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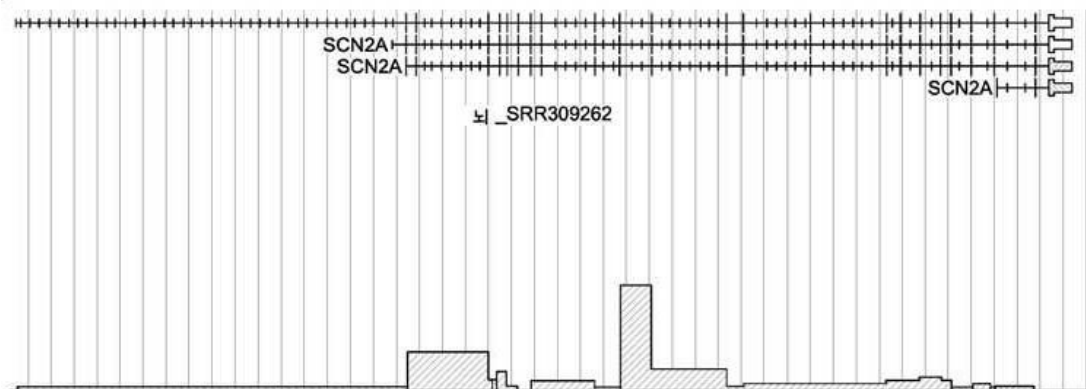
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(54) 발명의 명칭 SCN2A에서 기능-상실 돌연변이와 연관된 장애를 치료하기 위한 조성물 및 방법

(57) 요약

인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시키는 안티센스 올리고뉴클레오타이드와 세포를 접촉시키는 단계를 포함하여, 세포에서 SCN2A 단백질의 수준을 증가시키는 방법이 본원에 제공되며, 여기서 보유된 인트론은 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24 중에서 선택되고 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA에서 표적 영역에 상보적인 핵염기의 서열을 포함한다. 또한 이러한 방법에 사용하기 위한 안티센스 올리고뉴클레오타이드가 제공된다. 또한 이러한 안티센스 올리고뉴클레오타이드를 대상체에게 투여하는 것을 포함하여, SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 장애를 치료하는 방법이 제공된다.

대표도



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명세서

청구범위

청구항 1

인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시키는 안티센스 올리고뉴클레오타이드와 세포를 접촉시키는 단계를 포함하여 세포에서 SCN2A 단백질의 수준을 증가시키는 방법으로서, 여기서 상기 보유된 인트론은 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24 중에서 선택되고, 상기 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA에서 표적 영역에 상보적인 핵염기의 서열을 포함하는, 방법.

청구항 2

인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시키는 안티센스 올리고뉴클레오타이드를 대상체에게 투여하는 단계를 포함하여 대상체에서 SCN2A 단백질의 수준을 증가시키는 방법으로서, 여기서 상기 보유된 인트론은 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24 중에서 선택되고, 상기 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA에서 표적 영역에 상보적인 핵염기의 서열을 포함하는, 방법.

청구항 3

제2항에 있어서, 상기 대상체가 SCN2A에서 이형접합 기능-상실 돌연변이를 갖는, 방법.

청구항 4

제2항 또는 제3항에 있어서, 상기 대상체가 SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 장애를 갖는, 방법.

청구항 5

제4항에 있어서, SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 상기 장애가 유전성 간질, 발달성 및 간질성 뇌병증, 지적 장애, 자폐 스펙트럼 장애 또는 정신분열증인, 방법.

청구항 6

인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시키는 안티센스 올리고뉴클레오타이드를 대상체에게 투여하는 단계를 포함하여 SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 장애를 치료하는 방법으로서, 여기서 상기 보유된 인트론은 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24 중에서 선택되고, 상기 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA에서 표적 영역에 상보적인 핵염기의 서열을 포함하는, 방법.

청구항 7

제6항에 있어서, SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 상기 장애가 유전성 간질, 발달성 및 간질성 뇌병증, 지적 장애, 자폐 스펙트럼 장애 또는 정신분열증인, 방법.

청구항 8

제1항 내지 제7항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 인트론 스플라이싱 사일런서(ISS)에 결합하거나 이에 인접하거나, G-사중체 내의 뉴클레오타이드에 결합하거나, RNA 2차 구조를 갖는 뉴클레오타이드에 결합하는, 방법.

청구항 9

제8항에 있어서, 상기 ISS가 이중 핵 리보핵단백질(hnRNP)에 의해 인식되는, 방법.

청구항 10

제9항에 있어서, 상기 hnRNP가 hnRNPA1 또는 hnRNP I인, 방법.

청구항 11

제1항 내지 제10항 중 어느 한 항에 있어서, 상기 보유된 인트론이 인트론 2인, 방법.

청구항 12

제11항에 있어서, 상기 ISS는 인트론 2의 5' 스플라이스 부위에 대해 위치 +8 내지 +12, +32 내지 +36, +33 내지 +36, 또는 +60 내지 +65 및 인트론 2의 3' 스플라이스 부위에 대해 위치 -84 내지 -88 및 -82 내지 -87에 있는, 방법.

청구항 13

제1항 내지 제12항 중 어느 한 항에 있어서, 상기 보유된 인트론이 인트론 2이고 상기 표적 영역이 인트론 2의 5' 스플라이스 부위에 대해 위치 -4 내지 +14, -3 내지 +15, -2 내지 +16, +6 내지 +24, +7 내지 +25, +8 내지 +26, +9 내지 +27, +10 내지 +28, +14 내지 +32, +19 내지 +37, +20 내지 +38, +21 내지 +39, +22 내지 +40, +23 내지 +41, +24 내지 +42, +25 내지 +43, +26 내지 +44, +27 내지 +45, +28 내지 +46, +29 내지 +47, +30 내지 +48, +31 내지 +49, +32 내지 +50, +33 내지 +51, +34 내지 +52, +35 내지 +53, +43 내지 +61, +45 내지 +63, +46 내지 +64, +49 내지 +67, +52 내지 +70, +59 내지 +77, +64 내지 +82, +65 내지 +83, +68 내지 +86, +69 내지 +87, +70 내지 +88, +71 내지 +89, +72 내지 +90 및 +73 내지 +91에 걸쳐 있는, 방법.

청구항 14

제1항 내지 제13항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 서열번호 115 내지 142 중 어느 하나에 제시된 서열에 대해 적어도 또는 약 70%, 80% 또는 90% 서열 동일성을 갖는 서열 또는 서열번호 115 내지 142 중 어느 하나에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오타이드를 갖는 서열을 포함하는, 방법.

청구항 15

제1항 내지 제14항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 서열번호 126에 제시된 서열 또는 서열번호 126에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오타이드를 포함하는 서열을 포함하는, 방법.

청구항 16

제1항 내지 제10항 중 어느 한 항에 있어서, 상기 보유된 인트론이 인트론 2이고 상기 표적 영역이 인트론 2의 3' 스플라이스 부위에 대해 위치 -19 내지 -1, -21 내지 -3, -30 내지 -12, -31 내지 -13, -32 내지 -14, -62 내지 -44, -64 내지 -46, -67 내지 -49, -76 내지 -58, -77 내지 -59, -78 내지 -60, -79 내지 -61 -82 내지 -64, -83 내지 65, -84 내지 -66, -85 내지 -67, -86 내지 -68, -87 내지 -69, -95 내지 -77, -97 내지 -79, -100 내지 -82, -101 내지 -83, -102 내지 -84, -103 내지 -85, -107 내지 -89, -109 내지 -91, -111 내지 -93, -113 내지 -95 및 -114 내지 -96에 걸쳐 있는, 방법.

청구항 17

제14항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 서열번호 143 내지 205 중 어느 하나에 제시된 서열에 대해 적어도 또는 약 70%, 80% 또는 90% 서열 동일성을 갖는 서열 또는 서열번호 143 내지 205 중 어느 하나에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오타이드를 갖는 서열을 포함하는, 방법.

청구항 18

제1항 내지 제17항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 8 내지 50, 8 내지 40, 8 내지 35, 8 내지 30, 8 내지 25, 8 내지 20, 8 내지 15, 9 내지 50, 9 내지 40, 9 내지 35, 9 내지 30, 9 내지 25, 9 내지 20, 9 내지 15, 10 내지 50, 10 내지 40, 10 내지 35, 10 내지 30, 10 내지 25, 10 내지 20, 10 내

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

청구항 19

제1항 내지 제18항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 표적 영역에 적어도 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 또는 99% 상보적인, 방법.

청구항 20

제1항 내지 제19항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 표적 영역에 100% 상보적인 적어도 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 또는 20개의 인접 핵염기를 포함하는, 방법.

청구항 21

제1항 내지 제20항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 적어도 하나의 변형을 포함하는, 방법.

청구항 22

제21항에 있어서, 상기 변형이 핵염기 변형, 올리고뉴클레오타이드 백본의 변형 또는 리보스 당의 변형인, 방법.

청구항 23

제1항 내지 제22항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 2'-O-메틸(2OMe), 2'-O-메톡시-에틸(MOE), 잠금 핵산(LNA), 2'-플루오로 또는 S-제한된-에틸(cEt) 중에서 선택되는 변형된 당을 포함하는, 방법.

청구항 24

제1항 내지 제23항 중 어느 한 항에 있어서, 안티센스 올리고뉴클레오타이드가 포스포로티오에이트를 포함하는 백본을 포함하는, 방법.

청구항 25

제1항 내지 제24항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 RNase H를 활성화시키는, 방법.

청구항 26

제2항 내지 제25항 중 어느 한 항에 있어서, 상기 대상체가 SCN2A에서 이형접합 기능-상실 돌연변이를 갖는 것으로 결정되는, 방법.

청구항 27

제2항 내지 제26항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드는 비경구 투여 또는 비강 투여에 의해 대상체에 투여되는, 방법.

청구항 28

제27항에 있어서, 상기 비경구 투여가 피하 투여, 정맥내 투여, 근육내 투여, 동맥내 투여, 복강내 투여 또는 두개내 투여 중에서 선택되는, 방법.

청구항 29

제28항에 있어서, 두개내 투여가 척수강내 또는 뇌실내 투여인, 방법.

청구항 30

제2항 내지 제29항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드 또는 조성물이 대상체에게 약

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12개월 또는 그 이상마다 투여되는, 방법.

청구항 31

제2항 내지 제30항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드 또는 조성물이 대상체에게 약 3개월마다 투여되는, 방법.

청구항 32

인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 표적 영역에 상보적인 핵염기의 서열을 포함하는 안티센스 올리고뉴클레오타이드로서, 여기서 상기 표적 영역은 보유 인트론에 있고, 상기 보유 인트론은 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24 중에서 선택되는, 안티센스 올리고뉴클레오타이드.

청구항 33

제32항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 인트론 스플라이싱 사일런서(ISS)에 결합하거나 이에 인접하거나, G-사중체 내의 뉴클레오타이드에 결합하거나, RNA 2차 구조를 갖는 뉴클레오타이드에 결합하는, 안티센스 올리고뉴클레오타이드.

청구항 34

제33항에 있어서, 상기 ISS가 이중 핵 리보핵단백질(hnRNP)에 의해 인식되는, 안티센스 올리고뉴클레오타이드.

청구항 35

제34항에 있어서, 상기 hnRNP가 hnRNPA1 또는 hnRNP I인, 안티센스 올리고뉴클레오타이드.

청구항 36

제34항 또는 제35항에 있어서, 상기 보유된 인트론이 인트론 2이고 상기 ISS가 인트론 2의 5' 스플라이스 부위에 대해 위치 +8 내지 +12, +33 내지 +36 또는 +60 내지 +65 및 인트론 2의 3' 스플라이스 부위에 대해 위치 -84 내지 -88 및 -82 내지 -87에 있는, 안티센스 올리고뉴클레오타이드.

청구항 37

제32항 내지 제36항 중 어느 한 항에 있어서, 상기 보유된 인트론이 인트론 2이고 상기 표적 영역이 인트론 2의 5' 스플라이스 부위에 대해 위치 -4 내지 +14, -3 내지 +15, -2 내지 +16, +6 내지 +24, +7 내지 +25, +8 내지 +26, +9 내지 +27, +10 내지 +28, +14 내지 +32, +19 내지 +37, +20 내지 +38, +21 내지 +39, +22 내지 +40, +23 내지 +41, +24 내지 +42, +25 내지 +43, +26 내지 +44, +27 내지 +45, +28 내지 +46, +29 내지 +47, +30 내지 +48, +31 내지 +49, +32 내지 +50, +33 내지 +51, +34 내지 +52, +35 내지 +53, +43 내지 +61, +45 내지 +63, +46 내지 +64, +49 내지 +67, +52 내지 +70, +59 내지 +77, +64 내지 +82, +65 내지 +83, +68 내지 +86, +69 내지 +87, +70 내지 +88, +71 내지 +89, +72 내지 +90 및 +73 내지 +91에 걸쳐 있는, 안티센스 올리고뉴클레오타이드.

청구항 38

제32항 내지 제37항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 서열번호 115 내지 205 중 어느 하나에 제시된 서열에 대해 적어도 또는 약 70%, 80% 또는 90% 서열 동일성을 갖는 서열 또는 서열번호 115 내지 205 중 어느 하나에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오타이드를 갖는 서열을 포함하는, 안티센스 올리고뉴클레오타이드.

청구항 39

제32항 내지 제38항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 서열번호 126, 서열번호 138 또는 서열번호 155에 제시된 서열 또는 서열번호 126, 서열번호 138 또는 서열번호 155에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오타이드를 포함하는 서열을 포함하는, 안티센스 올리고뉴클레오타이드.

청구항 40

제32항 내지 제35항 중 어느 한 항에 있어서, 상기 보유된 인트론이 인트론 2이고 상기 표적 영역이 인트론 2의 3' 스플라이스 부위에 대해 위치 -19 내지 -1, -21 내지 -3, -30 내지 -12, -31 내지 -13, -32 내지 -14, -62 내지 -44, -64 내지 -46, -67 내지 -49, -76 내지 -58, -77 내지 -59, -78 내지 -60, -79 내지 -61 -82 내지 -64, -83 내지 65, -84 내지 -66, -85 내지 -67, -86 내지 -68, -87 내지 -69, -95 내지 -77, -97 내지 -79, -100 내지 -82, -101 내지 -83, -102 내지 -84, -103 내지 -85, -107 내지 -89, -109 내지 -91, -111 내지 -93, -113 내지 -95, 및 -114 내지 -96에 걸쳐 있는, 안티센스 올리고뉴클레오타이드.

청구항 41

제40항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 서열번호 143 내지 205중 어느 하나에 제시된 서열 또는 서열번호 143 내지 205 중 어느 하나에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오타이드를 포함하는 서열을 포함하는, 안티센스 올리고뉴클레오타이드.

청구항 42

제32항 내지 제41항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드는 8 내지 50, 8 내지 40, 8 내지 35, 8 내지 30, 8 내지 25, 8 내지 20, 8 내지 15, 9 내지 50, 9 내지 40, 9 내지 35, 9 내지 30, 9 내지 25, 9 내지 20, 9 내지 15, 10 내지 50, 10 내지 40, 10 내지 35, 10 내지 30, 10 내지 25, 10 내지 20, 10 내지 15, 11 내지 50, 11 내지 40, 11 내지 35, 11 내지 30, 11 내지 25, 11 내지 20, 11 내지 15, 12 내지 50, 12 내지 40, 12 내지 35, 12 내지 30, 12 내지 25, 12 내지 20, 또는 12 내지 15개의 핵염기로 이루어지는, 안티센스 올리고뉴클레오타이드.

청구항 43

제32항 내지 제42항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 표적 영역에 적어도 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 또는 99% 상보적인, 안티센스 올리고뉴클레오타이드.

청구항 44

제36항 내지 제43항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 표적 영역에 100% 상보적인 적어도 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 또는 20개의 인접 핵염기를 포함하는, 안티센스 올리고뉴클레오타이드.

청구항 45

제36항 내지 제44항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 적어도 하나의 변형을 포함하는, 안티센스 올리고뉴클레오타이드.

청구항 46

제45항에 있어서, 상기 변형이 핵염기 변형, 올리고뉴클레오타이드 백본의 변형 또는 리보스 당의 변형인, 안티센스 올리고뉴클레오타이드.

청구항 47

제32항 내지 제46항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 2'-O-메틸(2OMe), 2'-O-메톡시-에틸(MOE), 잠금 핵산(LNA), 2'-플루오로 또는 S-제한된-에틸(cEt) 중에서 선택되는 변형된 당을 포함하는, 안티센스 올리고뉴클레오타이드.

청구항 48

제32항 내지 제47항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 포스포로티오에이트를 포함하는 백본을 포함하는, 안티센스 올리고뉴클레오타이드.

청구항 49

제32항 내지 제48항 중 어느 한 항에 있어서, 상기 안티센스 올리고뉴클레오타이드가 RNase H를 활성화시키는, 안

티센스 올리고뉴클레오타이드.

청구항 50

제32항 내지 제49항 중 어느 한 항의 안티센스 올리고뉴클레오타이드를 포함하는 조성물.

청구항 51

SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 장애의 치료를 위한 안티센스 올리고뉴클레오타이드의 용도로서, 여기서 상기 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시키고, 상기 보유된 인트론은 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24 중에서 선택되고, 상기 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA의 표적 영역에 상보적인 핵염기의 서열을 포함하는, 용도.

발명의 설명

기술 분야

[0001] 본 개시내용은 일반적으로 SCN2A에서 기능-상실 돌연변이와 연관된 장애를 치료하기에 적합한 조성물 및 방법에 관한 것이다. 더 구체적으로, 본 개시내용은 SCN2A에 특이적인 안티센스 올리고뉴클레오타이드 및 SCN2A의 이형접합 기능-상실 돌연변이와 연관된 장애를 치료하기 위한 이의 용도에 관한 것이다.

배경 기술

[0002] SCN2A 단백질(나트륨 전압-개폐 채널 알파 서브유닛 2, 뇌 Nav1.2 전압-개폐 나트륨 채널, SCN2A1, SCN2A2, Nav1.2, HBA, NAC2, BFIC3, BFIS3, BFNIS, HBSCI, EIEE11 또는 HBSCI이라고도 지칭됨)은 2q24.3에서 인간 염색체 2 상의 SCN2A 유전자에 의해 인코딩된다(HGNC:10588; NCBI 유전자:6326; NCBI 참조 서열: NG_008143.1). SCN2A는 활동 전위의 개시 및 전파와 신경 및 뇌 흥분성의 조절에 중요한 역할을 한다. SCN2A 발현은 뇌와 신경에서 검출되지만, 이의 발현은 주로 뇌에 있다.

[0003] 뇌에서 SCN2A는 이질적으로 발현되며, 돌연변이, 기능장애 및/또는 단백질 또는 기능성 단백질 수준의 조절장애는 다양한 신경발달 장애와 연관이 있다. 최근 몇 년 동안 수행된 대규모 시퀀싱 연구를 통해, SCN2A는 신경유전 장애의 주요 단일 유전자 원인으로 부상했다. SCN2A 돌연변이는 인간 집단에서 종종 유전성 간질(발달성 및 간질성 뇌병증(DEE)을 포함함)의 두 번째로 흔한 원인이며(Nakamura et al, 2013; Howell et al, 2015), 지적 장애(Rauch et al, 2012; Li et al, 2016), 자폐 스펙트럼 장애(Iossifov et al, 2014; Sanders et al, 2015; Wang et al, 2016) 및 정신분열증(Carroll et al, 2016)과도 자주 연관된다.

[0004] SCN2A 1차 전사체는 대체적으로 스플라이싱되어 다수의 mRNA 전사체 변이체를 생성하며, 이들 중 일부는 발생적으로 조절되는 것으로 알려져 있다. 일부 형태의 간질성 뇌병증은 SCN2A의 변이체와 연관이 있다. SCN2A에서의 기능-상실 돌연변이 또는 변경(예를 들어, 넌센스 돌연변이, 큰 결실 및 프레임시프트 돌연변이)은 기능성 단백질 수준을 감소시키거나 절단된 전사체를 형성하여 반수체기능부전을 야기할 수 있다. SCN2A 유전자는 대립유전자 중 하나 또는 둘 다에 미스센스 또는 넌센스 돌연변이를 가질 수 있다. 일부 경우에는 SCN2A의 발현이 1차 장애 또는 유전자 또는 조절 영역의 돌연변이에 의해 조절되지 않아 단백질 또는 기능성 단백질의 수준이 감소할 수 있다. 다른 경우에, SCN2A 수준은 변경되지 않지만 기능성 SCN2A 단백질 수준이 증가하면 신경 장애 또는 정신 장애에 대한 치료 효과를 제공한다.

[0005] SCN2A 또는 SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 임의의 다른 장애를 가진 환자에게 현재 이용가능한 유일한 요법은 장애의 증상을 치료하는 것, 예를 들어 간질 발작을 치료하기 위한 작용제 또는 개입(예를 들어, 언어 치료, 물리치료, 직업치료 등)을 통해 행동 또는 발달 증상, 또는 지적 장애를 치료하는 것이다. 결과적으로, MRD5 또는 SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 임의의 다른 장애를 치료하기 위한 작용제, 조성물 및 방법에 대한 필요성이 남아 있다.

발명의 내용

해결하려는 과제

과제의 해결 수단

- [0006] 본 개시내용은 적어도 부분적으로는 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24를 포함하는 다수의 인트론이 뇌 조직의 성숙한 SCN2A mRNA에 보유된다는 결정에 근거한다. 특히 인트론 2 및 17이 상대적으로 보유 비율이 높다.
- [0007] 인트론 보유는 인트론-보유 전사체를 넌센스-매개 붕괴로 지시하여 유전자 발현을 감소시키는 역할을 하는 유전자 조절의 한 형태이다(Kurosaki & Maquat, 2016, J Cell Sci. 129(3): 461-467). 인트론-보유 전사체는 또한 발현이 필요할 때마다 스플라이싱 및 번역을 겪는 RNA의 저장고 역할을 하는 것으로 나타났다(Jacob & Smith, 2017, Hum Genet. 136(9): 1043-1057). 1 내지 4 kb/분의 속도로 발생하는 전사 과정은 신경 자극에 따른 신경 활성화에 대해 특히 속도-제한적이다(Darzacq et al, 2007, Nat Struct. Mol. Biol. 14, 796-806). 대조적으로, 보유된 인트론의 스플라이싱은 훨씬 더 빠른 과정으로, 단 몇 초에서 몇 분이 소요된다(Bayer and Osheim, 1988, Genes Dev. 2, 754-765; Singh and Padgett, 2009, Nat. Struct. Mol. Biol. 16, 1128-1133). 결과적으로, 뉴런은 새로운 전사 및 번역과 비교하여 인트론 보유 및 후속 스플라이싱 및 번역을 사용하여 더 빠른 유전자 조절 모드를 달성할 수 있다. 인트론 보유는 핵에 보유되는 폴리아데닐화된 전사체 풀에서 발생하는 것으로 입증되었다. 신경 자극 후 인트론 절제를 받고 추가 처리를 위해 세포질로 이동하여 더 빠른 유전자 조절을 돕는다.
- [0008] 본원에서 입증된 바와 같이, SCN2A mRNA 또는 pre-mRNA(예를 들어, 폴리아데닐화된 SCN2A mRNA 또는 세포 핵 내의 pre-mRNA 전사체)에 보유된 인트론은 안티센스 올리고뉴클레오타이드로 표적화될 수 있어서, 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시켜 완전히 스플라이싱된 SCN2A mRNA의 양을 증가시킬 수 있다. 결과적으로, 본원에 제공된 안티센스 올리고뉴클레오타이드는 세포에 의해 생성된 SCN2A의 양을 증가시키는 데 유용하다. 따라서 본원에서 제공되는 안티센스 올리고뉴클레오타이드는 또한 SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 질병 또는 장애, 예를 들어 인간 집단에서 중증 유전성 간질(발달성 및 간질성 뇌병증(DEE)을 포함함)의 치료를 위한 치료제로서도 유용하며, 또한 지적 장애, 자폐 스펙트럼 장애 및 정신분열증과 자주 연관되며, SCN2A 단백질 수준을 증가시키면 치료 효과를 제공할 수 있다.
- [0009] 따라서, 일 양태에서, 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시키는 안티센스 올리고뉴클레오타이드와 세포를 접촉시키는 단계를 포함하여, 세포에서 SCN2A 단백질의 수준을 증가시키는 방법이 제공되며, 여기에서 보유된 인트론은 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24 중에서 선택되고, 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA의 표적 영역에 상보적인 핵염기의 서열을 포함한다.
- [0010] 일 구현예에서, 보유된 인트론은 서열번호 12에 제시된 뉴클레오타이드 서열을 포함하는 인트론 2이다.
- [0011] 또 다른 양태에서, 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시키는 안티센스 올리고뉴클레오타이드를 대상체에게 투여하는 단계를 포함하여, 대상체에서 SCN2A 단백질의 수준을 증가시키는 방법이 제공되며, 여기서 보유된 인트론은 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24 중에서 선택되고 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA의 표적 영역에 상보적인 핵염기의 서열을 포함한다. 일부 구현예에서, 대상체는 SCN2A에서 이형접합 기능-상실 돌연변이를 갖는다.
- [0012] 일부 구현예에서, 대상체는 유전성 간질(발달성 및 간질성 뇌병증(DEE)을 포함함), 지적 장애, 자폐 스펙트럼 장애 및 정신분열증과 같은 SCN2A에서의 이형접합 기능-상실 돌연변이와 연관된 장애를 갖는다.
- [0013] 또한, 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시키는 안티센스 올리고뉴클레오타이드를 대상체에게 투여하는 단계를 포함하여, SCN2A에서의 이형접합 기능-상실 돌연변이와 연관된 장애를 치료하는 방법이 제공되며, 여기에서 보유된 인트론은 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24 중에서 선택되고, 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA의 표적 영역에 상보적인 핵염기의 서열을 포함한다. 특정 예에서, 유전성 간질(발달성 및 간질성 뇌병증(DEE)을 포함함), 지적 장애, 자폐 스펙트럼 장애 및 정신분열증.
- [0014] 본 개시내용의 방법의 일부 구현예에서, 안티센스 올리고뉴클레오타이드는 인트론 스플라이싱 사일런서(ISS)에 결합하거나 이에 인접하고; G-사중체(quadruplex) 내의 뉴클레오타이드에 결합하거나; 또는 RNA 2차 구조를 갖는 뉴클레오타이드에 결합한다. ISS는 hnRNP A1 또는 hnRNP I과 같은 이종 핵 리보핵단백질(hnRNP, heterogeneous nuclear ribonucleoprotein)에 의해 인식될 수 있다.

- [0015] 일 예에서, 보유된 인트론은 인트론 2이고 ISS는 인트론 2의 5' 스플라이스 부위에 대해 위치 +8 내지 +12, +33 내지 +36 또는 +60 내지 +65에 있다. 선택적으로, ISS는 서열번호 12의 첫 번째 뉴클레오티드에 대해 위치 +8 내지 +12, +33 내지 +36 또는 +60 내지 +65에 있다.
- [0016] 특정 구현예에서, 보유된 인트론은 인트론 2이고 표적 영역은 인트론 2의 5' 스플라이스 부위에 대해, 선택적으로 서열번호 12의 첫 번째 뉴클레오티드에 대해 위치 -6 내지 +12, -5 내지 +13, -4 내지 +14, -3 내지 +15, -2 내지 +16, -1 내지 +19, 0 내지 +18, +1 내지 +19, +2 내지 +20, +3 내지 21, +4 내지 22, +5 내지 +23, +6 내지 +24, +7 내지 +25, +8 내지 26, +9 내지 +27, +10 내지 +28, +11 내지 +29, +12 내지 +30, +13 내지 +31, +14 내지 +32, +15 내지 +33, +16 내지 +34, +17 내지 +35, +18 내지 +36, +19 내지 +37, +20 내지 +38, +21 내지 +39, +22 내지 +40, +23 내지 +41, +24 내지 +42, +25 내지 +43, +26 내지 +44, +27 내지 +45, +28 내지 +46, +29 내지 +47, +30 내지 +48, +31 내지 +49, +32 내지 +50, +33 내지 +51, +34 내지 +52, +35 내지 +53, +36 내지 +54, +37 내지 +55, +38 내지 +56, +39 내지 +57, +40 내지 +58, +41 내지 +59, +42 내지 +60, +43 내지 +61, +44 내지 +62, +45 내지 +63, +46 내지 +64, +47 내지 +65, +48 내지 +66, +49 내지 +67, +50 내지 +68, +51 내지 +69, +52 내지 +70, +53 내지 +71, +54 내지 +72, +55 내지 +73, +56 내지 +74, +57 내지 +75, +58 내지 +76, +59 내지 +77, +60 내지 +78, +61 내지 +79, +62 내지 +80, +63 내지 +81, +64 내지 +82, +65 내지 +83, +66 내지 +84, +67 내지 +85, +68 내지 +86, +69 내지 +87, +70 내지 +88, +71 내지 +89, +72 내지 +90, +73 내지 +91, +74 내지 +92, +75 내지 +93, +76 내지 +94, +77 내지 +95, +78 내지 +96, +79 내지 +97, +80 내지 +98, +81 내지 +99에 걸쳐 있다. 일부 구현예에서, 안티센스 올리고뉴클레오티드는 서열번호 115 내지 142 중 어느 하나에 제시된 서열에 대해 적어도 또는 약 70%, 80%, 또는 90% 서열 동일성을 갖는 서열, 또는 서열번호 115 내지 142 중 어느 하나에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오티드를 갖는 서열을 포함한다. 일 구현예에서, 안티센스 올리고뉴클레오티드는 서열번호 126 또는 서열번호 138에 제시된 서열, 또는 서열번호 126 또는 서열번호 138에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오티드를 포함하는 서열을 포함한다.
- [0017] 추가 구현예에서, 보유된 인트론은 인트론 2이고 표적 영역은 인트론 2의 3' 스플라이스 부위에 대해, 선택적으로 서열번호 12의 마지막 뉴클레오티드에 대해 위치 -19 내지 -1, -20 내지 -2, -21 내지 -3, -22 내지 -4, -23 내지 -5, -24 내지 -6, -25 내지 -7, -26 내지 -8, -27 내지 -9, -28 내지 -10, -29 내지 -11, -30 내지 -12, -31 내지 -13, -32 내지 -14, -33 내지 -15, -38 내지 -20, -39 내지 -21, -48 내지 -30, -49 내지 -31, -51 내지 -33, -52 내지 -34, -53 내지 -35, -54 내지 -36, -62 내지 -44, -64 내지 -46, -65 내지 -47, -66 내지 -48, -67 내지 -49, -76 내지 -58, -77 내지 -59, -78 내지 -60, -79 내지 -61, -82 내지 -64, -83 내지 65, -84 내지 -66, -85 내지 -67, -86 내지 -68, -87 내지 -69, -90 내지 -72, -91 내지 -73, -92 내지 -74, -95 내지 -77, -97 내지 -79, -98 내지 -80, -99 내지 -81, -101 내지 -83, -102 내지 -84, -103 내지 -85, -104 내지 -86, -105 내지 -87, -106 내지 -88, -107 내지 -89, -108 내지 -90, -109 내지 -91, -110 내지 -92, -111 내지 -93, -112 내지 -94, -113 내지 -95, -114 내지 -96, -115 내지 -97, -116 내지 -98, -117 내지 -99에 걸쳐 있다. 일부 구현예에서, 안티센스 올리고뉴클레오티드는 서열번호 143 내지 205 중 어느 하나에 제시된 서열에 대해 적어도 또는 약 70%, 80%, 또는 90% 서열 동일성을 갖는 서열, 또는 서열번호 143 내지 205 중 어느 하나에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오티드를 갖는 서열을 포함한다. 일 구현예에서, 안티센스 올리고뉴클레오티드는 서열번호 155에 제시된 서열, 또는 서열번호 155에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오티드를 포함하는 서열을 포함한다.
- [0018] 본 개시내용의 방법에 있어서, 상기 안티센스 올리고뉴클레오티드는, 예를 들어 8 내지 50, 8 내지 40, 8 내지 35, 8 내지 30, 8 내지 25, 8 내지 20, 8 내지 15, 9 내지 50, 9 내지 40, 9 내지 35, 9 내지 30, 9 내지 25, 9 내지 20, 9 내지 15, 10 내지 50, 10 내지 40, 10 내지 35, 10 내지 30, 10 내지 25, 10 내지 20, 10 내지 15, 11 내지 50, 11 내지 40, 11 내지 35, 11 내지 30, 11 내지 25, 11 내지 20, 11 내지 15, 12 내지 50, 12 내지 40, 12 내지 35, 12 내지 30, 12 내지 25, 12 내지 20 또는 12 내지 15개의 핵염기로 이루어질 수 있다. 일부 구현예에서, 안티센스 올리고뉴클레오티드는 표적 영역에 적어도 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% 또는 99% 상보적이다. 특정 구현예에서, 안티센스 올리고뉴클레오티드는 표적 영역에 100% 상보적인 적어도 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 또는 20개의 연속 핵염기를 포함한다.
- [0019] 본 개시내용의 방법에서 사용되는 안티센스 올리고뉴클레오티드는 적어도 하나의 변형, 예를 들어 핵염기 변형, 올리고뉴클레오티드 백본의 변형 또는 리보스 당의 변형을 포함할 수 있다. 일 구현예에서, 안티센스 올리고뉴

클레오티드는 2'-O-메틸(2OMe), 2'-O-메톡시-에틸(MOE), 잠금 핵산(LNA), 2'-플루오로 또는 S-제약된-에틸(cEt)로부터 선택되는 변형된 당을 포함한다. 추가 구현예에서, 안티센스 올리고뉴클레오티드는 포스포로티오에이트를 포함하는 백본을 포함한다.

- [0020] 안티센스 올리고뉴클레오티드를 대상체에게 투여하는 단계를 포함하는 본 개시내용의 방법에서, 대상체는 먼저 SCN2A에서 이형접합 기능-상실 돌연변이를 갖는 것으로 결정될 수 있다. 특정 구현예에서, 대상체는 SCN2A에서 이형접합 기능-상실 돌연변이를 식별하기 위해 유전자형이 결정되었다. 안티센스 올리고뉴클레오티드는 비경구 투여(예를 들어, 피하 투여, 정맥내 투여, 근육내 투여, 동맥내 투여, 복강내 투여 또는 두개내 투여) 또는 비강내 투여(예를 들어, 척수강내 또는 뇌실내 투여)에 의해 대상체에게 투여될 수 있다. 일부 구현예에서, 안티센스 올리고뉴클레오티드 또는 조성물은 대상체에게 약 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12개월 또는 그 이상마다 투여된다.
- [0021] 추가 양태에서, 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 표적 영역에 상보적인 핵염기의 서열을 포함하는 안티센스 올리고뉴클레오티드가 본원에 제공되며, 여기서 표적 영역은 보유한 인트론에 있고 여기서 보유한 인트론은 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24 중에서 선택된다.
- [0022] 일부 구현예에서, 안티센스 올리고뉴클레오티드는 인트론 스플라이싱 사일런서(ISS)에 결합하거나 이에 인접하거나; G-사중체 내의 뉴클레오티드에 결합하거나; RNA 2차 구조를 갖는 뉴클레오티드에 결합한다. 특정 구현예에서, ISS는 이중 핵 리보핵단백질(hnRNP), 예를 들어 hnRNP A1 또는 hnRNP I에 의해 인식된다.
- [0023] 일 구현예에서, 표적 영역이 존재하는 보유한 인트론은 인트론 2이고 ISS는 인트론 2의 5' 스플라이스 부위에 대해 위치 +8 내지 +12, +33 내지 +36 또는 +60 내지 +65에 있다. 선택적으로, ISS는 서열번호 12의 첫 번째 뉴클레오티드에 대해 +8 내지 +12, +33 내지 +36 또는 +60 내지 +65에 있다.
- [0024] 특정 구현예에서, 보유한 인트론은 인트론 2이고 표적 영역은 인트론 2의 5' 스플라이스 부위에 대해, 선택적으로 서열번호 12의 첫 번째 뉴클레오티드에 대해 위치 -4 내지 +14, -3 내지 +15, -2 내지 +16, +6 내지 +24, +7 내지 +25, +8 내지 26, +9 내지 +27, +10 내지 +28, +14 내지 +32, +19 내지 +37, +20 내지 +38, +21 내지 +39, +22 내지 +40, +23 내지 +41, +24 내지 +42, +25 내지 +43, +26 내지 +44, +27 내지 +45, +28 내지 +46, +29 내지 +47, +30 내지 +48, +31 내지 +49, +32 내지 +50, +33 내지 +51, +34 내지 +52, +35 내지 +53, +43 내지 +61, +45 내지 +63, +46 내지 +64, +49 내지 +67, +52 내지 +70, +59 내지 +77, +64 내지 +82, +65 내지 +83, +68 내지 +86, +69 내지 +87, +70 내지 +88, +71 내지 +89, +72 내지 +90 및 +73 내지 +91에 걸쳐 있다. 일부 구현예에서, 안티센스 올리고뉴클레오티드는 서열번호 115 내지 142 중 어느 하나에 제시된 서열에 대해 적어도 또는 약 70%, 80%, 또는 90% 서열 동일성을 갖는 서열, 또는 서열번호 115 내지 142 중 어느 하나에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오티드를 갖는 서열을 포함한다. 일 구현예에서, 안티센스 올리고뉴클레오티드는 서열번호 126 또는 서열번호 138에 제시된 서열, 또는 서열번호 126 또는 서열번호 138에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오티드를 포함하는 서열을 포함한다.
- [0025] 추가 구현예에서, 보유한 인트론은 인트론 2이고 표적 영역은 인트론 2의 3' 스플라이스 부위에 대해, 선택적으로 서열번호 12의 마지막 뉴클레오티드에 대해 위치 -19 내지 -1, -21 내지 -3, -30 내지 -12, -31 내지 -13, -32 내지 -14, -62 내지 -44, -64 내지 -46, -67 내지 -49, -76 내지 -58, -77 내지 -59, -78 내지 -60, -79 내지 -61 -82 내지 -64, -83 내지 65, -84 내지 -66, -85 내지 -67, -86 내지 -68, -87 내지 -69, -95 내지 -77, -97 내지 -79, -100 내지 -82, -101 내지 -83, -102 내지 -84, -103 내지 -85, -107 내지 -89, -109 내지 -91, -111 내지 -93, -113 내지 -95, 및 -114 내지 -96에 걸쳐 있다. 일부 구현예에서, 안티센스 올리고뉴클레오티드는 서열번호 143 내지 205 중 어느 하나에 제시된 서열에 대해 적어도 또는 약 70%, 80%, 또는 90% 서열 동일성을 갖는 서열, 또는 서열번호 143 내지 205 중 어느 하나에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오티드를 갖는 서열을 포함한다. 일 구현예에서, 안티센스 올리고뉴클레오티드는 서열번호 155에 제시된 서열, 또는 서열번호 155에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오티드를 포함하는 서열을 포함한다.
- [0026] 일부 구현예에서, 상기 안티센스 올리고뉴클레오티드는, 예를 들어 8 내지 50, 8 내지 40, 8 내지 35, 8 내지 30, 8 내지 25, 8 내지 20, 8 내지 15, 9 내지 50, 9 내지 40, 9 내지 35, 9 내지 30, 9 내지 25, 9 내지 20, 9 내지 15, 10 내지 50, 10 내지 40, 10 내지 35, 10 내지 30, 10 내지 25, 10 내지 20, 10 내지 15, 11 내지 50, 11 내지 40, 11 내지 35, 11 내지 30, 11 내지 25, 11 내지 20, 11 내지 15, 12 내지 50, 12 내지 40, 12 내지 35, 12 내지 30, 12 내지 25, 12 내지 20 또는 12 내지 15개의 핵염기로 이루어질 수 있다. 일부 구현예에

서, 안티센스 올리고뉴클레오타이드는 표적 영역에 적어도 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% 또는 99% 상보적이다. 특정 구현예에서, 안티센스 올리고뉴클레오타이드는 표적 영역에 100% 상보적인 적어도 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 또는 20개의 연속 핵염기를 포함한다.

[0027] 안티센스 올리고뉴클레오타이드는 적어도 하나의 변형, 예를 들어 핵염기 변형, 올리고뉴클레오타이드 백본의 변형 또는 리보스 당의 변형을 포함할 수 있다. 일 구현예에서, 안티센스 올리고뉴클레오타이드는 2'-O-메틸(2OMe), 2'-O-메톡시-에틸(MOE), 잠금 핵산(LNA), 2'-플루오로 또는 S-제약된-에틸(cEt)로부터 선택되는 변형된 당을 포함한다. 추가 구현예에서, 안티센스 올리고뉴클레오타이드는 포스포로티오에이트를 포함하는 백본을 포함한다.

[0028] 또한 본 개시내용의 안티센스 올리고뉴클레오타이드를 포함하는 조성물, 예를 들어 약제학적 조성물이 제공된다.

[0029] 추가 양태에서, SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 장애의 치료를 위한 안티센스 올리고뉴클레오타이드의 용도가 제공되며, 여기서 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시키고, 보유된 인트론은 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24 중에서 선택되고 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA의 표적 영역에 상보적인 핵염기의 서열을 포함한다.

[0030] 또 다른 양태에서, SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 장애의 치료를 위한 안티센스 올리고뉴클레오타이드의 용도가 제공되며, 여기서 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시키고, 여기서 보유된 인트론은 인트론 2 및 17 중에서 선택되며, 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA의 표적 영역에 상보적인 핵염기의 서열을 포함한다.

도면의 간단한 설명

[0031] 도 1은 IRBase로부터 얻은 정보로부터 분석된 바와 같은, SCN2A에서의 인트론 보유의 도식적 표현이다. 상단 패널은 UCSC 브라우저에서 SCN2A 유전자의 게놈 지도를 나타낸다. 가는 선은 인트론을 나타내고 두꺼운 선/블록은 엑손에 해당한다. 하단 패널은 게놈 지도에서 인트론에 해당하는 인트론-보유 이벤트를 나타낸다. 막대의 높이는 기록된 이벤트의 수를 나타낸다.

도 2는 SCN2A 서열에 걸친 각각의 엑손-인트론 쌍에 대한 프라이머의 설계를 나타내는 개략도이다. A. 인트론-보유 전사체에 특이적인 프라이머: 정방향 프라이머는 앞선 엑손의 서열로부터 설계하고 역방향 프라이머는 엑손에 대해 하류의 인트론 서열로부터 설계하였다. B. 스플라이싱된 전사체에 특이적인 프라이머: 프라이머 중 하나는 인접한 2개의 엑손의 연결부에 걸쳐 있도록 설계되었고, 다른 하나는 그에 따라 선행 또는 후속 엑손의 서열로부터 설계되었다.

도 3은 2개의 상업적 공급원으로부터 얻은 전체 뇌 SCN2A mRNA에서 인트론의 상대적인 발현을 나타낸다. 전체 전사체에 걸친 개별 인트론의 발현을 평균 엑손 발현과 비교하였다. 결과는 표시된 평균의 표준 오차와 함께 세 가지 실험을 나타낸다. a. 출처 1인 Ambion의 mRNA. b. 출처 2인 Takara의 mRNA.

도 4는 세포주로부터의 SCN2A mRNA에서 인트론의 상대적인 발현을 나타낸다. 전체 전사체에 걸친 개별 인트론의 발현을 평균 엑손 발현과 비교하였다. 결과는 표시된 평균의 표준 오차와 함께 세 가지 실험을 나타낸다. a. SH-SY5Y 세포로부터의 mRNA. b. SK-N-AS 세포로부터의 mRNA.

도 5는 SCN2A 인트론 2의 2차 구조 예측의 개략도이다.

도 6은 안티센스 올리고뉴클레오타이드의 존재 하에 SCN2A 전사체 발현의 조절을 도식적 및 개략적으로 나타낸 것이다. 형질주입 및 인큐베이션 후, SCN2A의 발현을 qPCR로 분석하였다. 모의 형질주입된 세포를 음성 대조군으로 사용하였다. 하우스키핑 유전자 HPRT1을 정규화에 사용했다. 생물학적 복제 수의 범위는 3 내지 6이다. 빨간색과 녹색으로 강조 표시된 영역은 각각 HnRNP1 및 HnRNP1에 대한 예상 결합 부위이다. a. 이의 발현에 대한 SCN2A 인트론 2의 5' 말단을 표적으로 하는 안티센스 올리고뉴클레오타이드의 효과. b. 이의 발현에 대한 SCN2A 인트론 2의 3' 말단을 표적으로 하는 안티센스 올리고뉴클레오타이드의 효과.

도 7은 엑손 2 및 3(A), 및 엑손 18 및 19(B)의 엑손에서 엑손 경계까지 교차하는 두 세트의 프라이머를 사용하여 SCN2A의 발현에 대한 안티센스 올리고뉴클레오타이드 INT2 +6(서열번호 126; 인트론 2의 5' 하류의 6개 염기쌍 영역을 표적화함)의 효과를 나타낸다. 이와 비교하여, 안티센스 올리고뉴클레오타이드 INT2 +35(서열번호 142; 인

트론 2의 5' 하류의 35개 염기쌍 영역을 표적화함)는 SCN2A 전사체를 증가시키지 않는다. 모의 형질주입된 세포를 음성 대조군으로 사용하였다.

발명을 실시하기 위한 구체적인 내용

- [0032] 달리 정의되지 않는 한, 본원에 사용된 모든 기술 및 과학 용어는 개시내용이 속하는 기술 분야의 당업자에 의해 일반적으로 이해되는 것과 동일한 의미를 갖는다. 모든 특허, 특허 출원, 공개된 출원 및 간행물, 데이터베이스, 웹사이트 및 전체 개시내용에 전반에 걸쳐 언급된 기타 공개된 자료는 달리 언급되지 않는 한 전체가 참조로 포함된다. 용어에 대한 복수의 정의가 있는 경우 이 섹션의 정의가 우선한다. URL 또는 기타 그러한 식별자 또는 주소에 대한 언급이 있는 경우, 그러한 식별자는 변경될 수 있고 인터넷의 특정 정보가 변할 수 있지만 인터넷을 검색하여 동등한 정보를 찾을 수 있음을 이해한다. 식별자에 대한 참조는 해당 정보의 가용성 및 대중 보급을 증명한다.
- [0033] 본원에서 사용되는 단수 형태("a", "an" 및 "the")는 또한 문맥이 명백히 다르게 지시하지 않는 한 복수 양태(즉, 적어도 하나 또는 하나 초과)를 포함한다. 따라서, 예를 들어 "폴리펩티드"에 대한 언급은 단일 폴리펩티드뿐만 아니라 2개 이상의 폴리펩티드를 포함한다.
- [0034] 본 명세서의 맥락에서, 용어 "약"은 당업자가 동일한 기능 또는 결과를 달성하는 맥락에서 인용된 값과 동등하다고 간주할 수 있는 숫자의 범위를 지칭하는 것으로 이해된다.
- [0035] 문맥상 달리 요구되지 않는 한, 하기 본 명세서 및 청구범위 전체에 걸쳐, 단어 "포함하다", 및 "포함한" 및 "포함하는"과 같은 변형은 명시된 정수 또는 단계 또는 정수 또는 단계의 군을 포함하지만 다른 정수 또는 단계 또는 정수 또는 단계의 군을 제외하지 않음을 의미하는 것으로 이해될 것이다.
- [0036] "안티센스 올리고뉴클레오타이드"는 표적 핵산의 상응하는 영역 또는 분절에 대한 혼성화를 허용하는 서열을 갖는 단일-가닥 올리고뉴클레오타이드를 지칭한다. 안티센스 올리고뉴클레오타이드에 대한 언급은 변형되지 않은 안티센스 올리고뉴클레오타이드와 변형된 안티센스 올리고뉴클레오타이드 둘 다에 대한 언급을 포함하며, 여기서 변형된 안티센스 올리고뉴클레오타이드는 적어도 하나의 변형된 뉴클레오타이드 및/또는 변형된 뉴클레오타이드간 연결을 포함한다.
- [0037] 본원에 사용된 "상보적"은 안티센스 올리고뉴클레오타이드에서 핵염기 및 SCN2A mRNA 또는 pre-mRNA의 핵염기 사이와 같은 두 개의 핵염기 사이의 정확한 쌍형성 능력을 지칭한다. 안티센스 올리고뉴클레오타이드 및 mRNA 또는 pre-mRNA는 각각의 분자에서 충분한 수의 상응하는 위치가 서로 수소 결합할 수 있는 핵염기에 의해 점유될 때 서로 상보적이다. 따라서, "상보적인"은 안티센스 올리고뉴클레오타이드 및 mRNA 또는 pre-mRNA 사이에 안정적인 결합이 발생하도록 충분한 수의 뉴클레오타이드에 대한 충분한 정도의 정확한 쌍형성을 나타내는 데 사용된다. 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA의 표적 영역에 혼성화하기 위해 100% 상보적일 필요는 없다는 것이 이해된다. 또한, 올리고뉴클레오타이드는 개재 또는 인접 분절이 혼성화 이벤트에 관여하지 않도록 하나 이상의 분절에 걸쳐 혼성화되거나, 이에 상보적일 수 있다. 따라서 본원에서 사용되는 "상보적인"은 적어도 또는 약 70%, 75%, 80%, 85%, 90% 또는 95% 서열 상보성과 같이 100% 미만의 상보성에 대한 언급을 포함한다.
- [0038] 본원에 사용된 바와 같이, "SCN2A에서 기능-상실 돌연변이와 연관된 장애"는 SCN2A에서의 돌연변이와 연관되거나, 부분적으로 또는 완전히 이에 의해 유발되거나, 부분적으로 또는 완전히 이에 의해 유발되는 하나 이상의 증상을 갖는 장애를 지칭하며, 이는 기능 상실 표현형, 즉 SCN2A의 수준(또는 양) 또는 활성 감소를 초래한다.
- [0039] 본원에서 사용되는 "SCN2A의 발현"은 SCN2A로부터의 mRNA의 전사 또는 SCN2A mRNA로부터의 단백질의 번역을 지칭한다. SCN2A 발현은 노던 블롯, 웨스턴 블롯 및 qRT-PCR을 포함하나 이에 제한되지 않는 당업계에 공지된 임의의 방법을 사용하여 평가될 수 있다.
- [0040] 본원에서 사용되는 "기능-상실 돌연변이"는 인코딩된 SCN2A 단백질의 발현 및/또는 활성을 감소시키는 SCN2A에서의 돌연변이이다. 인코딩된 SCN2A 단백질의 발현은 웨스턴 블롯과 같은 표준 검정을 사용하여 평가될 수 있다. 통상적으로, 기능-상실 돌연변이는 인코딩된 SCN2A 단백질의 발현 및/또는 활성의 적어도 또는 약 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% 또는 그 이상의 감소를 초래한다. 일부 예에서, 예를 들어 기능-상실 돌연변이가 번역되지 않거나 비-기능적 단백질로 번역되는 잘린 전사체의 형성을 초래하는 돌연변이(예를 들어, 넌센스 돌연변이, 큰 결실 또는 프레임시프트 돌연변이)인 경우, 기능-상실 돌연변이는 인코딩된 SCN2A 단백질의 발현 또는 활성의 완전한(즉, 100%) 상실을 초래한다. SCN2A에서 "이종접합 기능-상실 돌연변이"는 세포 내

SCN2A의 한 카피에만 존재하는 돌연변이(즉, 하나의 대립유전자는 야생형 대립유전자임)이며 반수체기능부전을 야기할 수 있다.

- [0041] 본원에서 지칭되는 "갭머(gapmer)"는 RNase H 절단을 지원하는 복수의 뉴클레오타이드를 갖는 내부 영역이 하나 이상의 뉴클레오타이드를 갖는 외부 영역 사이에 위치하는 키메라 안티센스 올리고뉴클레오타이드이며, 여기서 내부 영역을 포함하는 뉴클레오타이드는 외부 영역을 포함하는 뉴클레오타이드 또는 뉴클레오타이드와 화학적으로 구별된다.
- [0042] 본원에 사용된 바와 같이, "혼성화" 또는 "결합" 또는 이의 문법적 변형은 본 개시내용의 안티센스 올리고뉴클레오타이드 및 SCN2A mRNA 또는 pre-mRNA 사이에서와 같이 실질적으로 상보적인 핵산 가닥의 쌍을 형성하는 것을 의미한다. 쌍형성의 한 메커니즘은 핵산 가닥의 상보적 핵염기 사이의 수소 결합을 포함하는데, 이는 왓슨-크릭(Watson-Crick), 후그스틴(Hoogsteen) 또는 역 후그스틴(Hoogsteen) 수소 결합일 수 있다. 예를 들어, 아데닌과 티민 또는 우라실은 수소 결합 형성을 통해 쌍을 형성하는 상보적인 뉴클레오타이드이다. 혼성화는 다양한 상황에서 발생할 수 있다. 본원에 사용된 "혼성화하다" 또는 "결합하다"에 대한 언급은 안티센스 올리고뉴클레오타이드가 안티센스 올리고뉴클레오타이드 및 표적 영역 사이의 서열 상보성에 의해 SCN2A mRNA 또는 pre-mRNA의 표적 영역에 혼성화하거나 결합하고, 비표적 영역에 크게 결합하지 않는 것을 의미한다.
- [0043] 용어 "연결된" 및 "부착된"은 상호교환적으로 사용되며 안티센스 올리고뉴클레오타이드 및 모이어티(예를 들어, 세포 투과 펩티드)와 같은 2개의 실체를 연결하는 임의의 유형의 상호작용에 관한 것이며, 공유 결합 또는 비-공유 결합, 예를 들어 소수성/친수성 상호작용, 반 데르 발스 힘, 이온 결합 또는 수소 결합을 포함한다.
- [0044] 용어 "엑손"은 성숙한 형태의 mRNA에 존재하는 유전자의 일부를 지칭한다. 엑손에는 5' 및 3' UTR(비번역 영역)뿐만 아니라 단백질을 인코딩하는 서열인 ORF(열린 해독틀)가 포함된다. UTR은 단백질 번역에 중요하다. 알고리즘 및 컴퓨터 프로그램은 DNA 서열에서 엑손을 예측하는 데 사용될 수 있다(예를 들어, 엑손-인트론 접합부를 결정하기 위한 Grail, Grail 2 및 Genscan, 및 US 20040219522).
- [0045] 용어 "인트론"은 야생형 단백질로 번역되지 않는 유전자의 일부를 지칭하며, 게놈 DNA 및 pre-mRNA에 존재하지만 일반적으로 스플라이싱에 의해 성숙한 mRNA의 형성에서 제거된다.
- [0046] 용어 "메신저 RNA" 또는 "mRNA"는 게놈 DNA로부터 전사되고 단백질 합성을 위한 코딩 서열을 보유하는 RNA를 지칭한다. 용어 "전구체 mRNA" 또는 "pre-mRNA"는 하나 이상의 인트론을 함유하고 DNA로부터 직접 전사되는 메신저 리보핵산(mRNA)의 미성숙 단일 가닥을 지칭하며; 본 개시내용의 목적을 위해, 폴리(A)가 첨가되고 5' 및 3' 변형이 일어날 때까지는 pre-mRNA로 간주된다. pre-mRNA는 세포핵의 DNA 주형에서 RNA 중합효소에 의해 전사되며 인트론과 엑손의 교대 서열로 구성된다. 진핵생물에서 pre-mRNA는 mRNA로 가공되며, 이는 인트론 제거, 즉 "스플라이싱", 및 5' 및 3' 말단에 대한 변형(예를 들어, 폴리아데닐화)을 포함한다. mRNA는 통상적으로 5'에서 3'으로; 5' 캡(변형 구아닌 뉴클레오타이드), 5' UTR(번역되지 않은 영역), 코딩 서열(시작 코돈으로 시작하여 정지 코돈으로 끝남), 3' UTR 및 폴리(A) 테일을 포함한다. 진핵 세포의 pre-mRNA는 mRNA로 가공되기 전에 일시적으로만 존재한다. 본원에 기재된 바와 같이, 세포의 핵에서 폴리아데닐화된 전사체는 1차 전사체의 초기 스플라이싱 및 폴리(A) 테일의 추가 후에도 하나 이상의 보유된 인트론을 가질 수 있다. 본 개시내용의 목적을 위해, 이들 전사체는 보유된 인트론을 갖는 mRNA로 간주된다. pre-mRNA가 mRNA로 적절하게 가공되면, 핵 밖으로 내보내지고 세포질의 리보솜에 의해 단백질로 번역된다. 본원에서 사용되는 용어 "완전히 스플라이싱된 mRNA"는 mRNA가 어떠한 인트론도 포함하지 않거나 본 개시내용에 따른 안티센스 올리고뉴클레오타이드 및 방법에 의해 표적이 되는 인트론을 함유하지 않는다는 것을 의미한다.
- [0047] 본원에서 사용되는 "핵염기"는 또 다른 핵산의 염기와 쌍을 형성할 수 있는 헤테로사이클릭 모이어티를 의미하고, 예를 들어 아데닌(A), 구아닌(G), 시토신(C), 티민(T) 및 우라실(U)을 포함한다. 본원에서 핵염기에 대한 언급은 또한 변형된 핵염기에 대한 언급을 포함한다.
- [0048] 본원에서 사용되는 "뉴클레오타이드"는 당에 연결된 핵염기를 지칭한다. 본원에서 뉴클레오타이드에 대한 언급은 또한 변형된 당 모이어티 또는 변형된 핵염기를 갖는 변형된 뉴클레오타이드에 대한 언급을 포함한다. "뉴클레오타이드 모방체"는 당 또는 당 및 염기를 대체하는 데 사용되는 구조를 포함하고, 반드시 올리고머 화합물의 하나 이상의 위치에서의 연결을 포함하는 것이 아니며, 예를 들어 모르폴리노, 사이클로헥세닐, 사이클로헥실, 테트라하이드로피라닐, 바이사이클로 또는 트리사이클로 당 모방체, 예를 들어 비 푸라노스 당 단위를 갖는 뉴클레오타이드 모방체를 포함한다.
- [0049] 본원에서 사용되는 "뉴클레오타이드"는 뉴클레오타이드의 당 부분에 공유적으로 연결된 포스페이트 기를 갖는 뉴클

레오시드를 지칭한다. 본원에서 뉴클레오티드에 대한 언급은 또한 변형된 당 모이어티, 변형된 뉴클레오시드간 연결 또는 변형된 핵염기를 갖는 변형된 뉴클레오티드에 대한 언급을 포함한다. "뉴클레오티드 모방체"는, 예를 들어 펩티드 핵산 또는 모르폴리노(-N(H)-C(=O)-O- 또는 기타 비-포스포디에스테르 연결에 의해 연결된 모르폴리노)와 같은 올리고머 화합물의 하나 이상의 위치에서 뉴클레오시드 및 연결을 대체하는 데 사용되는 구조를 포함한다.

[0050] 용어 "스플라이싱"은 인트론이 제거되고 엑손이 연결되는 전사 후 pre-mRNA의 변형을 지칭한다. pre-mRNA 스플라이싱에는 두 가지 순차적인 생화학 반응이 포함된다. 두 반응 모두 RNA 뉴클레오티드 사이의 스플라이스좀에 스테르코환(spliceosomal transesterification)을 포함한다. 첫 번째 반응에서, 인트론 내의 특정 분기점 뉴클레오티드의 2'-OH는 스플라이스좀 조립 중에 정의되며, 5' 스플라이스 부위에서 인트론의 첫 번째 뉴클레오티드에 대해 친핵성 공격을 수행하여 래리어트(lariat) 중간체를 형성한다. 두 번째 반응에서, 방출된 5' 엑손의 3'-OH는 3' 스플라이스 부위에 있는 인트론의 마지막 뉴클레오티드에서 친핵성 공격을 수행하여 엑손을 결합하고 인트론 래리어트를 방출한다.

[0051] 본원에서 사용되는 용어 "서열 동일성" 또는 "% 동일" 또는 문법적 변형은 특정 영역에 대한 두 서열의 비교에서 두 서열이 동일한 위치에서 동일한 잔기의 특정 수 또는 백분율을 갖는다는 것을 의미한다. 서열은 당업자에게 공지된 임의의 방법에 의해 정렬될 수 있다. 이러한 방법은 통상적으로 일치율을 최대화하고 수동 정렬 사용 및 사용가능한 수많은 정렬 프로그램 사용과 같은 방법을 포함한다.

[0052] 용어 "스플라이스 부위"는 pre-mRNA 분자에서 엑손과 인트론 사이의 접합부("스플라이스 접합부"로도 알려져 있음)를 지칭한다. "스플라이스 부위 서열"은 세포의 스플라이싱 기구에 의해 인식될 수 있는 스플라이스 부위를 둘러싸는 서열이다. 5' 스플라이스 부위(스플라이스 공여체 부위로도 지칭됨)는 인트론의 시작과 이전 엑손 서열과의 경계를 표시하는 인트론의 5' 말단에 있는 스플라이스 부위이다. 3' 스플라이스 부위(스플라이스 수용체 부위로도 지칭됨)는 인트론의 말단과 다음 엑손 서열과의 경계를 표시하는 인트론의 3' 말단에 있는 스플라이스 부위이다. 따라서 인트론의 5' 스플라이스 부위와 관련하여 본원에서 사용된 넘버링은 또한 인트론의 첫 번째 뉴클레오티드를 기준으로 한다. 따라서, 예를 들어, 인트론의 5' 스플라이스 부위에 대한 위치 +1에 대한 언급은 인트론 서열의 첫 번째 뉴클레오티드에 대한 언급이고, 예를 들어 인트론 2의 5' 스플라이스 부위에 대한 위치 +1에 대한 언급은 인트론 2 서열의 뉴클레오티드 위치 1, 예를 들어 서열번호 12의 위치 1에 대한 언급이다. 또 다른 예에서, 인트론의 5' 스플라이스 부위에 대한 위치 +18 내지 +27에 대한 언급은 인트론 서열의 18번째에서 27번째까지 뉴클레오티드 위치에 대한 언급, 예를 들어 서열번호 12에 제시된 인트론 2의 위치 18에서 위치 27까지에 대한 언급이다. 인트론의 5' 스플라이스 부위에 대한 위치 -1에 대한 언급은 인트론 서열에 대한 상류의 첫 번째 뉴클레오티드에 대한 언급이고, 예를 들어 인트론 2의 5' 스플라이스 부위에 대한 위치 -1에 대한 언급은 인트론 2 서열 상류의 뉴클레오티드 위치 1, 즉 인접한 엑손의 마지막 뉴클레오티드에 대한 언급이다. 또 다른 예에서, 인트론 2 서열의 3' 스플라이스 부위에 대한 위치 -1에 대한 언급은 인트론 2의 최종 뉴클레오티드, 예를 들어 서열번호 12의 최종 뉴클레오티드에 대한 언급이다. 또 다른 예에서, 인트론의 3' 스플라이스 부위에 대한 위치 -10 내지 -1에 대한 언급은 3' 스플라이스 부위 상류의 10번째에서 1번째까지 뉴클레오티드 위치 또는 인트론의 최종 뉴클레오티드 10개 뉴클레오티드에 대한 언급이다.

[0053] 본원에서 사용되는 용어 "치료하는" 또는 "치료"는 병태 또는 증상을 치료하거나, 병태 또는 질병의 확립을 예방하거나, 달리 어떤 식으로든 병태나 질병 또는 기타 바람직하지 않은 증상의 진행을 예방, 방해, 지연 또는 역전시키는 임의의 및 모든 사용을 지칭한다. 따라서 용어 "치료하는"은 가장 넓은 맥락에서 고려되어야 한다. 예를 들어, 치료는 반드시 환자가 완전히 회복될 때까지 치료받는 것을 의미하지는 않는다. 다수의 증상을 나타내거나 이에 의해 특징지어지는 병태에서, 치료 또는 예방은 상기 증상 모두를 반드시 치료, 예방, 방해, 지연 또는 역전시킬 필요는 없지만 상기 증상 중 하나 이상을 예방, 방해, 지연 또는 역전시킬 수 있다. 본 개시내용의 맥락에서, 개선, 역전, 예방, 지연 또는 연관될 수 있는 증상은 발작 및 경련을 포함하지만 이에 제한되지 않는다.

[0054] 본원에서 사용되는 용어 "대상체"는 동물, 특히 포유동물, 보다 구체적으로 하등 영장류를 포함하는 영장류, 훨씬 더 구체적으로 본 개시내용의 프로토콜로부터 혜택을 받을 수 있는 인간을 지칭한다. 인간 또는 비인간 동물 또는 배아 여부에 관계없이 대상체는 개체, 대상체, 동물, 환자, 숙주 또는 수용체로 지칭될 수 있다.

[0055] SCN2A을 위한 안티센스 올리고뉴클레오티드

[0056] 본원에서 입증된 바와 같이, 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24는 뇌 조직의 성숙한 SCN2A mRNA에 보유된다. 특히 인트론 2와 17은 상대적으로 보유 비율이 높다. 본원에서 입증된 바와 같이, SCN2A mRNA 또는 pre-

RNA에 보유된 인트론은 안티센스 올리고뉴클레오타이드로 표적화될 수 있어서, 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시켜 완전히 스플라이싱된 SCN2A mRNA(즉, 임의의 인트론을 포함하지 않는 SCN2A mRNA)의 양을 증가시킬 수 있다. 따라서 이러한 안티센스 올리고뉴클레오타이드는 세포에 의해 생성된 SCN2A의 양을 증가시키는 데 유용하고, 따라서 유전성 간질(발달성 및 간질성 뇌병증(DEE)을 포함함), 지적 장애, 자폐 스펙트럼 장애 및 정신분열증과 같은 SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 장애의 치료를 위한 치료제로서 유용하며, 여기서 SCN2A 단백질 수준을 증가시키면 치료 효과를 제공할 수 있다.

[0057] 따라서, 인트론-보유 SCN2A mRNA 또는 pre-mRNA, 예컨대 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24에서 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시키는 안티센스 올리고뉴클레오타이드가 본원에서 제공된다. 특정 예에서, 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 인트론 2 또는 인트론 17의 스플라이스 부위에서 스플라이싱을 향상시킨다.

[0058] 안티센스 올리고뉴클레오타이드는 많은 방법 중 하나로 스플라이싱을 향상시키는 기능을 할 수 있다. 일 예에서, 안티센스 올리고뉴클레오타이드는 인트론 스플라이싱 사일런서(IS)(ISS 부위 또는 ISS 모티프로도 지칭됨)에 결합하거나 이에 인접한다. ISS는 스플라이스 부위에서 스플라이싱을 침묵시키거나 억제하는 역할을 하는 RNA의 시스(*cis*)-작용 요소(즉, 서열)이다. ISS는 사일런싱 억제자 역할을 하는 RNA-결합 단백질(RBP)에 의해 결합된다. 억제자로 작용하는 예시적인 RBP는 hnRNP A1, A2/B1, C1/C2, E1/E2/E3/E4, F, G, H, I, K, L, M, P, Q1/Q2/Q3 및 U 및 hnRNP A2와 같은 이종 핵 리보핵단백질(hnRNP)을 포함한다. hnRNP에 의해 인식되고 결합된 모티프는 반드시 고전적 의미에서 엄격한 컨센서스 서열일 필요는 없지만, 반복 요소(예를 들어, hnRNP L1의 경우, CA 반복-풍부한 요소를 인식) 또는 짧고 퇴화된 서열일 수 있다. 또한, hnRNP는 hnRNP F의 경우와 같이 선형 서열 모티프가 아닌 특정 구조를 인식할 수 있다(검토를 위해, 예를 들어 문헌[Gunes *et al.*, 2016, Hum Genet. 135:851-867; Dvinge, 2018, FEBS Letters, 592:2987-3006] 참조). 그럼에도 불구하고 알고리즘은 RNA 분자에서 hnRNP 결합 모티프를 예측하는 데 사용될 수 있다(Piva *et al.*, 2009, Bioinformatics; Piva *et al.*, 2012, Hum Mutat. 2012 Jan;33(1):81-85). ISS에 또는 이에 인접한 안티센스 올리고뉴클레오타이드의 결합은 RBP 억제자(예를 들어, hnRNP A1과 같은 hnRNP)의 결합을 방지하거나 억제하여, 인트론 1, 2, 3, 4, 5, 11, 13, 17, 또는 24와 같은 보유된 인트론의 스플라이스 부위에서 스플라이싱을 향상시킨다. 다른 예에서, 안티센스 올리고뉴클레오타이드는 RNA 2차 구조(예를 들어, 스템, 헤어핀 루프, 유사매듭(pseudoknot), 별지, 내부 루프 또는 멀티루프)를 형성하는 경향이 있는 SCN2A mRNA 또는 pre-mRNA의 한 부위에 결합하여, 구조의 형성을 감소시키고 스플라이싱 인자의 효율적인 모집을 촉진하여 보유-인트론의 스플라이싱을 향상시킨다. 추가 예에서, 안티센스 올리고뉴클레오타이드는 G-사중체의 형성에 관여하는 서열에 결합하며, 이는 G-사중체를 안정화시키고 스플라이싱을 향상시킬 수 있다(예를 들어, 문헌[Rouleau *et al.*, 2015, Nucleic Acids Res. 43(1): 595-606; Ribeiro *et al.*, 2015, Hum Genet. 134(1):37-44] 참조). 당업자는 SCN2A의 추정 사중체 형성 G-풍부 서열(QGRS, Quadruplex forming G-Rich Sequences)의 구성 및 분포가 컴퓨터 도구 QGRS 매퍼(Mapper)를 사용하여 예측될 수 있음을 인식할 것이며, 여기서 통상적으로 19 초과의 G 점수를 갖는 서열은 안정적인 G4 구조에 대한 경향이 더 높은 것으로 간주된다.

[0059] 일부 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 표적화된 인트론, 즉 향상된 스플라이싱이 수행될 인트론, 예를 들어 인트론 1, 2, 3, 4, 5, 11, 13, 17 및 24 내의 뉴클레오타이드(또는 표적 영역)에 결합한다. 다른 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 표적 인트론의 스플라이스 부위에서 스플라이싱을 여전히 향상시키면서 인접한 엑손의 뉴클레오타이드(또는 표적 영역)에 결합한다.

[0060] 일 예에서, 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 인트론 2 내의 표적 영역에 결합한다. 따라서, 일부 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 SCN2A pre-mRNA의 인트론 2, 예를 들어 다음과 같이 제시된 인트론 2 내의 뉴클레오타이드 서열에 상보적인 핵염기 서열을 갖는다:

GTGAGTTCTTAGTCAAGTTGCCITCACTGCCTATTTACTAATTGGTTCTGGGCTAGTCCCAGGGATGAT
GGTGAAGAAGGCTGGCCTCCTTCCCTCTGTCTAAAGTATCACTAAGATGCTGGATGGGCTGACCGTGTA
ATGGACCAATGATCCTAGAAAGTCTTTTGAAGCACTCATTTGAACCTGCATTTGTGAGACAGGCAGAGAA
CTGGTGAGGCATCCTCCAGCGCGGGAATTAAGGAAGGACAAAAGCCTATTACCTTCTTGAATACAAATT
ATATGCTTAAACCAGTGTAATTTGACCCTGATTCCCTAATAATGTTGAGAAGCAAAAAGTGTAACTAGG
AGTCTATTTAAATTTTATTTTATATTTGCAGGAGTAGTATCTAAATTCCTCTTTATAGTCTCTAGCTC
TCCATAAGTCACTTTGATCTTCAGTGGGTTTAAATTATTCCTTTATACCATACTTTCTCCTTTCTATTGCT
CTCCACAGAAGGAATAATAGCAGGTGACTTGTAGGTGCCAAATAAGATTCTGAGCAAAAGAACACACCTGG
AAAACCTTGAAGTTCTCATGAGAAAATTTCTAACCAAAAAAAAAAATCAAAGCCTCAATTTTGTGCTTT
ATGTGAATTATAAATGCGGTTTTAAATACTTACATTAAAACCTGATAAAGTTGCTAAGAATTCCTATGG
CATTGATCACAAATTTTCTTAATAATCCTCATGTCATTTATCAAATTTAGGAAAGTTTATAGTGCTCAGA
AAAAAAGCATCTATCTTCATGTCATATGATGGTAATTATTATGTTATACACTATTTTACAGGGCAATA
TTTATAAATAATGGTTTTACTTTTCTCTTAAATATTCTTAATATATATTCTAAGTTTTATTTTATGTGT
TGTGTTTTCTTTTTCAG

(서열번호 12)

[0061]

[0062]

일부 예에서, 안티센스 올리고뉴클레오티드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 인트론 2의 표적 영역에 결합(즉, 이에 상보적인 서열을 포함)하며, 여기서 표적 영역은 인트론 2의 5' 스플라이스 부위에 대해 위치 -6 내지 +12, -5 내지 +13, -4 내지 +14, -3 내지 +15, -2 내지 +16, -1 내지 +19, 0 내지 +18, +1 내지 +19, +2 내지 +20, +3 내지 21, +4 내지 22, +5 내지 +23, +6 내지 +24, +7 내지 +25, +8 내지 26, +9 내지 +27, +10 내지 +28, +11 내지 +29, +12 내지 +30, +13 내지 +31, +14 내지 +32, +15 내지 +33, +16 내지 +34, +17 내지 +35, +18 내지 +36, +19 내지 +37, +20 내지 +38, +21 내지 +39, +22 내지 +40, +23 내지 +41, +24 내지 +42, +25 내지 +43, +26 내지 +44, +27 내지 +45, +28 내지 +46, +29 내지 +47, +30 내지 +48, +31 내지 +49, +32 내지 +50, +33 내지 +51, +34 내지 +52, +35 내지 +53, +36 내지 +54, +37 내지 +55, +38 내지 +56, +39 내지 +57, +40 내지 +58, +41 내지 +59, +42 내지 +60, +43 내지 +61, +44 내지 +62, +45 내지 +63, +46 내지 +64, +47 내지 +65, +48 내지 +66, +49 내지 +67, +50 내지 +68, +51 내지 +69, +52 내지 +70, +53 내지 +71, +54 내지 +72, +55 내지 +73, +56 내지 +74, +57 내지 +75, +58 내지 +76, +59 내지 +77, +60 내지 +78, +61 내지 +79, +62 내지 +80, +63 내지 +81, +64 내지 +82, +65 내지 +83, +66 내지 +84, +67 내지 +85, +68 내지 +86, +69 내지 +87, +70 내지 +88, +71 내지 +89, +72 내지 +90, +73 내지 +91, +74 내지 +92, +75 내지 +93, +76 내지 +94, +77 내지 +95, +78 내지 +96, +79 내지 +97, +80 내지 +98, +81 내지 +99에 걸쳐 있다. 특정 구현예에서, 안티센스 올리고뉴클레오티드는 인트론 2에서 ISS에 결합하거나 인접한다. 본원에서 결정된 바와 같이, hnRNP1에 의해 인식되는 추정 ISS는 인트론 2의 5' 스플라이스 부위에 대해 위치 8 내지 +12(CTTAG), +33 내지 +36(ATTTA) 또는 +60 내지 +65(CAGGGA)(상기 서열번호 12에서 굵은 글꼴로 표시되어 있음)에 있다. 따라서, 일부 구현예에서, 안티센스 올리고뉴클레오티드는 인트론 2의 5' 스플라이스 부위에 대해 +8 내지 +12, +33 내지 +36 또는 +60 내지 +65 위치에서 뉴클레오티드에 결합하거나 인접한다. 예를 들어, 안티센스 올리고뉴클레오티드는 인트론 2의 5' 스플라이스 부위에 대해 위치 +4, +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, +30, +31, +32, +33, +34, +35, +36, +37, +38, +39, 또는 +40에서 하나 이상의 뉴클레오티드에 결합할 수 있거나, 인트론 2의 5' 스플라이스 부위에 대해 위치 +50, +51, +52, +53, +54, +55, +56, +57, +58, +59, +60, +61, +62, +63, +64, +65, +66, +67, +68, +69, 또는 +70 에서 하나 이상의 뉴클레오티드에 결합할 수 있다.

[0063]

일 예에서, 안티센스 올리고뉴클레오티드는 인트론 2(예를 들어, 서열번호 12에 제시된 인트론 2)의 5' 스플라이스 부위에 대해 위치 -6 내지 +12, -5 내지 +13, -4 내지 +14, -3 내지 +15, -2 내지 +16, -1 내지 +19, 0 내지 +18, +1 내지 +19, +2 내지 +20, +3 내지 21, +4 내지 22, +5 내지 +23, +6 내지 +24, +7 내지 +25, +8 내지 26, +9 내지 +27, +10 내지 +28, +11 내지 +29, +12 내지 +30, +13 내지 +31, +14 내지 +32, +15 내지 +33, +16 내지 +34, +17 내지 +35, +18 내지 +36, +19 내지 +37, +20 내지 +38, +21 내지 +39, +22 내지 +40, +23 내지 +41, +24 내지 +42, +25 내지 +43, +26 내지 +44, +27 내지 +45, +28 내지 +46, +29 내지 +47, +30 내지 +48, +31 내지 +49, +32 내지 +50, +33 내지 +51, +34 내지 +52, +35 내지 +53, +36 내지 +54, +37 내지 +55, +38 내지 +56, +39 내지 +57, +40 내지 +58, +41 내지 +59, +42 내지 +60, +43 내지 +61, +44 내지 +62, +45 내지 +63, +46 내지 +64, +47 내지 +65, +48 내지 +66, +49 내지 +67, +50 내지 +68, +51 내지 +69, +52 내지 +70, +53 내지 +71, +54 내지 +72, +55 내지 +73, +56 내지 +74, +57 내지 +75, +58 내지 +76, +59 내지 +77, +60 내지 +78, +61 내지 +79, +62 내지 +80, +63 내지 +81, +64 내지 +82, +65 내지 +83, +66 내지 +84, +67 내지 +85, +68 내지 +86, +69 내지 +87, +70 내지 +88, +71 내지 +89, +72 내지 +90, +73 내지 +91, +74 내지 +92, +75 내지 +93, +76 내지 +94, +77 내지 +95, +78 내지 +96, +79 내지 +97, +80 내지 +98, +81 내지 +99에 걸쳐 있거나 상기 위치 내에 있는 표적 영역에 결합하고, 즉, 안티센스 올리고뉴클레오티드는 상기 언급

한 영역 중 적어도 하나에 상보적인 서열을 갖는다.

[0064] 다른 예에서, 안티센스는 인트론 2(예를 들어, 서열번호 12에 제시된 인트론 2)의 3' 스플라이스 부위에 대해 위치 -19 내지 -1, -20 내지 -2, -21 내지 -3, -22 내지 -4, -23 내지 -5, -24 내지 -6, -25 내지 -7, -26 내지 -8, -27 내지 -9, -28 내지 -10, -29 내지 -11, -30 내지 -12, -31 내지 -13, -32 내지 -14, -33 내지 -15, -38 내지 -20, -39 내지 -21, -48 내지 -30, -49 내지 -31, -51 내지 -33, -52 내지 -34, -53 내지 -35, -54 내지 -36, -62 내지 -44, -64 내지 -46, -65 내지 -47, -66 내지 -48, -67 내지 -49, -76 내지 -58, -77 내지 -59, -78 내지 -60, -79 내지 -61, -82 내지 -64, -83 내지 65, -84 내지 -66, -85 내지 -67, -86 내지 -68, -87 내지 -69, -90 내지 -72, -91 내지 -73, -92 내지 -74, -95 내지 -77, -97 내지 -79, -98 내지 -80, -99 내지 -81, -101 내지 -83, -102 내지 -84, -103 내지 -85, -104 내지 -86, -105 내지 -87, -106 내지 -88, -107 내지 -89, -108 내지 -90, -109 내지 -91, -110 내지 -92, -111 내지 -93, -112 내지 -94, -113 내지 -95, -114 내지 -96, -115 내지 -97, -116 내지 -98, -117 내지 -99에 걸쳐 있거나 상기 위치 내에 있는 표적 영역에 결합하고, 즉, 안티센스 올리고뉴클레오타이드는 상기 언급한 영역 중 적어도 하나에 상보적인 서열을 갖는다.

[0065] 일부 예에서, 안티센스 올리고뉴클레오타이드는 인트론 2의 5' 스플라이스 부위에 대해 위치 -4 내지 +14, -3 내지 +15, -2 내지 +16, +6 내지 +24, +7 내지 +25, +8 내지 26, +9 내지 +27, +10 내지 +28, +14 내지 +32, +19 내지 +37, +20 내지 +38, +21 내지 +39, +22 내지 +40, +23 내지 +41, +24 내지 +42, +25 내지 +43, +26 내지 +44, +27 내지 +45, +28 내지 +46, +29 내지 +47, +30 내지 +48, +31 내지 +49, +32 내지 +50, +33 내지 +51, +34 내지 +52, +35 내지 +53, +43 내지 +61, +45 내지 +63, +46 내지 +64, +49 내지 +67, +52 내지 +70, +59 내지 +77, +64 내지 +82, +65 내지 +83, +68 내지 +86, +69 내지 +87, +70 내지 +88, +71 내지 +89, +72 내지 +90 및 +73 내지 +91에 결합하고 따라서 상기 위치에 상보적인 서열을 포함한다. 일부 구현예에서, 안티센스 올리고뉴클레오타이드는 서열번호 115 내지 142 중 어느 하나에 제시된 서열에 대해 적어도 또는 약 70%, 80%, 또는 90% 서열 동일성을 갖는 서열, 또는 서열번호 115 내지 142 중 어느 하나에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오타이드를 갖는 서열을 포함한다. 특정 구현예에서, 서열은 서열번호 126 또는 서열번호 138 중 어느 하나에 제시된 서열에 대해 적어도 또는 약 70%, 80%, 또는 90% 서열 동일성을 갖거나, 또는 서열번호 126 또는 서열번호 138에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오타이드를 갖는 서열을 갖는다.

[0066] 추가 예에서, 안티센스 올리고뉴클레오타이드는 인트론 2의 3' 스플라이스 부위에 대해 위치 -19 내지 -1, -21 내지 -3, -30 내지 -12, -31 내지 -13, -32 내지 -14, -62 내지 -44, -64 내지 -46, -67 내지 -49, -76 내지 -58, -77 내지 -59, -78 내지 -60, -79 내지 -61 -82 내지 -64, -83 내지 65, -84 내지 -66, -85 내지 -67, -86 내지 -68, -87 내지 -69, -95 내지 -77, -97 내지 -79, -100 내지 -82, -101 내지 -83, -102 내지 -84, -103 내지 -85, -107 내지 -89, -109 내지 -91, -111 내지 -93, -113 내지 -95, 및 -114 내지 -96에 결합하고 따라서 상기 위치에 상보적인 서열을 포함한다. 안티센스 올리고뉴클레오타이드는 서열번호 143 내지 205 중 어느 하나에 제시된 서열, 또는 서열번호 143 내지 205 중 어느 하나에 제시된 서열로부터 적어도 8, 9, 10, 11, 12, 13, 14 또는 15개의 연속 뉴클레오타이드를 포함하는 서열을 포함한다.

[0067] 일 구현예에서, 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 인트론 17 내의 뉴클레오타이드에 결합한다. 따라서, 일부 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 SCN2A의 인트론 17 내의 뉴클레오타이드 서열에 상보적인 핵염기 서열을 갖는다(NCBI 참조 서열: NG_008143.1의 뉴클레오타이드 120271 내지 130741).

[0068] 일 구현예에서, 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 인트론 1 내의 뉴클레오타이드에 결합한다. 따라서, 일부 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 SCN2A의 인트론 1 내의 뉴클레오타이드 서열에 상보적인 핵염기 서열을 갖는다(NCBI 참조 서열: NG_008143.1의 뉴클레오타이드 5240 내지 61371).

[0069] 다른 구현예에서, 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 인트론 3 내의 뉴클레오타이드에 결합한다. 따라서, 일부 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 SCN2A의 인트론 3 내의 뉴클레오타이드 서열에 상보적인 핵염기 서열을 갖는다(NCBI 참조 서열: NG_008143.1의 뉴클레오타이드 62735 내지 73446).

[0070] 또 다른 구현예에서, 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 인트론 4 내의 뉴클레오타이드에 결합한다. 따라서, 일부 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 SCN2A의 인트론 4 내의 뉴클레오타이드 서열에 상보적인 핵염기 서열을 갖는다(NCBI 참조 서열: NG_008143.1의 뉴클레오타이드

73537 내지 74264).

- [0071] 일 구현예에서, 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 인트론 5 내의 뉴클레오타이드에 결합한다. 따라서, 일부 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 SCN2A의 인트론 5 내의 뉴클레오타이드 서열에 상보적인 핵염기 서열을 갖는다(NCBI 참조 서열: NG_008143.1의 뉴클레오타이드 74394 내지 74950).
- [0072] 일 구현예에서, 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 인트론 11 내의 뉴클레오타이드에 결합한다. 따라서, 일부 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 SCN2A의 인트론 11 내의 뉴클레오타이드 서열에 상보적인 핵염기 서열을 갖는다(NCBI 참조 서열: NG_008143.1의 뉴클레오타이드 81358 내지 88754).
- [0073] 일 구현예에서, 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 인트론 13 내의 뉴클레오타이드에 결합한다. 따라서, 일부 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 SCN2A의 인트론 13 내의 뉴클레오타이드 서열에 상보적인 핵염기 서열을 갖는다(NCBI 참조 서열: NG_008143.1의 뉴클레오타이드 92584 내지 96928).
- [0074] 또 다른 구현예에서, 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 인트론 24 내의 뉴클레오타이드에 결합한다. 따라서, 일부 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 SCN2A의 인트론 24 내의 뉴클레오타이드 서열에 상보적인 핵염기 서열을 갖는다(NCBI 참조 서열: NG_008143.1의 뉴클레오타이드 146329 내지 146691).
- [0075] 본 개시내용의 안티센스 올리고뉴클레오타이드는 안티센스 올리고뉴클레오타이드와 접촉하는 세포 또는 세포 집단에서 완전히 스플라이싱된 SCN2A mRNA의 양 또는 수준, 또는 SCN2A 단백질의 양 또는 수준을, 본 개시내용의 안티센스 올리고뉴클레오타이드와 접촉하지 않은 세포 또는 세포 집단에서 완전히 스플라이싱된 SCN2A mRNA 또는 SCN2A 단백질의 양 또는 수준과 비교하여 적어도 또는 약 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 600%, 700% 이상 증가되도록 스플라이싱을 향상시킬 수 있다. 따라서, 일부 경우에, 본 개시내용의 안티센스 올리고뉴클레오타이드에 노출된 후 세포 또는 세포 집단에서 완전히 스플라이싱된 SCN2A mRNA 또는 SCN2A 단백질의 양 또는 수준은, 본 개시내용의 안티센스 올리고뉴클레오타이드에 노출되지 않은 세포 또는 세포 집단에서 완전히 스플라이싱된 SCN2A mRNA 또는 SCN2A 단백질의 양 또는 수준에 비해 1.2X, 1.3X, 1.4X, 1.5X, 1.6X, 1.7X, 1.8X, 1.9X, 2X, 2.1X, 2.2X, 2.3X, 2.4X, 2.5X, 3X, 3.5X, 4X, 4.5X, 5X, 6X, 7X 이상이다. 일부 예에서, 완전히 스플라이싱된 SCN2A mRNA는 NCBI 참조 서열: NM_001040142.2(서열번호 1)로 기재되고/되거나 NCBI 참조 서열: NG_008143.1(서열번호 2)의 뉴클레오타이드 서열로부터 유래되고/되거나 SCN2A 단백질은 NCBI 참조 서열: NP_001035232.1(서열번호 3)로 기재된 단백질이다. 다른 예에서, 완전히 스플라이싱된 SCN2A mRNA는 NCBI 참조 서열: NM_001040143.2(서열번호 4)로 기재된 것이고/이거나 SCN2A 단백질은 NCBI 참조 서열: NP_001035233.1(서열번호 5)로 기재된 것이다. 다른 예에서, SCN2A mRNA는 NCBI 참조 서열: NM_001371246.1(서열번호 6)로 기재된 것이고/이거나 SCN2A 단백질은 NCBI 참조 서열: NP_001358175.1(서열번호 7)로 기재된 것이다. 다른 예에서, SCN2A mRNA는 NCBI 참조 서열: NM_001371247.1(서열번호 8)로 기재된 것이고/이거나 SCN2A 단백질은 NCBI 참조 서열: NP_001358176.1(서열번호 9)로 기재된 것이다. 또 다른 예에서, SCN2A mRNA는 NCBI 참조 서열: NM_021007.3(서열번호 10)으로 기재된 것이고/이거나 SCN2A 단백질은 NCBI 참조 서열: NP_066287.2(서열번호 11)로 기재된 것이다.
- [0076] 본 개시내용의 안티센스 올리고뉴클레오타이드는 통상적으로 8 내지 50개의 핵염기 길이, 예컨대 8 내지 50, 8 내지 40, 8 내지 35, 8 내지 30, 8 내지 25, 8 내지 20, 8 내지 15, 9 내지 50, 9 내지 40, 9 내지 35, 9 내지 30, 9 내지 25, 9 내지 20, 9 내지 15, 10 내지 50, 10 내지 40, 10 내지 35, 10 내지 30, 10 내지 25, 10 내지 20, 10 내지 15, 11 내지 50, 11 내지 40, 11 내지 35, 11 내지 30, 11 내지 25, 11 내지 20, 11 내지 15, 12 내지 50, 12 내지 40, 12 내지 35, 12 내지 30, 12 내지 25개, 12 내지 20 또는 12 내지 15개의 핵염기 길이이다. 따라서, 특정 예에서, 안티센스 올리고뉴클레오타이드는 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, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79 또는 80개의 핵염기 길이이다.
- [0077] 안티센스 올리고뉴클레오타이드는 전체 길이에 걸쳐 인트론-보유 SCN2A mRNA 또는 pre-mRNA의 표적 영역에 대해 100% 상보적일 수 있거나 100% 미만으로 상보적일 수 있다. 통상적으로, 안티센스 올리고뉴클레오타이드는 상기

인트론 5, 8, 9, 12, 13 또는 14에서 확인된 영역과 같은 인트론-보유 SCN2A mRNA 또는 pre-mRNA의 표적 영역에 대해 적어도 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% 또는 99% 상보적이다. 안티센스 올리고뉴클레오타이드는 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 표적 영역에 상보적인, 예를 들어 적어도 8, 적어도 9, 적어도 10, 적어도 11, 적어도 12, 적어도 13, 적어도 14, 적어도 15, 적어도 16, 적어도 17, 적어도 18, 적어도 19개 또는 적어도 20개의 연속 핵염기를 함유할 수 있다. 안티센스 올리고뉴클레오타이드가 100% 상보적이지 않은 경우, 미스매치된 또는 비-상보적 핵염기(들)는 상보적 핵염기로 클러스터링되거나 산재될 수 있으며 서로 인접할 필요는 없다. 비-상보적 핵염기(들)는 안티센스 화합물의 5' 말단 및/또는 3' 말단에 위치할 수 있다. 대안적으로, 비-상보적인 핵염기(들)는 안티센스 올리고뉴클레오타이드의 내부 위치에 있을 수 있다. 2개 이상의 비-상보적 핵염기가 존재하는 경우, 이들은 인접적이거나 비인접적일 수 있다.

[0078] 특정 구현예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 길이가 최대 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 또는 30개의 핵염기이고 인트론-보유 SCN2A mRNA 또는 pre-mRNA에서 표적 영역에 대해 6, 5, 4, 3, 2 또는 1개 이하의 비-상보적 핵염기를 포함한다.

[0079] 본 개시내용의 안티센스 올리고뉴클레오타이드는 당업계에 공지된 임의의 방법을 사용하여 생성될 수 있다. 통상적으로, 안티센스 올리고뉴클레오타이드는 화학적 합성 방법을 사용하여 생성된다. 안티센스 올리고뉴클레오타이드는 변형되지 않을 수 있지만, 보다 통상적으로 본 개시내용의 안티센스 올리고뉴클레오타이드는 하나 이상의 변형을 포함한다. 이러한 변형은, 예를 들어 안티센스 올리고뉴클레오타이드의 안정성을 증가시키고(예를 들어, 뉴클레아제에 의한 분해에 대한 안티센스 올리고뉴클레오타이드의 저항성을 증가시키고), 표적 mRNA 또는 pre-mRNA에 대한 안티센스 올리고뉴클레오타이드의 친화도를 증가시키고, 안티센스 올리고뉴클레오타이드에 의한 입체 장애를 증가시키고, RNase H 활성을 증가시키고/시키거나 세포내 흡수를 개선시키는 기능을 할 수 있다. 당업자에게 잘 알려진 예시적인 변형은 핵염기의 변형, 백본 포스페이트 연결 변형(예를 들어, 포스포디에스테르, 포스포르아미데이트 또는 포스포로티오에이트(PS) 변형), 리보스 당의 변형(예를 들어, 2'-O-메틸(2OMe), 2'-O-메톡시-에틸(MOE), 잠금 핵산(LNA), 2'-플루오로 및 S-제약된-에틸(cEt) 변형) 및 펩티드 핵산(PNA)을 생산하기 위한 전체 당 포스페이트 백본의 폴리아미드 연결로의 대체 및 리보스 고리 대신 모르폴린 고리의 사용 및 포스포로디아미데이트 모르폴리노 올리고머(PMO)를 생산하기 위한 포스포로아미데이트 서브유닛간 연결과 같은 기타 변형을 포함하지만 이에 제한되지 않는다(예를 들어, 문헌[Sardone *et al.* (2017) *Molecules* 22(4): 563 Evers *et al.* (2015) *Adv Drug Del Rev* 87:90-103; Kole *et al.* (2012) *Nat Rev Drug Discov.* 11(2): 125-140]에서 광범위하게 검토됨).

[0080] 특정 구현예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 하나 이상의 변형된 핵염기를 함유한다. 이들은, 예를 들어 안티센스 올리고뉴클레오타이드의 안정성 또는 결합 친화도를 증가시키는 기능을 할 수 있다. 예시적인 변형된 핵염기는 N^6 -메틸아데닌, N^2 -메틸구아닌, 하이포크산틴, 7-메틸구아닌, 5-메틸시토신, 5-하이드록시메틸시토신, 슈도우라실, 4-티오우라실, 2,6-디아미노퓨린, 오로트산, 아그마티딘, 리시딘, 2-티오피리미딘(예를 들어, 2-티오우라실, 2-티오티민), G-클램프 및 이의 유도체, 5-치환 피리미딘(예를 들어, 5-할로우라실, 5-프로피닐우라실, 5-프로피닐시토신, 5-아미노메틸우라실, 5-하이드록시메틸우라실, 5-아미노메틸시토신, 5-하이드록시메틸시토신, 슈퍼 T), 7-테아자구아닌, 7-테아자아데닌, 7-아자-2,6-디아미노퓨린, 8-아자-7-테아자구아닌, 8-아자-7-테아자아데닌, 8-아자-7-테아자-2,6-디아미노퓨린, 슈퍼 G, 슈퍼 A 및 N^4 -에틸시토신 또는 이들의 유도체; N^2 -사이클로펜틸구아닌(cPent-G), N2-사이클로펜틸-2-아미노퓨린(cPent-AP) 및 N^2 -프로필-2-아미노퓨린(Pr-AP), 슈도우라실 또는 이들의 유도체; 및 2,6-디플루오로톨루엔과 같은 축퇴성 또는 보편적 염기 또는 무염기 부위와 같은 부재 염기(예를 들어, 1-데옥시리보스, 1,2-디데옥시리보스, 1-데옥시-2-O-메틸리보스; 또는 고리 산소가 질소로 대체된 피롤리딘 유도체(아자리보스))를 포함하지만, 이에 제한되지 않는다. 특정 구현예에서, 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA에 대한 안티센스 올리고뉴클레오타이드의 결합 친화도를 증가시키는 하나 이상의 변형된 핵염기, 예컨대 5-메틸시토신(5-me-C), 5-치환된 피리미딘, 6-아자피리미딘 및 2 아미노프로필아데닌, 5-프로피닐우라실 및 5-프로피닐시토신을 포함하는 N-2, N-6 및 0-6 치환된 퓨린을 함유한다.

[0081] 본 개시내용의 안티센스 올리고뉴클레오타이드는 변형된 당 모이어티를 포함할 수 있다. 예시적인 당 모이어티 변형은 2'-O-메틸(2OMe), 2'-O-메톡시-에틸(MOE), 잠금 핵산(LNA), 2'-플루오로 및 S-제약된-에틸(cEt) 변형을 포함한다.

[0082] 특정 구현예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드의 백본은 포스포로티오에이트, 키랄 포스포로티

오에이트, 포스포로디티오에이트, 포스포트리에스테르, 아미노알킬포스포트리에스테르, 메틸 또는 3'알킬렌 포스포네이트를 포함하는 다른 알킬 포스포네이트 또는 키랄 포스포네이트, 포스페네이트, 3'-아미노 포스포르아미데이트 및 아미노알킬포스포르아미데이트를 포함하는 포스포르아미데이트, 티오노포스포르아미데이트, 티오노알킬포스포네이트, 티오노알킬포스포트리에스테르 또는 보라노포스페이트를 포함한다. 다른 구현예에서, 백본은 인 원자를 갖지 않는다. 인 원자를 포함하지 않는 예시적인 올리고뉴클레오타이드 백본은 단쇄 알킬 또는 사이클로알킬 뉴클레오시드간 연결, 혼합 헤테로원자 및 알킬 또는 사이클로알킬 뉴클레오시드간 연결, 또는 하나 이상의 단쇄 헤테로원자 또는 헤테로사이클릭 뉴클레오시드간 연결에 의해 형성되는 것들을 포함한다. 이들은 모르폴리노 연결을 갖는 것(부분적으로 뉴클레오시드의 당 부분에서 형성된 것; 예를 들어, 미국 특허 5,698,685, 5,217,866, 5,142,047, 5,034,506, 5,166,315, 5,185,444, 5,521,063, 5,506,337, 8,076,476, 8,299,206 및 7,943,762 참조); 실록산 백본; 설파이드, 설폭사이드 및 설폰 백본; 포름아세틸 및 티오포름아세틸 백본; 메틸렌 포름아세틸 및 티오포름아세틸 백본; 알켄 함유 백본; 설파메이트 백본; 메틸렌이미노 및 메틸렌하이드라지노 백본; 설포네이트 및 설폰아미드 백본; 아마이드 백본; 및 N, O, S 및 CH₂ 성분 부분이 혼합된 기타를 포함한다.

[0083] 일 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 펩티드 핵산(PNA)이다. PNA 화합물에서, 올리고뉴클레오타이드의 당-백본은 아마이드 함유 백본, 특히 아미노에틸글리신 백본으로 대체된다. 핵염기는 보유하고 백본의 아마이드 부분의 아자 질소 원자에 직접 또는 간접적으로 결합된다(예를 들어, 미국 특허 5,539,082; 5,714,331; 및 5,719,262 참조).

[0084] 특정 구현예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 RNase H에 대해 부분적으로 또는 완전히 저항성이다. 이러한 안티센스 올리고뉴클레오타이드는 2'-O-메틸 유도체 및/또는 포스포로티오에이트 백본을 포함할 수 있으며, 둘 다 뉴클레아제 분해에 대해 저항성이다. 추가 예에서, 안티센스 올리고뉴클레오타이드는 통상적으로 안티센스 올리고뉴클레오타이드 및 SCN2A mRNA 또는 pre-mRNA를 함유하는 이중체 분자에 대한 RNase H의 결합을 입체적으로 방해하거나 방지하는 하나 이상의 구조적 변형의 존재로 인해 RNase H를 활성화시키지 않는다. 예를 들어, 이러한 안티센스 올리고뉴클레오타이드는 뉴클레오타이드간 가교 포스페이트 잔기의 적어도 하나 또는 전부가 변형된 포스페이트, 예컨대 메틸 포스포네이트, 메틸 포스포로티오에이트, 포스포로모르폴리데이트, 포스포로피페라지데이트 및 포스포르아미데이트인 것을 포함한다. 예를 들어, 뉴클레오타이드간 가교 포스페이트 잔기 중 두 개마다 하나는 기재된 바와 같이 변형될 수 있다. 또 다른 비제한적 예에서, 이러한 안티센스 분자는 적어도 하나 또는 모든 뉴클레오타이드가 2' 저급 알킬 모이어티(예를 들어, C₁-C₄, 선형 또는 분지형, 포화 또는 불포화 알킬, 예컨대 메틸, 에틸, 에테닐, 프로필, 1-프로페닐, 2-프로페닐 및 이소프로필)를 함유하는 분자이다.

[0085] 다른 예에서, 본 개시내용의 안티센스 올리고뉴클레오타이드는 SCN2A mRNA 또는 pre-mRNA와 DNA-RNA 이중체를 형성할 때 Rnase H를 활성화시킨다. 이러한 안티센스 올리고뉴클레오타이드의 예는 갭머이며, 이는 뉴클레아제 분해에 대한 증가된 저항성, 증가된 세포 흡수, 표적 핵산에 대한 증가된 결합 친화도 및 Rnase H에 대한 기질로서 작용하는 제2 영역을 부여하도록 변형된 적어도 하나의 영역을 함유하는 키메라 분자이다. 갭머는 Rnase H 절단을 지원하는 복수의 뉴클레오타이드가 있는 내부 영역을 가지고 있다. 이 내부 영역은 내부 영역의 뉴클레오시드와 화학적으로 구별되고, 예를 들어 안티센스 올리고뉴클레오타이드의 안정성을 증가시키고 뉴클레아제 분해로부터 이를 보호하는 역할을 하는 복수의 뉴클레오타이드를 갖는 외부 영역 사이에 위치한다. 특정 구현예에서, 갭머의 외부 영역은 β 리보뉴클레오시드, β 데옥시리보뉴클레오시드, 2'-변형 뉴클레오시드(예를 들어, 특히 2'-MOE 및 2'-O-CH₃), 가교 핵산(BNA) 또는 잠긴 핵산(LNA)을 함유한다.

[0086] 본 개시내용의 안티센스 올리고뉴클레오타이드는 또한 올리고뉴클레오타이드의 활성화, 세포 분포 또는 세포 흡수를 향상시키는 하나 이상의 하나 이상의 모이어티에 연결될 수 있다. 그러한 모이어티는 지질 모이어티, 예컨대 콜레스테롤 모이어티, 콜산, 티오에테르, 예를 들어 핵실-5-트리틸티올, 티오펜콜레스테롤, 지방족 사슬, 예를 들어 도데칸디올 또는 운데실 잔기, 인지질, 예를 들어 디-핵사데실-rac-글리세롤 또는 트리에틸암모늄 1,2-디-O-핵사데실-rac-글리세로-3-H-포스포네이트, 폴리아민 또는 폴리에틸렌 글리콜 사슬, 또는 다만탄 아세트산, 팔미틸 모이어티, 또는 옥타데실아민 또는 핵실아미노-카르보닐-옥시콜레스테롤 모이어티, 탄수화물, 인지질, 비오틴, 페나진, 엽산, 페난트리딘, 안트라퀴논, 아크리딘, 플루오레세인, 로다민, 쿠마린 및 다양한 염료를 포함하지만, 이에 제한되지 않는다.

[0087] 특정 구현예에서, 안티센스 올리고뉴클레오타이드는 화합물의 세포로의 수송을 향상시키는데 효과적인 세포 침투 펩티드(CPP)에 연결된다. 수송 모이어티는 안티센스 올리고뉴클레오타이드의 어느 한쪽 말단에 부착될 수 있고,

전신 투여시 생체내 다중 조직 내에서 거대분자 전좌 및 세포 내로의 안티센스 올리고뉴클레오타이드의 침투 증가를 초래할 수 있다. 일 구현예에서, 세포-투과 펩티드는 아르기닌-풍부 펩티드 수송체이다. 아르기닌-풍부한 CPP와 연결된 안티센스 올리고뉴클레오타이드는 혈액-뇌 장벽을 통과할 수 있었고 전신 전달 후 야생형 마우스의 뇌 전체에 널리 분포되었다(Du *et al.* Hum. Mol. Genet., 20(2011), pp. 3151-3160). 또 다른 구현예에서, 세포 침투 펩티드는 페네트라틴(Penetratin) 또는 Tat 펩티드일 수 있다. 이들 펩티드는 당업계에 잘 알려져 있고, 예를 들어 미국 공개 20100016215에 개시되어 있다. 상기 기재된 수송 모이어티는 부착된 수송 모이어티의 부재 하에서의 올리고머의 흡수에 비해 부착된 올리고머의 세포 진입을 크게 향상시키는 것으로 나타났다. 예를 들어, 아르기닌-풍부 CPP와 연결된 안티센스 올리고뉴클레오타이드는 혈액-뇌 장벽을 통과할 수 있었고 전신 전달 후 야생형 마우스의 뇌 전체에 널리 분포되었다(Du *et al.* Hum. Mol. Genet., 20 (2011), pp. 3151-3160). 흡수는 접합되지 않은 화합물에 비해 적어도 10배 또는 적어도 20배 향상될 수 있다. 다른 예에서, 안티센스 올리고뉴클레오타이드는, 예를 들어 미국 특허 9193969에 기재된 바와 같이 도파민 재흡수 억제제(DRI), 선택적 세로토닌 재흡수 억제제(SSRI), 노르아드레날린 재흡수 억제제(NRI), 노르에피네프린-도파민 재흡수 억제제(NDRI) 또는 세로토닌-노르에피네프린-도파민 재흡수 억제제(SNDRI)에 결합된다. 추가 예에서, 안티센스 올리고뉴클레오타이드는 저밀도 지단백질 수용체-관련 단백질-1(LRP-1)을 사용하여 수용체-매개 통과세포외배출(transcytosis)에 의해 혈액-뇌 장벽을 통과할 수 있는 총칭하여 "안지오펩(angiopep)"으로 알려진 펩티드에 접합되고, 전신 투여된 안티센스-펩티드 접합체의 뇌로의 전달을 허용한다(예를 들어, WO200979790 참조).

[0088] 안티센스 올리고뉴클레오타이드는 또한, 예를 들어 뉴클레아제 안정성과 같은 특성을 향상시키기 위해 하나 또는 두 말단에 일반적으로 부착되는 하나 이상의 안정화 기를 갖도록 변형될 수 있다. 안정화 기에는 캡 구조가 포함된다. 이들 말단 변형은 말단 핵산을 갖는 안티센스 화합물을 엑소뉴클레아제 분해로부터 보호하고, 세포 내 전달 및/또는 국소화를 도울 수 있다. 캡은 5'-말단(5'-cap) 또는 3'-말단(3'-cap)에 존재할 수 있거나 양쪽 말단에 존재할 수 있다. 캡 구조는 당업계에 잘 알려져 있으며, 예를 들어 반전된 데옥시 무염기성 캡을 포함한다.

[0089] **안티센스 올리고뉴클레오타이드의 평가**

[0090] 본 개시내용의 안티센스 올리고뉴클레오타이드는 인트론의 특정 영역 또는 부위(예를 들어, ISS, G-사중체 또는 2차 구조 성향을 갖는 영역)를 표적화하도록 및/또는 전체 인트론 또는 인트론 영역만 덮는 안티센스 마이크로위크 또는 타일링과 같은 방법에 의해 합리적으로 설계될 수 있다. 예를 들어, 안티센스 워크에 사용되는 안티센스 올리고뉴클레오타이드는 보유한 인트론의 5' 스플라이스 부위의 약 100개 뉴클레오타이드 상류(예를 들어, 선행 엑손에서)로부터 5' 스플라이스 부위의 약 100개 뉴클레오타이드 하류까지 및/또는 보유한 인트론의 3' 스플라이스 부위의 약 100개 뉴클레오타이드 상류로부터 표적/보유된 인트론의 3' 스플라이스 부위의 약 100개 뉴클레오타이드 하류(예를 들어, 다음 엑손에서)까지 1, 2, 3, 4, 5, 6, 7, 8, 9 또는 10개 뉴클레오타이드마다 타일링될 수 있다. 그런 다음 이러한 안티센스 올리고뉴클레오타이드의 활성은 당업계에 공지된 다양한 기법을 사용하여 평가되고 확인될 수 있다. 예를 들어, 스플라이싱을 향상시켜 완전히 스플라이싱된 SCN2A mRNA 및/또는 SCN2A 단백질의 생산을 증가시키는 안티센스 올리고뉴클레오타이드의 능력은 안티센스 올리고뉴클레오타이드가 본 개시내용의 방법에 사용하기에 적합하다는 것을 확인하기 위해 시험관내 검정을 사용하여 평가될 수 있다. 마우스 모델은 안티센스 올리고뉴클레오타이드가 생체내에서 완전히 스플라이싱된 SCN2A mRNA 및/또는 SCN2A 단백질의 수준 또는 양을 증가시키는 능력을 평가할 뿐만 아니라 이형접합 기능-상실 SCN2A 돌연변이와 연관된 증상을 개선시키는 데 사용될 수 있다.

[0091] 일 예에서, 포유동물 뉴런 세포(예를 들어, SH-SY5Y 또는 SK-N-AS 세포)와 같은 세포는 본 개시내용의 안티센스 올리고뉴클레오타이드로 형질주입된다. 완전히 스플라이싱된 SCN2A mRNA 및 인트론-보유 SCN2A mRNA의 수준은 당업계에 잘 알려진 바와 같이 qRT-PCR 또는 노던 블롯을 사용하여 평가될 수 있다. SCN2A 단백질 수준은 또한 예컨대 총 세포 용해물 또는 분획에 대한 웨스턴 블롯에 의해 평가될 수 있다.

[0092] 세포가 본 개시내용의 안티센스 올리고뉴클레오타이드에 노출되었을 때 관찰된 완전히 스플라이싱된 SCN2A mRNA, 인트론-보유 SCN2A mRNA 및/또는 SCN2A 단백질의 수준은 본 개시내용의 안티센스 올리고뉴클레오타이드로 인한 변화를 결정하기 위해 세포가 음성 대조군 안티센스 올리고뉴클레오타이드에 노출되었을 때 관찰된 각각의 수준과 비교된다. 통상적으로, 완전히 스플라이싱된 SCN2A mRNA 및/또는 SCN2A 단백질의 수준은 적어도 또는 약 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 100%, 110%, 120%, 125%, 30%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 200%, 250%, 300%, 350%, 400% 이상 증가된다. 그러한 경우에, 본 개시내용의 안티센스 올리고뉴클레오타이드는 SCN2A에서 이형접합 기능-상실

돌연변이와 연관된 질병 또는 병태를 치료하기 위해 사용될 수 있다.

[0093] 마우스 모델은 또한 본 개시내용의 안티센스 올리고뉴클레오타이드의 활성을 평가하고 확인하기 위해 사용될 수 있다. 예를 들어, 안티센스 올리고뉴클레오타이드는 SCN2A-관련 지적 장애가 있는 환자에서 관찰되는 것과 유사한 신체적 및 행동적 특성을 나타내는 이형접합 SCN2A 녹아웃 마우스에 투여될 수 있다(예를 들어, 문헌[Nakajima *et al.* 2019, Neuropsychopharmacol Rep. 39(3):223-237; Guo *et al.*, 2009, Neuropsychopharmacol. 2009 34(7):1659-72] 참조). 그런 다음 스플라이싱을 향상시키고/시키거나, 완전히 스플라이싱된 SCN2A mRNA 및/또는 SCN2A 단백질의 수준을 증가시키고/시키거나 SCN2A 돌연변이와 연관된 임의의 증상을 개선시키는 본 개시내용의 안티센스 올리고뉴클레오타이드의 능력이 평가될 수 있다. 특정 예에서, 뇌, 특히 뉴런에서 SCN2A mRNA 및/또는 단백질 수준이 평가된다. 본 개시내용의 안티센스 올리고뉴클레오타이드로 인한 변화를 결정하기 위해, 본 개시내용의 안티센스 올리고뉴클레오타이드의 투여 후 완전히 스플라이싱된 SCN2A mRNA, 인트론-보유 SCN2A mRNA 및/또는 SCN2A 단백질의 수준을 음성 대조군 안티센스 올리고뉴클레오타이드를 마우스에 투여했을 때 관찰된 각각의 수준과 비교한다. 통상적으로, 완전히 스플라이싱된 SCN2A mRNA 및/또는 SCN2A 단백질의 수준은 적어도 또는 약 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 100%, 110%, 120%, 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 165%, 170%, 175%, 180%, 185%, 190%, 200%, 250%, 300%, 350%, 400% 또는 그 이상 증가된다. 또 다른 예에서, 마우스의 신체적 및/또는 행동 특성에 대한 본 개시내용의 안티센스 올리고뉴클레오타이드의 투여 효과가 평가된다. 예를 들어, 마우스의 기억 및 발작에 대한 행동 및 전기생리학 적 측정은 문헌[Creson *et al.*, eLife 2019;8:e46752]에 기재된 바와 같이 평가될 수 있다.

[0094] 조성물

[0095] 본 개시내용은 상기 및 본원에 기재된 안티센스 올리고뉴클레오타이드를 포함하는 조성물을 제공한다. 특정 예에서, 안티센스 올리고뉴클레오타이드 및 약제학적으로 허용되는 담체를 포함하는 약제학적 조성물이 제공된다. 조성물은 또한 담체, 희석제, 안정화제 및 부형제와 같은 추가 성분을 포함할 수 있다. 조성물은 하나 이상의 안티센스 올리고뉴클레오타이드(예를 들어, 동일하거나 상이한 인트론을 표적으로 하는 둘 이상의 안티센스 올리고뉴클레오타이드)를 포함할 수 있고, 하나 이상의 다른 치료제를 추가로 포함할 수 있다.

[0096] 담체, 희석제, 안정화제 및 부형제는 포스페이트, 시트레이트 또는 다른 유기산과 같은 완충제; 아스코르브산과 같은 항산화제; 저분자량 폴리펩티드(예를 들어, 약 10개 미만의 잔기); 혈청 알부민, 젤라틴 또는 면역글로불린과 같은 단백질; 폴리비닐피롤리돈과 같은 친수성 중합체; 글리신, 글루타민, 아스파라긴, 아르기닌 또는 라이신과 같은 아미노산; 글루코스, 만노스 또는 텍스트린을 포함하는 당당류, 이당류 및 기타 탄수화물; EDTA와 같은 킬레이트제; 만니톨 또는 소르비톨과 같은 당 알코올; 나트륨과 같은 염-형성 반대이온; 및/또는 TweenTM, PluronicTM 또는 폴리에틸렌 글리콜(PEG)과 같은 비이온성 계면활성제를 포함할 수 있다. 일부 구현예에서, 생리학적으로 허용되는 담체는 pH 완충 수용액이다.

[0097] 안티센스 올리고뉴클레오타이드는 또한 본 개시내용의 안티센스 올리고뉴클레오타이드를 세포에 도입하기 위해 리포솜, 나노입자, 미세입자, 미세구, 지질 입자, 소포 등을 포함하는 조성물로 제형화될 수 있다. 구현예에서, 안티센스 올리고뉴클레오타이드의 효율적인 전달을 수행하기 위해, 예를 들어 세포막을 통한 확산을 돕기 위해 및/또는 친유성 약물의 투과성을 향상시키기 위해 침투 증강제가 포함된다. 일부 구현예에서, 침투 증강제는 계면활성제, 지방산, 담즙산염, 킬레이트제, 또는 비-킬레이트 비계면활성제이다.

[0098] 구현예에서, 안티센스 올리고뉴클레오타이드는 바이러스 벡터(예를 들어, 아데노-연관 바이러스(AAV) 벡터)의 맥락에서 제형화되며, 여기서 벡터는 본 개시내용의 안티센스 올리고뉴클레오타이드를 인코딩하는 계놈을 포함한다.

[0099] 안티센스 올리고뉴클레오타이드를 포함하는 조성물은 임의의 약제학적으로 허용되는 염, 에스테르, 또는 이러한 에스테르의 염, 또는 인간을 포함하는 동물에게 투여시(직접 또는 간접적으로) 생물학적 활성 대사산물 또는 이의 잔류물을 제공할 수 있는 임의의 다른 올리고뉴클레오타이드를 포함하는 조성물을 포함한다. 따라서, 예를 들어, 본 개시내용은 또한 본원에 기재된 안티센스 올리고뉴클레오타이드의 약제학적으로 허용되는 염 및 다른 생물학적 등가물을 제공한다. 적합한 약제학적으로 허용되는 염은 나트륨 및 칼륨 염을 포함하지만 이에 제한되지 않는다.

[0100] 방법

[0101] 상기 및 본원에 기재된 안티센스 올리고뉴클레오타이드는 세포(예를 들어, 뉴런 세포) 또는 대상체에서 완전히 스플라이싱된 SCN2A mRNA 및/또는 SCN2A 단백질의 수준을 증가시키기 위해 사용될 수 있다. 결과적으로, 상기 및

본원에 기재된 안티센스 올리고뉴클레오타이드는 SCN2A에서 이형접합 기능-상실 돌연변이와 연관된 장애, 예를 들어 간질성 뇌병증 또는 SCN2A의 이형접합 기능-상실 돌연변이와 연관된 자폐증 또는 지적 장애를 치료하는데 사용될 수 있다. 따라서 본 개시내용의 방법은 세포를 본 개시내용의 안티센스 올리고뉴클레오타이드에 접촉시키는 단계 및/또는 본 개시내용의 안티센스 올리고뉴클레오타이드를 대상체에게 투여하는 단계를 포함한다. 이해할 수 있는 바와 같이, 어구 "안티센스 올리고뉴클레오타이드를 투여하는"은 및 이의 문법적 변형은 안티센스 올리고뉴클레오타이드를 포함하는 조성물이 대상체에게 투여되는 구현예, 및 안티센스 올리고뉴클레오타이드를 인코딩하는 적용제(예를 들어, 바이러스 벡터)를 포함하는 조성물이 대상체에게 투여되는 구현예를 포함한다. 후자의 구현예에서, 안티센스 올리고뉴클레오타이드는 생체 내에서 발현됨으로써 대상체에게 안티센스 올리고뉴클레오타이드의 투여를 수행하는 것으로 이해된다.

[0102] 일부 예에서, SCN2A에서 이형접합 기능-상실 돌연변이와 연관될 수 있는 질병 또는 병태를 나타내는 대상체는 안티센스 올리고뉴클레오타이드 및 이의 조성물의 투여 전에 유전자형을 분석하여 SCN2A에서 알려진 이형접합 기능-상실 돌연변이의 존재를 확인한다. 예를 들어, 대상체에 대해 전체 엑솜 시퀀싱을 수행할 수 있다. SCN2A에서 알려진 이형접합 기능-상실 돌연변이에는 문헌[Vlaskamp *et al.*, *Neurology*, 2019, 92(2):e96-e97]에 기재된 것을 포함할 수 있지만 이로 제한되지 않는다. 다른 예에서, 대상체는 먼저 SCN2A에서 돌연변이의 존재를 확인하기 위해 유전자형이 분석된 다음, 예를 들어 SCN2A mRNA 또는 단백질의 수준을 평가함으로써, 이 돌연변이가 기능-상실 돌연변이인 것으로 확인된다.

[0103] 대상체에 투여되는 안티센스 올리고뉴클레오타이드의 정확한 양 또는 투여량은, 예를 들어 안티센스 올리고뉴클레오타이드의 효능, 다른 모이어티(예를 들어, CCP)의 존재, 투여 경로, 투여되는 투여량의 수, 및 대상체의 체중, 연령 및 일반적인 상태와 같은 기타 고려 사항에 따라 다르다. 특정 투여량 및 투여 프로토콜은, 예를 들어 동물 모델에서의 연구 또는 인간에서의 이전 연구로부터 경험적으로 결정되거나 추정될 수 있거나, 그렇지 않으면 표준 절차를 사용하여 당업자에 의해 결정될 수 있다.

[0104] 안티센스 올리고뉴클레오타이드는 숙련된 기술자에 의해 적합하다고 이해되는 임의의 방법 및 경로에 의해 투여될 수 있다. 통상적으로, 안티센스 올리고뉴클레오타이드는 피하 투여, 정맥내 투여, 근육내 투여, 동맥내 투여, 복강내 투여, 또는 두개내 투여, 예를 들어 척수강내 또는 뇌실내 투여와 같은 비경구로 투여된다. 다른 구현예에서, 안티센스 올리고뉴클레오타이드는 비강내로 전달된다. 본원에 기재된 방법에서 안티센스 올리고뉴클레오타이드의 투여는 바람직하게는 안티센스 올리고뉴클레오타이드를 중추신경계로 전달하게 된다. 특정 구현예에서, 안티센스 올리고뉴클레오타이드는 척수강내 투여 또는 뇌실내 투여에 의해 투여된다. 본 개시내용의 방법은 임의의 2개 이상의 경로의 임의의 조합을 수반할 수 있다.

[0105] 안티센스 올리고뉴클레오타이드는 2, 3, 4, 5회 이상을 포함하여 1회 또는 1회 초과로 대상체에게 투여될 수 있다. 안티센스 올리고뉴클레오타이드가 1회 초과로 투여되는 경우, 투여량 투여 사이의 시간은, 예를 들어 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12주 이상, 또는 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12개월 이상일 수 있다. 최적의 프로토콜을 선택하는 것은 당업자의 기술 수준 내에 있으며, 예를 들어 안티센스 올리고뉴클레오타이드의 반감기 및 병태의 중증도에 따라 달라질 수 있다. 특정 구현예에서, 안티센스 올리고뉴클레오타이드는 약 3개월마다 투여된다.

[0106] 안티센스 올리고뉴클레오타이드는, 원하는 경우 하나 이상의 단위 투여량 형태를 포함할 수 있는 바늘이 있는 주사기, 또는 바이알 및 바늘이 있는 주사기와 같은 패키지, 키트 또는 디스펜서 장치로 제공될 수 있다. 키트 또는 디스펜서 장치에는 투여 지침이 수반될 수 있다.

[0107] 본 개시내용이 쉽게 이해되고 실질적인 효과를 발휘할 수 있도록 하기 위해, 특정한 예시적인 구현예가 이제 다음의 비제한적인 예를 통해 설명될 것이다.

[0108] 임의의 이전 간행물(또는 그로부터 파생된 정보) 또는 알려진 모든 문제에 대한 본 명세서의 참조는 이전 간행물(또는 그로부터 파생된 정보) 또는 알려진 사항이 본 명세서와 관련된 노력의 분야에서 일반적인 일반 지식의 일부를 형성한다는 승인 또는 인정 또는 임의의 형태의 제안으로 간주되지 않으며, 간주되어서는 안 된다.

[0109] **실시예**

[0110] **실시예 1 - 재료 및 방법**

[0111] 세포 배양

[0112] 세포주 SH-SY5Y 및 SK-N-AS는 ECACC(European Collection of Authenticated Cell Cultures; 인증된 세포 배양

의 유럽 컬렉션)로부터 입수하였다. 인간 뇌 RNA는 Ambion(미국) 및 Takara-Bio(미국)에서 구입했다. 세포를 10% FBS가 보충된 돌베코의 변형된 이글 배지(Dulbeccos modified Eagle's medium, DMEM)에서 성장시켰다. 세포를 37℃ 및 5% CO₂에서 유지하였다. 세포의 동결 스톱을 계대 6 내에서 만들고 액체 질소에 저장하였다. 계대 수 20 미만의 세포를 실험에 사용하였다.

[0113] 세포 형질주입

[0114] 세포를 웰당 5,000 내지 10,000개 세포의 밀도로 96웰 플레이트에 플레이팅하였다. 18 내지 24시간의 인큐베이션 후에 형질주입을 수행하였다. Thermofisher Scientific의 리포펙타민 3000 형질주입 시약과 함께 제공된 지침을 따랐다. 간략하게, 형질주입 시약을 하나의 튜브에서 OptiMEM과 혼합하고, ASO를 다른 튜브에서 동일한 것과 혼합하였다. 두 튜브의 내용물을 함께 부드럽게 혼합하고 인큐베이션 후 형질주입 복합체를 신선한 배지를 함유하는 세포에 층을 이루게 했다. 그런 다음 세포를 필요한 시간 동안 인큐베이션했다.

[0115] RNA 분리

[0116] Qiagen Rneasy 미니키트를 사용하여 필요한 기간 동안 ASO로 처리된 세포로부터 전체 RNA를 추출하였다. 간단히 말해, 세포를 펠릿화하고 제조업체의 지침에 따라 RNA를 분리했다. 게놈 DNA 용출 컬럼을 사용하여 오염된 게놈 DNA를 RNA에서 제거했다. RNA 양과 무결성은 나노드롭(nanodrop)에 의해 결정되었다.

[0117] 역전사

[0118] 키트 지침에 따라 Promega M-MLV 역전사효소 효소를 사용하여 총 500 ug RNA를 cDNA로 역전사했다. 성숙한 폴리 아데닐화 RNA 전사체만 역전사되도록 보장하는 OligodT 프라이머를 사용하여 cDNA의 첫 번째 가닥 합성을 수행하였다.

[0119] 인트론 보유 분석

[0120] Promega에서 제공하는 GoTaq[®] 그린 키트를 사용하여 다양한 조직/세포 샘플의 cDNA를 분석했다. cDNA를 반응 마스터믹스에 첨가하고 qPCR 플레이트에 피펫팅했다. 그런 다음 각각의 엑손-엑손 및 엑손-인트론 쌍에 대한 프라이머를 웰에 피펫팅했다. 각각의 반응에는 기술적 복제물과 물 제어가 있었다. RT 마이너스 반응으로 게놈 DNA 오염이 없음을 확인했다.

[0121] Taqman[®] Fast Advanced Cells to Ct 키트를 사용한 안티센스 올리고뉴클레오타이드(ASO) 스크리닝

[0122] 세포를 필요한 밀도로 96웰 플레이트에 시딩했다. 다음 18 내지 24시간 내에 형질주입을 수행하고 세포를 스크리닝 시점까지 인큐베이션하였다. 추가 실험 절차를 제조업체의 지침에 따라 수행하였다. 간략하게, 배지를 흡인하고 세포를 차가운 PBS로 세척하였다. Dnase-함유 용해 용액을 세포에 첨가하고 실온에서 5분 동안 인큐베이션하였다. 그런 다음 정지 용액을 세포에 첨가하고 세포를 실온에서 2분 동안 인큐베이션하여 용해를 정지시켰다. 역전사를 위한 키트 구성 요소를 사용하여 cDNA로 전환하는 데 총 20%의 용해물을 사용하였다. 그런 다음 키트에 제공된 qPCR용 성분을 사용하여 만든 마스터 믹스에 cDNA를 25% 농도로 첨가했다. 검정을 위해 Taqman[®] 프라이머를 사용했다. 각각의 반응은 발현의 웰-내 정규화를 위해 하우스키핑 유전자로 증폭되었다. ASO 처리된 세포를 모의 형질주입된 세포로 정규화하였다.

[0123] 상대 유전자 발현 검정

[0124] 형질주입 24시간 후에 세포 용해물을 수집하고, taqman 기반 유전자 발현 검정에 사용하였다. Cells to Ct 키트(Invitrogen) 실시간 RT-PCR 키트를 사용하여 SCN2A 발현 수준을 측정하였다. SCN2A 엑손 2-3 경계를 표적으로 하는 Taqman 프로브를 사용하였으며 하우스키핑 HPRT(하이포크산틴 구아닌 포스포리보실트랜스퍼라제) 유전자를 대조군으로 사용하였다.

[0125] 실시예 2 - 인간 SCN2A에서 인트론 보유

[0126] IRBase(Middleton *et al.*, 2017, Genome Biol. 18: 51)는 2000개가 넘는 인간 샘플의 RNA 시퀀싱 자원이며, 여기에서 특정 조직에서 유전자의 특정 인트론 보유 이벤트가 평가될 수 있다. 이 데이터베이스를 사용하여, 뇌 조직에서 SCN2A의 인트론 보유 이벤트를 분석했다. 도 1에 나타난 바와 같이, SCN2A의 여러 인트론은 보유를 나타내며 인트론 11은 가장 많은 수의 이벤트를 나타낸다(가는 선은 인트론을 나타내고 두꺼운 선/블록은 엑손에 해당함; 막대의 높이는 인트론 보유의 기록된 이벤트 수를 나타냄).

- [0127] 정량적 PCR에 의해 인트론에 상응하는 서열의 수준을 분석함으로써 인트론 보유의 시험관내 검증을 수행하였다. 이를 달성하기 위해, 프라이머는 이에 축적하는 엑손에 비해 유지된 인트론의 존재를 특이적으로 검출하도록 설계되었다. DNase-처리된 폴리아데닐화 RNA에서 역전사된 cDNA를 사용하여 게놈 DNA가 아닌 pre-mRNA 전사체가 프라이머에 의해 검출되도록 했다.
- [0128] 성숙한 SCN2A mRNA에서 인트론 보유 이벤트를 실시간 PCR로 분석하였다. 27개의 엑손과 26개의 인트론으로 이루어진 SCN2A 서열(NG_008143.1; 서열번호 2)에 걸쳐 각각의 엑손-인트론 쌍에 대해 2세트의 프라이머를 설계했다:
- [0129] A: 인트론-보유 전사체에 특이적인 프라이머: 정방향 프라이머는 선행 엑손의 서열로부터 설계되었고 역방향 프라이머는 엑손에 대해 하류의 인트론 서열로부터 설계되었다(도 2A).
- [0130] B: 스플라이싱된 전사체에 특이적인 프라이머: 프라이머 중 하나는 인접한 2개의 엑손의 접합부에 걸쳐 있도록 설계되었고, 다른 하나는 이에 따라 선행 또는 후속 엑손의 서열로부터 설계되었다(도 2B).
- [0131] 프라이머는 하기 표 1에 제시되어 있다.

표 1

인트론 보유 분석을 위한 프라이머

프라이머 명칭	프라이머 서열	서열번호
SCN2A E1-E2F	CTTTATTTTCAGCACTTTCTTATGCAAG	13
SCN2A E1-E2R	TCTTCTGCAATGCGTTGTTC	14
SCN2A E1-I1F	TTTTCCCTCCCTGTTTCTG	15
SCN2A E1-I1R	TCTTCTGCAATGCGTTGTTC	16
SCN2A E2-E3F	GACCCCTACTATATCAATAAGAAAACG	17
SCN2A E2-E3R	AGGGTTGAAGGGAGTTAAAATG	18
SCN2A E2-I2F	AGAAAGTGAGTTCCTAGTCAAGTTGC	19
SCN2A E2-I2R	TGCAGGTTCAAATGAGTGCT	20
SCN2A E3,4,5F	GGTACATTCTTTATTCAATATGCTCATTA	21
SCN2A E3,4,5R	TCTAAACAAAAGCCCCTTGC	22
SCN2A E3-I3F	GGTACATTCATATCCTTTTTCAAATC	23
SCN2A E3-I3R	CACTTAGAAGACGGGGGAAA	24
SCN2A I4-E4F	ATATCGTAGGGGGACCAACC	25
SCN2A I4-E4R	TCTAAACAAAAGCCCCTTGC	26
SCN2A E5-E6F	CACAGTCATTACTTTTGCATATGTG	27
SCN2A E5-E6R	TGTCTGAAATTGTTTCAATGCT	28
SCN2A I5-E6F	AAGGTGGTACGCCCCCTTATATC	29
SCN2A I5-E6R	TTGTTTTCAATGCTCGGAGA	30
SCN2A E6-E7F	ATTCCAGACCTGAAGACCATT	31
SCN2A E6-E7R	GAGGCCATTGGAACATTTA	32
SCN2A E6-I6F	TTCTCCGAGCATTGAAAACA	33
SCN2A E6-I6R	GCAAAGAATCAAATCCATCCA	34
SCN2A E7-E8F	CCTGAAGACCATTGTGGGGG	35
SCN2A E7-E8R	AAAGTGACTTTTATCCTC	36

[0132]

SCN2A E7-I7F	TGTTTGCAATGGCCTCCAGA	37
SCN2A E7-I7R	AGTCTAAGTCACTTGATTCACATCT	38
SCN2A E8-E9F	AGATGCACGCCAGTGTCTCT	39
SCN2A E8-E9R	CAGTTGATAAAGGTTTTCCAGAGA	40
SCN2A E8-I8F	TAGAGGGGCAAAATGATGCT	41
SCN2A E8-I8R	TGGAGGGTTTTGAGTGAGGA	42
SCN2A E9-E10F	CTTTATCAACTGACACTACGTGCT	43
SCN2A E9-E10R	CCTTCTGTTCAGCCTCTTCC	44
SCN2A E9-I9F	ACCCCAACTATGGCTACACG	45
SCN2A E9-I9R	GCAATTCTCTTGAGTTCGGTGT	46
SCN2A E10-E11F	CAAGAAGAAGCTCAGGCGG	47
SCN2A E10-E11R	CGATTTTCGGACTCTGTCAAT	48
SCN2A E10-I10F	AGGTTTCCGTTTTCTCTGG	49
SCN2A E10-I10R	AAGGCGGTTTTCTCTCCATT	50
SCN2A E11-E12F	CTCCACACCAGTCTTACTGAGC	51
SCN2A E11-E12R	TTGTCCTCAAAGGTGCTGTG	52
SCN2A E11-I11F	AGGTTTCCGTTTTCTCTGG	53
SCN2A E11-I11R	AAGGCGGTTTTCTCTCCATT	54
SCN2A E12-E13F	CTACCAGAGGGCACAACACTG	55
SCN2A E12-E13R	CATTGCTCTTTGCCTTGATG	56
SCN2A E12-I12F	GACTGCAATGGTGTGGTCTC	57
SCN2A E12-I12R	TGGGCAACAACTCAACAAA	58
SCN2A E13-E14F	CATGGAAGAAGTTGAAGAATCCA	59
SCN2A E13-E14R	TGGGATAGTGCTCCATAGCC	60
SCN2A E13-I13F	TTTTGACCAACACCATGGAA	61
SCN2A E13-I13R	GCAGCTTTCTGGAGGCTAAA	62
SCN2A E14-E15F	TGGAAACCTGGTCTTCACAG	63
SCN2A E14-E15R	CGGAATGATCGGAGAACTG	64
SCN2A E14-I14F	CCATCACCATCTGCATTGTC	65
SCN2A E14-I14R	GGGATGCTAAACATTTGATGTC	66
SCN2A E15-E16F	GGCTGCTCCGAGTTTTCAA	67
SCN2A E15-E16R	ACACGATCAGGAAGGAGTGG	68
SCN2A E15-I15F	GGCAAATGTGGAAGGATTGT	69
SCN2A E15-I15R	TGGTGGTATGGCAATCGAAT	70
SCN2A E16-E17F	GGAAATCTAGTGCTTCTGAACCTC	71
SCN2A E16-E17R	GCTTTCTGCTTCCTAACAAAGG	72
SCN2A E16-I16F	GCCAAACCATGTGCCTTACT	73
SCN2A E16-I16R	GACAATAGGAAGTGGCCTTGA	74
SCN2A E17-E18F	GCAAAGAGAAGCTAAATGCAAC	75
SCN2A E17-E18R	GCCTTCAGGTTCAAGGGATT	76
SCN2A E17-I17F	CCAAATGCTGTTGGAGAATC	77
SCN2A E17-I17R	ACAGGAAGGAAACACGCAAT	78
SCN2A E18-E19F	CTGTTTTACAGAAGACTGTGTACGG	79
SCN2A E18-E19R	GCTCAGCAGAATGATGAAGACA	80
SCN2A E18-I18F	TGAGGAATCCCTTGAACCTG	81

[0133]

SCN2A E18-I18R	TTCAAACAGCAAATGCAAAAA	82
SCN2A E19-E20F	GGGCTCTCGCCTTTGAAGATA	83
SCN2A E19-E20R	AGGAAGTCTAGCCAGCACCA	84
SCN2A E19-I19F	CAAAGGGAAACTCTGGTGGA	85
SCN2A E19-I19R	TTGAAGGGAAAGGGAAAAGA	86
SCN2A E20-21F	CTGATTGTTGATGTCTCACTGG	87
SCN2A E20-21R	ATTCCTTCAAACCGGGACA	88
SCN2A E20-I20F	ATGCTGCTAAAGTGGGTTGC	89
SCN2A E20-I20R	TGTCAGGCACCATTTGCTAGA	90
SCN2A E21-E22F	GAATGAGGGTTGTTGTAAATGC	91
SCN2A E21-E22R	ACCTGGCAGTTTGATTGCTC	92
SCN2A E21-I21F	AGGCCACTGAGAGCTTTGTC	93
SCN2A E21-I21R	GACTCCAGCAGTGCCAAGTA	94
SCN2A E22,23,24F	GTAGCCACGTTTAAGGGATG	95
SCN2A E22,23,24R	GACACCAATGAAAAGATTCAAGG	96
SCN2A E22-I22F	GCCAGGTGGAAAAATGTGAA	97
SCN2A E22-I22R	TTACATCCCTGCCAACAATG	98
SCN2A E23-E24F	CACGAAATGTAGAATTACAACCC	99
SCN2A E23-E24R	GACACCAATGAAAAGATTCAAGG	100
SCN2A E23-I23F	CACGTTTAAGGGATGGATGG	101
SCN2A E23-I23R	CAGAGTGATTAGAGAAAATCATGCTATG	102
SCN2A E24-E25F	GGTGTTCATCATTTTGGAGGTC	103
SCN2A E24-E25R	CGAGGTATGGGTTTTTGTGG	104
SCN2A E24-I24F	CAACCCAAGTATGAAGACAACC	105
SCN2A E24-I24R	TTTGGTGATTGGATTTTGA	106
SCN2A E25-E26F	CGACCTGCTAACAAATCCAAG	107
SCN2A E25-E26R	TTCAGCACACATTCTCCAGTG	108
SCN2A E25-I25F	AACCCATACCTCGACCTGCT	109
SCN2A E25-I25R	AGGCTAGGTAGTCCCATTTGT	110
SCN2A E26-E27F	CATTGTAGGAATGTTTCTGGC	111
SCN2A E26-E27R	ACATCCCAAAGATGGCGTAG	112
SCN2A E26-I26F	TGTGGTGGTCATTCTCTCCA	113
SCN2A E26-I26R	CTCATCATTTCAAGCACAGCA	114

[0134]

[0135]

인간 뇌 RNA를 사용하여 SCN2A mRNA에서 인트론 보유를 확인했다. 더 넓은 스펙트럼의 RNA 샘플을 포함하기 위해, 두 개의 상업적 공급업체(Ambion 및 Takara)로부터 RNA를 얻었다. 프라이머가 인트론에 걸쳐 있기 때문에, RNA와 함께 분리된 임의의 게놈 DNA가 검출되어 인트론 보유의 과대평가를 야기할 수 있었다. 따라서 RNA를 역전사 전에 DNase로 처리하였다. 또한 역전사효소 효소가 없는 반응물을 포함하여 게놈 DNA가 없는지 확인했다. RNA를 cDNA로 역전사하는 동안, 성숙(폴리아데닐화)되고 보유된 인트론을 갖는 전사체의 선택을 선호하기 위해 oligodT 프라이머를 사용했다. 이것은 인트론이 스플라이싱 과정에 있는 미성숙 RNA 전사체의 역전사를 방지했다.

[0136]

상기 기재한 전략을 사용하여, 인간 전체 뇌 RNA(Ambion 제공)의 제1 샘플에서 다양한 보유 수준을 나타내는 다수의 인트론이 검출되었으며, 그 수준은 엑손 발현의 10% 미만에서 최대 40% 범위이었다. 가장 높은 보유를 보이는 인트론에는 인트론 2 및 17이 포함되었다(도 3a). 유사한 인트론 보유 프로파일이 인간 뇌 RNA의 두 번째 공급원(Takara 제공)에서 관찰되었으며, 여기서 인트론 2 및 17은 또한 엑손에 대해 가장 높은 보유를 나타냈다(도 3b).

[0137]

SCN2A 성숙 mRNA 전사체에서 다수의 인트론을 다양한 정도로 보유한다. 상이한 실험 코호트의 대표로서 2개의 상이한 RNA 공급원 사이의 보유를 비교하면, 이들 코호트의 인트론 보유 프로파일의 차이에도 불구하고, 코호트들 다에서 보유를 나타내는 공통 인트론이 있다.

[0138]

2개의 뉴런-유사 세포주, 즉 SH-SY5Y 및 SK-N-AS에서의 인트론 보유를 상기 기재된 바와 같이 평가하였다. SH-SY5Y 및 SK-N-AS는 전이성 골종양에서 유래된 형질전환된 뉴런-유사 세포주이며 뉴런 기능 연구에 널리 사용된다.

[0139]

뇌 조직에서 관찰된 인트론 보유 프로파일과 유사하게, 인트론 2는 SH-SY5Y 세포를 평가했을 때 엑손 발현 수준의 35% 수준으로 가장 높게 보유된 인트론이었다(도 4a). SK-N-AS 세포는 인트론 2와 17의 높은 보유율을 나타냈고, 생물학적 복제물 사이에 높은 변동이 있었지만, 이러한 인트론의 보유 수준은 엑손 발현의 75%였다.

보유를 나타낸 이 세포주의 다른 인트론은 인트론 1, 3, 4, 5, 13 및 24였다(도 4b).

- [0140] 요약하면, SCN2A mRNA에 보유한 인트론 중에서, 인트론 2(서열번호 12) 및 인트론 17은 테스트된 상이한 세포주 및 공급원 중에서 가장 높은 보유 수준을 나타낸다.
- [0141] **실시예 3 - 인트론 2의 보유를 감소시키기는 안티센스 올리고뉴클레오타이드의 확인**
- [0142] 특정 서열에 대한 ASO의 표적화는 핵산 분자에 대한 스플라이스좀과 같은 단백질의 접근을 입체적으로 차단할 수 있다. 마찬가지로, ASO는 스플라이싱 인핸서 또는 사일런서 서열과 같은 부위를 차단하여 서열의 스플라이싱 성향을 변경하는 데 사용될 수 있다. 보유한 인트론에서 인트론 스플라이싱 사일런서(ISS) 부위를 차단하면 사실상 스플라이싱이 유도된다. 따라서 스플라이싱을 유도하여 단백질로 번역하기 위해 세포질로 수송되는 스플라이싱된 전사체의 수준을 증가시키기 위해 보유한 인트론을 가지고 ISS 부위를 차단하는 SCN2A 전사체 풀을 표적으로 하는 ASO를 확인하기 위해 다양한 도구와 연구를 수행하였다.
- [0143] ISS 서열이 이상적인 안티센스 표적 역할을 하지만, 긴 인트론에서는 종종 눈에 띄지 않는다. 표적화되었을 때 잠재적으로 스플라이싱을 증가시키는 인트론 서열의 부분(들)을 확인하기 위해, 인트론 서열의 인실리코(*in silico*) 분석에 사용가능한 예측 도구(Human Splicing Finder, SpliceAid2, RBP Map, PESXs 및 RegRNA 2.0)를 활용했다.
- [0144] 이들 온라인 도구는 컨센서스 서열에 기반하여 엑손 스플라이싱 인핸서(ESE), 엑손 스플라이싱 사일런서(ESS), 인트론 스플라이싱 인핸서(ISE) 및 인트론 스플라이싱 사일런서(ISS)를 포함하는 서열에서 스플라이싱 부위를 예측한다. 사용가능한 도구에는 Human Splicing Finder, SpliceAid, RBP Map, PESXs, RegRNA 2.0이 포함된다.
- [0145] 불량한 스플라이싱 인자 모집은 약해진 스플라이싱을 초래하고, 이는 pre-mRNA의 2차 구조에 의해 영향을 받는 것으로 보고되었다(Buratti *et al.*, 2004, Mol. Cell Biol. 24(24), 10505-10514). pre-mRNA 2차 구조의 인실리코 예측은 Mfold 및 RNA fold와 같은 예측 도구를 사용하여 수행되었다. 도 5는 SCN2A 인트론 2의 2차 구조 예측을 나타낸다. ASO는 2차 구조에 대한 경향이 높은 표적 영역에 대해 설계될 수 있다.
- [0146] 공개된 보고서에 기반하여, 스플라이싱 메커니즘에 대한 스플라이스 억제자인 hnRNP A1 및 hnRNP I의 역할의 중요성이 추론되었다(Yimin Hua *et al.*, 2008, Am. J. Hum. Genet. 82, 834-848). 예측 도구인 SpliceAid2 (<http://www.introni.it/splicing.html>)를 사용하여 이러한 스플라이싱 사일런서에 의해 결합될 수 있는 5' 및 3' 스플라이스 부위 근처의 인트론 서열 영역을 좁혔다. HnRNP A1 부위는 인트론 2의 5' 스플라이스 부위에 대해 위치 +8 내지 +12(CTTAG), +33 내지 +36(ATTTA) 및 +59 내지 +65(CAGGGA)에 있고 인트론 2의 3' 스플라이스 부위에 대해 위치 -84 내지 -88(ATTTA) 및 -82 내지 -87(TTTATA)에 있을 것으로 예측되었다. HnRNP A1 부위도 또한 인트론 17에 대해 예측되었고, 인트론 17의 5' 스플라이스 부위에 대해 위치 +21 내지 +26(AGATAT) 및 인트론 17의 3' 스플라이스 부위에 대해 위치 -53 내지 -58(TTTATA), -49 내지 -53(ATTTA) 및 -18 내지 -24(TTTTTT)에서 발견되었다.
- [0147] ASO는 스플라이싱 억제자인 hnRNP A1 및 hnRNP I에 대한 결합 부위의 예측을 기반으로 ASO가 이들 부위를 표적화하도록 설계되었다. ASO는 포스포로티오에이트(PS) 백본(안정성 증가) 및 2'-O-메톡시에틸리보스(2'-MOE) 당 변형(결합 친화도 증가 및 독성 감소)이 있는 18개 뉴클레오타이드 길이의 완전히 변형된 올리고뉴클레오타이드였다. 마이크로워크 전략을 사용하여 인트론 2의 5' 말단에서 시작하여 ASO를 설계하였다(도 6b 참조). 표적-외 결합 부위가 있는 ASO는 제외되었다.
- [0148] 표 2는 인트론 2의 5' 말단에 있는 ASO를 제시한다.

표 2

ASO 명칭	서열	서열번호
SCN2A-EX2-6	CTAAGAACTCACTTTCTT	115
SCN2A-EX2-5	ACTAAGAACTCACTTTCT	116
SCN2A-EX2-4	GACTAAGAACTCACTTTC	117
SCN2A-EX2-3	TGACTAAGAACTCACTTT	118
SCN2A-EX2-2	TTGACTAAGAACTCACTT	119
SCN2A-EX2-1	CTTGACTAAGAACTCACT	120
SCN2A-INT2+1	ACTTGACTAAGAACTCAC	121
SCN2A-INT2+2	AACCTTGACTAAGAACTCA	122
SCN2A-INT2+3	CAACTTGACTAAGAACTC	123
SCN2A-INT2+4	GCAACTTGACTAAGAACT	124
SCN2A-INT2+5	GGCAACTTGACTAAGAAC	125
SCN2A-INT2+6	AGGCAACTTGACTAAGAA	126
SCN2A-INT2+8	GAAGGCAACTTGACTAAG	127
SCN2A-INT2+9	TGAAGGCAACTTGACTAA	128
SCN2A-INT2+10	GTGAAGGCAACTTGACTA	129
SCN2A-INT2+11	AGTGAAGGCAACTTGACT	130
SCN2A-INT2+12	CAGTGAAGGCAACTTGAC	131
SCN2A-INT2+13	GCAGTGAAGGCAACTTGA	132
SCN2A-INT2+14	GGCAGTGAAGGCAACTTG	133
SCN2A-INT2+15	AGGCAGTGAAGGCAACTT	134
SCN2A-INT2+16	TAGGCAGTGAAGGCAACT	135
SCN2A-INT2+17	ATAGGCAGTGAAGGCAAC	136
SCN2A-INT2+18	AATAGGCAGTGAAGGCAA	137
SCN2A-INT2+19	AAATAGGCAGTGAAGGCA	138
SCN2A-INT2+20	TAAATAGGCAGTGAAGGC	139
SCN2A-INT2+21	GTAAATAGGCAGTGAAGG	140
SCN2A-INT2+22	AGTAAATAGGCAGTGAAG	141
SCN2A-INT2+35	CCCAGAACCAATTAGTAA	142

[0149]

[0150] 표 3은 인트론 2의 3' 말단에 있는 ASO를 제시한다.

표 3

ASO 명칭	서열(3' 말단)	서열번호
SCN2A-INT2-1	CTGAAAAAGAAAACACAA	143
SCN2A-INT2-2	TGAAAAAGAAAACACAAC	144
SCN2A-INT2-3	GAAAAAGAAAACACAACA	145
SCN2A-INT2-4	AAAAAGAAAACACAACAC	146
SCN2A-INT2-5	AAAAGAAAACACAACACA	147
SCN2A-INT2-6	AAAGAAAACACAACACAT	148
SCN2A-INT2-7	AAGAAAACACAACACATA	149
SCN2A-INT2-8	AGAAAACACAACACATAA	150
SCN2A-INT2-9	GAAAACACAACACATAAA	151
SCN2A-INT2-10	AAAAACACAACACATAAAA	152
SCN2A-INT2-11	AAACACAACACATAAAAT	153
SCN2A-INT2-12	AACACAACACATAAAATA	154
SCN2A-INT2-13	ACACAACACATAAAATAA	155
SCN2A-INT2-14	CACAACACATAAAATAAA	156
SCN2A-INT2-15	ACAACACATAAAATAAAA	157
SCN2A-INT2-20	ACATAAAATAAACTTAG	158
SCN2A-INT2-21	CATAAAATAAACTTAGA	159
SCN2A-INT2-30	AACTTAGAATATATATT	160
SCN2A-INT2-31	AACTTAGAATATATATTA	161
SCN2A-INT2-33	CTTAGAATATATATTAAG	162
SCN2A-INT2-34	TTAGAATATATATTAAGA	163
SCN2A-INT2-35	TAGAATATATATTAAGAA	164
SCN2A-INT2-36	AGAATATATATTAAGAAT	165
SCN2A-INT2-44	TATTAAGAATATTTAAG	166
SCN2A-INT2-46	TTAAGAATATTTAAGAG	167
SCN2A-INT2-47	TAAGAATATTTAAGAGA	168

[0151]

SCN2A-INT2-48	AAGAATATTTTAAGAGAA	169
SCN2A-INT2-49	AGAATATTTTAAGAGAAA	170
SCN2A-INT2-58	TAAGAGAAAAGTAAAACC	171
SCN2A-INT2-59	AAGAGAAAAGTAAAACCA	172
SCN2A-INT2-60	AGAGAAAAGTAAAACCAT	173
SCN2A-INT2-61	GAGAAAAGTAAAACCATT	174
SCN2A-INT2-64	AAAAGTAAAACCATTATT	175
SCN2A-INT2-65	AAAGTAAAACCATTATTT	176
SCN2A-INT2-66	AAGTAAAACCATTATTTA	177
SCN2A-INT2-67	AGTAAAACCATTATTTAT	178
SCN2A-INT2-68	GTAAAACCATTATTTATA	179
SCN2A-INT2-69	TAAAACCATTATTTATAA	180
SCN2A-INT2-72	AACCATTATTTATAAATA	181
SCN2A-INT2-73	ACCATTATTTATAAATAT	182
SCN2A-INT2-74	CCATTATTTATAAATATT	183
SCN2A-INT2-77	TTATTTATAAATATTGCC	184
SCN2A-INT2-79	ATTTATAAATATTGCCCT	185
SCN2A-INT2-80	TTTATAAATATTGCCCTG	186
SCN2A-INT2-81	TTATAAATATTGCCCTGT	187
SCN2A-INT2-82	TATAAATATTGCCCTGTA	188
SCN2A-INT2-83	ATAAATATTGCCCTGTAA	189
SCN2A-INT2-84	TAAATATTGCCCTGTAAA	190
SCN2A-INT2-85	AAATATTGCCCTGTAAAA	191
SCN2A-INT2-86	AATATTGCCCTGTAAAAT	192
SCN2A-INT2-87	ATATTGCCCTGTAAAATA	193
SCN2A-INT2-88	TATTGCCCTGTAAAATAG	194
SCN2A-INT2-89	ATTGCCCTGTAAAATAGT	195
SCN2A-INT2-90	TTGCCCTGTAAAATAGTG	196

SCN2A-INT2-91	TGCCCTGTAAAATAGTGT	197
SCN2A-INT2-92	GCCCTGTAAAATAGTGTA	198
SCN2A-INT2-93	CCCTGTAAAATAGTGTAT	199
SCN2A-INT2-94	CCTGTAAAATAGTGTATA	200
SCN2A-INT2-95	CTGTAAAATAGTGTATAA	201
SCN2A-INT2-96	TGTAAAATAGTGTATAAC	202
SCN2A-INT2-97	GTAAAATAGTGTATAACA	203
SCN2A-INT2-98	TAAAATAGTGTATAACAT	204
SCN2A-INT2-99	AAAATAGTGTATAACATA	205

[0152]

[0153]

[0154] ASO를 200 nM 농도의 리포펙타민 3000으로 SH-SY5Y 세포에 형질주입시키고, 24시간 후에 세포를 용해시켰다. Taqman® Fast Advance Cells to Ct 키트(Invitrogen, Thermofisher Scientific)를 사용하여 실시예 1에 기재된 바와 같이 역전사 및 qPCR 분석을 수행하였다. 복제물 사이에 유도된 변이에 대해 정규화하기 위해 Taqman 프라이머는 하우스키핑 유전자와 이중체를 형성하였다. ASO 처리 후 세포에서 SCN2A의 발현을 모의-형질주입된 세포로 형질주입된 세포와 비교하고 정규화하였다.

[0155] 도 6에 나타난 바와 같이, 테스트된 인트론 2 ASO 중에서 ASO SCN2A-INT2+6은 모의-형질주입된 세포와 비교할 때 일관되게 1.37배의 SCN2A mRNA의 상향 조절을 초래하였다. 1배 초과 상향 조절을 제공한 다른 ASO는 5-프라임 말단에서 SCN2A-INT2 -4, -3, -2, +6, +8, +9, +10, +14, +19, +20, +21, +22, +23, +24, +25, +26, +28, +29, +30, +33, +34, +43, +45, +46, +49, +52, +59, +64, +65, +68, +69, +70, +71, +72, +73을 포함하고, 3-프라임 말단에서 SCN2A-INT2 -1, -3, -12, -13, -14, -44, -46, -49, -58, -59, -60, -61, -64, -65, -66, -67, -68, -69, -77, -79, -82, -83, -84, -85, -89, -91, -93, -95, -96을 포함한다. 생물학적 복제물 사이의 변이는 형질주입의 다양한 효율성을 나타내는 것일 수 있다.

[0156] SCN2A 전사체의 상향 조절에 대한 인트론 2 ASO의 작용 메커니즘을 규명하기 위해, 인트론 2 없이 전사체만을 증폭시키는 프라이머를 사용하였다. 이 프라이머는 인트론 2-측접 엑손, 즉 엑손 2와 3에 결합하는 반면, 프로브는 이들 사이의 접합부에 걸쳐 있다. 이들은 아래 표 4에 나와 있다.

표 4

프라이머 명칭	서열	서열번호
APDJZ44 SCN2A E2-E3 프로브(FAM)	AGTGCCACCCCTGC	206
APDJZ44 SCN2A-E2-E3F	TCTGGACCCCTACTATATCAATAAGAAAA	207
APDJZ44 SCN2A-E2-E3R	GGGTTGAAGGGAGTTAAATGTAAAG	208

[0158] 도 7에 나타난 바와 같이, SCN2A mRNA의 가장 높은 상향 조절을 유발하는 SCN2A-INT2+6으로 형질주입된 세포는 모의 및 어떠한 상향 조절을 유발하지 않은 ASO(SCN2A-INT2+ 35)와 비교하여 증가된 SCN2A 전사체의 양을 가졌다. 이는 SCN2A mRNA의 상향 조절을 유발하는 ASO가 보유한 인트론 2로부터의 스플라이싱에 의한 제거를 통해 효과를 발휘한다는 것을 시사하였다.

[0159] 본 개시내용에서 언급된 다른 서열

[0160] 서열번호 1; SCN2A 전사체 2(NM_001040142.2):

AACAGACATTGGGTACCATTGCAATGACTGTGTCAGAACAGAAAGCTAAGGCCAAGGAGGAGGATGCTGTGG
TCATCCTTTCTTGTGTTTTTTTCTTCTTTAATGAGGATAGAGCACATGTGAGATTTTACTTTTCTACTCCAGT
AAAAATTCTGAAGAATTGCATTGGAGACTGTTATATTCAACACATACGTGGATTCTGTGTTATGATTTAC
ATTTTCTTTTATTTCAGCATTCTTATGCAAGGAGCTAAACAGTGTATTAAAGGAGCAGGATGAAAAGAT
GGCACAGTCAGTGTGGTACCGCCAGGACCTGACAGCTTCCGCTTCTTTACCAGGGAATCCCTTGTGTGCT
ATTGAACAACCGATTGCAAGAGAGAAAGCTAAGAGACCCAAACAGGAACGCAAGGATGAGGATGATGAAA
ATGGCCCAAGCCAAACAGTGTGACTTGAAGCAGGAAATCTCTCCATTATTATTGAGAGACATTCCTCC
AGAGATGGTGTGAGTCCCTGGAGGATCTGGACCCCTACTATATCAATAAGAAAACGTTTATAGTATTG
AATAAAGGGAAGCAATCTCTCGATTGAGTCCACCCCTGCCCCCTTACATTTTAACTCCCTTCAACCCCTA
TTAGAAAATTAGCTATTAGATTGTTGGTACATTCTTTATTCAATATGCTCATTTATGTGCACGATTCTTAC
CAACTGTGTATTTATGACCATGAGTAACCCCTCCAGACTGGACAAAGAAATGTGGAGTATACCTTTACAGGA
ATTTATACTTTTGAATCACTTATTAATAATCTTGCAAGGGGCTTTGTTTAGAAGATTTACATTTTAC
GGGATCCATGGAATTGGTTGGATTTCACAGTCATTACTTTTGCATATGTGACAGATTTGTGGACCTGGG
CAATGTCTCAGCGTTGAGAACATTGAGATTCTCCGAGCATTGAAAACAATTTGAGTATTCAGGCTG
AAGACCATTTGGGGGCTGATCCAGTCAGTGAAGAAGCTTTCTGATGTGATGATCTTGACTGTGTCT
GTCTAAGCGTGTGTCGCTTAATAGGATTGCAAGTGTGTCATGGGCAACCTACGAAATAAATGTTTGAATG
GCCTCCAGATAATCTCTCTTTGAAATAAATATCACTTCTCTTTAAACAATTCATTGGATGGGAATGGT
ACTACTTTCAATAGGACAGTGAAGATTTAACTGGGATGAATATATTGAGGATAAAAGTCACTTTTATT
TTTTAGAGGGGCAAAATGATGCTCTGCTTTGTGGCAACAGCTCAGATGAGGCGAGTGTCTGAAGGATA
CATCTGTGTGAAGGCTGGTAGAAACCCCACTATGGCTACACGAGCTTTGACACCTTTAGTTGGGCTTT
TTGTCTTATTTCTGCTCTATGACTCAAGACTTCTGGGAAACCTTTATCAACTGACACTACGTGCTGCTG
GGAAAACGTACATGATATTTTGTGCTGTGCTATTTCTTGGGCTCATCTATCTAATAAATTTGATCTT
GGCTGTGGTGGCCATGGCTATGAGGAACAGAATCAGGCCACATTTGAAGAGGCTGAACAGAAGGAAGCT
GAATTTGAGCAGATGCTGCAAGCTTGAAGAAAGCAAGAAAGAGCTCAGGCGGAGCTGACAGCCGAT
CTGCTGAATCAAGAGACTTCAGTGGTGTGCTGGGATAGGAGTTTTCAGAGAGTTCTTCAGTAGCATC
TAAGTTGAGCTCCAAAGTGAAAAGAGCTGAAAACAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGCTCT
GGAGAAAGAGAGAAAATGACAGAGTCCGAAAATCGGAATCTGAAGACAGCATTAAGAAAGAAAGTTTCC
GTTTTCTCTTGAAGGAAGTAGGCTGACATATGAAAAGAGATTTTCTTCTCCACACCGACTCTTACTGAG
CATCCGTGGCTCCCTTTTCTCTCAAGACGCAACAGTAGGGCGAGCCCTTTTCACTTCAGAGGTCGAGCA
AAGGACATTTGGCTGAGAAATGACTTTGCTGATGATGAGCACAGCCTTTGAGGACAAATGACAGCCGAA
GAGACTCTCTGTTCTGTCGCGCAGCAGATGGAGAACGGCGCCACAGCAATGTGAGCCAGGCCAGCCGTGC
CTCCAGGGTGTCTCCCATCTGCCCATGAATGGGAAGATGATAGCGCTGTGGAGTGCATGGTGTGGTCT
TCCTTGGTGGGGGCTCTTACCTTCACATCTGCTGGGAGCTCTTACAGAGGGCACTACTGAAA

[0161]

CAGAAATAAGAAAGAGACGGTCCAGTTCTTATCATGTTTCCATGGATTATTGGAAGATCCTACATCAAG
GCAAAAGAGCAATGAGTATAGCCAGTATTTTGACCAACCATGGAAGAACTTGAAGAATCCAGACAGAAA
TGCCACCATGCTGGTATAAAATTTGCTAATATGTGTTTGATTGGGACTGTTGTAACCATGGTTAAAGG
TGAAACACCTTGTCAACCTGGTTGTAAATGGACCCATTGTTGACCTGGCCATCACCATCTGCATTGTCTT
AAATACACTCTTCATGGCTATGAGGACCTATCCCATGACGGAGCAGTTACGACGTGTACTGTCTGTGGGA
AACCTGGTCTTCACAGGGATCTTCACAGCAGAAATGTTTCTCAAGATAATTGCCATGGATCCATATTATT
ACTTTCAAGAAGGCTGGAATATTTTGTGATGGTTTTATTGTGAGCCTTAGTTTAATGGAACCTGGTTTGGC
AAATGTGGAAGGATTGTCACTTCTCCGATCATTCCGGCTGTCCGAGTTTCAAGTTGGCAAAATCTTGG
CCAACCTAAATATGCTAATTAAGATCATTGGCAATTTCTGTGGGGGCTCTAGGAAACCTCACCTTGGTAT
TGGCCATCATCGTCTTCATTTTGTGCTGGTGGCATGCACTCTTTGGTAAGAGCTACAAAGAATGTGT
CTGCAAGATTTCGAATGATTGTGAACCTCCACGCTGGCAGATGATGACTTTTCCACTCCTTCTGTGATC
GTGTTCCGCGTGTGTGTGGAGAGTGGATAGAGACCATGTGGGACTGTATGGAGGTGCTGGCCAAACCA
TGTGCTTACTGTCTTCATTTTGTGCTGGTGGCATGCACTCTTTGGTAAGAGCTACAAAGAATGTGT
GCTTTTGAGTTCTCTCAGTTCTGACAATCTTGTGCTGCACTGATGATGATAACGAAATGAATAATCTCCAG
ATTGCTGTGGGAAGGATGCAAGAAAGGAATCGATTGTTTAAAGAAAAATACGTGAATTTATTCAGAAAG
CCTTTGTAGGAAGCTGGAATATTTAGATGAAATTAACCGCTTGAAGATCTAATAATAAAAAAGACAG
CTGTATTTCCAAACCATACCCCATAGAAATAGGCAAGACCTCAATTATCTCAAAGACGGAAATGGAAC
ACTAGTGGCATAGGACGAGTGTAGAAAAATATGTGCTGGATGAAAGTGATTACATGTCATTTATAACA
ACCTTAGCCTCAGCTGTGACAGTACCAATTGCTGTTGGAGAACTGACTTTGAAATTTAAATACGAAGA
ATTACAGCAGCGAGTCAAGATATGGAGGAAAGCAAGAGAAGCTAAATGCAACTAGTTTCTGTAAGGCGAGC
ACGGTTGATATTGGAGCTCCCGCCGAGGGAGAACAGCCTGAGGTTGAACCTGAGGAATCCCTTGAACCTG
AAGCCTGTTTACAGAAAGCTGTGTACGGAAAGTTCAAGTGTGTGACAGATAAGCATAGAAGAGGCAAGG
GAAACTCTGGTGGAAATTTGAGGAAACATGCTATAAGATAGTGGAGCACAATTTGGTTGAAACCTTCATT
GTCTTCATGATTCTGTGAGCAGTGGGGCTCTGGCCTTTGAAGATATATACATTGAGCAGCGAAAAACCA
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TCACTGGTTAGCTTAACCTGCAAAATGCCTTGGGTTACTCAGAACTTGGTGGCATCAAAATCCCTCAGAACAC
TAAGAGCTCTGAGGCCACTGAGAGCTTTGTCCCGGTTTGAAGGAATGAGGGTTGTTGTAATGCTCTTTT
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GGAGTGAATCTCTTTGCTGGCAAGTTTTACCATGTATTAATTACACCACCTGGAGAGATGTTGATGTAA
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GAAAGTAACTTTGATTAACCTAGGACTTGGATATCTGTCTACTTCAAGTAGCCACGTTTAAAGGATGG
ATGGATATTATGTATGCAGCTGTTGATTACGAAATGTAGAATTACAACCCAAGTATGAAGACAACCTGT
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AAGAAATACTACAATGCAATGAAAAAACTGGGTTCAAGAAACCAAAAAACCATACCTCGACCTGCTA
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CTGCTTTAAGATGGTCAACCATGATGGTGGAAACCGATGACAGAGTCAAGAAATGACAAACATTCTGTAC
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GGCTGAACCTGATAGAAAGTATTTTGTGTCCCTTACCTGTTCCGAGTGTATCCGCTCTTCCAGGATTGGC
CGAATCCTACGCTGTATCAAGAGGACAAAGGGGATCCGACGCTGCTCTTTGCTTTGATGATGTCCCTTC
CTGCGTTGTTTAAACATCGGCCCTCTCTTTTCTGGTCAATGTTCACTACGCCATCTTTGGGATGTCCAA
TTTTGCTATGTTAAGAGGGAGTTGGGATCGATGACATGTTCAACTTTGAGACCTTTGGCAACAGCATG
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ATCCTGGAGAACTTCAGTGTGTCTACTGAAGAAAGTGCAGAGCCTCTGAGTGAGGATGACTTTGAGATGT
TCTATGAGTTTGGGAGAAATTTGATCCCGATGCGACCCAGTTTATAGAGTTTGCCAAACCTTCTGATTT
TGCAGATGCCCTGGATCCTCTCTCTCATAGCAAAACCAACAAAGTCCAGCTCATTGCCATGGATCTG
CCCATGGTGAGTGGTGACCGGATCCACTGTCTTGACATCTTATTTGCTTTTACAAAGCGTGTTTTGGGTG
AGAGTGGAGAGATGGATGCCCTTCGAATACAGATGGAAGAGCGATTGATGGCATCAAAACCCCTCCAAAGT
CTCTTATGAGCCCATACGACACGTTGAAACGCAAAACAGAGGAGGTGTCTGTATTATTATCCAGAGG
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AAATTTGAAAAAGACAAATCAGAAAAAGGAGACAAAGGGAAGATATCAGGGAAGTAAAAAGTAAAAAG
AAACCAAGAAATTTTCCATTTTGTGATCAATTTTACAGCCCGTGTGTTGATGTGTTTGTGTCAACAGG
ACTCCACAGGAGGTCTATGCCAACTGACTGTTTTACAAATGTATACTTAAGGTGAGTCCCTATAACA
AGACAGAGACCTCTGCTCAGCAAACTGGAACCTCAGTAACTGGAGAAATAGTATCGATGGGAGGTTTCTA
TTTTCAACACAGCTGACACTGCTGAAGAGCAGAGCGTAATGGCTACTCAGACGATAGGAACCAATTTA
AAGGGGGAGGGGAAGTTAAATTTTTATGTAATTCACATGTGACACTTGATAATAGTAATTGTCAACAG

[0162]

TGTTTAAGTTTAACTGCCACACCTGCCATATTTTACAAAACGTGTGCTGGAATTTATCACTTTCTT
TTAAATTCACAGGTGTTTACTATTATATGTGACTATTTTGTAAATGGGTTTGTGTTTGGGAGAGGGA
TTAAAGGGAGGGAATTCACATTTCTCTATTGTATGTATAACGGATATATTTAAATGGAGGCATGCT
GCAATTCCTATTACACATATAAAAAATCACATCACAAAAGGGAAGAGTTTACTCTTGTGTTTCAGGATGTT
TTTAGATTTTGGAGGTGCTTAAATAGCTATTCTGATTTTAAAGGTGCTCATCCAGAAAAAATTTAATGT
GCCTGTAATGTTCCATAGAAATCACAAGCATTAAAGAGTTGTTTATTTTACATAACCCATTAAATGTA
CATGTATATATGTATATATGTATATGTGCGTGTATATACATATATATGTATACACACATGCACACACAGA
GATATACACATACCATTAATTTGCTATTCACAGTCCAGCAGCATGACTATCACATTTTGTAAAGTGTCT
CTTTGGCATAAAAAATAAATATCTATCAGTCTTTCTAAGAAGCCTGAATTGACCAAAAAACATCCCA
CCACCACTTTTATAAGGTGATTCTGCTTTATCCTGCAGTATTGTTTAGCCATCTCTGCTCTTGGTAAGG
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TGTTTGAGCAACAAAAATGATGATTTAAGCACACTACTTATTCATCAAAATATGTACCACAGTAAGTAT
AGTTTGCAAGCTTTCAACAGGTAATATGATGTAATTTGGTTCATTATAGTTTGAAGCTGTCACTGCTGCA
TGTTTATCTTGCCATGCTGCTGTATCTTATTCCTTCCACGTTCAGAACTCAATATGGGAAGCCATAT
ATCAGTGGTAAAGTGAAGCAAAATGTTCTACCAAGACCTCATTCTCATGTCTTAAAGCAATAGGTGCA
GCAAAACAGGAAGAGCTTCTTGCTTTTATTCTTCCAACTTAAATGAACATCAATGATGAAAAGCCCG
ACTGTACAAACATGTTGCAAGCTGCTTAAATCTGTTTAAATATATGTTTAGAGTTTCTAAGAAAAATAT
AAATACGTGAAAAAGTTTCAATTTTATTTTATTTTTCAGCCTTTTGTACGTAAAAATGAGAAATTAAGTAT
CTTCAGGTGGATGTCACAGTCACTATTGTTAGTTTCTGTTCCAGCCTTTTAAATGAAGCACTTCACA
AAATAAGAAAGCAAGGACTAGGATGCAAGTGTAGGTTTCTGCTTTTATTAGTACTGTAACTTGACACACA
TTTCAATGTGAACAAATCTCAAACTGAGTTCAATGTTTATTGCTTCAATAGTAATGCTTATCATTTG
AAAGAGGCTTAAAGAAAAAATAAATCAGCTGATACTCTTGGCATTGCTTGAATCCAATGTTTCCACCTAG
TCTTTTATTCAGTAATCATCAGTCTTTTCCAATGTTTGTTCACACAGATAGATCTTATGACCCATATG
GCATAGAACTGTATCAGATATATAATATGGGATCCAGCTTTTTCCTCTCCACAAAAACAGGTAGTGA
AGTTATATTACAGTTACAGCAAAATACCTTTGTTTTCACAAGCAACAAATAATGTAGATTTCTTTATCT
GAAGCTATTGACTTGTAGTGTGTTGGTGAATGCATGCAGGAAATGCTGTTACCATAAAGAACGGTAAA
CCACATTAACAATCAAGCCAAAGAAATAAAGGTTTCGCTTTTGTATTTAATGTTGCTTTGTTT
CTATCTTTGAAATGCCATTTAAAGGTAGATTTCTATCATGTAAAAATAATCTATCTGAAAAACAAATGTA
AAGAACACACATTAATTTACTATAATTCATCTTTCAATTTTTCATGGAATGGAAGTTAATTAAGAAAGGT
GTATTGGATAACTATTAAATTTGGCCAAAAAGCTAGATATGGCATCAGGTAGACTAGTGGAAAGTTAC
AAAAATTAATAAAAAATTTGACTAACA

[0163]

[0164] 서열번호 2; SCN2A RefSeqGene 뉴클레오티드 서열(NG_008143.1):

AAGACTTCCATCTGCCCTCCGTAGGTTCAACTTTAATGCTTCCTACAGATGGGCAACAGGGCTATCTGCT
CAGTGCAACCCCAAGAAATTCATTTGCTGTGCTGTCTCCACTCCATACCATAACTAAGCCTAGGAAGCG
CCTCCTGAAGTCTTCTCACTGGGCTGAATTAAGAACAGGATCATCGTTCTCTATCTGGGTGTTTCTACCC
CACTGAGAGAGGTGAATTTGTCAGAACAAAAATTAGGAATTTACCAATGCAGTTTTTTTTGGCTGAT
AATTACTATTATTAACAATAATGACAAATGATAAGCTTAGAAAAATTAACACTAAACAATGGCTGCCGTA
AGAACACTGAAATGACTTTATAGTTTACAATCAGTCTTTCTGCTTTTCAGGGATATTTTGTGGACATCTG
GTGCCACATTTTAGTATGGGTTTCAGAGAAATATCAATTTACCTGTTTCTTCATCATGCTATATTGAGTTA
TTTACTCCACAATAAAGGTAGACCTACTGATGAGAAATGTCAGGGCAACCTTTGTGTATTTCAAGTTACCC
TCAGCAAGGTGCTACCACTAGCATTTAGTGTATTTCTGCTGTTGGTAAACAGGATCATGTGAGCCTTGTAG
ATGAGAACTCCTTTGCTCTCAGGGGATCATGGAATGCTGCTCTTTTCAGACAGCCATCTTCGGGGAAGA
GAAGGGTCTCTGGCAGAGCTCTATGGGTCTGCACCTCTCTTTCACTCTCTGCTTGTCTTTCACTCA
TTAATTTCTTGACAAATTTATTTTCTTAATAACTCTAGTTCCCTCTCTTTATCTTCTCTGTCATCTCTT
GCATCCATTGCAAAATCCATCACTGCTCTGAAATACCACACTGAAGGGAGGTACTCCTAAAGACCTTCTCT
TCTTTTCCACTCTTGTTTTACCCTTGGCTTACACAACCTGTTGAACAACCTGGAGCTGAGGACAAAAAGCAC
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TTAGCATATTTTAAAGGTGACTAAAACTGTACAAATGAAATGGCAATTTAAAAATGTCTGTTTCAATTTGCTT
TTCTCCAATGCAGATATTTTAAATAAGAAAGAGTGAGGCAAGAAAAATGTAACAAATATTAGGTATAAA
CTAGGAACCTTAGTCTTTTATTTTCTGAATCAGATGGATGATTTTAAAGCTGGGTGAATGATAAATGG

[0165]

– 37 –

TGGGGAAATGACAACAAAATAACAACAGTTCTCTTAAGCATATATGAAGGTAGTGCTTGAAATATCATGA
 AAAAAAGAAAGGGAGATTATGATAATGTTATGATAAGAGAAGACTGACACTATCCCTCTTCTCTGAATAA
 CTGATATCAAAATGTGAAATTTCTAAGGAATGAAGCAAGCTGGTCAGGGCAATGGTGTGCTGCTCTGTGA
 TCAGCCCGACATTTCCAGCTAGGATATTTTCCCTCTTTCTGTGTGATACAGTTTGGCTCTGTATCCTCA
 CCCAAATCTCACCTTGAATTTGTAGTTCCCATAACTCTCCACGTGTTGTGGGAGGGACCTGTGGGAGATAA
 TTTAATCAGGGGGTGGTTACCCCTCCTGCTGTTCTTGTGATAGTGAGTGAGTTCTCACTCGATCTGATGG
 TTTTATAGGGGTTTCTTCCCTTTTCTTGGACTTCTCTGTCCTGACACCTTGAGAAGAAGGATGTGTTT
 GCTTACCCCTTCCGCCATGATTGTAATTTCTGAGGCCCTTCCAGCCATGCAGCACTGTGAGTCAATTAA
 ACCTCTTTCTTTATAAATTAACCAATCTTGGGTATTTCTTCACAGCAGCATGAGAAGTGAATAACACAC
 TATGCTTCCCTGGATTGAGCAAAATGAGGGAGCCAGCAATTTGACGGCAGCTAGGATCTGTGAGGAGTG
 CTTAGACCTACACTTACTATTTCTGAAATTAACAGTCTAGAAGACAGGAGCCTAGTTATACAGACAGGGT
 GACCATAAAATGTGTCATCTAAACCAGGATTCACTTTGTTAATAAATTTCTCCACAACGACAGGTATAAAT
 CAAGATGGTTCCAGGTAAACTGGGATGCACAGTCACTCCACTTCTAAGGGTCAAGAAATCTTACATTATA
 ATCAGTCTATTCAATTAATTTGTTTCATGATTTTGTGCTAAGTGTAGAAAGTTAAATGTGAAATGAC
 CTATCAATAGACAAAGGGCATTCACTAAGCTGCTGCAATTAAGTGGCTTGATAAATCCATCAACCGAA
 ATCTCTAATGATTTCTGTTGAATCTTAAAGGAGTTTTCATGAATGTGTAATCTTGAAGATAGGAATTTAGCT
 CCAAGAACACTGGAAATTTCTCCACTGGCCTTTATACCCAAATCTTGGTAAATGTCAATATGCATATGTA
 TGGCCTCTTAACAGACTACCTGTAAAGTAGTTAAATTTCTTCCCAATGATCATGTTTTTTTTTACCC
 TTCTTGAGACATCCACTCCATGGTTTTATCTTGAAACTCTTCATGGCCAGTAACCTGCGGCTGCCATA
 ATCCTTCCCTTCATACATCCACACCTTTCTAGCGCATTTCTACAACTCCAAATTCATTCTCTTTAA
 TCCCACAGGCCCTCTAAATCACTGATCTCGTCATACTTTGATTATCTCTCACTAAATGCTCTTTCTCT
 TCATTAGATAAATTCACAGTTTATCCATAAAAGTCACTCCTTGATATACATACATACCTCACAACTCC
 TCGCTCAGTGTTTTTGTGTTGGTGGTTTTTGTGAACGTGCTGGCTAAATCTTCATCCTCATATTCA
 GTTCCACCTTACTTCTGCTGCACCTTTTGACGCTGGACGATGCAAAAGTGGGTCTTCATGATGCC
 AGCAATCATGCCACATGTCCCTAGATTGATGCCAGCAACCATGCCACATGTCCCTGGATCACTTACTCT
 CCGAGTTCTTGACTTAGATTTCATACCTTTCTTTCTCTCAAACCAACATACCTCCTACCTTGAC
 CTCACCTCAGTTGACAACCTTTGTTTCTAAATCAGTGAGAAAATGAAACAATCAGGAGAGATTTTTCC
 CCACAGGTTTCTATCACTGCATGTGCCCTTCTACCAAGTTCTGCACCCACATAACCTGACCTCCACCCA
 TTACCTTAATCAACTATCCCTGTTTCTATCTAAATCATTCCTGCACTGTGCACTAGATCCTATTCTT
 TCTTACTTACACAAGGGCATCTCTAGAGCATTTTATTTCTCTGCTCTCTGCAATTAGTAATTTAGCTC
 TTTATTATATTTGCATACAGCATTTTATTATTGCTCTTGTAATTTGAATTTTTTACTTCTTCAACTAT
 CTTTTTTTTATTTTTTGTGCTTTGCAATAAATCTTAATGAAAGTGTGCTGTATTCTCTGTATCTAT
 GACTCTCTTTCTATTCTCTTTTAAATTTAGGTTAGTCAAGTTTTTACTCTTACCAATTCACCAATCAGC
 TCTGATAGAAATCTGATGACACCTTATTGGGTGCTGATTCGTGCTGATTTCTTTCTTTCTTTATCTAC
 ACCATCCAGCAATATTGGAGACAGTTGATGCTGCTCCGCTTGATACACTGCTTCCGTGGATTGCA
 GGATAGCACACAATTTGACTCTTCTCTTAGCTTATTGTTCACTCCTTCACAGTCACTTTACTCTTTACT
 CTGCTTCCAGACTTCATTATTGGGTGCTGATTCGTGCTGATTTCTTTCTTTCTTTATCTAC
 TTCTTTTGATCCATTGAGTTTTATGGCGTTAAATGCCATATAAATCATGATGACATCCAAATATTAC
 CTCCAGTTTTCTGCCAACTGAAGTTGTGTCTAACTGCTTATTGGCTTGGATATCTAGAAGACACTG
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[0168]

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[0169]

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[0170]

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[0171]

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[0172]

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[0173]

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[0174]

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[0175]

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[illegible]

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[0186]

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[0187]

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[0188]

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[0189]

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[0194]

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[0195]

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[0196]

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[0197]

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[0198]

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[0199]

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[0200]

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[0205]

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GCCGAGGGAGAACAGCCTGAGGTTGAACCTGAGGAATCCCTTGAACCTGAAGCCTGTTTTACAGAAGACT
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GAAAACATGCTATAAGATAGTGGAGCACAATTTGTTTCAAAACCTTCATTGTCTTCATGATTCTGCTGAGC
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[0206]

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[0207]

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TAACA

[0208]

[0209] 서열번호 5; SCN2A 단백질 이소폼 2(NP_001035233.1):

MAQSVLVPPGPDSEFRFFTTRESLAAIEQRIAEKAKRKPQERKDEDDENGPKPNSDLEAGKSLPFIYGDIP
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TNCVFMTMSNPDPWTKNVEYTFGTGIYTFESLIKILARGFCLEDFTLRDPWNWLDFTVITFAYVTEFVNL
GNVSALRTFRVLRALKTIISVIPGLKTIIVGALIQSVKKLSVDMILTIVFCLSVFALIGLQLFMGNLRNKLQ
WPPDSSFEINITSFNNSLDNGNTTFNRTVSIFNWDDEYIEDKSHFYFLEBQNDALLCGNSSDAGQCPEG
YICVKAGRNPNGYTSFDTFSWAFSLSLFRLMTQDFWENLYQLTLRAAGKTYMIFFFVLVIFLGSFYLINLI
LAVVAMAYEEQNQATLEEAQKEAEFQQMLEQLKKQEEAQAASAESESRDFSGAGGIGVFSESSVA
SKLSSKSEKELKNRRKKKKQKEQSGEEKNDRVRKSESEDSIRRKGFRRFSLGSRITYEKRFSSPHQSL
SIRGSLFSPRRNSRASLSFSRGRAKDIGSENDFADDEHSTFEDNDSRRDSLFPVPHRHGERRHSNVSQASR
ASRVLPILPMNGKMHSAVDCNGVSVLVGGPSTLTSAGQLLPEGTTTTETEIRKRSSSYHVSMDLLEDPTS
RQRAMSIASILINTMEELLESROKCPWCWYKFANMCLIWDCCKPWLKVKHLVNLVVMDFVDLAIITICIV
LNTLFMAHEHYPMTEQFSSVSLVGNLVFTGIFTAEMFLKIIAMDPIYFYFQEGWNIFDGFIVSLSLMELGL
ANVEGLSVLRSLRLLRVFLKAKSWPTLNMLIKIIGNSVGALGNLTVLALIVFIFAVVGMQLFGKSYKEC
VCKISNDCELPRWHMDFHSHFLIVFRVLCGEWETMWDCEMVAGQTMCLTVFMMVMVIGNLVVLNLF
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GKLWNNLRKTCYKIVEHNWFETFIVFIMILLSSGALAFEDIYIEQRKTIKTMLEYADKVFTYIFILEMLLK
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LGAIPSIIMNVLLVCLIFWLFISIMGNLFPAGKFYHCINYYTGMDFVSVVNNYSECKALIESNQATARW
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VGIFFFVSVYIIISFLVVVMYLAIVILENFSVATEESAELSEDDFEMFYEVWEKFDPDATQFIEFAKLS
FADALDPPLLIAPKNVQLIAMDLMVSGDRIHCLDILFAFTKRVLGESGEMDALRIQMEERFMAFNPSK
VSYEPITTTLRKKQEEVSAIIQRAYRRYLLKQKVKVSSIIYKKDKGKECDGTPIKEDTLIDKLNENSTP
EKTDMTPTSTSPSYDSVTKEPEKEFEKDKSEKEDKDKDIRESKK

[0210]

[0211] 서열번호 6; SCN2A 전사체 변이체 4(NM_001371246.1)

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[0212]

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[0213]

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ACTGTACAAACATGTTGCAAGCTGCTTAAATCTGTTTAAAAATATAGGTTAGAGTTTTCTAAGAAAAATAT
AAATCTGTAAAAAGTTTCATTTTATTTTATTTTTCAGCCTTTGTACGTAAATGAGAAATTAAGAGTAT
CTTCAGGTGGATGTACAGTCACTATTGTTAGTTTCTGTTCTAGCACTTTTAAATTGAAGCACTTCACA
AAATAAGAAAGCAAGGACTAGGATGCAGTGTAGGTTTCTGCTTTTTTATTAGTACTGTAACTTGACACACA
TTTCAATGTGAACAAATCTCAAACTGAGTTCATGTTTATTTGCTTTCAATAGTAATGCCTTATCATTG
AAAGAGGCTTAAAGAAAAATAAAATCAGCTGATACTCTTGGCATTGCTTGAATCCAATGTTTCCACCTAG
TCTTTTTATTTCAGTAATCATCAGTCTTTTCCAATGTTTGTTCACAGATAGATCTTATTGACCCATATG
GCACTAGAACTGTATCAGATATAATATGGGATCCCAGCTTTTTTCTCTCCCACAAAACAGGTAAGTGA
AGTTATATTACAGATTACAGCAAAATATCTTTGTGTTTCAACAGCAACAAATAATGTAGATTCTTTATAT
GAAGCTATTGACTTGTAGTGTGTTGGTGAATGCATGCAGGAAATGCTGTTACCATAAAGAACGGTAA
CCACATTACAATCAAGCCAAAAGAAATAAGGTTTCGCTTTTGTGTTTTGTTATTTAATTGTTGCTTTGTTT
CTATCTTTGAAATGCCATTTAAAGGTAGATTCTATCATGTAAAAATAATCTATCTGAAAAACAAATGTA
AAGAACACACATTAATTACTATAATTCTATCTTTCAATTTTTCATGGAATGGAAGTTAATTAAGAAGAGT
GTATTGGATAACTACTTTAATATTGGCCAAAAGCTAGATATGGCATCAGGTAGACTAGTGGAAAGTTAC
AAAAATTAATAAAAAATTGACTAACA

[0214]

[0215]

서열번호 7; SCN2A 단백질 이소폼 2(NP_001358175.1):

MAQSVLVPPGPDSEFRFFTTRESLAAIEQRIAEKAKRPKQERKDEDDENGPKPNSDLEAGKSLPFIYGDIP
PEMVSVPLEDLDPYYINKKTFIVLNKGKALSRFSATPALYILTPFNPIRKLAIKILVHSLFNMLIMCTIL
TNCVFMMSNPDPWTKNVEYTFGTGYTFESLIKILARGFCLEDFTLRDPWNWLDFTVITFAYVTEFVNL
GNVSLRTRFVRLALKTISVIGPKTIVGALIQSVKKLSDVMILTVPFLCSVFALIGLQLFMGNLRNKLQ
WPDNSSFNINITSFFNNSDNGNTTFNRTVSIFNWEYIEDKSHFYFLEGQNDALLCGNSSDAGCQPEG
YICVKAGRPNPNYGYTSFDTFSWAFLSLFLRLMTQDFWENLYQLTLRAAGKTYMIFVVLVIFLGSFYLINLI
LAVVAMAYEEONATLEEAEOKEAEFOQMLEQLKKOQEEAQAASAEASRDFSGAGGIGVFSESSVA
SKLSSKSEKELKNRRKKQKEQSGGEEKNDRVRKSESEDSIRRKGRFSLSEGRSLTYEKRFSSPHQSL
SIRGSLFSPRRNSRASLSFSRGRAKDIGSENDADDEHSTFEDNDSRRDSLFPVPHRGERRHSNVQASR
ASRVLPILPMNGKMHSAVDNCGVSVLVGGPSTLTSAQQLLPEGTTTETETIRKRSSSYHVSMDLLEDPTS
RQRAMSIASILNTMTEEEESRQKCPWCYKFANMCLIWDCCKPWLKVKHLVNLVMDPFVDLATTICIV
LNTLFMAMEHYPMTEQFSSVLSVGNLVFTGIFTAEMFLKIIAMDPIYFQEGWNIFDGFIVSLSLMELGL
ANVEGLSVLRSLRLLRVFKLAKSNPTLNMILIKIIGNSVGALGNLTTLVLAIVFIFAVVGMQLFGKSYKEC

[0216]

VCKISNDCELPRWHMHDFHSLFIVFRVLCGEWIETMWDCEVAGQTMCLTVFMMVMVIGNLVVNLFLA
LLLSFSFSDNLAATDDDNEMNQLIAGVRMQKGIDFVKRKIREFIQKAFVRKQKALDEIKPLEDLNNKKD
SCISNHTTIEIGKDLNLYKDNNGTSGIGSSVEKYVVDSESYMFIINPSTLTVTVPIAVGESDFENLNT
EFSSESDMEESKEKLNATSSSEGSTVDIGAPAEQEPEVEPEESLEPEACTEDCVRKFKCCQISIEEGK
GKLWNLRLKTCYKIVEHNWFETFIVFMILLSSGALAFEDIYIEQRKTIKTMLEYADKVFTYIFILEMLLK
WVAYGFQVYFTNAWCWLDLFLVDVSLVSLTANALGYSELGAIKSLRTLRLRPLRLALSREFEGMRVVNAL
LGAIPSIMNVLLVCLIFWLIFSIMGVNLFAKFYHCINYTTGEMFDVSVVNNYSECKALIESNQARWKN
VKVNFNDVGLGLYLSLLQVATFKGWMIMYAAVDSRNVELQPKYEDNLYMYLYFVIFIIIFGSFFTLLNFIG
VIIIDNFNQKKKFGGQDIFMTEEQKKYINAMKKLGSKPKQKPIPRANKFQGMVDFVTKQVFDISIMIL
ICLNMVIMMVETDDQSQEMTNILYWINLVFIVLFTGECVLKILSLRYYYFTIGWNIFDFVVLVLSIVGMF
LAELEKYFVSPTLFRVIRLARIGRILRLIKGAKGIRTLFALMMSLPALFNIGLLFLVMFIYAIFGMS
NFAYVKREVIDDMFNFTFGNSMICLFIQITTSAGWDGLLAPILNSGPPDCDPDKDHPGSSSVKGDCCNPS
VGIFFFVSYIIISFLVVNNYIAVLENFSAVTEESAEPLESDDFEMFYEVWEKFDPDATQFIEFAKLS
FADALDPPLLIAPKNVQLIAMDLPMVSGDRIHCLDILFAFTKRVLVGESGEMDALRIQMEERFMAFNPSK
VSYEPIITTLKRQKEVSAIIIQRAYRRYLLKQKVKVSSIIYKKDKGKECDGTPIKEDTLIDKLNENSTP
EKTDMPTSTTSPPSYDSVTKPEKEKEKDKSEKEDKDKDIRESKK

[0217]

[0218] 서열번호 8; SCN2A 전사체 변이체 5(NM_001371247.1):

ATAGCAGTAACACAATTACCCCTCTAGTGTGAACATATCAGGATGGCATAGACCAGCACCTTTCTTATGCAA
GGAGCTAAACAGTGATTAAAGGAGCAGGATGAAAAGATGGCACAGTCAGTGCTGGTACCGCCAGGACCTG
ACAGCTTCCGCTTCTTTACCAGGGAATCCCTTGCTGCTATTGAACAACGCATTGCAGAAGAGAAAGCTAA
GAGACCCAAACAGGAACGCAAGGATGAGGATGATGAAAATGGCCCAAGCCAAACAGTGACTTGGAAAGCA
GGAAAATCTCTTCCATTATTTATGGAGACATTCTCCAGAGATGGTGTGAGTGCCCTGGAGGATCTGG
ACCCCTACTATATCAATAAGAAAACGTTTATAGTATTGAATAAAGGGAAAGCAATCTCTCGATTTCAGTGC
CACCCCTGCCCTTTACATTTTAACTCCCTTCAACCCCTATTAGAAAATTAGCTATTAAAGATTTTGGTACAT
TCTTTATTCAATATGCTCATTATGTGCACGATTCTTACCAACTGTGTATTTATGACCATGAGTAACCCCTC
CAGACTGGACAAAGAATGTGGAGTATACCTTTACAGGAATTTATACTTTTGAATCACTTATTAATAATACT
TGCAAGGGGCTTTTGTTTAGAAAGATTTCACATTTTACGGGATCCATGGAATTGGTTGGATTTCACAGTC
ATTACTTTTGCATATGTGACAGAGTTTGTGGACCTGGGCAATGTCTCAGCGTTGAGAACATTGAGAGTTC
TCCGAGCATTGAAAACAATTTTCAGTCATTCCAGGCCGTAAGACCATTGTGGGGGCCCTGATCCAGTCAGT
GAAGAAGCTTCTGATGTGATGATCTTGACTGTGTTCTGTCTAAGCGTGTTCGCGCTAATAGGATTGCAG
TTGTTGATGGGCAACCTACGAAATAAATGTTTGCAATGGCCTCCAGATAAATCTTCCCTTTGAAATAAATA
TCACTTCTCTTTTAAACAATTCATTGGATGGGAATGGTACTACTTTCAATAGGACAGTGAGCATATTTAA
CTGGGATGAATATATTGAGGATAAAAGTCACTTTTATTTTTAGAGGGGCAAAATGATGCTCTGCTTTGT
GGCAACAGCTCAGATGCGAGGCCAGTGTCTTGAAGGATACATCTGTGTGAAGGCTGGTAGAAACCCCACT
ATGGCTACACGAGCTTTGACACCTTTAGTTGGGCCCTTTTGTCTTATTTTCTGCTCATGACTCAAGACTT
CTGGGAAAACCTTTATCACTGACACTACGTGCTGCTGGGAAAACGTAATGATATTTTTTGTGCTGGTC
ATTTCTTGGGCTCATTCTATCTAATAAATTTGATCTTGGCTGTGGTGGCCATGGCCTATGAGGAACAGA
ATCAGGCCACATTGGAAGAGGCTGAACAGAAAGGCTGAATTTTCAGCAGATGCTCGAACAGTTGAAAAA
GCAACAAGAAGAAGCTCAGGCGGAGCTGCAGCCGATCTGCTGAATCAAGAGACTTCAGTGGTGTGCTGT
GGGATAGGAGTTTTTTCAGAGAGTTCTTCAGTAGCATCTAAGTTGAGCTCCAAAAGTGAAAAAGAGCTGA
AAAAAG
ATCGGAATCTGAAGACAGCATAAGAAGAAAAGTTTCCGTTTTTCTTGGAAAGGAGTAGGCTGACATAT
GAAAAGAGATTTTCTTCCACACCCAGTCTTACTGAGCATCCGTGGCTCCCTTTTCTCTCCAAGACGCA
ACAGTAGGGCGAGCCTTTTCAGCTTCAGAGGTGAGCAAGGACATTGGCTCTGAGAATGACTTTGCTGA
TGATGAGCACAGCACCTTTGAGGACAATGACAGCCGAAAGAGACTCTCTGTTCTGTCGCCACAGACATGGA
GAACGGCGCCACAGCAATGTGAGCCAGGCCAGCCGTGCTCCAGGGTGTCTCCCATCTGCCATGAATG
GGAAGATGATAGCGCTGTGGACTGCAATGGTGTGCTCTCCCTGGTCGGGGGCCCTTCTACCCTCACATC
TGCTGGGAGCTCTTACCAGAGGCGACAACACTGAAACAGAAATAAGAAAGAGACGGTCCAGTTCTTAT
CATGTTTCCATGGATTTATTGGAAGATCCTACATCAAGGCAAGAGCAATGAGTATAGCCAGTATTTTGA
CCAACACCATGGAAGAACTTGAAGAAATCCAGACAGAAATGCCACCATGCTGGTATAAATTTGCTAATAT
GTGTTTGATTGGGACTGTGTAAACCATGGTTAAAGGTGAAACACCTTGTCACCTGGTTGTAATGGAC
CCATTTGTTGACCTGGCCATCACCATCTGCATTGTCTTAAATACACTCTTCATGGCTATGGAGCACTATC
CCATGACGGAGCAGTTTCAGCAGTGTACTGTCTGTGGAAACCTGGTCTTCACAGGGATCTTCACAGCAGA
AATGTTTCTCAAGATAATTGCCATGGATCCATATTATTACTTTCAAGAAGGCTGGAATATTTTTGATGGT
TTTATTGTGAGCCTTAGTTTAAATGGAACCTTGGTTTGGCAATGTGGAAGGATTGTGAGTTCTCCGATCAT
TCCGGCTGCTCCGAGTTTTCAAGTTGGCAAAATCTTGGCCAACCTCTAAATATGCTAATTAAGATCATTGG
CAATTCGTGGGGGCTCTAGGAAACCTCACCTTGGTATTGGCCATCATCGTCTTCATTTTGTCTGTGGTC
GGCATGCACTCTTTGGTAAGAGCTACAAAGAATGTGTCTGCAAGATTCCAATGATTGTGAACCTCCAC
GCTGGCACATGATGACTTTTTCCACTCCTTCTGATCGTGTCCCGCTGCTGTGTGGAGAGTGGATAGA

[0219]

GACCATGTGGGACTGTATGGAGGTGCGCTGGCCAAACCATGTGCCCTTACTGTCTTCATGATGGTCATGGTG
ATTGGAATCTAGTGGTCTGTAACCTCTCTCTGGCCCTTGGCTTTTGGTTCCTTCAGTCTGACAACTCTTG
CTGCCACTGATGATGATAACGAATGAATAATCTCCAGATTGCTGTGGGAAGGATGCAGAAAGGAATCGA
TTTTGTTAAAAGAAAAATACGTGAATTTATTCAGAAAGCCTTTGTTAGGAAGCAGAAAGCTTTAGATGAA
ATTAACCGCTTGAAGATCTAAATAATAAAAAAGACAGCTGTATTTCCAAACCATACCACCATAGAAATAG
GCAAAAGACCTCAATTATCTCAAAGACGGAAATGGAACCTACTAGTGGCATAGGCAGCAGTGTAGAAAAATA
TGTCTGGATGAAAGTGATTACATGTCATTTATAAACCAACCTAGCCTCAGTGTGACAGTACCAATTGCT
GTTGGAGAACTGACTTTGAAATTTAAATACTGAAGAATTGAGCAGCGAGTCAGATATGGAGGAAAGCA
AAGAGAAGCTAAATGCAACTGATTCATCTGAAGGCAGCACGGTTGATATTGGAGCTCCCGCCGAGGGAGA
ACAGCCTGAGGTTGAACCTGAGGAATCCCTTGAACCTGAAGCCTGTTTTACAGAAGACTGTGTACGGAAAG
TTCAAGTGTGTGACAGATAAGCATAGAAGAGGCAAGGGAAGCTCTGGTGGAAATTTGAGGAAAAACATGCT
ATAAGATAGTGGAGCACAATTGGTTCGAAACCTTCATTGTCTTCATGATTCTGCTGAGCAGTGGGGCTCT
GGCCTTTGAAGATATATACATTGAGCAGCGAAAAACCATTAAGACCATGTTAGAATATGCTGACAAGGTT
TTCATTACATATTCATTCTGGAATGCTGCTAAAGTGGGTTGCATATGGTTTTCAAGTGTATTTTACCA
ATGCTGTGGTCTGGCTGGCTGCTGATTTGATTTGATGCTCTCACTGGTTAGCTTAACTGCAATGCCTTGGG
TTACTCAGAACTTGGTGGCATCAAAATCCCTCAGAACACTAAGAGCTCTGAGGCCACTGAGAGCTTTGTCC
CGGTTTGAAGGAATGAGGGTGTGTTGAAATGCTCTTTTAGGAGCCATTCCATCTATCATGAATGACTTCT
TGGTTTGGCTGATCTTTTGGCTAAATATTTCAGTATCATGGGAGTGAATCTCTTTGGTGGCAAGTTTACCA
TTGTATTAATTACACCACCTGGAGAGATGTTTGTATGAAGCGTGGTCAACAACCTACAGTGAGTGCAAGGCT
CTCATTTGAGAGCAATCAAACTGCCAGGTGGAAAAATGTGAAAGTAACTTTGATAACGTAGGACTTGGAT
ATCTGTCTCTCATCTCAAGTCAAGTCCAGCTTTAAGGGATGGATGGATATTATGTATGCAGCTGTTGATTACG
AAATGTAGAATTACAACCCGAAGTATGAAGACAACCTGTACATGTATCTTTATTTTGTCTCTTTATTATT
TTTGGTTCATTCTTTACCTTTGAATCTTTTCATTGGTGTCTCATAGATAAACTTCAACCAACAGAAAAAGA
AGTTTGGAGGTCAGAGCTCAAGAAATTTATGACAGAGAAGACAGAGAATACTACAATGCAATGAAAAAAGTGG
TTCAAGAAACCCACAAAAACCCATACCTCGACCTGCTAACAAATTTCAAGGAATGGTCTTTGATTTTGTGTA
ACCAAAACAGTCTTTGATATCAGCATCATGATCTCTCATCTGCTTAACTAGGTCACCATGATGGTGGAA
CCGATGACCCAGAGTCAAGAAATGACAAACATCTGTACTGGATTAATCTGCTGTTTATTGTTCTGTTTCTC
TGGAGAATGTGTGCTGAACTGATCTCTCTTCTGTTACTACTATTTCACTATTGGATGGAATATTTTGTAT
TTTGTGGTGGTCTATCTCTCTCTGTTAGGAATGTTTCTGGCTGAACCTGATAGAAAAGTATTTTGTGCTCC
CTACCTGTTTCCGATGATCCGCTTCTGCGAGGATGGCGGAATCTTACGCTGTGATCAAGGAGCAAAAGG
GATCCGCGACGCTGCTCTTTGCTTTGATGATGTCCTTCTGCGTTGTTTAACTCGGCTCTCTCTTTTCT
CTGGTCATGTTTCATCTACGCCATCTTTGGGATGTCCAATTTTGCTATGTTAAGAGGGAAGTTGGGATCG
ATGACATGTTCAACTTTTGAAGCTTTTGGCAACAGCATGATCTGCTGTTTCCAATTTACAACCTCTGCTGG
CTGGGATGGATTGCTAGCCCTATTCTTAATAGTGGACCTCCAGACTGTGACCTGACAAAGATCACCCCT
GGAAGCTCAGTTAAAGGAGACTGTGGGAACCCATCTGTTGGGATTTTCTTTTTTGTGATGATCATCATCA
TATCTTCTCTGTTGCTGTTGATCATGATCATCATCTGCGCTCATCTGGAGAACCTTCAAGTGTGCTACTGAAGA
AAGTGCAGAGCCTCTGAGTGAGGATGACTTTGAGATGTTCTATGAGGTTTGGGAGAAGTTTGATCCCGAT
GCGACCCAGTTTATAGAGTTTGGCAAACTTTCTGATTTTGCAGATGCCCTGGATCCTCCTCTTCTCATAG
CAAAACCAAGAGAGGTTGCTGATTTGCTATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
TGACATCTTATTTGCTTTTACAAAGCGTGTTTTGGGTGAGAGTGGAGAGATGGATGCCCTTCGAATACAG
ATGGAAGAGCGATTGATGGCATCAAAACCCCTCCAAAGTCTCTTATGAGCCCATAGCAGCCAGTGAAGC
GCAAAACAGAGAGGTTGCTGCTGATTTATTTATTTATTTATTTATTTATTTATTTATTTATTTATTTATTT
TAAAAAGGTATCAAGTATATACAAGAAAGACAAGGCAAGGAATGTGATGGAACACCCATCAAGGAAGAT
ACTCTCATTGATAAAGTGAATGAGAATCAACTCCAGAGAAAACCGATGACGCTTCCACCACGCTCTC
CACCTCGTATGATAGTGTGACCAAAACCCAGAAAAGAAAAATTTGAAAAAGACAAATCAGAAAAAGGAAGA
CAAAAGGGAAGATATCAGGGAAGTAAAAAGTAAAAAGAAACCAAGAAATTTCCATTTTGTGATCAATTG
TTTACAGCCCGTGTGATGATGTTTGTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTG
TTTTTCAAAATGATATCTTAAAGTCAAGTGCCTGCTTATAACAGACAGAGACCTCTGGTCAAGCAACTGGA
CAGTAACTGGAGAAATAGTATCGATGGGAGGTTTCTATTTTCAACACAGCTGACACTGCTGAAGAGCA
GAGGCGTAAATGCTACTCAGACGATAGGAACCAATTTAAAGGGGGAGGGAAGTTAAATTTTATGTAATA
TTCAACATGTGACATCTGATATGATAATTGTCACAGTGTATTATGTTTAACTGCCACACCTGCCATAT
TTTTACAAAACGTTGCTGTGTAATTTATCACTTTTCTTTTAAATTCACAGGTTGTTTACTATTATATGTG
ACTATTTTGTAAATGGGTTTGTGTTTGGGGAGAGGGAATAAAGGGAGGGAATTTACATTTCTCTATTG
TATGTATAACTGGATATATTGTTTAAATGGAGGATGCTGCAATTTCTATTACACATATAAAAAATCACAT
CACAAAAGGGAAGAGTTTACTTCTTGTGTTTCAAGGATGTTTTAGATTTTGTAGGTGCTTAAATAGCTATT
GTATTTTAAAGTGCTCATCAGAAAAAATTTAATGTGCTGTAAATGTTCCATAGAAATCACAAAGCATT
AAAGAGTTGTTTTATTTTACATAACCCATTAAATGTACATGTATATATGTATATATGTATATGTGCTG
TATATACATATATATGTATACACACATGACACACAGAGATATACATATACATTACATTGTCAATTCACA
GTCCAGCAGCATGACTATCACATTTTGTAAAGTGTCTTTGGCATAAAATAAAAATATCCTATCAGTC
CTTTTGAAGAGCCTGAATTTGACCAAAAAACATCCCAACCACTTTTAAAGTTGATTCTGCTTTATC
CTGCAGTATTGTTTAGCCATCTTCTGCTTTGGTAAAGTTGACATAGTATATGTCAATTTAAAAAATAAA
AGTCTGCTTTGTAAATAGTAATTTTACCAGTGGTGCATGTTTGGAGCAACAAAAATGATGATTTAAGCA

[0220]

CACACTTATTTGCATCAAAATATGTACCACAGTAAGTATAGTTTGCAAGCTTTCAACAGGTAATATGATGT
AATTGGTTCATTATAGTTTGAAGCTGTCACTGCTGCATGTTTATCTTGCCTATGCTGCTGTATCTTATT
CCTTCCACTGTTTCAGAGCTAATATGGGAAGCATATATCAGTGGTAAAGTGAAGCAAAATGTTCTACC
AAGACCTCATCTTCATGCCATTAAGCAATAGGTTGCAGCAAAACAAGGAAGAGCTTCTTGTCTTTTATTC
TTCCAACCTTAATTGAACACTCAATGATGAAAAGCCGACTGTACAAACATGTTGCAAGCTGCTTAAATC
TGTTTAAATATATGTTAGAGTTTCTAAGAAAAATATAAATACTGTAAAGAGTTCAATTTATTTTATTT
TTCAGCCTTTTGTACGTAAATGAGAAATTAAGATATCTTCAGGTGGATGTCACAGTCACTATTGTTAG
TTTCTGTTCTAGCACTTTTAAATGAAGCACTTCACAAAATAAGAAGCAAGGACTAGGATGCAAGTGTAG
GTTTCTGCTTTTTATTAGTACTGTAACTTGCACACATTTCAATGTGAAACAAATCTCAAACTGAGTTC
AATGTTTATTTGCTTTCAATAGTAATGCTTATCAATTGAAAGAGGCTTAAAGAAAAAATAATCAGCTGA
TACTCTTGGCATTGCTTGAATCCAATGTTTCCACCTAGTCTTTTATTTCAGTAATCAICAGTCTTTTCCA
ATGTTTGTTCACAGATAGATCTTATGACCATATGGCACTAGAAGTGTATCAGATATAATATGGGAT
CCAGCTTTTTTCTCTCCCAAAACAGGATAGTGAAGTTATATACCAGTTACAGCAAAATACTTTG
TGTTTCACAGCAACAATAAATGTAGATTCTTTTACTGAAGCTATTGACTGTAGTGTGTTGGTGAAT
GCATGCAGGAAATGCTGTTACCAATAAGAACCGTAAACCAATTACAATCAAGCCAAAAAGAAATAAAGGT
TTCGCTTTTGTGTTTAAATGTTGCTTTGTTTCTATCTTTGAAATGCCATTTAAAGGTAGATT
CTATCATGTAAAAATAATCTATCTGAAAAACAAATGTAAAGAACACACATTAACTACTATAATTCATCTT
TCAATTTTTTGCATGGAATGGAAGTTAATTAAGAGAGTGTATTGGATAACTACTTTAATATTGGCCAAAA
AGCTAGATATGCATCAGGTAGACTAGTGGAAAGTTACAAAAATTAATAAAAAATTTGACTAACA

[0221]

[0222] 서열번호 9; SCN2A 단백질 이소폼 1(NP_001358176.1):

MAQSVLVPPGPDSEFRFFTTRESLAAIEQRIAEKAKRPKQERKDEDDENGPKPNSDLEAGKSLPFIYGDIP
PEMVSVPLEDDLPYYINKKTFVVLNKGKATSRFSATPALYILTFNPIRKLAIKILVHSLFNMLIMCTIL
TNCVFMTMSNPDPWTKNVEYFTFTGIYTFESLIKILARGFCLEDFTFLRDPWNWLDFTVITFAYVTEFVDL
GNVSALRTFRVLRALKTISVIPGLKTIIVGALIQSVKKLSVDMILTVCFLSVFALIGLQLFMGNLRNKCLO
WPPDSSSEINTTSFFNNSLDGNGTTFNRTVSIWNWDEYIEDKSHFYFLEGQNDALLCGNSSDAGQCPEG
YICVKAGRNPNGYTSFDTFSWAFSLFRMLTQDFWENLYQLTLRAAGKTYMIFVFLVIFLGSFYLINLI
LAVVAMAYEEQNQATLEEAQEAEAFQOMLEQLKKQEEAQAASAESESRDFSGAGGIGVFSESSVA
SKLSSKSEKELKNRRKKKKQKQSGGEBEKNDRVRKSESED SIRKGFRRFSLGSRITYEKRFSPPHQSLL
SIRGSLFSPRRNSRASLSFRGRAKDIGSENDPADDEHSTFEDNDSRRDSLFPVPHRGERRHSNVSQASR
ASRVLPILPMNGKMHSAVDCMGVSVLVGGPSTLTSAQGLLPEGTTTETEIRKRRSSSYHVSMDLLEDPTS
RQRAMSIASILLNTMEELEESQKCPWCYKFFANMCLIWDCCKPWLKVKHLVNLVVMDDPFVDLAIITCIV
LNTLFMAMHYPMTQESSVLSVGNLVFTGIFTAEMFLKIAMDPIYFYQEGWNIFDGFIVSLSLMELGL
ANVEGLSVLRFRLLRVFKLAKSWPTLNMLIKIIGNSVGGALGNLTLLVLAIVFIFAVVGMQLFGKSYKEC
VCKISNDCELPWHMHDFPHSLIVFRVLCGEWETMWDCEVAGQTMCLTVFMMVMVIGNLVVLNLFLLA
LLSSFSSENLAATDDNEMNMLQIAVGRMKGIDFVKRKIREFIQKAFVRKQKALDEIKPLEDLNNKKD
SCISNHTTIEIGKDLNLYLKDNGTTSIGIGSSVEKYVVDSESDYMSFINNPSLTIVTPIAVGESDFENLNT
EFSSESDMEESKEKLNATSSSEGSTVDIGAPAEQEPEVEPEESLEPEACFTEDCVRKFKCCQISIEEGK
GKLWNNLRKTCYKIVEHNWFETFIVFMILLSSGALAFEDIYIEQRKTIKTMLEYADKVFTYIFILEMLLK
WVAYGFQVYFTNAWCWLDLIVDVSLVSLTANALGYSELGAIKSLRTRLRALRPLRALSRFEGMRVVNAL
LGAIPSIMNVLLVCLIFWLIFSIMGVNLFPAGKFYHCINYTGEMFDVSVNNYSECKALIESNQATARWKN
VKVNFNDVGLGYLSLLQVATFKGWMIMYAAVDSRNVLPQKYEDNLYMYLYFVIFIIFGSFTLNLFIG
VIIDNFNQKKKFGGDIPTMTEEQKKYYNAMKKLGSKKPQKPIPRANKFQGMVDFVTKQVFDISIMIL
ICLNMTMMVETDDQSQEMTNILYWINLVFIVLFTGECVLKLISLRYFYFTIGWNIFDFVVLISIVGMF
LAELIEKYFVSPTLFRVIRLARIGRILRLIKGAKGIRTLFALMMSLPALFNIGLLFLVMFIYAFGMS
NFAYVKREVGIDDMFNFTFTGNSMICLFQITTSAGWDGLLAPILNSGPPDCDPDKDHPGSSVKGDCGNPS
VGIFFFVSYIIISFLVVNMVYIAVILENFSVATEESAEPLEDDFEMFYEVWEKFDPDATQFIEFAKLS
FADALDPPLLIAPKNVQLIAMDLPMVSGDRIHCLDILFAFTKRVLGESGEMDALRIQMEERFMAFNPSK
VSYEPITTTTLKRRQEEVSAIIIQRAYRRYLLKQKVKVSSIIYKKDKKECDGTPIKEDTLIDKLNENSTP
EKTDMPTSTTSPFSYDSVTKEPEKEFEKDKSEKEDKGDIRESKK

[0223]

[0224] 서열번호 10; SCN2A 전사체 변이체 1(NM_021007.3):

AAGCATGATGGAATTTTAGCTGCAGTCTTCTTGGTGCCAGCTTATCAATCCCAAACCTCTGGGTGTAAAAG
ATTCTACAGGGCACTTTCTTATGCAAGGAGCTAAACAGTGATTAAAGGAGCAGGATGAAAAGATGGCACA
GTCAGTGCTGGTACCGCCAGGACCTGACAGCTTCCGCTTCTTTACCAGGGGAATCCCTTGCTGCTATTGAA
CAACGCATTGCAGAGAGAGAAAGCTAAGAGACCCAAACAGGAACGCAAGGATGAGGATGATGAAAATGGCC
CAAAGCCAAACAGTGACTTGGAAGCAGGAAAATCTCTTCCATTTATTATGGAGACATTCTCCAGAGAT
GGTGTCAGTGCCCTGGAGGATCTGGACCCCTACTATATCAATAAGAAAACGTTTATAGTATTGAATAAA

[0225]

GGGAAAGCAATCTCTCGATTTCAGTGCCACCCCTGCCCTTTACATTTTAACTCCCTTCAACCCCTATTAGAA
AATTAGCTATTAAAGATTTTGGTACATTTCTTTATTCATATATGCTCATTATGTGCACGATTCTTACCAACTG
TGTATTTATGACCATAGTAACCCCTCCAGACTGGACAAAGAATGTGGAGTATACCTTTACAGGAATTTAT
ACTTTTGAATCAGTTATTTAAATATCTTGAAGGGGCTTTTGGTTTGAAGATTTTACATTTTACGGGATC
CATGGAAATGGTTGGATTTTACAGTCAATTACTTTTGCATATGTGACAGAGTTTGTGGACCTGGGCAATGT
CTCAGCGTTGAGAACATTCAGAGTTCTCCGAGCATTGAAAACAATTTTCACTCATTCCAGGCCCTGAAGACC
ATTGTGGGGGCCCTGATCCAGTCAGTGAAGAAGCTTTCTGATGTCATGATCTTGACTGTGTTCTGTCTAA
GGGTGTTTGGCTTAATAGGATTTGCAGTTGTTTCATGGGCAACCTACGAAATAAATGTTTGAATGGCTCC
AGATAATTTCTTCTTTGAAATAAATATCACTTCTCTCTTTTAAACAATTCATTGGATGGGAATGGTACTACT
TTCAATAGGACAGTGAACATATTTAACTGGGATGAATATATTGAGGATAAAAGTCACTTTTATTTTTTAG
AGGGGCAAAATGATGCTCTGCTTTTGTGGCAACAGCTCAGATGCCAGCCAGTGTCTTGAAGGATACATCTG
TGTGAAGGCTGGTAGAAACCCCACTATGGCTACACAGAGCTTTGACACCTTTAGTTGGGCCCTTTTGTCTC
TTATTTGCTCTCATGACTCAAGACTTCTGGGAAAACCTTTATCAACTGACACTACGTGCTGCTGGGAAAA
CGTACATGATATTTTTTGTGCTGGTCAATTTTCTGGGCTCATTCTATCTAATAAATTTGATCTTGGCTGT
GGTGGCCATGGCTTATGAGGAACAGAATCAGGCCACATTGGAAGAGGCTGAACAGAAGGAAGCTGAATTT
CAGCAGATGCTGCAACAGTTGAAAAAGCAACAAGAAGAAGCTCAGGCCGACGTGACAGCCGATCTGCTG
AATCAAGAGACTTCAGTGGTGTGGTGGGATAGGAGTTTTTTCAGAGAGTTCTTCAGTAGCATCTAAGTT
GAGCTCCAAAAGTGAAAAAGAGCTGAAAAACAGAAGAAAAAGAAACAGAAAGAAGCAGTCTGGAGAA
GAAGAGAAAAATGACAGAGTCCGAAAATCGGAATCTGAAGACAGCATAAGAAGAAAAAGTTTCCGTTTTT
CCTTGAAGGAAGTAGGCTGACATATGAAAAAGAGATTTTCTTCCACACCAGTCTTACTGAGCATCCG
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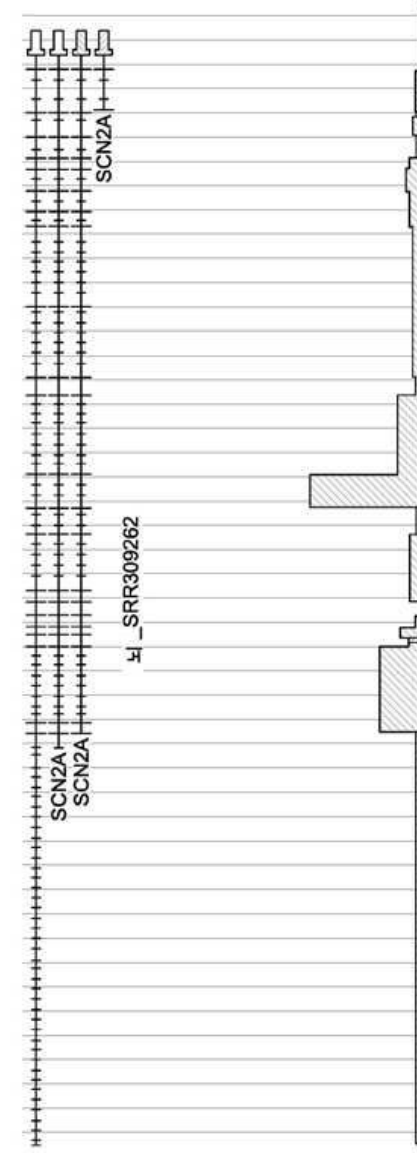
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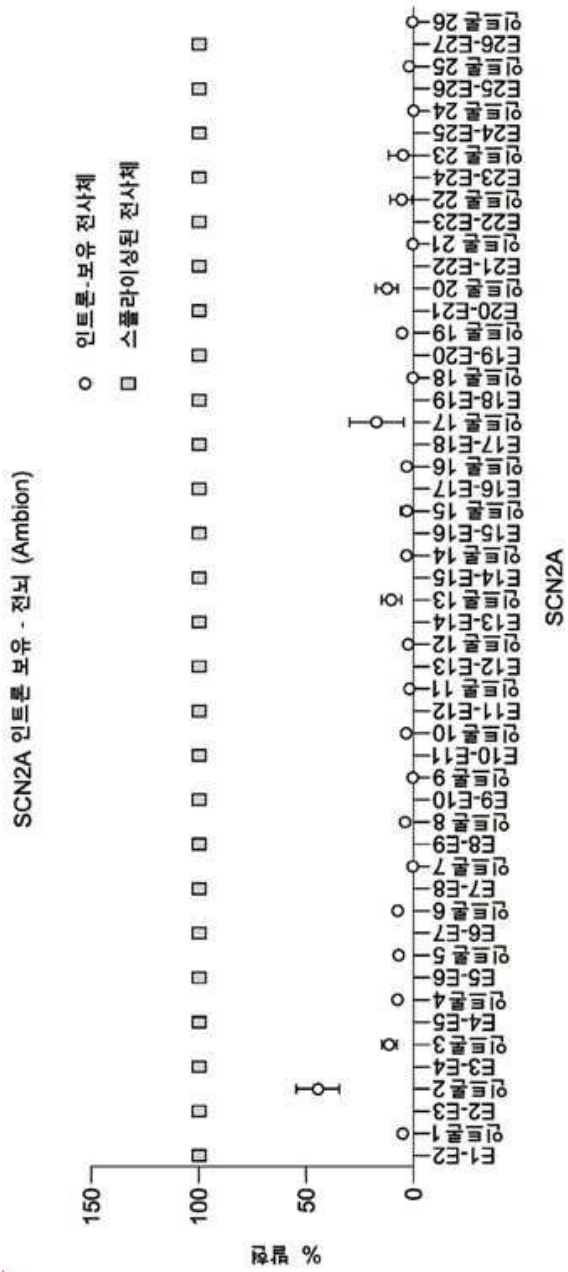
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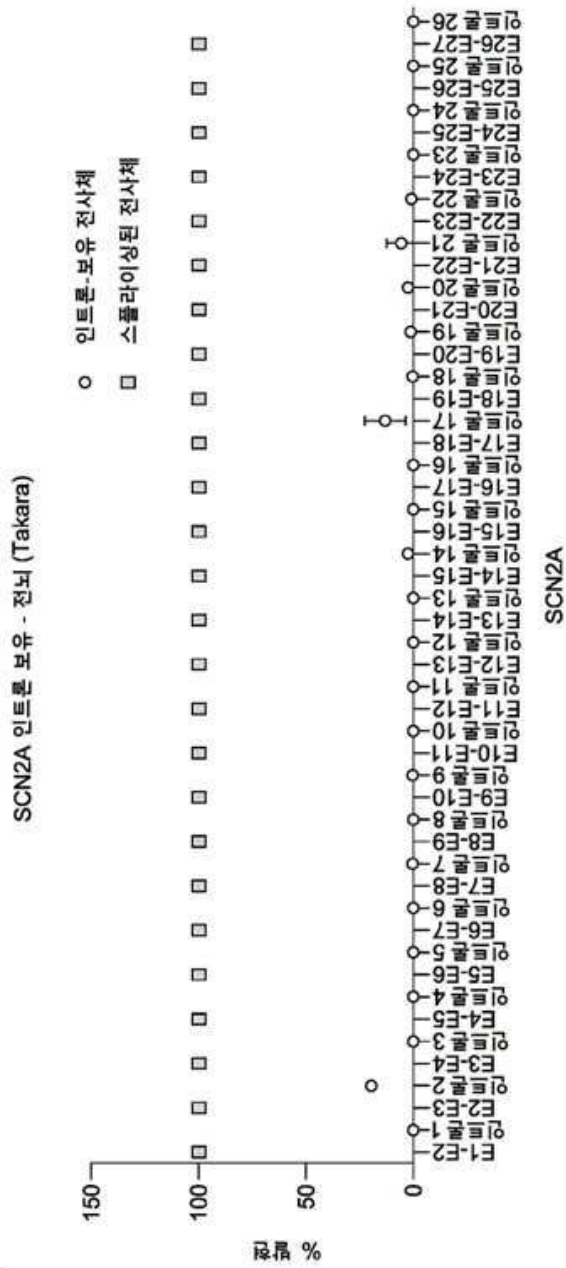
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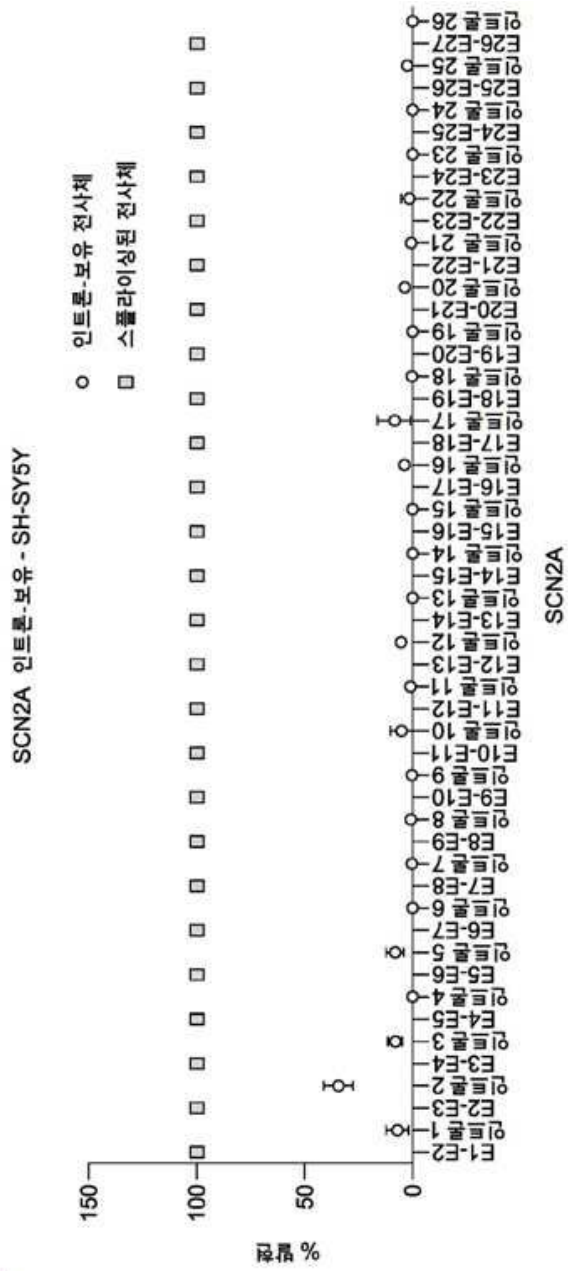
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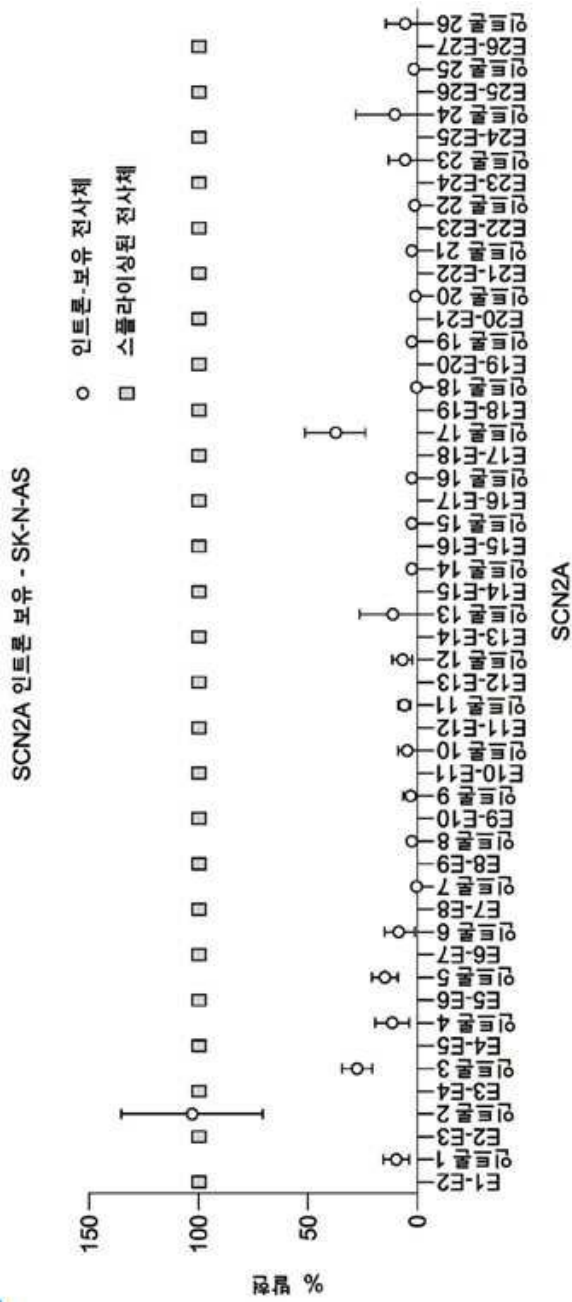
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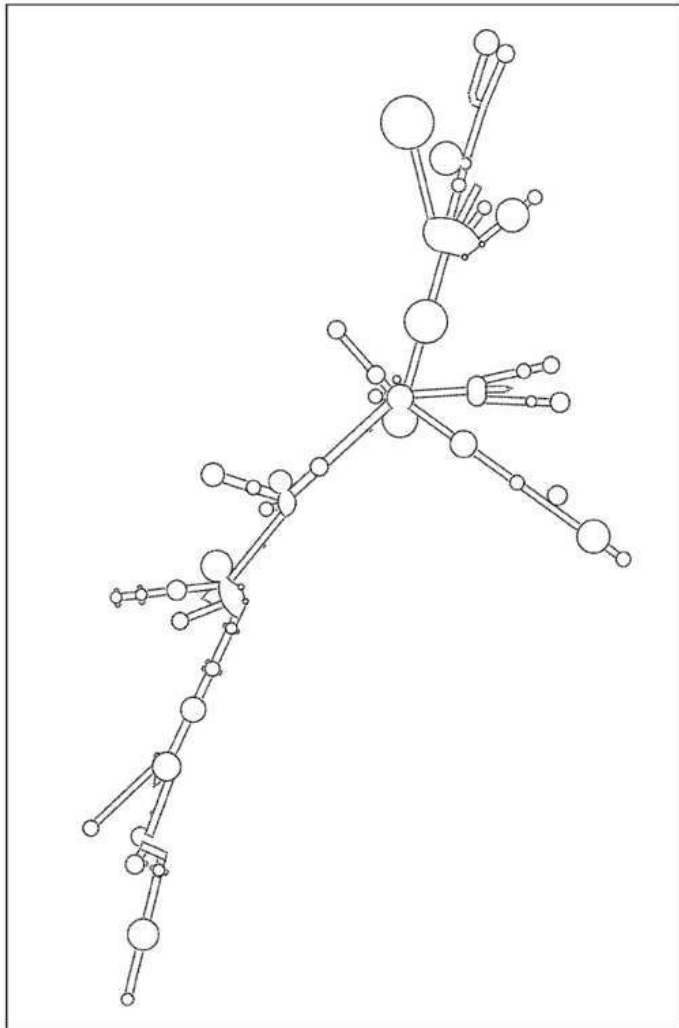
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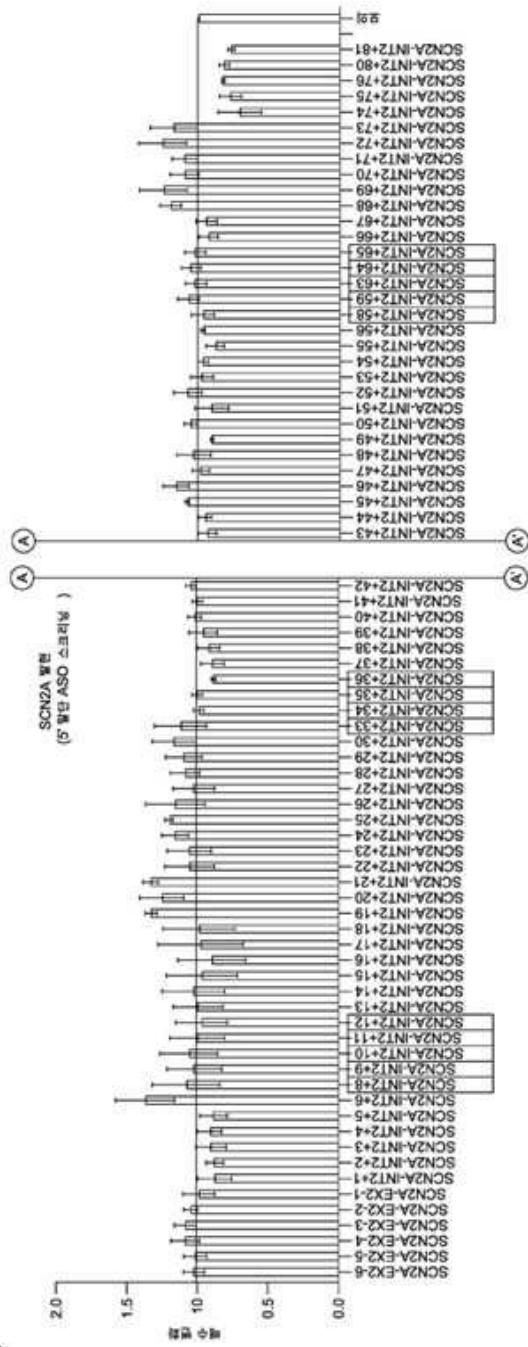
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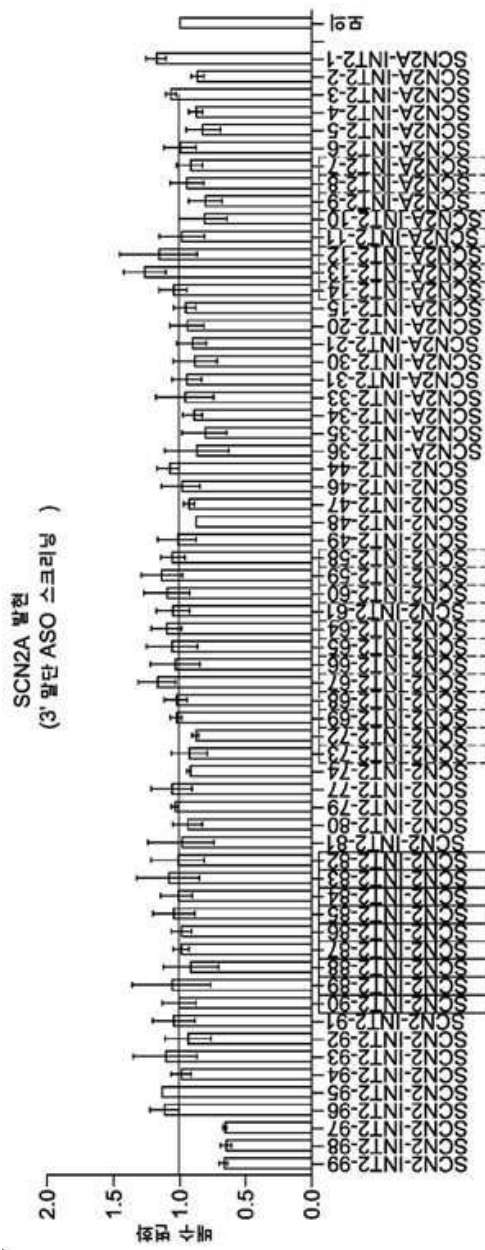
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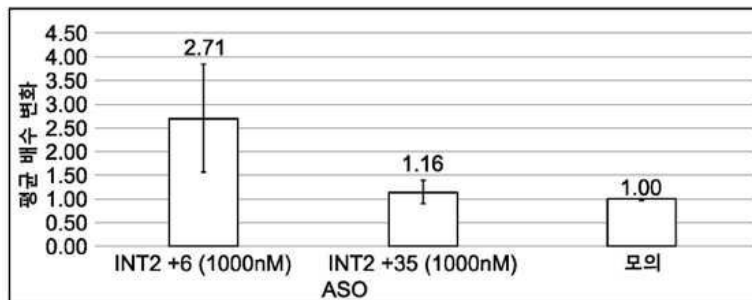
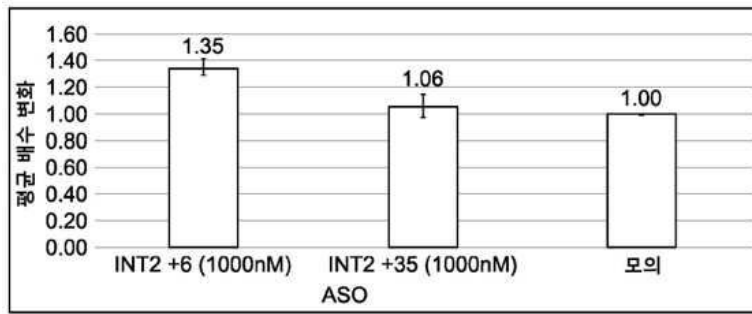
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575

Leu Phe Ser Phe Arg Gly Arg Ala Lys Asp Ile Gly Ser Glu Asn Asp

580

585

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Phe Ala Asp Asp Glu His Ser Thr Phe Glu Asp Asn Asp Ser Arg Arg

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Asp Ser Leu Phe Val Pro His Arg His Gly Glu Arg Arg His Ser Asn

610

615

620

Val Ser Gln Ala Ser Arg Ala Ser Arg Val Leu Pro Ile Leu Pro Met

625

630

635

640

Asn Gly Lys Met His Ser Ala Val Asp Cys Asn Gly Val Val Ser Leu

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Val Gly Gly Pro Ser Thr Leu Thr Ser Ala Gly Gln Leu Leu Pro Glu

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665

670

Gly Thr Thr Thr Glu Thr Glu Ile Arg Lys Arg Arg Ser Ser Ser Tyr

675

680

685

His Val Ser Met Asp Leu Leu Glu Asp Pro Thr Ser Arg Gln Arg Ala

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Met Ser Ile Ala Ser Ile Leu Thr Asn Thr Met Glu Glu Leu Glu Glu

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Ser Arg Gln Lys Cys Pro Pro Cys Trp Tyr Lys Phe Ala Asn Met Cys

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Leu Ile Trp Asp Cys Cys Lys Pro Trp Leu Lys Val Lys His Leu Val

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1940	1945	1950
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Gly Pro Lys Pro Asn Ser Asp Leu Glu Ala Gly Lys Ser Leu Pro Phe

50 55 60

Ile Tyr Gly Asp Ile Pro Pro Glu Met Val Ser Val Pro Leu Glu Asp

65 70 75 80

Leu Asp Pro Tyr Tyr Ile Asn Lys Lys Thr Phe Ile Val Leu Asn Lys

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Gly Lys Ala Ile Ser Arg Phe Ser Ala Thr Pro Ala Leu Tyr Ile Leu

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785 790 795 800

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Tyr Tyr Phe Gln Glu Gly Trp Asn Ile Phe Asp Gly Phe Ile Val Ser

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Phe His Ser Phe Leu Ile Val Phe Arg Val Leu Cys Gly Glu Trp Ile

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945 950 955 960

Thr Val Phe Met Met Val Met Val Ile Gly Asn Leu Val Val Leu Asn

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Gly Pro Lys Pro Asn Ser Asp Leu Glu Ala Gly Lys Ser Leu Pro Phe

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Ile Tyr Gly Asp Ile Pro Pro Glu Met Val Ser Val Pro Leu Glu Asp

65 70 75 80

Leu Asp Pro Tyr Tyr Ile Asn Lys Lys Thr Phe Ile Val Leu Asn Lys

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Gly Lys Ala Ile Ser Arg Phe Ser Ala Thr Pro Ala Leu Tyr Ile Leu

100 105 110

Thr Pro Phe Asn Pro Ile Arg Lys Leu Ala Ile Lys Ile Leu Val His

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Ser Leu Phe Asn Met Leu Ile Met Cys Thr Ile Leu Thr Asn Cys Val

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1820	1825	1830

Pro Asn Lys Val Gln Leu Ile Ala Met Asp Leu Pro Met Val Ser
1835 1840 1845

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1850 1855 1860

Arg Val Leu Gly Glu Ser Gly Glu Met Asp Ala Leu Arg Ile Gln
1865 1870 1875

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1880 1885 1890

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1895 1900 1905

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1910 1915 1920

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1955	1960	1965
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