
242	SCN1A-IVS20X+13	UUAAAGGUAGCAAAAGGG
243	SCN1A-IVS20X+18	AAGGAUUAAAGGUAGCAA
244	SCN1A-IVS20X+23	AGUGCAAGGAUUAAAGGU
245	SCN1A-IVS20X+28	GUCACAGUGCAAGGAUUA
246	SCN1A-IVS20X+33	CAUAAGUCACAGUGCAAG
247	SCN1A-IVS20X+38	CUACACAUAAGUCACAGU
248	SCN1A-IVS20X+43	CCCCACUACACAUAAGUC
249	SCN1A-IVS20X+48	CCUCACCCCACUACACAU
250	SCN1A-IVS20X+53	CCCUCCCUCACCCCACUA
251	SCN1A-IVS20X+58	CCAAUCCCUCCCUCACCC
252	SCN1A-IVS20X+63	CCUUCCCAAUCCCUCCCU
253	SCN1A-IVS20X+68	AGUACCCUUCCCAAUCCC

[0469]

서열 번호	서열 명칭	ASO 서열
254	SCN1A-IVS20X+73	AUAAUAGUACCCUUCCCA
255	SCN1A-IVS20X+78	GUGCAAUAAUAGUACCCU
256	SCN1A-IVS20X+83	CUGUGGUGCAAUAAUAGU

[0470]

[0471] [표 6a]

마우스 *SCN1A* 를 표적화하는 ASO 의 서열

서열 번호	서열 명칭	ASO 서열
68	mScn1a-IVS20X-81	GATGCTCACTGCCTGTTT
69	mScn1a-IVS20X-76	TATATGATGCTCACTGCC
70	mScn1a-IVS20X-71	TTTTGTATATGATGCTCA
71	mScn1a-IVS20X-66	TTACTTTTTGTATATGAT
72	mScn1a-IVS20X-61	TGGTGTTACTTTTTGTAT
73	mScn1a-IVS20X-56	ACATTTGGTGTTACTTTT
74	mScn1a-IVS20X-51	ACAGAACATTTGGTGTTA
75	mScn1a-IVS20X-46	ATATGACAGAACATTTGG
76	mScn1a-IVS20X-41	AGCTGATATGACAGAACA
77	mScn1a-IVS20X-36	TAGAAAGCTGATATGACA
78	mScn1a-IVS20X-31	TTAGTTAGAAAGCTGATA
79	mScn1a-IVS20X-26	TATTATTAGTTAGAAAGC
80	mScn1a-IVS20X-21	TAGTTTATTATTAGTTAG
81	mScn1a-IVS20X-16	ATATATAGTTTATTATTA
82	mScn1a-IVS20X-11	TAGAAATATATAGTTTAT
83	mScn1a-IVS20X-6	TAAAATAGAAATATATAG
84	mScn1a-IVS20X-3	ATATAAAATAGAAATATA
85	mScn1a-IVS20X-1	CTATATAAAATAGAAATA
86	mScn1a-I20X/E21X+2	TCCTATATAAAATAGAAA
87	mScn1a-I20X/E21X+4	TATCCTATATAAAATAGA
88	mScn1a-I20X/E21X+6	ATTATCCTATATAAAATA
89	mScn1a-Ex21X+1	AGTTGGAGCAAGATTATC
90	mScn1a-Ex21X+6	ATCCAAGTTGGAGCAAGA
91	mScn1a-Ex21X+11	ACCCCATCCAAGTTGGAG
92	mScn1a-Ex21X+16	GCTCCACCCCATCCAAGT
93	mScn1a-Ex21X+21	CCACCGCTCCACCCCATC
94	mScn1a-Ex21X-24	GAACCACCGCTCCACCCC
95	mScn1a-Ex21X-19	GGGAGGAACCACCGCTCC
96	mScn1a-Ex21X-3	ATAATAAAGGGCTGAGGG
97	mScn1a-Ex21X-1	CCATAATAAAGGGCTGAG
98	mScn1a-E21X/I21X-6	GTAATACAGTACCCATAA
99	mScn1a-E21X/I21X-4	GGGTAATACAGTACCCAT
100	mScn1a-IVS21X+13	TTAAAGGTAGCAAAAGGG
101	mScn1a-IVS21X+18	AAGGATTAAAGGTAGCAA
102	mScn1a-IVS21X+23	AGTGCAAGGATTAAAGGT
103	mScn1a-IVS21X+28	GTCACAGTGCAAGGATTA

[0472]

서열 번호	서열 명칭	ASO 서열
104	mScn1a-IVS21X+33	CATAAGTCACAGTGCAAG
105	mScn1a-IVS21X+38	CTACACATAAGTCACAGT
106	mScn1a-IVS21X+43	TCCCACTACACATAAGTC
107	mScn1a-IVS21X+48	CTCAATCCCACTACACAT
108	mScn1a-IVS21X+53	CCTCCCTCAATCCCACTA
109	mScn1a-IVS21X+58	CACTCCCTCCCTCAATCC
110	mScn1a-IVS21X+63	CTTCCCACTCCCTCCCTC
111	mScn1a-IVS21X+68	GTACCCTTCCCACTCCCT
112	mScn1a-IVS21X+73	CAATTGTACCCTTCCCAC
113	mScn1a-IVS21X+78	TGGTGCAATTGTACCCTT
114	mScn1a-IVS21X+83	TACTGTGGTGCAATTGTA

[0473]

[0474] [표 6b]

마우스 *SCN1A* 를 표적화하는 ASO 의 서열

서열 번호	서열 명칭	ASO 서열
257	mScn1a-IVS20X-81	GAUGCUCACUGCCUGUUU
258	mScn1a-IVS20X-76	UAUAUGAUGCUCACUGCC
259	mScn1a-IVS20X-71	UUUUGUAUAUGAUGCUCA
260	mScn1a-IVS20X-66	UUACUUUUUGUAUAUGAU
261	mScn1a-IVS20X-61	UGGUGUUACUUUUUGUAU
262	mScn1a-IVS20X-56	ACAUUUGGUGUUACUUUU
263	mScn1a-IVS20X-51	ACAGAACAUUUGGUGUUA
264	mScn1a-IVS20X-46	AUAUGACAGAACAUUUGG
265	mScn1a-IVS20X-41	AGCUGAUAUGACAGAACA
266	mScn1a-IVS20X-36	UAGAAAGCUGAUAUGACA
267	mScn1a-IVS20X-31	UUAGUUAGAAAGCUGAUA
268	mScn1a-IVS20X-26	UAUUAUUAGUUAGAAAGC
269	mScn1a-IVS20X-21	UAGUUUAUUAUUAGUUAG
270	mScn1a-IVS20X-16	AUAUAUAGUUUAUUAUUA
271	mScn1a-IVS20X-11	UAGAAAUAUAUAGUUUAU
272	mScn1a-IVS20X-6	UAAAAUAGAAAUAUAUAG
273	mScn1a-IVS20X-3	AUAUAAAAUAGAAAUAUA
274	mScn1a-IVS20X-1	CUAUAUAAAAUAGAAAUA
275	mScn1a-I20X/E21X+2	UCCUAUAUAAAAUAGAAA
276	mScn1a-I20X/E21X+4	UAUCCUAUAUAAAAUAGA
277	mScn1a-I20X/E21X+6	AUUAUCCUAUAUAAAAUA
278	mScn1a-Ex21X+1	AGUUGGAGCAAGAUUAUC
279	mScn1a-Ex21X+6	AUCCAAGUUGGAGCAAGA
280	mScn1a-Ex21X+11	ACCCCAUCCAAGUUGGAG

[0475]

서열 번호	서열 명칭	ASO 서열
281	mScn1a-Ex21X+16	GCUCCACCCCAUCCAAGU
282	mScn1a-Ex21X+21	CCACCGCUCCACCCCAUC
283	mScn1a-Ex21X-24	GAACCACCGCUCCACCCC
284	mScn1a-Ex21X-19	GGGAGGAACCACCGCUCC
285	mScn1a-Ex21X-3	AUAAUAAAGGGCUGAGGG
286	mScn1a-Ex21X-1	CCAUAAUAAAGGGCUGAG
287	mScn1a-E21X/I21X-6	GUAAUACAGUACCCAUAA
288	mScn1a-E21X/I21X-4	GGGUAAUACAGUACCCAU
289	mScn1a-IVS21X+13	UUAAAGGUAGCAAAAGGG
290	mScn1a-IVS21X+18	AAGGAUUAAAGGUAGCAA
291	mScn1a-IVS21X+23	AGUGCAAGGAUUAAAGGU
292	mScn1a-IVS21X+28	GUCACAGUGCAAGGAUUA
293	mScn1a-IVS21X+33	CAUAAGUCACAGUGCAAG
294	mScn1a-IVS21X+38	CUACACAUAAGUCACAGU
295	mScn1a-IVS21X+43	UCCCACUACACAUAAGUC
296	mScn1a-IVS21X+48	CUCAAUCCCACUACACAU
297	mScn1a-IVS21X+53	CCUCCCUCAAUCCCACUA
298	mScn1a-IVS21X+58	CACUCCCUCCCUCAAUCC
299	mScn1a-IVS21X+63	CUUCCCACUCCCUCCCUC
300	mScn1a-IVS21X+68	GUACCCUUCCCACUCCCU
301	mScn1a-IVS21X+73	CAAUUGUACCCUUCCCAC
302	mScn1a-IVS21X+78	UGGUGCAAUUGUACCCUU
303	mScn1a-IVS21X+83	UACUGUGGUGCAAUUGUA

[0476]

[0477] **실시예 4: RT-PCR에 의해 평가된 SCN1A 엑손 20x 영역 ASO 워크**

[0478] ASO 워크 서열은 예를 들어 RT-PCR에 의해 평가할 수 있다. [도 5a]에서, 대표적인 PAGE는 짐노틱 흡수에 의해 RenCell VM 세포에서 모의 처리된(Sham), SMN-대조군 ASO 처리된(SMN), 또는 실시예 및 [도 4]의 설명에서 본원에 기재된 바와 같이 엑손 20x 영역을 표적화하는 20 μM 농도의 2'-MOE ASO로 처리된 *SCN1A*의 SYBR-세이프 염색된 RT-PCR 생성물을 보여준다. 엑손 20x 포함(상부 밴드) 및 전장(엑손 20x 배제, 하부 밴드)에 해당하는 2개의 생성물을 정량화하였고 퍼센트 엑손 20x 포함을 막대그래프로 플로팅한다(도 5b). 검은 선은 Sham에 대하여 변화없음을 나타낸다. 또한, 전장 생성물을 *RPL32* 내부 대조군에 정규화하였고 Sham에 대한 배수 변화를 막대그래프로 플로팅한다(도 5c). 검은 선은 Sham에 대한 1의 비율 및 변화없음을 나타낸다.

[0479] **실시예 5: RT-qPCR에 의해 평가된 *SCN1A* 엑손 20x 영역 ASO 워크.**

[0480] [도 6]에 도시된 바와 같이 SYBR-세이프 RT-PCR에 의해 평가된 동일한 ASO 흡수 실험을 사용하여 얻은, RPL32로 정규화된 SYBR-그린 RT-qPCR *SCN1A* 증폭 결과는 Sham에 대한 배수 변화로서 플로팅하며, 이는 SYBR-세이프 RT-PCR 결과를 확인해준다. 검은 선은 1의 비율(Sham에 대하여 변화없음)을 나타낸다.

[0481] **실시예 6: CXH 처리된 세포에서 선택된 ASO의 용량 의존적 효과.**

[0482] [도 8a]에서, 대표적인 PAGE는 RNAiMAX 형질 감염에 의해 Neuro 2A(마우스 신경 모세포종) 세포에서 모의 처리된(Sham, RNAiMAX 단독), 또는 엑손 21x(마우스 명명법, 인간 엑손 20x에 해당)를 표적화하는 30nM, 80nM, 및 200nM 농도의 Ex21x+1 2'-MOE ASO로 처리된 마우스 *Scn1a*의 SYBR-세이프 염색된 RT-PCR 생성물을 나타낸다. Ex21x+1(마우스 명명법)과 Ex20x+1(인간 명명법)은 동일하다. 엑손 20x 포함(상부 밴드) 및 전장(엑손 20x 배제, 하부 밴드)에 해당하는 2개의 생성물을 정량화하였고 퍼센트 엑손 21x 포함을 막대그래프로 플로팅한다(도 8b). 또한, 전장 생성물은 HPRT 내부 대조군에 정규화하였고 Sham에 대한 배수 변화를 막대그래프로 플로팅한다(도 8c). 검은 선은 Sham에 대하여 1의 비율 및 변화없음을 나타낸다.

[0483] **실시예 7: 선택된 ASO의 안내(IVT) 주사.**

[0484] [도 9a]는 PBS 주사된(1μL) 좌안(-) 또는 10mM 농도의 IVS20x-21, Ex21x+1, IVS21x+18, IVS21x+33 또는 Cep290(음성 대조군 ASO; Gerard et al, *Mol. Ther. Nuc. Ac.*, 2015) 2'-MOE ASO 주사된(1μL) 우안(+)으로부

터 마우스 *Scn1a*의 SYBR-세이프 염색된 RT-PCR 생성물의 PAGE 겔을 나타낸다. Ex21x+1, IVS21x+18, 및 IVS21x+33(마우스 명명법)과 Ex20x+1, IVS20x+18, 및 IVS20x+33(인간 명명법)은 동일하다. 엑손 21x 포함(상부 밴드) 및 전장(엑손 21x 배제, 하부 밴드)에 해당하는 2개의 생성물을 정량화하였고 퍼센트 엑손 21x 포함을 [도 9b]에 플로팅한다. 흰색 막대는 ASO 주사된 눈에 해당하고 회색 막대는 PBS 주사된 눈에 해당한다(각 그룹에서 n = 5). 전장 생성물을 GAPDH 내부 대조군에 정규화하였고 PBS 주사된 눈에 대한 ASO 주사된 눈의 배수 변화를 [도 9c]에 나타낸다. 검은 선은 PBS에 대하여 1의 비율 및 변화없음을 나타낸다(각 그룹에서 n = 5).

[0485] **실시예 8: 선택된 ASO의 뇌실내(ICV) 주사.**

[0486] [도 10a]는 주사되지 않은(-, ASO 없는 대조군), 또는 300 μg의 Cep290(음성 대조군 ASO; Gerard et al, *Mol. Ther. Nuc. Ac.*, 2015), Ex21x+1, IVS21x+18, IVS21x+33 2'-MOE ASO 주사된 뇌로부터 마우스 *Scn1a*의 SYBR-세이프 염색된 RT-PCR 생성물의 PAGE 겔을 나타낸다. Ex21x+1, IVS21x+18, 및 IVS21x+33(마우스 명명법)과 Ex20x+1, IVS20x+18, 및 IVS20x+33(인간 명명법)은 동일하다. 엑손 21x 포함(상부 밴드) 및 전장(엑손 21x 배제, 하부 밴드)에 해당하는 2개의 생성물을 정량화하였고 퍼센트 엑손 21x 포함을 [도 10b]에 플로팅한다(n = 6(각 표적화 ASO), n = 5(Cep290 ASO), n = 1(주사되지 않음, ASO 없는 대조군)). 엑손 21 및 22 접합부에 걸친 2개의 상이한 프로브를 사용하여 태크맨 qPCR을 수행하고 생성물을 GAPDH 내부 대조군에 정규화하고 Cep290 주사된 뇌에 대한 ASO 주사된 뇌의 배수 변화를 [도 10c]에 막대그래프로 플로팅하였다. 검은 선은 Cep290에 대하여 1의 비율 및 변화없음을 플로팅한다(n = 6(각 표적화 ASO), n = 5(Cep290 ASO), n = 1(주사되지 않음, ASO 없는 대조군)).

[0487] [도 11]은 C57BL6J 마우스(수컷, 3개월령)에서 선택된 ASO의 뇌실내(ICV) 주사의 예시적인 용량 의존적 반응을 도시한다. [도 11a]는 300ug의 Cep290(음성 대조군 ASO; Gerard *et al, Mol. Ther. Nuc. Ac.*, 2015), 또는 33ug, 100ug, 및 300ug의 Ex21x+1 2'-MOE ASO 주사된 뇌로부터 마우스 *Scn1a*의 SYBR-세이프 염색된 RT-PCR 생성물의 PAGE 겔을 나타낸다. Ex21x+1(마우스 명명법)과 Ex20x+1(인간 명명법)은 동일하다. 엑손 21x 포함(상부 밴드) 및 전장(엑손 21x 배제, 하부 밴드)에 해당하는 2개의 생성물을 정량화하였다. [도 11b]는 [도 11a]의 데이터로부터 퍼센트 엑손 21x 포함을 플로팅한 그래프를 도시한다(각 그룹 n = 5). [도 11c]는 엑손 21 및 22 접합부에 걸친 2개의 상이한 프로브를 사용하여 수행된 태크맨 qPCR 분석 결과의 그래프를 도시한다. 생성물을 *Gapdh* 내부 대조군에 정규화하였고 Cep290 주사된 뇌에 대한 ASO 주사된 뇌의 배수 변화를 플로팅한다. 검은 선은 Cep290에 대하여 1의 비율 및 변화없음을 나타낸다(각 그룹 n = 5).

[0488] [도 12]는 C57BL6J 마우스(생후 2일)에서 선택된 ASO의 뇌실내(ICV) 주사의 예시적인 결과를 도시한다. [도 12a]는 주사되지 않은(-, ASO 없는 대조군), 또는 20ug의 Ex21x+1 2'-MOE ASO 주사된 뇌로부터 마우스 *Scn1a*의 SYBR-세이프 염색된 RT-PCR 생성물의 PAGE 겔을 나타낸다. 엑손 21x 포함(상부 밴드) 및 전장(엑손 21x 배제, 하부 밴드)에 해당하는 2개의 생성물을 정량화하였다. Ex21x+1(마우스 명명법)과 Ex20x+1(인간 명명법)은 동일하다. [도 12b]는 [도 12a]의 데이터로부터 퍼센트 엑손 21x 포함을 나타내는 그래프를 플로팅한다(각 그룹 n = 4). [도 12c]는 엑손 21 및 22 접합부에 걸친 2개의 상이한 프로브를 사용하여 수행된 태크맨 qPCR 분석 결과의 그래프를 도시한다. 생성물을 *Gapdh* 내부 대조군에 정규화하였고 ASO 없는 대조군 뇌에 대한 ASO 주사된 뇌의 배수 변화를 플로팅한다. 검은 선은 ASO 없는 대조군에 대하여 1의 비율 및 변화없음을 나타낸다(각 그룹 n = 4).

[0489] **실시예 9: 드라벳 증후군의 치료를 위한 핵 유전자 출력의 표적화된 증강**

[0490] 드라벳 증후군(DS)은 중증 발작, 인지 및 운동 장애 및 사망을 특징으로 하는 파괴적인 소아기 유전 질환이다. DS의 주요 원인은 나트륨 전압 게이팅 채널 1형 알파 서브유닛(Nav1.1)의 발현 감소이다. *SCN1* 비생산적인 스플라이싱 이벤트는 인간과 마우스 사이에서 보존된다. [도 13a]는 지시된 마우스 CNS 샘플에서 퍼센트 엑손 21x 포함을 플로팅한 그래프를 도시한다. [도 13b]는 지시된 인간 CNS 샘플에서 퍼센트 엑손 20x 포함을 플로팅한 그래프를 도시한다. 이 연구에서, 안티센스 올리고뉴클레오티드(ASO) 요법을 이용하여 생산적인 Scn1a mRNA를 증가시키고 결과적으로 Nav1.1 단백질의 수준을 회복시켰다.

[0491] [도 14a]는 지시된 용량에서 엑손 21x 포함의 퍼센트 감소를 플로팅한 그래프를 도시한다(그룹당 n = 3 내지 6). [도 14b]는 지시된 용량에서 Scn1a mRNA의 퍼센트 증가를 플로팅한 그래프를 도시한다(그룹당 n = 3 내지 6). [도 14c]는 지시된 용량에서 Nav 1.1 단백질 수준의 퍼센트 증가를 플로팅한 그래프를 도시한다(그룹당 n = 2).

[0492] [도 15a]는 지시된 용량에서 엑손 21x 포함의 퍼센트 감소를 플로팅한 그래프를 도시한다(그룹당 n = 4). [도

15b]는 지시된 용량에서 Scn1a mRNA의 퍼센트 증가를 플로팅한 그래프를 도시한다(그룹당 n = 4).

[0493] [도 16]은 표적 선택도를 평가하기 위해 생후 2일 마우스에서 ICV 주사를 통해 10ug 용량으로 투여된 선택된 *Scn1a* 표적화 ASO를 주사 후 5일에 *SCN1A*, *SCN2A*, *SCN3A*, *SCN4A*, *SCN5A*, *SCN7A*, *SCN8A*, *SCN9A*, *SCN10A*, 및 *SCN11A*의 태크맨 qPCR에 의해 평가하여 도시한다. Ex20x+1 ASO를 사용하여 얻은, *Gapdh*에 정규화된 태크맨-qPCR 증폭 결과는 PBS 주사된 마우스에 대한 배수 변화로서 플로팅한다(그룹당 n = 3 내지 6).

[0494] [도 17]은 야생형(WT) 또는 129S-Scn1a^{tm1Kea} x C57BL/6J 교배의 이종 접합 드라벳 마우스(HET) F1 마우스에서 지시된 용량의 선택된 ASO의 출생 2일 후 뇌실 내(ICV) 주사로부터의 주사 3일 후의 예시적인 결과를 도시한다(그룹당 n = 9 내지 14). [도 17a]는 엑손 21 및 22에 걸친 프로브를 사용하여 수행된 태크맨 qPCR 분석 결과의 그래프를 도시한다. 생성물을 *Gapdh* 내부 대조군에 정규화하였고 PBS 주사된 뇌에 대한 ASO 주사된 뇌의 배수 변화를 플로팅한다. [도 17b]는 항-Nav1.1 항체를 사용하여 수행된 웨스턴 블롯의 결과의 그래프를 도시한다. 생성물을 폰소 염색된 밴드에 정규화하였고 PBS 주사된 뇌에 대한 ASO 주사된 배수 변화를 플로팅한다.

[0495] [도 19]는 *SCN1A* 표적화 ASO의 ICV 주사 후 시간 경과에 따른 마우스의 관상 뇌 슬라이스에서 *Scn1a* mRNA의 수준증가를 플로팅한 그래프이다. 도시된 바와 같이, 태크맨 qPCR에 의해 정량화된 *Scn1a* mRNA의 수준증가는 주사 후 적어도 80일 동안 유지되었다(그룹당 n = 3 내지 9).

[0496] [도 20]은 드라벳 마우스 모델에서 *SCN1A* 표적화 ASO에 의해 제공되는 100% 생존 이점을 입증하는 예시적인 생존 곡선이다. WT, 및 129S-scn1a^{tm1Kea} x C57BL/6J 교배의 F1 자손인 이형 접합 드라벳 마우스(+/-)는 생후 2일에 20 μg PBS 또는 가려진 ASO(A 또는 B로 표시된 처리)의 단일 용량의 ICV 주사를 받았으며, 그들의 생존을 모니터링하였다. 도시된 바와 같이, A +/- 그룹의 마우스(PBS 처리를 받은 드라벳 마우스)는 대략 생후 16일부터 죽기 시작했던 반면, B +/- 그룹(ASO 처리를 받은 드라벳 마우스)을 포함하는 다른 세 그룹의 모든 마우스는 적어도 생후 35일까지 생존하였다(그룹당 n = 32 내지 39).

[0497] [도 18]은 자유 흡수를 통한 RenCell에서의 *SCN1A* 엑손 20x 영역 ASO 마이크로 워크의 예시적인 결과를 도시한다. ASO는 한 번에 1 뉴클레오티드를 이동 하거나(6 내지 41) ASO 17의 길이를 줄임으로써(1 내지 5) [도 6]에서 이전에 확인된 3개의 표적화 ASO(별표로 표시됨) 주위의 영역을 덮도록 설계하였다. 그래프는 SYBR-그린 qPCR에 의해 측정된 퍼센트 엑손 20x 포함을 도시한다. 검은 선은 ASO가 없는 것(-)에 대하여 변화없음을 나타낸다.

[0498] ASO의 서열은 표 7a 및 표 7b에 요약한다.

[0499] [표 7a]

인간 *SCN1A* 를 표적화하는 ASO 의 서열

ASO 번호	서열 5'-3'	서열 번호
1	TTGGAGCAAGATTATC	304
2	GTTGGAGCAAGATTATC	305
3	GTTGGAGCAAGATTAT	306
4	AGTTGGAGCAAGATTAT	307
5	AGTTGGAGCAAGATTA	308
6	GATTATCCTATACAAAAT	309
7	AGATTATCCTATACAAAA	310
8	AAGATTATCCTATACAAA	311
9	CAAGATTATCCTATACAA	312
10	GCAAGATTATCCTATACA	313
11	AGCAAGATTATCCTATAC	314
12	GAGCAAGATTATCCTATA	315
13	GGAGCAAGATTATCCTAT	316
14	TGGAGCAAGATTATCCTA	317
15	GTTGGAGCAAGATTATCC	318
16	TTGGAGCAAGATTATCCT	319
18	AAGTTGGAGCAAGATTAT	320
19	CAAGTTGGAGCAAGATTA	321
20	CCAAGTTGGAGCAAGATT	322
21	TCCAAGTTGGAGCAAGAT	323

[0500]

22	AGTACCCATAATAAAGGG	324
23	AATACAGTACCCATAATA	325
24	ATTAAAGGTAGCAAAAGG	326
25	GATTAAAGGTAGCAAAAG	327
26	GGATTAAAGGTAGCAAAA	328
27	AGGATTAAAGGTAGCAAA	329
29	CAAGGATTAAAGGTAGCA	330
30	GCAAGGATTAAAGGTAGC	331
31	TGCAAGGATTAAAGGTAG	332
32	GTGCAAGGATTAAAGGTA	333
33	AGTCACAGTGCAAGGATT	334
34	AAGTCACAGTGCAAGGAT	335
35	TAAGTCACAGTGCAAGGA	336
36	ATAAGTCACAGTGCAAGG	337
38	ACATAAGTCACAGTGCAA	338
39	CACATAAGTCACAGTGCA	339
40	ACACATAAGTCACAGTGC	340
41	TACACATAAGTCACAGTG	341

[0501]

[0502] [표 7b]

인간 *SCN1A* 를 표적화하는 ASO 의 서열

ASO 번호	서열 5'-3'	서열 번호
1_U	UUGGAGCAAGAUUAUC	342
2_U	GUUGGAGCAAGAUUAUC	343
3_U	GUUGGAGCAAGAUUAU	344
4_U	AGUUGGAGCAAGAUUAU	345
5_U	AGUUGGAGCAAGAUUA	346
6_U	GAUUAUCCUAUACAAAAU	347
7_U	AGAUUAUCCUAUACAAAA	348
8_U	AAGAUUAUCCUAUACAAA	349
9_U	CAAGAUUAUCCUAUACAA	350
10_U	GCAAGAUUAUCCUAUACA	351
11_U	AGCAAGAUUAUCCUAUAC	352
12_U	GAGCAAGAUUAUCCUAUA	353
13_U	GGAGCAAGAUUAUCCUAU	354
14_U	UGGAGCAAGAUUAUCCUA	355
15_U	GUUGGAGCAAGAUUAUCC	356
16_U	UUGGAGCAAGAUUAUCCU	357
18_U	AAGUUGGAGCAAGAUUAU	358
19_U	CAAGUUGGAGCAAGAUUA	359
20_U	CCAAGUUGGAGCAAGAUU	360
21_U	UCCAAGUUGGAGCAAGAU	361
22_U	AGUACCCAUAAUAAAGGG	362

[0503]

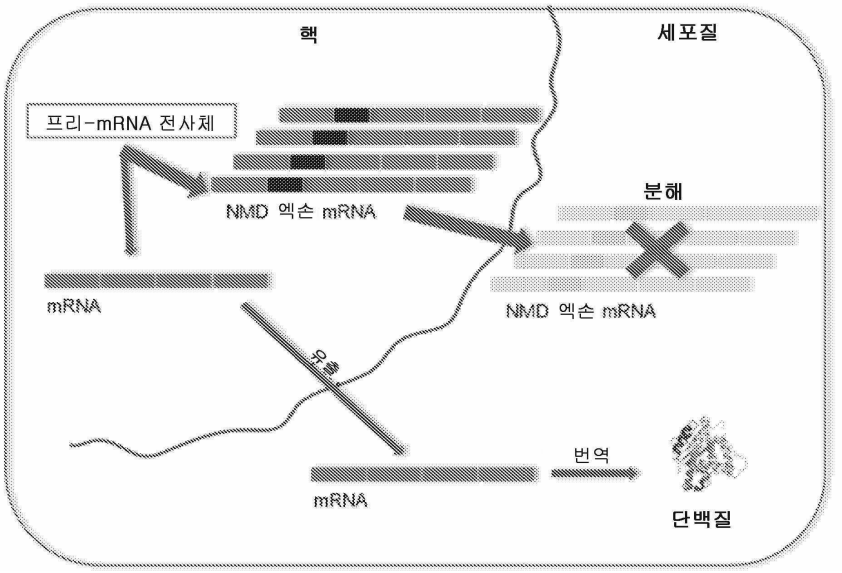
23_U	AAUACAGUACCCAUAAUA	363
24_U	AUUAAAGGUAGCAAAAGG	364
25_U	GAUUAAAGGUAGCAAAAG	365
26_U	GGAUUAAAGGUAGCAAAA	366
27_U	AGGAUUAAAGGUAGCAAA	367
29_U	CAAGGAUUAAAGGUAGCA	368
30_U	GCAAGGAUUAAAGGUAGC	369
31_U	UGCAAGGAUUAAAGGUAG	370
32_U	GUGCAAGGAUUAAAGGUA	371
33_U	AGUCACAGUGCAAGGAUU	372
34_U	AAGUCACAGUGCAAGGAU	373
35_U	UAAGUCACAGUGCAAGGA	374
36_U	AUAAGUCACAGUGCAAGG	375
38_U	ACAUAAGUCACAGUGCAA	376
39_U	CACAUAAGUCACAGUGCA	377
40_U	ACACAUAAGUCACAGUGC	378
41_U	UACACAUAAGUCACAGUG	379

[0504]

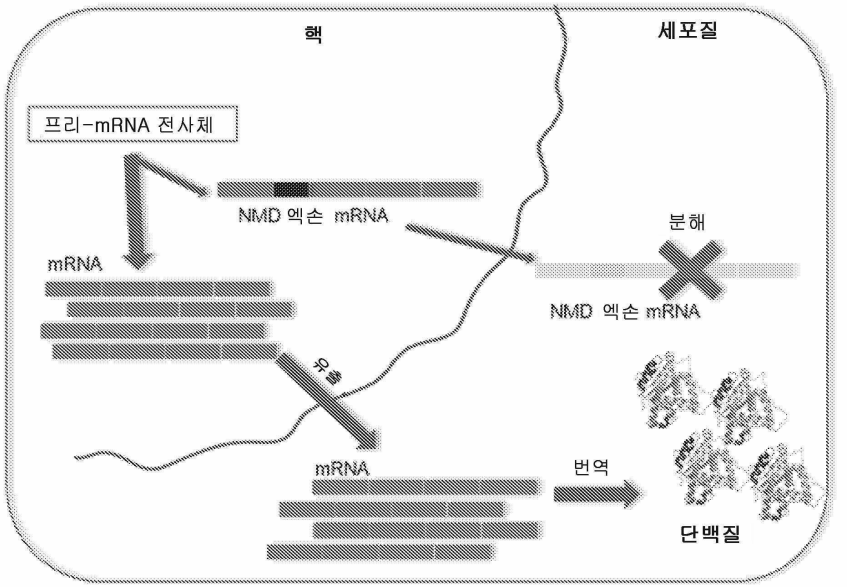
[0505] 본 발명의 바람직한 실시양태가 본원에 제시되고 설명되었지만, 이러한 실시양태는 단지 예로서 제공된다는 것은 당업자에게 명백할 것이다. 당업자는 본 발명을 벗어나지 않으면서 다수의 변형, 변경 및 치환을 생각해 낼 것이다. 본원에 기술된 본 발명의 실시양태에 대한 다양한 대안이 본 발명을 실시하는 데 사용될 수 있음을 이해해야 한다. 다음의 청구범위는 본 발명의 범위를 정의하고 이들 청구범위의 범위 내의 방법 및 구조 및 그 등가물은 이에 의해 포함되는 것으로 의도된다.

도면

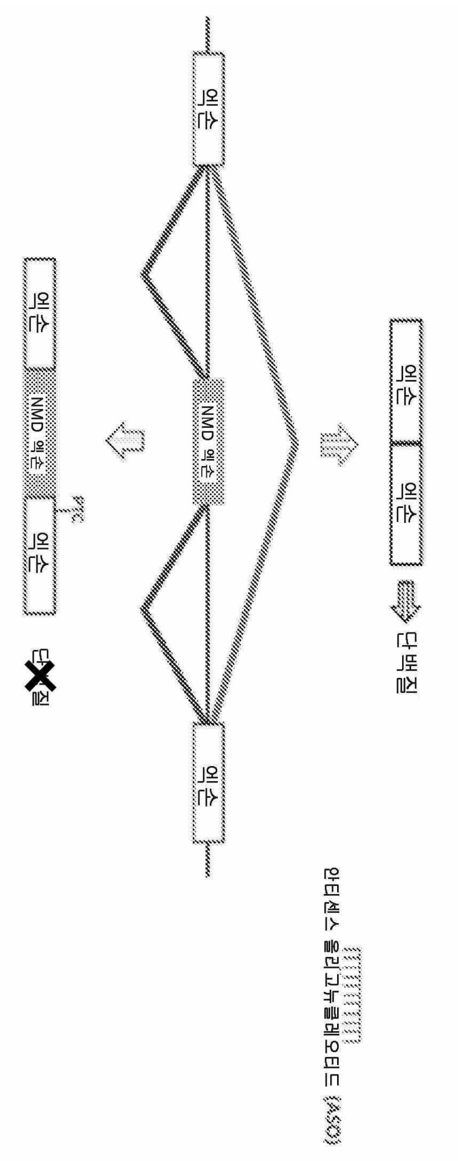
도면1a


핵
세포질
프리-mRNA 전사체
NMD 엑손 mRNA
분해
mRNA
NMD 엑손 mRNA
우출
번역
mRNA
단백질

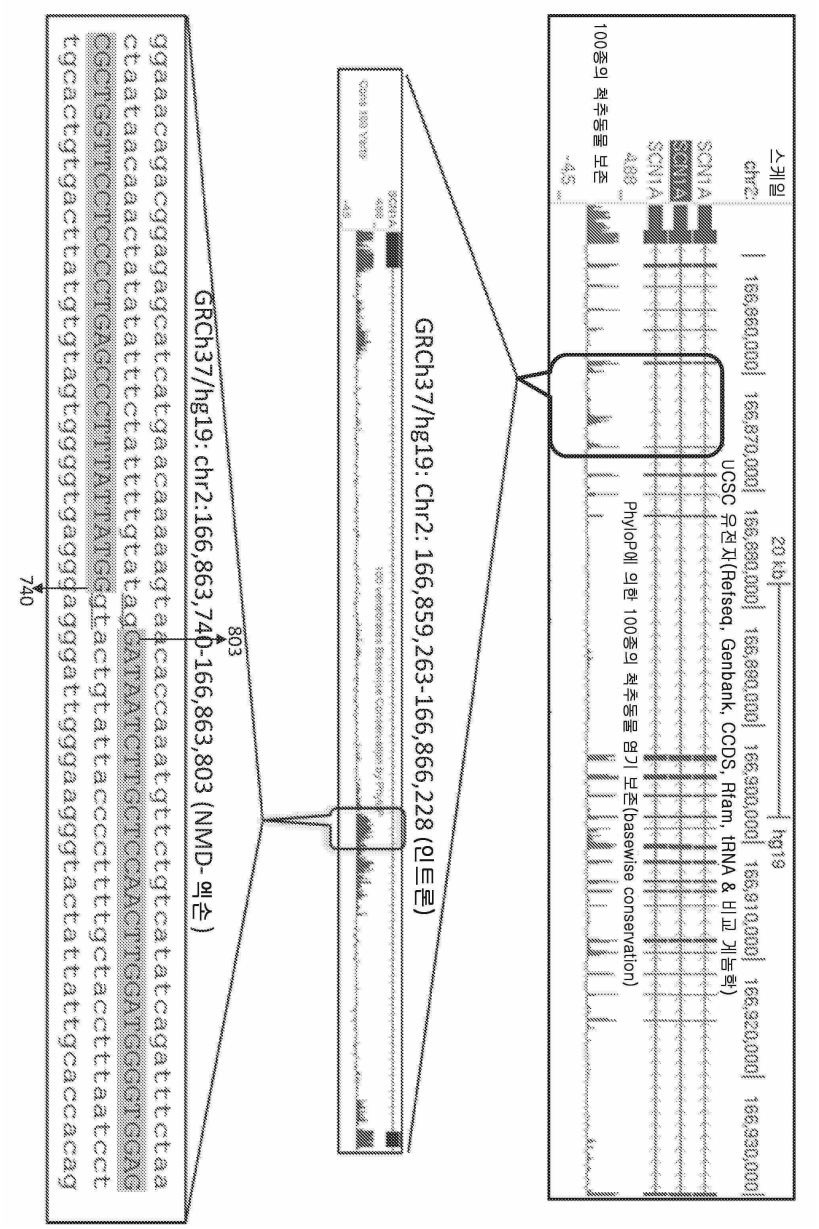
도면1b


핵
세포질
프리-mRNA 전사체
NMD 엑손 mRNA
분해
mRNA
NMD 엑손 mRNA
우출
mRNA
번역
단백질

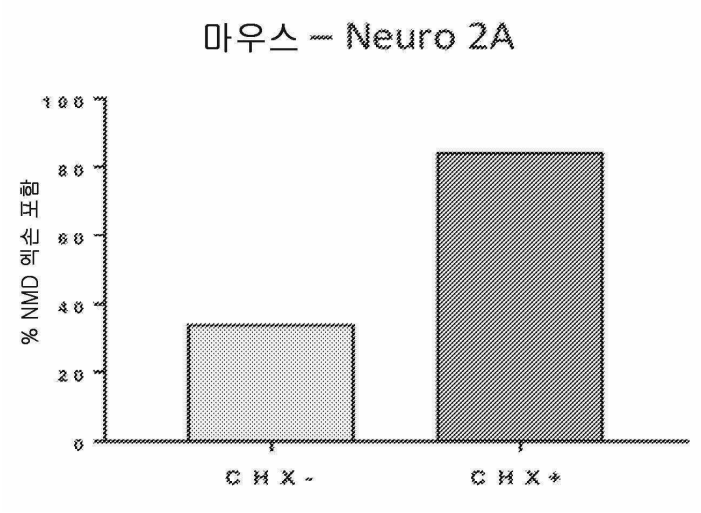
도면1c



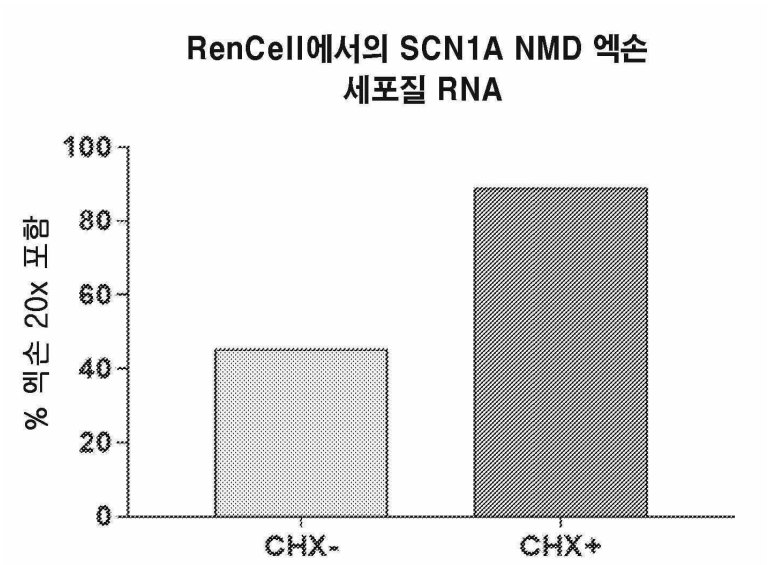
도면2


스케일
chr2:
166,860,000
166,870,000
166,880,000
166,890,000
166,900,000
166,910,000
166,920,000
166,930,000
20 kb
hg19
SCN1A
SCN1A
SCN1A
4.89
-4.5
100종의 척추동물 보존
UCSC 유전자(Refseq, Genbank, CCDS, Rfam, tRNA & 비교 게놈학)
PhyloP에 의한 100종의 척추동물 엽기 보존(basewise conservation)
GRCh37/hg19: Chr2: 166,859,263-166,866,228 (인트론)
GRCh37/hg19: chr2:166,863,740-166,863,803 (NMD- 엑손)
740
803
ggaaacagacggagagcatcatgaacaaaaagtaacaccaaatgttctgtcatatcagatttctaa
ctaataacaaactatatatttctatttttgtatagGATAYATCTTTGCTCCAACTTGGATGGGTGGAG
CGCTGGTTCGTCCCGTGAGCCCTTTATTATTGTgtactgtattaccccttttgctacctttaatcct
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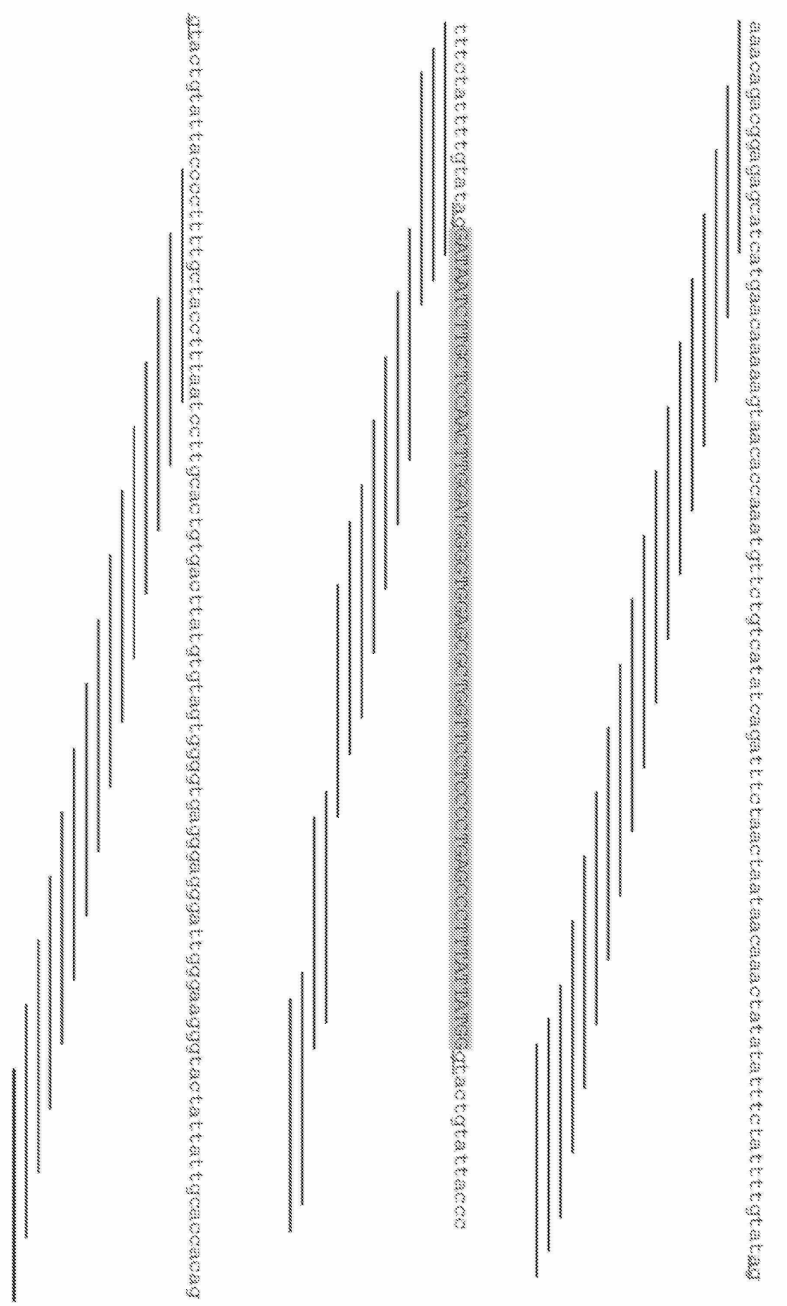
도면3a


마우스 – Neuro 2A
% NMD 엑손 포함
100
80
60
40
20
0
CHX-
CHX+

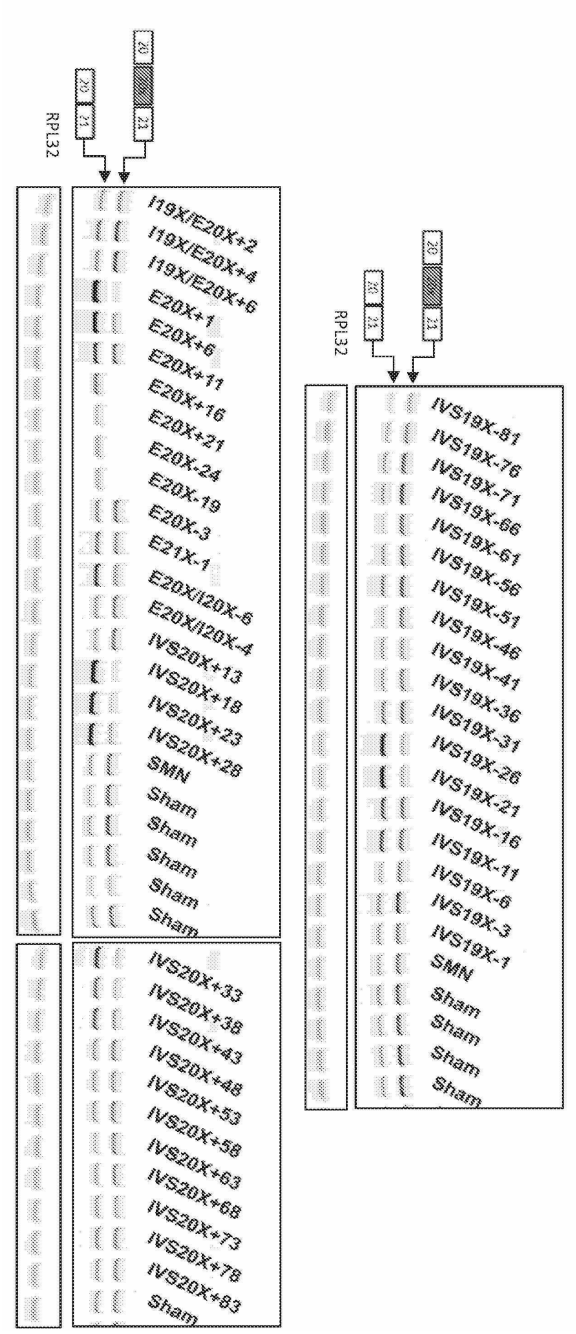
도면3b


RenCell에서의 SCN1A NMD 엑손
세포질 RNA
% 엑손 20x 포함
100
80
60
40
20
0
CHX-
CHX+

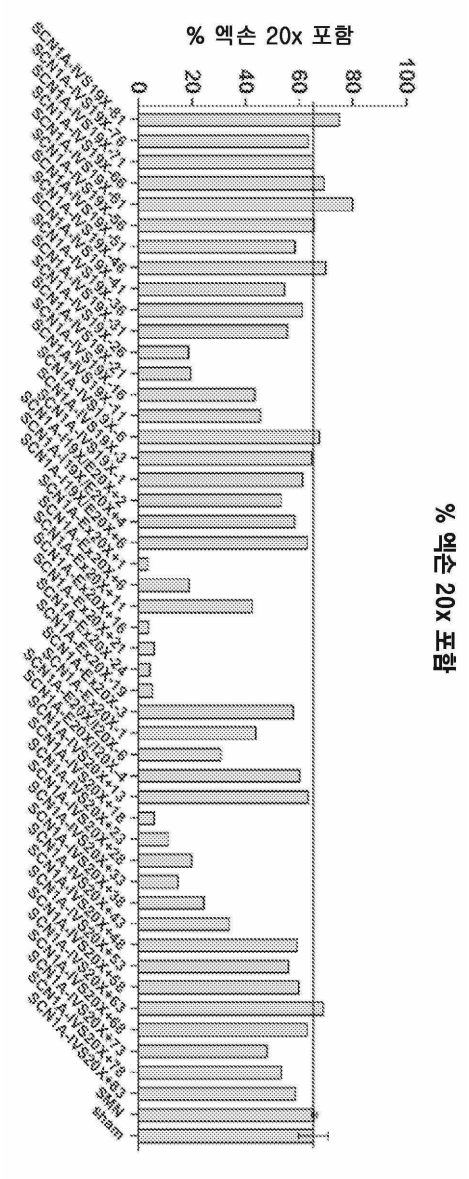
도면4



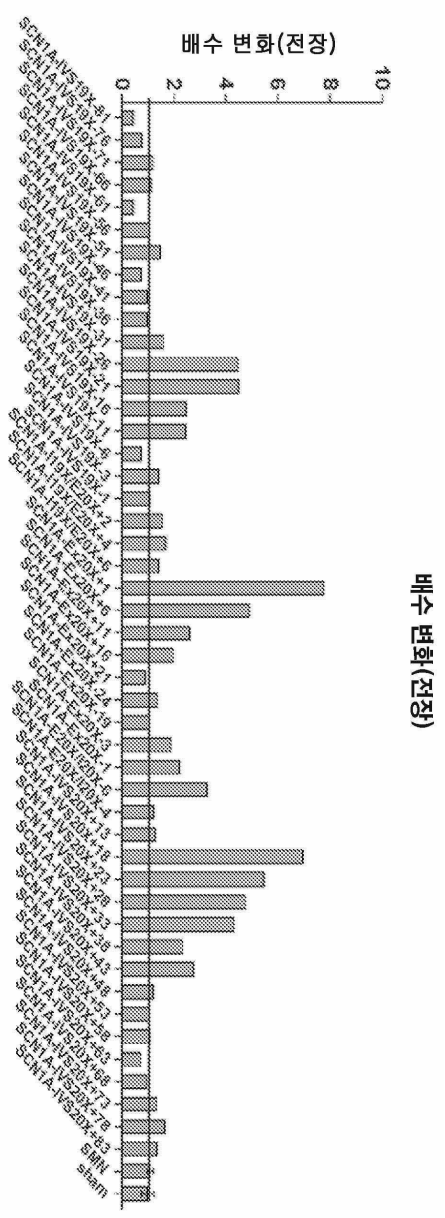
도면5a


RP132
I19X/E20X+2
I19X/E20X+4
I19X/E20X+6
E20X+1
E20X+6
E20X+11
E20X+16
E20X+21
E20X-24
E20X-19
E20X-3
E21X-1
E20X/I20X-6
E20X/I20X-4
IVS20X+13
IVS20X+18
IVS20X+23
IVS20X+28
SMN
Sham
Sham
Sham
Sham
Sham
IVS20X+33
IVS20X+38
IVS20X+43
IVS20X+48
IVS20X+53
IVS20X+58
IVS20X+63
IVS20X+68
IVS20X+73
IVS20X+78
IVS20X+83
Sham
RP132
IVS19X-81
IVS19X-76
IVS19X-71
IVS19X-66
IVS19X-61
IVS19X-56
IVS19X-51
IVS19X-46
IVS19X-41
IVS19X-36
IVS19X-31
IVS19X-26
IVS19X-21
IVS19X-16
IVS19X-11
IVS19X-6
IVS19X-3
IVS19X-1
SMN
Sham
Sham
Sham
Sham

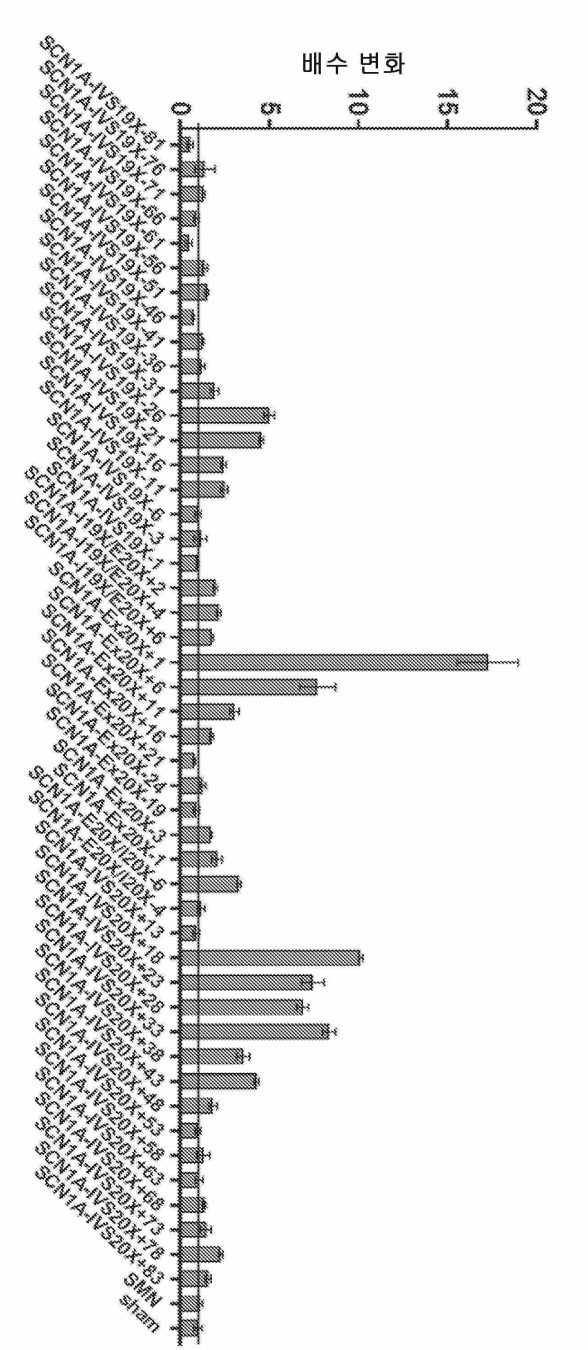
도면5b



도면5c



도면6


배수 변화
0
5
10
15
20
SCN1A-IVS19X-81
SCN1A-IVS19X-76
SCN1A-IVS19X-71
SCN1A-IVS19X-66
SCN1A-IVS19X-61
SCN1A-IVS19X-56
SCN1A-IVS19X-51
SCN1A-IVS19X-46
SCN1A-IVS19X-41
SCN1A-IVS19X-36
SCN1A-IVS19X-31
SCN1A-IVS19X-26
SCN1A-IVS19X-21
SCN1A-IVS19X-16
SCN1A-IVS19X-11
SCN1A-IVS19X-6
SCN1A-IVS19X-3
SCN1A-IVS19X-1
SCN1A-I19XE20X+2
SCN1A-I19XE20X+4
SCN1A-E20X+8
SCN1A-E20X+1
SCN1A-E20X+6
SCN1A-E20X+11
SCN1A-E20X+16
SCN1A-E20X+21
SCN1A-E20X+24
SCN1A-E20X+19
SCN1A-E20X-3
SCN1A-E20XI20X-1
SCN1A-IVS20X-6
SCN1A-IVS20X-4
SCN1A-IVS20X+13
SCN1A-IVS20X+18
SCN1A-IVS20X+23
SCN1A-IVS20X+28
SCN1A-IVS20X+33
SCN1A-IVS20X+38
SCN1A-IVS20X+43
SCN1A-IVS20X+48
SCN1A-IVS20X+53
SCN1A-IVS20X+58
SCN1A-IVS20X+63
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SCN1A-IVS20X+83
SMN
sham

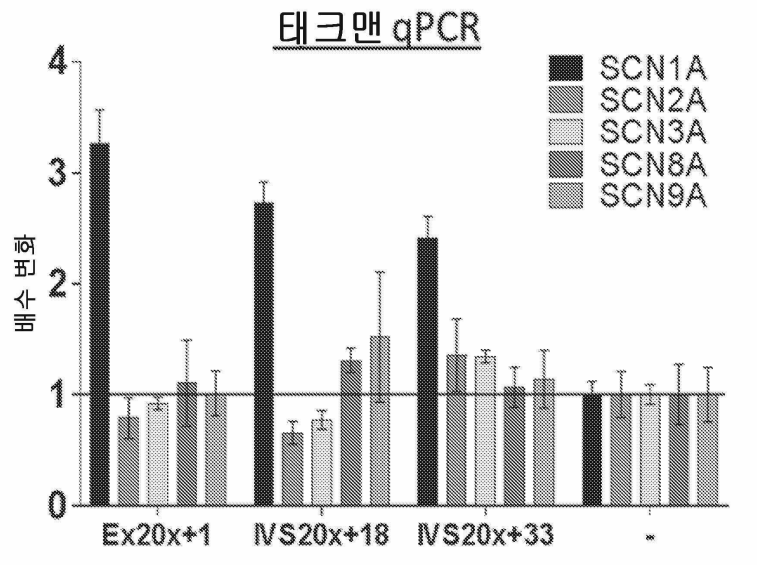
도면7a

나트륨 전압 게이팅 채널 알파 서브유닛

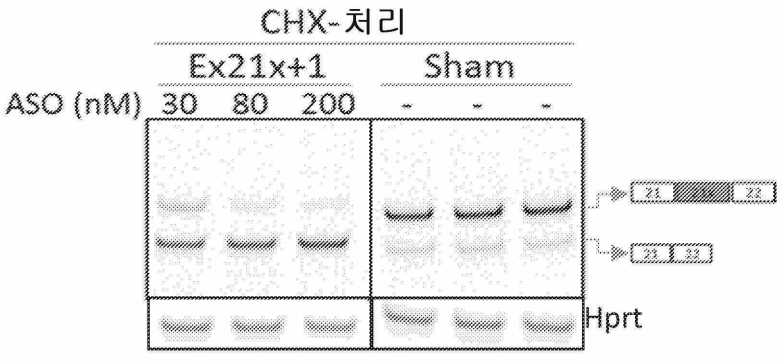
유전자	승인된 명칭	염색체	
SCN1A	나트륨 전압 게이팅 채널 알파 서브유닛 1	2q24.3	⬅
SCN2A	나트륨 전압 게이팅 채널 알파 서브유닛 2	2q24.3	⇦
SCN3A	나트륨 전압 게이팅 채널 알파 서브유닛 3	2q24.3	⇦
SCN7A	나트륨 전압 게이팅 채널 알파 서브유닛 7	2q24.3	X
SCN8A	나트륨 전압 게이팅 채널 알파 서브유닛 8	12q13.13	⇦
SCN9A	나트륨 전압 게이팅 채널 알파 서브유닛 9	2q24.3	⇦
SCN10A	나트륨 전압 게이팅 채널 알파 서브유닛 10	3p22.2	X
SCN11A	나트륨 전압 게이팅 채널 알파 서브유닛 11	3p22.2	X

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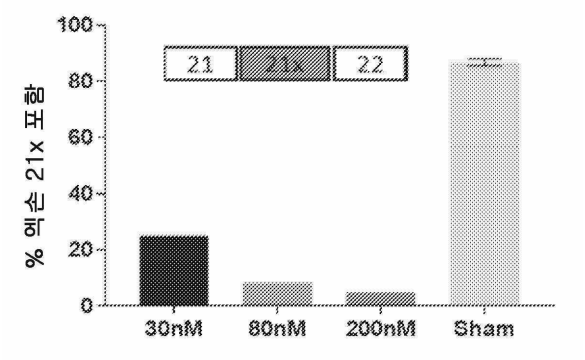
도면7b



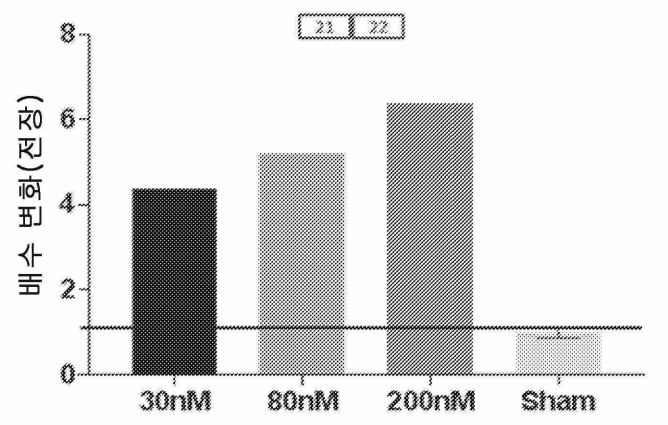
도면8a



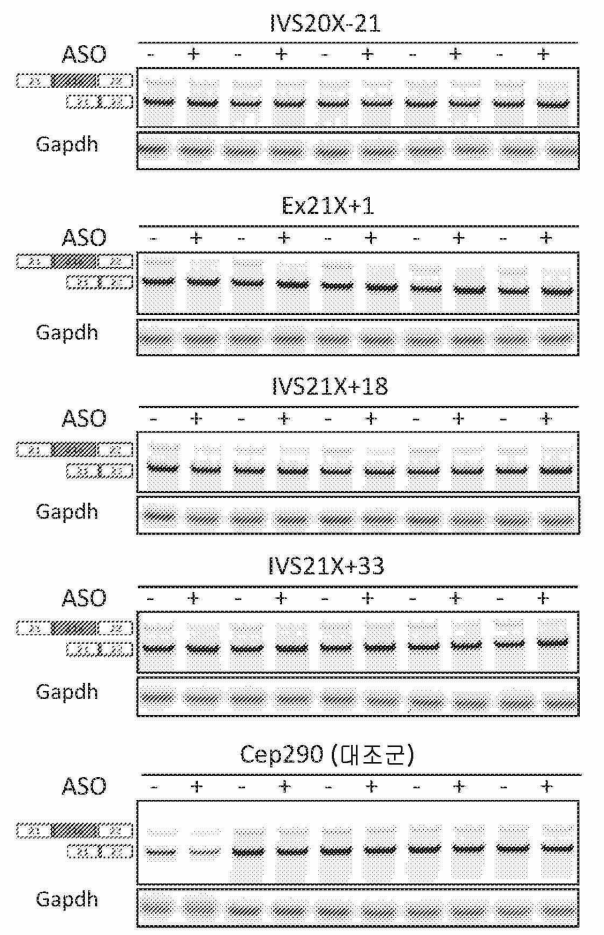
도면8b


100
80
60
40
20
0
% 엑손 21x 포함
21
21x
22
30nM
80nM
200nM
Sham

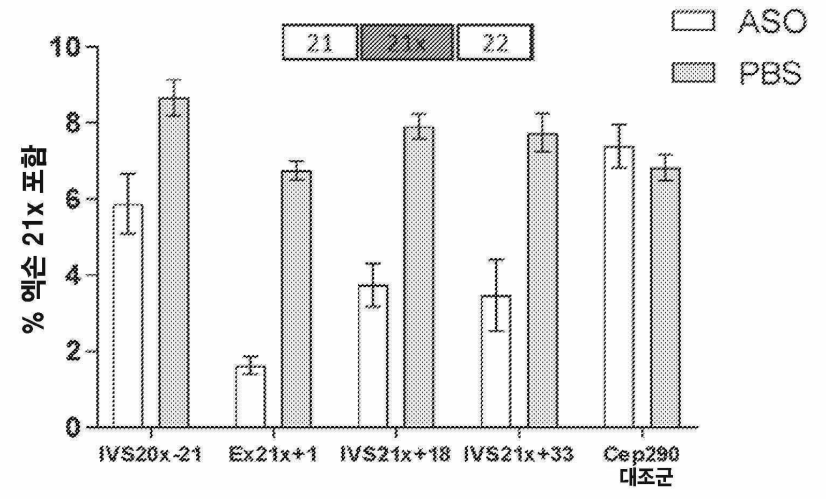
도면8c


8
6
4
2
0
배수 변화(전장)
21
22
30nM
80nM
200nM
Sham

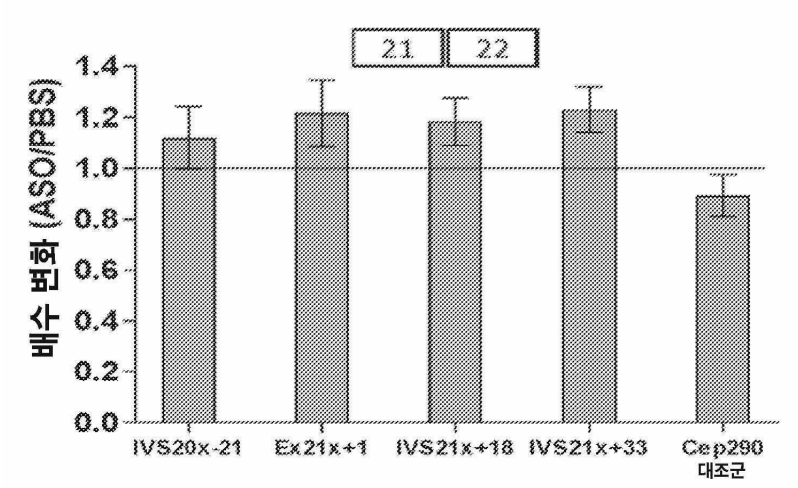
도면9a


IVS20X-21
ASO
- + - + - + - + - +
Gapdh
Ex21X+1
ASO
- + - + - + - + - +
Gapdh
IVS21X+18
ASO
- + - + - + - + - +
Gapdh
IVS21X+33
ASO
- + - + - + - + - +
Gapdh
Cep290 (대조군)
ASO
- + - + - + - + - +
Gapdh

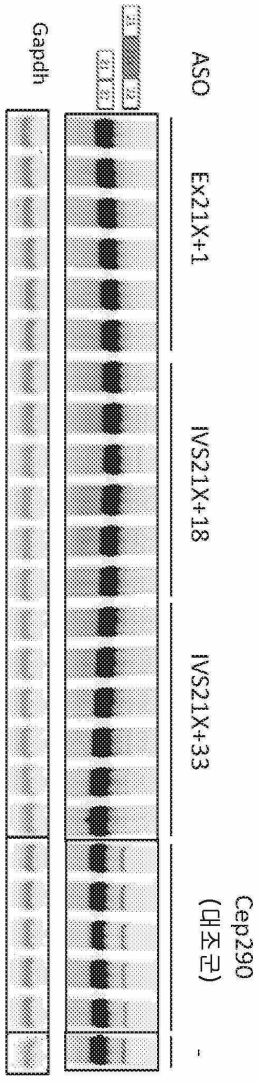
도면9b


ASO
PBS
21
21x
22
% 엑손 21x 포함
0
2
4
6
8
10
IVS20x-21
Ex21x+1
IVS21x+18
IVS21x+33
Cep290
대조군

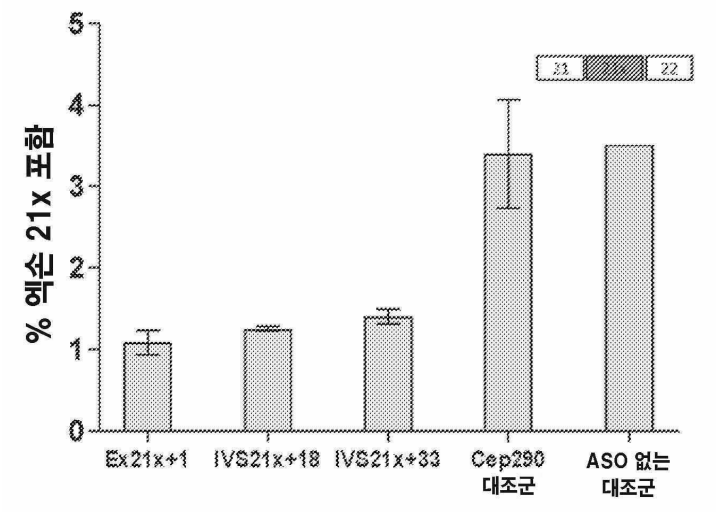
도면9c


21
22
배수 변화 (ASO/PBS)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
IVS20x-21
Ex21x+1
IVS21x+18
IVS21x+33
Cep290
대조군

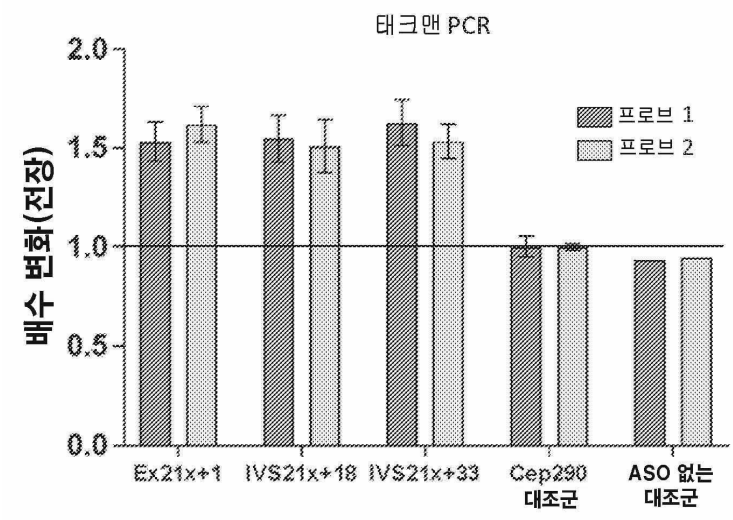
도면10a


Gapdh
ASO
Ex21X+1
IVS21X+18
IVS21X+33
Cep290
(대조군)
-

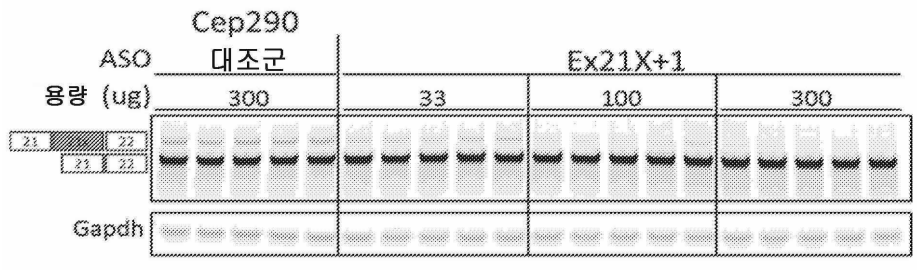
도면10b



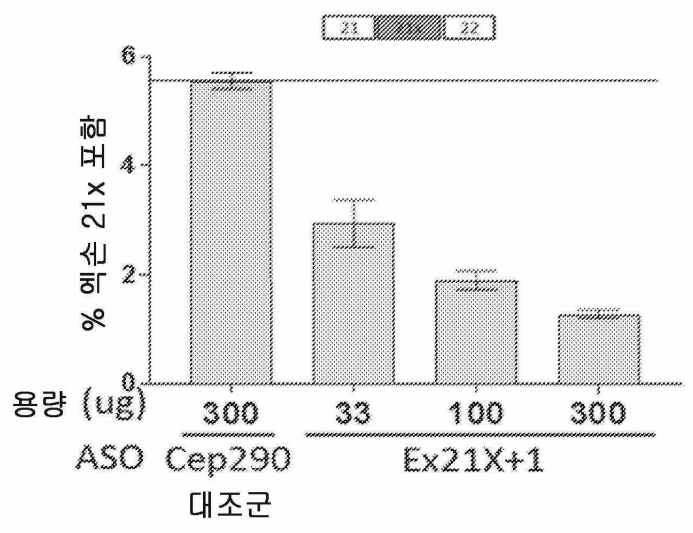
도면10c



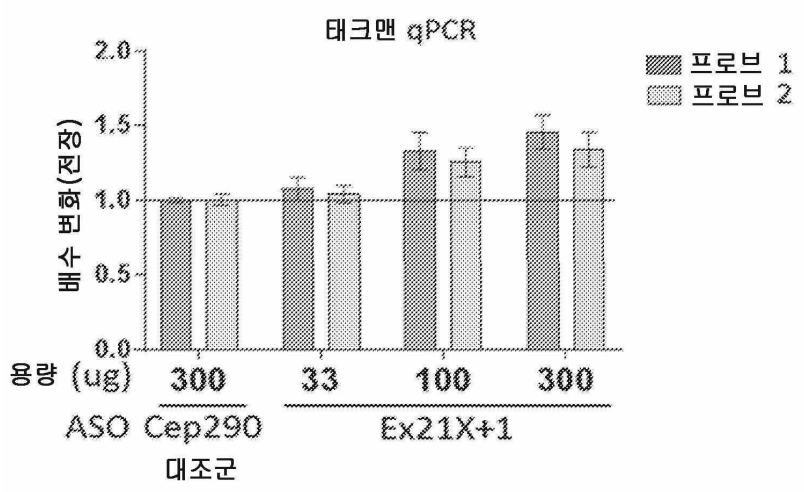
도면11a



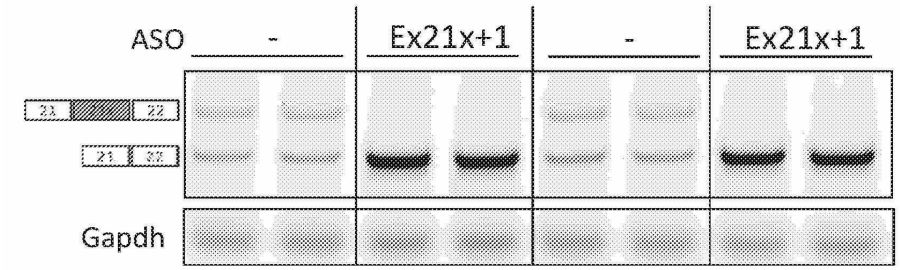
도면11b



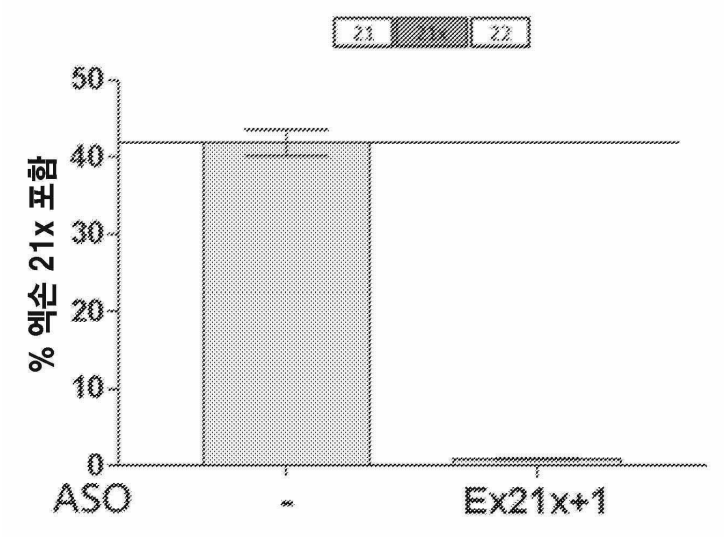
도면11c



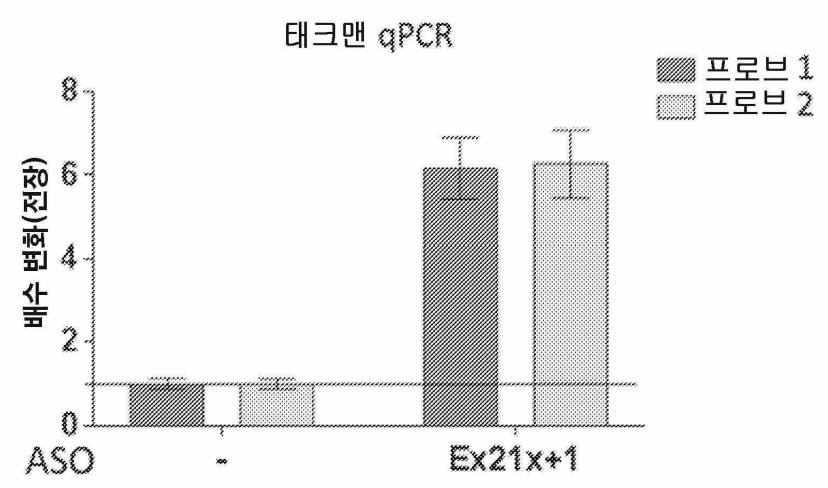
도면12a



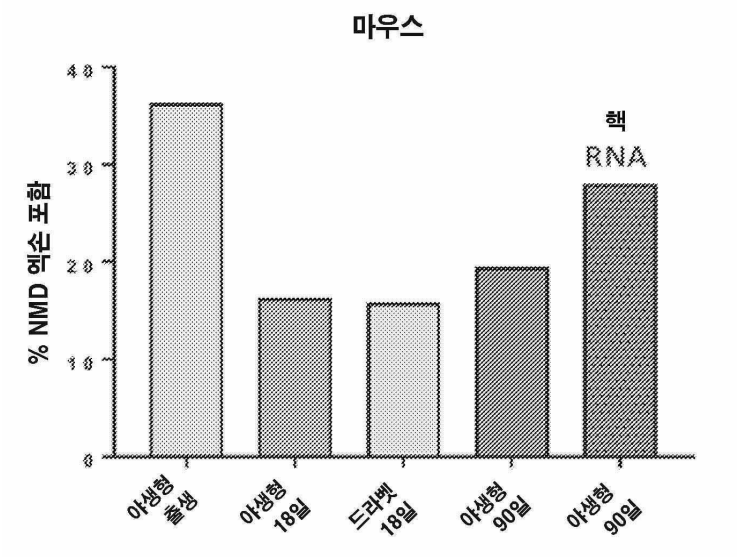
도면12b



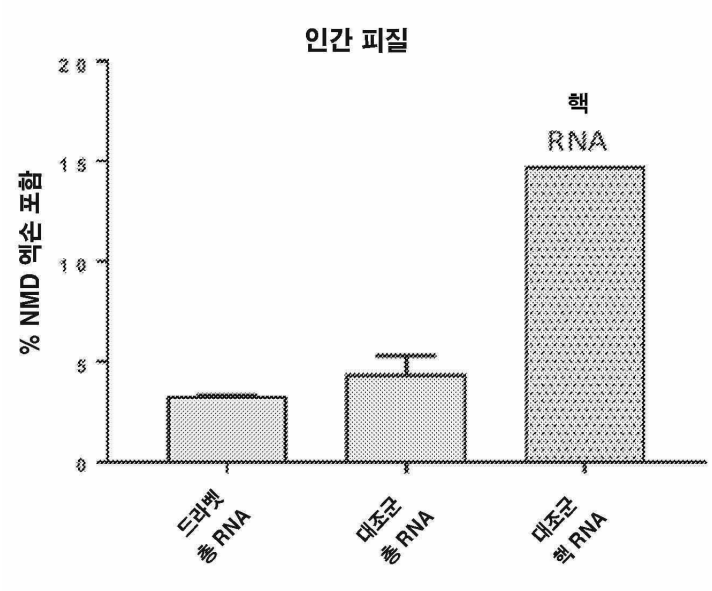
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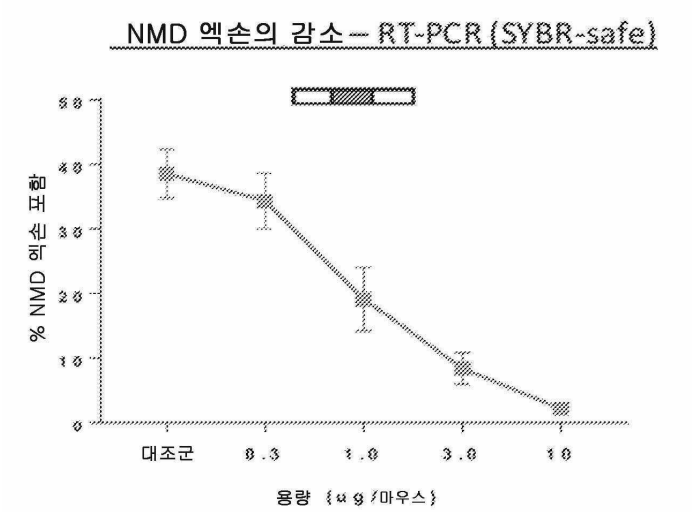
도면13a



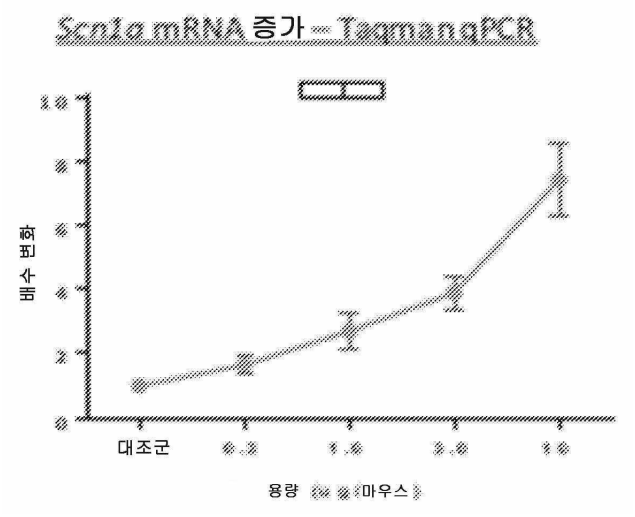
도면13b


인간 피질
% NMD 엑손 포함
20
15
10
5
0
핵
RNA
드라벳
총 RNA
대조군
총 RNA
대조군
핵 RNA

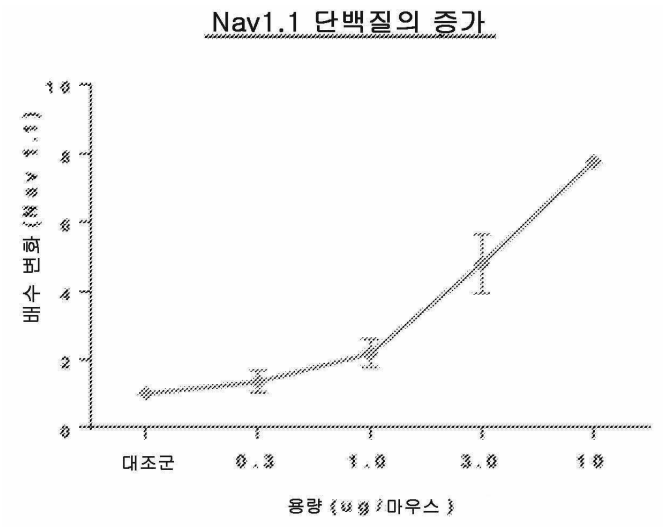
도면14a


NMD 엑손의 감소 – RT-PCR (SYBR-safe)
% NMD 엑손 포함
50
40
30
20
10
0
대조군
0.3
1.0
3.0
10
용량 (㎍/마우스)

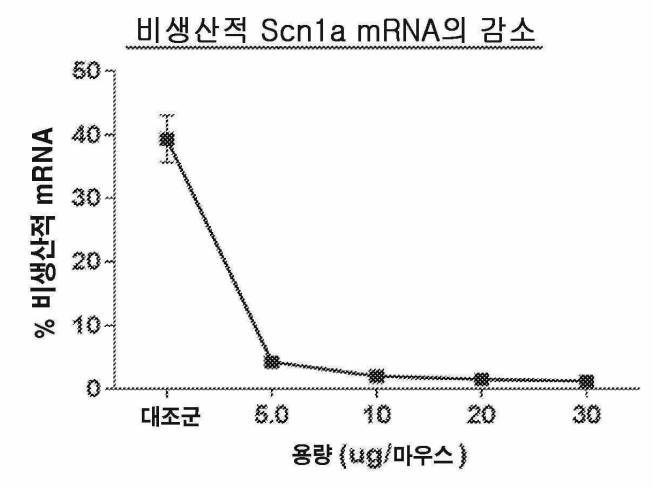
도면14b



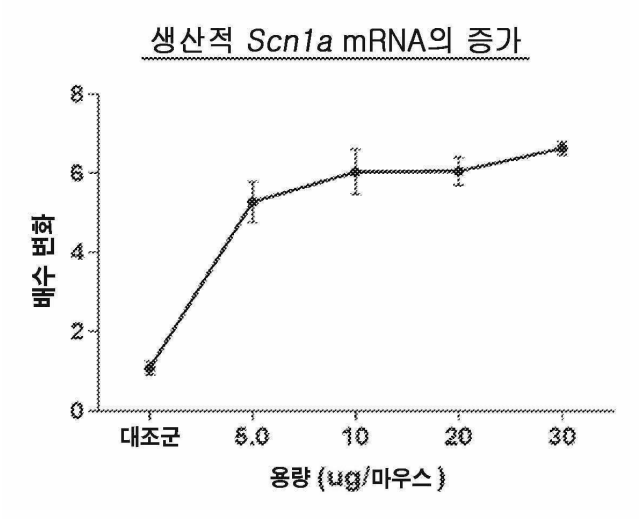
도면14c



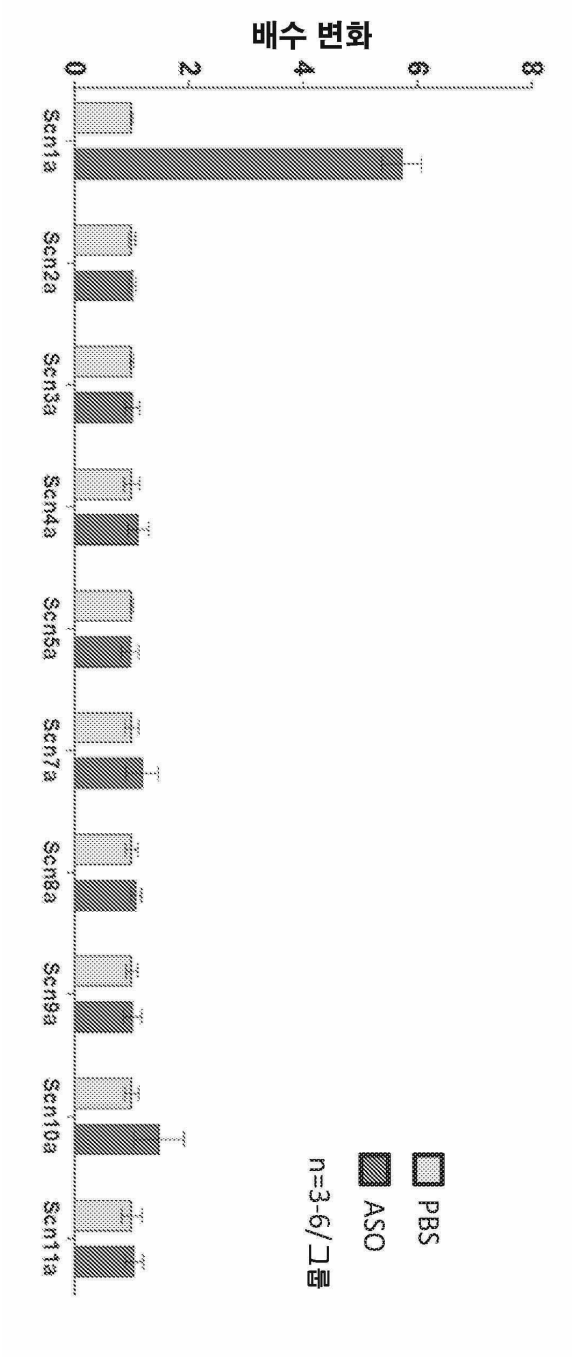
도면15a



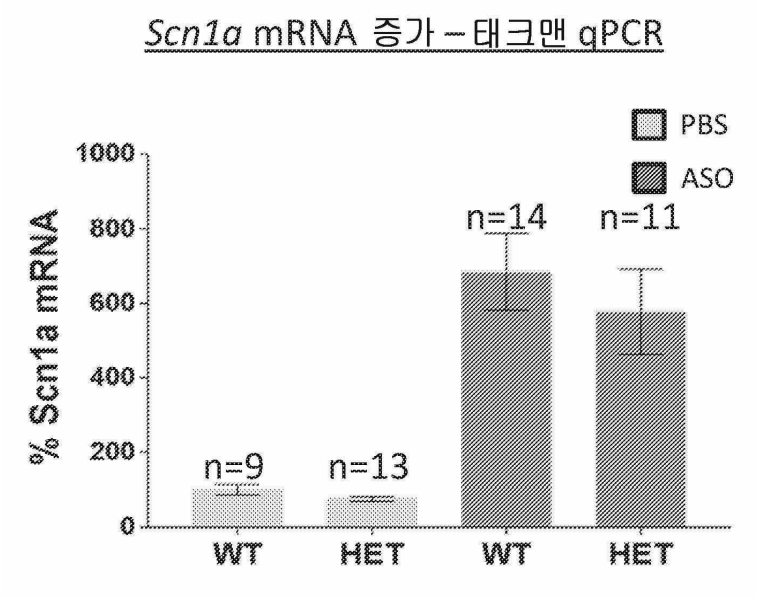
도면15b



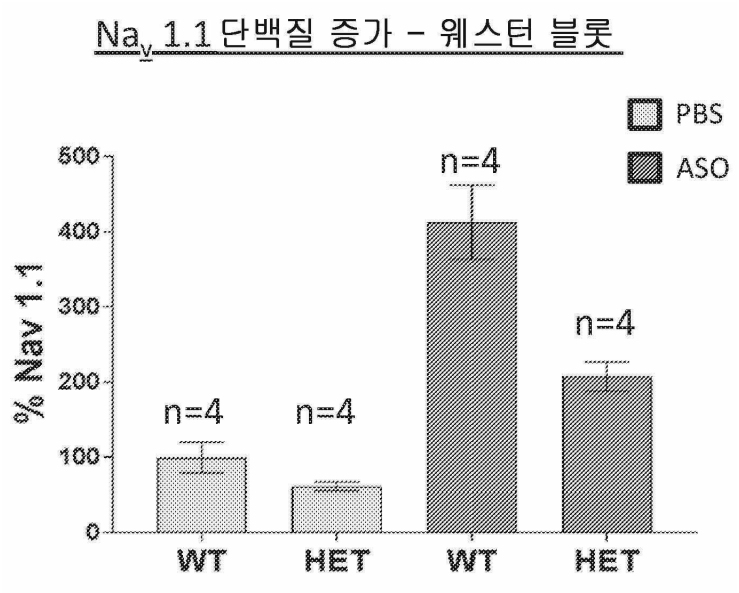
도면16


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2
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6
8
Scn1a
Scn2a
Scn3a
Scn4a
Scn5a
Scn7a
Scn8a
Scn9a
Scn10a
Scn11a
PBS
ASO
n=3-6/그룹

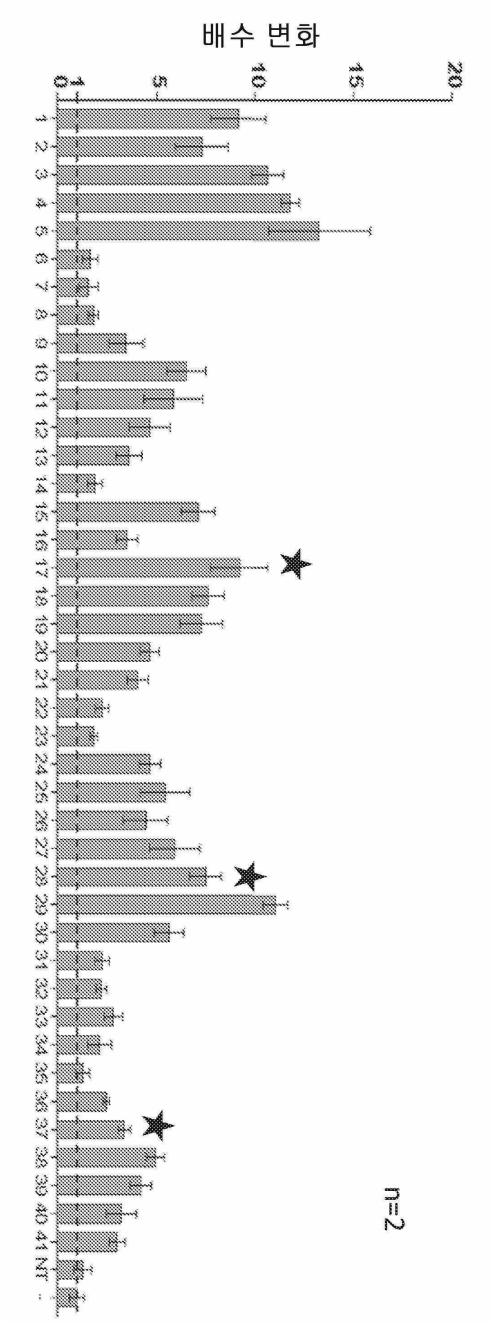
도면17a


Scn1a mRNA 증가 – 태크맨 qPCR
PBS
ASO
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n=14
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WT
HET
WT
HET

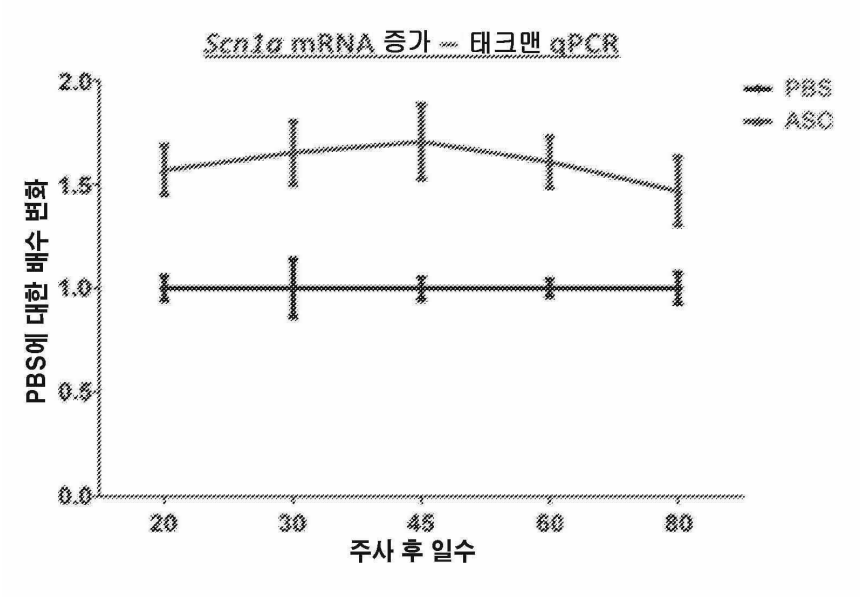
도면17b


Na$_V$1.1 단백질 증가 – 웨스턴 블롯
PBS
ASO
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300
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WT
HET
WT
HET

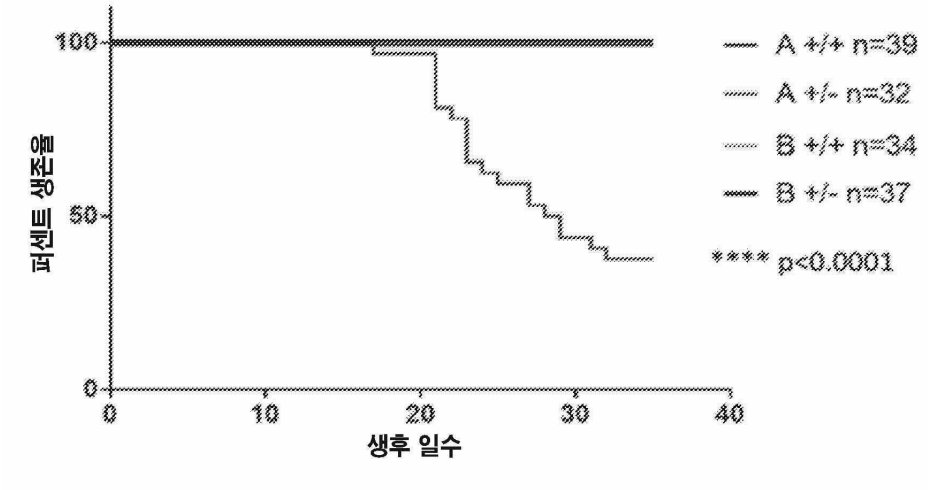
도면18


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NT
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도면19



도면20



서 열 목 록

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<120> ANTISENSE OLIGOMERS FOR TREATMENT OF CONDITIONS AND DISEASES

<130> 47991-719.601

<140> PCT/US2018/048031

<141> 2018-08-24

<150> 62/671,745

<151> 2018-05-15

<150> 62/667,356

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<210> 26

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cataagtcac agtgcaag 18

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gatggggtgg agcgctggtt cctcccctga gcccttttatt atgggtactg tattaccccct 180

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