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(54)发明名称

使用α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)抑制剂治疗认知障碍

(57)摘要

本公开提供了治疗患有认知障碍或处于发展出认知障碍的风险中的受试者的方法,以及鉴定具有增加的发展出认知障碍的风险的受试者的方法。

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1.一种治疗患有认知障碍或处于发展出认知障碍的风险中的受试者的方法,所述方法包括向所述受试者施用α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)抑制剂。

2.一种治疗患有髓鞘完整性降低或处于发展出髓鞘完整性降低的风险中的受试者的方法,所述方法包括向所述受试者施用α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)抑制剂。

3.一种治疗患有神经变性或处于发展出神经变性的风险中的受试者的方法,所述方法包括向所述受试者施用α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)抑制剂。

4.一种治疗患有灰质/白质对比度(GWC)降低或处于发展出GWC降低的风险中的受试者的方法,所述方法包括向所述受试者施用α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)抑制剂。

5.一种治疗患有轻度认知障碍转变为痴呆或处于轻度认知障碍转变为痴呆的风险中的受试者的方法,所述方法包括向所述受试者施用α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)抑制剂。

6.根据权利要求1至5中任一项所述的方法,其中所述ST6GALNAC5抑制剂包含抑制性核酸分子。

7.根据权利要求6所述的方法,其中所述抑制性核酸分子包含与ST6GALNAC5核酸分子杂交的反义核酸分子、小干扰RNA(siRNA)或短发夹RNA(shRNA)。

8.根据权利要求1至5中任一项所述的方法,其中所述ST6GALNAC5抑制剂包含与ST6GALNAC5基因组核酸分子内的指导RNA(gRNA)识别序列杂交的Cas蛋白和gRNA。

9.根据权利要求8所述的方法,其中所述Cas蛋白是Cas9或Cpf1。

10.根据权利要求8或权利要求9所述的方法,其中所述gRNA识别序列包括或接近对应于根据SEQ ID NO:1的位置176,867的位置。

11.根据权利要求8或权利要求9所述的方法,其中所述gRNA识别序列位于距对应于根据SEQ ID NO:1的位置176,867的位置约1000、约500、约400、约300、约200、约100、约50、约45、约40、约35、约30、约25、约20、约15、约10或约5个核苷酸处。

12.根据权利要求8或权利要求9所述的方法,其中原间隔序列邻近基序(PAM)序列在所述gRNA识别序列下游约2至约6个核苷酸处。

13.根据权利要求8至12中任一项所述的方法,其中所述gRNA包含约17个核苷酸至约23个核苷酸。

14.根据权利要求8至12中任一项所述的方法,其中所述gRNA识别序列包含根据SEQ ID NO:43-62中任一个的核苷酸序列。

15.根据权利要求1至14中任一项所述的方法,其还包括检测从所述受试者获得的生物样品中存在或不存在编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子。

16.根据权利要求15所述的方法,其还包括以标准剂量量向受试者施用治疗或抑制认知障碍的治疗剂,其中所述ST6GALNAC5错义变体核酸分子不存在于所述生物样品中。

17.根据权利要求15所述的方法,其还包括以同于或低于标准剂量量的剂量量向对于所述ST6GALNAC5错义变体核酸分子是杂合的受试者施用治疗或抑制认知障碍的治疗剂。

18.根据权利要求15至17中任一项所述的方法,其中所述ST6GALNAC5错义变体核酸分

子编码Val135Ala、Val45Ala、Val45Ala*或Val135Ala*。

19.根据权利要求15至17中任一项所述的方法,其中所述ST6GALNAC5错义变体核酸分子编码Val135Ala、Val45Ala、Val45Ala*或Val135Ala*。

20.根据权利要求18所述的方法,其中所述ST6GALNAC5错义变体核酸分子是:

基因组核酸分子,所述基因组核酸分子具有包含在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶的核苷酸序列;

mRNA分子,所述mRNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶;或

cDNA分子,所述cDNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶。

21.根据权利要求15至20中任一项所述的方法,其中所述检测步骤在体外进行。

22.根据权利要求15至21中任一项所述的方法,其中所述检测步骤包括对所述生物样品中的所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列的至少一部分进行测序,其中所述测序的部分包含对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置;

其中当所述生物样品中所述ST6GALNAC5基因组核酸分子的所述测序的部分包含在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶时,则所述生物样品中的所述ST6GALNAC5基因组核酸分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体基因组核酸分子。

23.根据权利要求15至21中任一项所述的方法,其中所述检测步骤包括对所述生物样品中的所述ST6GALNAC5 mRNA分子的核苷酸序列的至少一部分进行测序,其中所述测序的部分包含对应于以下的位置:根据SEQ ID NO:11或其互补序列的位置600;根据SEQ ID NO:12或其互补序列的位置639;根据SEQ ID NO:13或其互补序列的位置562;根据SEQ ID NO:14或其互补序列的位置515;根据SEQ ID NO:15或其互补序列的位置579;根据SEQ ID NO:16或其互补序列的位置416;根据SEQ ID NO:17或其互补序列的位置639;或根据SEQ ID NO:18或其互补序列的位置600;

其中当所述生物样品中的所述ST6GALNAC5 mRNA分子的所述测序的部分包含:在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶;在对应于根据

SEQ ID NO:17的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶时;则所述生物样品中的所述ST6GALNAC5 mRNA分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体mRNA分子。

24.根据权利要求15至21中任一项所述的方法,其中所述检测步骤包括对由所述生物样品中的mRNA分子产生的所述ST6GALNAC5 cDNA分子的核苷酸序列的至少一部分进行测序,其中所述测序的部分包含对应于以下的位置:根据SEQ ID NO:27或其互补序列的位置600;根据SEQ ID NO:28或其互补序列的位置639;根据SEQ ID NO:29或其互补序列的位置562;根据SEQ ID NO:30或其互补序列的位置515;根据SEQ ID NO:31或其互补序列的位置579;根据SEQ ID NO:32或其互补序列的位置416;根据SEQ ID NO:33或其互补序列的位置639;或根据SEQ ID NO:34或其互补序列的位置600;

其中当所述生物样品中的所述ST6GALNAC5 cDNA分子的所述测序的部分包含:在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶时;则由所述生物样品中的mRNA分子产生的所述ST6GALNAC5 cDNA分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体cDNA分子。

25.根据权利要求15至21中任一项所述的方法,其中所述检测步骤包括:

a)使所述生物样品与引物接触,所述引物与所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列的一部分杂交,所述部分接近对应于根据SEQ ID NO:2的位置176,867的位置;

b)将所述引物延伸至少通过所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列的对应于根据SEQ ID NO:2的位置176,867的位置;以及

c)确定所述引物的所述延伸产物是否包含在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶。

26.根据权利要求15至21中任一项所述的方法,其中所述检测步骤包括:

a)使所述生物样品与引物接触,所述引物与所述ST6GALNAC5mRNA分子或其互补序列的核苷酸序列的一部分杂交,所述部分接近对应于以下的位置:根据SEQ ID NO:11的位置600、根据SEQ ID NO:12的位置639、根据SEQ ID NO:13的位置562、根据SEQ ID NO:14的位置515、根据SEQ ID NO:15的位置579、根据SEQ ID NO:16的位置416、根据SEQ ID NO:17的位置639或根据SEQ ID NO:18的位置600;

b)将所述引物延伸至少通过所述ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列的对应于以下的位置:根据SEQ ID NO:11的位置600、根据SEQ ID NO:12的位置639、根据SEQ ID NO:13的位置562、根据SEQ ID NO:14的位置515、根据SEQ ID NO:15的位置579、根据SEQ ID NO:16的位置416、根据SEQ ID NO:17的位置639或根据SEQ ID NO:18的位置600;以及

c)确定所述引物的所述延伸产物是否包含:在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置

处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶。

27.根据权利要求15至21中任一项所述的方法,其中所述检测步骤包括:

a)使所述生物样品与引物接触,所述引物与所述ST6GALNAC5cDNA分子或其互补序列的核苷酸序列的一部分杂交,所述部分接近对应于以下的位置:根据SEQ ID NO:27的位置600、根据SEQ ID NO:28的位置639、根据SEQ ID NO:29的位置562、根据SEQ ID NO:30的位置515、根据SEQ ID NO:31的位置579、根据SEQ ID NO:32的位置416、根据SEQ ID NO:33的位置639或根据SEQ ID NO:34的位置600;

b)将所述引物延伸至少通过所述ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列的对应于以下的位置:根据SEQ ID NO:27的位置600、根据SEQ ID NO:28的位置639、根据SEQ ID NO:29的位置562、根据SEQ ID NO:30的位置515、根据SEQ ID NO:31的位置579、根据SEQ ID NO:32的位置416、根据SEQ ID NO:33的位置639或根据SEQ ID NO:34的位置600;以及

c)确定所述引物的所述延伸产物是否包含:在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶。

28.根据权利要求22至27中任一项所述的方法,其中所述检测步骤包括对整个核酸分子进行测序。

29.根据权利要求15至21中任一项所述的方法,其中所述检测步骤包括:

a)对所述生物样品中的所述ST6GALNAC5基因组核酸分子或其互补序列的至少一部分进行扩增,其中所述部分包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;

b)用可检测标记来标记所述扩增的核酸分子;

c)使所述标记的核酸分子与包含改变特异性探针的支持物接触,其中所述改变特异性探针包含在严格条件下与所述扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;以及

d)检测所述可检测标记。

30.根据权利要求15至21中任一项所述的方法,其中所述检测步骤包括:

a)对所述生物样品中的所述ST6GALNAC5 mRNA分子或其互补序列的至少一部分进行扩增,其中所述部分包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对

应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;

b)用可检测标记来标记所述扩增的核酸分子;

c)使所述标记的核酸分子与包含改变特异性探针的支持物接触,其中所述改变特异性探针包含在严格条件下与所述扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;以及

d)检测所述可检测标记。

31.根据权利要求15至21中任一项所述的方法,其中所述检测步骤包括:

a)对所述生物样品中的所述ST6GALNAC5 cDNA分子或其互补序列的至少一部分进行扩增,其中所述部分包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;

b)用可检测标记来标记所述扩增的核酸分子;

c)使所述标记的核酸分子与包含改变特异性探针的支持物接触,其中所述改变特异性探针包含在严格条件下与所述扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;以及

d)检测所述可检测标记。

32.根据权利要求31所述的方法,其中所述样品中的所述核酸分子是mRNA,并且在所述扩增步骤之前将所述mRNA逆转录成cDNA。

33.根据权利要求15至21中任一项所述的方法,其中所述检测步骤包括:

使所述生物样品中的所述ST6GALNAC5基因组核酸分子或其互补序列与包含可检测标记的改变特异性探针接触,其中所述改变特异性探针包含在严格条件下与所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列杂交的核苷酸序列,所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列包含在对应于根据SEQ ID NO:2或其互补序列的位置

176,867的位置处的胞嘧啶;以及

检测所述可检测标记。

34.根据权利要求15至21中任一项所述的方法,其中所述检测步骤包括:

使所述生物样品中的所述ST6GALNAC5 mRNA分子或其互补序列与包含可检测标记的改变特异性探针接触,其中所述改变特异性探针包含在严格条件下与所述ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列杂交的核苷酸序列,所述ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;以及

检测所述可检测标记。

35.根据权利要求15至21中任一项所述的方法,其中所述检测步骤包括:

使所述生物样品中的所述ST6GALNAC5 cDNA分子或其互补序列与包含可检测标记的改变特异性探针接触,其中所述改变特异性探针包含在严格条件下与所述ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列杂交的核苷酸序列,所述ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;以及

检测所述可检测标记。

36.一种用治疗或预防认知障碍的治疗剂治疗受试者的方法,其中所述受试者患有认知障碍或处于发展出认知障碍的风险中,所述方法包括:

通过以下方式确定所述受试者是否具有编码α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子:

从所述受试者获得或已经获得生物样品;并且

对所述生物样品进行或已经进行序列分析以确定所述受试者是否具有包含编码所述ST6GALNAC5预测的功能丧失多肽的所述ST6GALNAC5错义变体核酸分子的基因型;以及

以标准剂量量向作为ST6GALNAC5参考的受试者施用或继续施用所述治疗或抑制认知障碍的治疗剂,和/或向所述受试者施用ST6GALNAC5抑制剂;以及

以同于或低于标准剂量量的量向对于所述ST6GALNAC5错义变体核酸分子是杂合的受试者施用或继续施用所述治疗或抑制认知障碍的治疗剂,和/或向所述受试者施用ST6GALNAC5抑制剂;

其中具有编码所述ST6GALNAC5预测的功能丧失多肽的所述ST6GALNAC5错义变体核酸

分子的基因型的存在指示所述受试者具有降低的发展出认知障碍的风险。

37.根据权利要求36所述的方法,其中所述受试者是ST6GALNAC5参考,并且以标准剂量量向所述受试者施用或继续施用所述治疗或抑制认知障碍的治疗剂,并且施用ST6GALNAC5抑制剂。

38.根据权利要求36所述的方法,其中所述受试者对于ST6GALNAC5错义变体核酸分子是杂合的,并且以同于或低于标准剂量量的量向所述受试者施用或继续施用所述治疗或抑制认知障碍的治疗剂,并且施用ST6GALNAC5抑制剂。

39.根据权利要求36至38中任一项所述的方法,其中所述ST6GALNAC5错义变体核酸分子编码Val135Ala、Val45Ala、Val45Ala*或Val135Ala*。

40.根据权利要求36至38中任一项所述的方法,其中所述ST6GALNAC5错义变体核酸分子编码Val135Ala、Val45Ala、Val45Ala*或Val135Ala*。

41.根据权利要求39所述的方法,其中所述ST6GALNAC5错义变体核酸分子是:

基因组核酸分子,所述基因组核酸分子具有包含在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶的核苷酸序列;

mRNA分子,所述mRNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶;或

由mRNA分子产生的cDNA分子,其中所述cDNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶。

42.根据权利要求36至41中任一项所述的方法,其中所述序列分析包括对所述生物样品中的所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列的至少一部分进行测序,其中所述测序的部分包含对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置;

其中当所述生物样品中所述ST6GALNAC5基因组核酸分子或其互补序列的所述测序的部分包含在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶时,则所述生物样品中的所述ST6GALNAC5基因组核酸分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体基因组核酸分子。

43.根据权利要求36至41中任一项所述的方法,其中所述序列分析包括对所述生物样品中的所述ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列的至少一部分进行测序,其中所述测序的部分包含对应于以下的位置:根据SEQ ID NO:11或其互补序列的位置600;根据SEQ ID NO:12或其互补序列的位置639;根据SEQ ID NO:13或其互补序列的位置562;根

据SEQ ID NO:14或其互补序列的位置515;根据SEQ ID NO:15或其互补序列的位置579;根据SEQ ID NO:16或其互补序列的位置416;根据SEQ ID NO:17或其互补序列的位置639;或根据SEQ ID NO:18或其互补序列的位置600;

其中当所述生物样品中的所述ST6GALNAC5 mRNA分子的所述测序的部分包含:在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶时;则所述生物样品中的所述ST6GALNAC5 mRNA分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体mRNA分子。

44.根据权利要求36至41中任一项所述的方法,其中所述序列分析包括对所述生物样品中的所述ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列的至少一部分进行测序,其中所述测序的部分包含对应于以下的位置:根据SEQ ID NO:27或其互补序列的位置600;根据SEQ ID NO:28或其互补序列的位置639;根据SEQ ID NO:29或其互补序列的位置562;根据SEQ ID NO:30或其互补序列的位置515;根据SEQ ID NO:31或其互补序列的位置579;根据SEQ ID NO:32或其互补序列的位置416;根据SEQ ID NO:33或其互补序列的位置639;或根据SEQ ID NO:34或其互补序列的位置600;

其中当所述生物样品中的所述ST6GALNAC5 cDNA分子的所述测序的部分包含:在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶时;则所述生物样品中的所述ST6GALNAC5 cDNA分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体cDNA分子。

45.根据权利要求36至41中任一项所述的方法,其中所述序列分析包括:

a)使所述生物样品与引物接触,所述引物与所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列的一部分杂交,所述部分接近对应于根据SEQ ID NO:2的位置176,867的位置;

b)将所述引物延伸至少通过所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列的对应于根据SEQ ID NO:2的位置176,867的位置;以及

c)确定所述引物的所述延伸产物是否包含在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶。

46.根据权利要求36至41中任一项所述的方法,其中所述序列分析包括:

a)使所述生物样品与引物接触,所述引物与所述ST6GALNAC5mRNA分子或其互补序列的核苷酸序列的一部分杂交,所述部分接近对应于以下的位置:根据SEQ ID NO:11的位置600、根据SEQ IDNO:12的位置639、根据SEQ ID NO:13的位置562、根据SEQ ID NO:14的位置515、根据SEQ ID NO:15的位置579、根据SEQ ID NO:16的位置416、根据SEQ ID NO:17的位

置639或根据SEQ ID NO:18的位置600;

b)将所述引物延伸至少通过所述ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列的对应于以下的位置:根据SEQ ID NO:11的位置600、根据SEQ ID NO:12的位置639、根据SEQ ID NO:13的位置562、根据SEQ ID NO:14的位置515、根据SEQ ID NO:15的位置579、根据SEQ ID NO:16的位置416、根据SEQ ID NO:17的位置639或根据SEQ ID NO:18的位置600;以及

c)确定所述引物的所述延伸产物是否包含:在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶。

47.根据权利要求36至41中任一项所述的方法,其中所述序列分析包括:

a)使所述生物样品与引物接触,所述引物与所述ST6GALNAC5cDNA分子或其互补序列的核苷酸序列的一部分杂交,所述部分接近对应于以下的位置:根据SEQ ID NO:27的位置600、根据SEQ ID NO:28的位置639、根据SEQ ID NO:29的位置562、根据SEQ ID NO:30的位置515、根据SEQ ID NO:31的位置579、根据SEQ ID NO:32的位置416、根据SEQ ID NO:33的位置639或根据SEQ ID NO:34的位置600;

b)将所述引物延伸至少通过所述ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列的对应于以下的位置:根据SEQ ID NO:27的位置600、根据SEQ ID NO:28的位置639、根据SEQ ID NO:29的位置562、根据SEQ ID NO:30的位置515、根据SEQ ID NO:31的位置579、根据SEQ ID NO:32的位置416、根据SEQ ID NO:33的位置639或根据SEQ ID NO:34的位置600;以及

c)确定所述引物的所述延伸产物是否包含:在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶。

48.根据权利要求42至47中任一项所述的方法,其中所述序列分析包括对整个核酸分子进行测序。

49.根据权利要求36至41中任一项所述的方法,其中所述序列分析包括:

a)对所述生物样品中的所述ST6GALNAC5基因组核酸分子或其互补序列的至少一部分进行扩增,其中所述部分包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;

b)用可检测标记来标记所述扩增的核酸分子;

c)使所述标记的核酸分子与包含改变特异性探针的支持物接触,其中所述改变特异性探针包含在严格条件下与所述扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;以及

d)检测所述可检测标记。

50.根据权利要求36至41中任一项所述的方法,其中所述序列分析包括:

a)对所述生物样品中的所述ST6GALNAC5 mRNA分子或其互补序列的至少一部分进行扩增,其中所述部分包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;

b)用可检测标记来标记所述扩增的核酸分子;

c)使所述标记的核酸分子与包含改变特异性探针的支持物接触,其中所述改变特异性探针包含在严格条件下与所述扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;以及

d)检测所述可检测标记。

51.根据权利要求36至41中任一项所述的方法,其中所述序列分析包括:

a)对所述生物样品中的所述ST6GALNAC5 cDNA分子或其互补序列的至少一部分进行扩增,其中所述部分包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;

b)用可检测标记来标记所述扩增的核酸分子;

c)使所述标记的核酸分子与包含改变特异性探针的支持物接触,其中所述改变特异性探针包含在严格条件下与所述扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;以及

d)检测所述可检测标记。

52.根据权利要求51所述的方法,其中所述样品中的所述核酸分子是mRNA,并且在所述扩增步骤之前将所述mRNA逆转录成cDNA。

53.根据权利要求36至41中任一项所述的方法,其中所述序列分析包括:

使所述生物样品中的所述ST6GALNAC5基因组核酸分子或其互补序列与包含可检测标记的改变特异性探针接触,其中所述改变特异性探针包含在严格条件下与所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列杂交的核苷酸序列,所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;以及

检测所述可检测标记。

54.根据权利要求36至41中任一项所述的方法,其中所述序列分析包括:

使所述生物样品中的所述ST6GALNAC5 mRNA分子或其互补序列与包含可检测标记的改变特异性探针接触,其中所述改变特异性探针包含在严格条件下与所述ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列杂交的核苷酸序列,所述ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;以及

检测所述可检测标记。

55.根据权利要求36至41中任一项所述的方法,其中所述序列分析包括:

使所述生物样品中的所述ST6GALNAC5 cDNA分子或其互补序列与包含可检测标记的改变特异性探针接触,其中所述改变特异性探针包含在严格条件下与所述ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列杂交的核苷酸序列,所述ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;以及

检测所述可检测标记。

56.根据权利要求36至55中任一项所述的方法,其中所述核酸分子存在于从所述受试者获得的细胞内。

57.根据权利要求36至56中任一项所述的方法,其中所述ST6GALNAC5抑制剂包含抑制性核酸分子。

58.根据权利要求57所述的方法,其中所述抑制性核酸分子包含与ST6GALNAC5核酸分

子杂交的反义核酸分子、小干扰RNA(siRNA)或短发夹RNA(shRNA)。

59.根据权利要求36至56中任一项所述的方法,其中所述ST6GALNAC5抑制剂包含与ST6GALNAC5基因组核酸分子内的指导RNA(gRNA)识别序列杂交的Cas蛋白和gRNA。

60.根据权利要求59所述的方法,其中所述Cas蛋白是Cas9或Cpf1。

61.根据权利要求59或权利要求60所述的方法,其中所述gRNA识别序列包括或接近对应于根据SEQ ID NO:1的位置176,867的位置。

62.根据权利要求59或权利要求60所述的方法,其中所述gRNA识别序列位于距对应于根据SEQ ID NO:1的位置176,867的位置约1000、约500、约400、约300、约200、约100、约50、约45、约40、约35、约30、约25、约20、约15、约10或约5个核苷酸处。

63.根据权利要求59或权利要求60所述的方法,其中原间隔序列邻近基序(PAM)序列在所述gRNA识别序列下游约2至6个核苷酸处。

64.根据权利要求59至63中任一项所述的方法,其中所述gRNA包含约17至约23个核苷酸。

65.根据权利要求59至64中任一项所述的方法,其中所述gRNA识别序列包含根据SEQ ID NO:43-62中任一个的核苷酸序列。

66.一种鉴定具有增加的发展出认知障碍的风险的受试者的方法,所述方法包括:

确定或已经确定在从所述受试者获得的生物样品中存在或不存在编码α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子;

其中:

当所述受试者是ST6GALNAC5参考时,则所述受试者具有增加的发展出认知障碍的风险;并且

当所述受试者对于编码所述ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的或纯合的时,则所述受试者具有降低的发展出认知障碍的风险。

67.根据权利要求66所述的方法,其中所述ST6GALNAC5错义变体核酸分子编码Val135Ala、Val45Ala、Val45Ala*或Val135Ala*。

68.根据权利要求66所述的方法,其中所述ST6GALNAC5错义变体核酸分子编码Val135Ala、Val45Ala、Val45Ala*或Val135Ala*。

69.根据权利要求67所述的方法,其中所述ST6GALNAC5错义变体核酸分子是:

基因组核酸分子,所述基因组核酸分子具有包含在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶的核苷酸序列;

mRNA分子,所述mRNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶;或

由mRNA分子产生的cDNA分子,其中所述cDNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639

的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶。

70.根据权利要求66至69中任一项所述的方法,其中所述确定步骤在体外进行。

71.根据权利要求66至70中任一项所述的方法,其中所述确定步骤包括对所述生物样品中的所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列的至少一部分进行测序,其中所述测序的部分包含对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置;

其中当所述生物样品中所述ST6GALNAC5基因组核酸分子的所述测序的部分包含在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶时,则所述生物样品中的所述ST6GALNAC5基因组核酸分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体基因组核酸分子。

72.根据权利要求66至70中任一项所述的方法,其中所述确定步骤包括对所述生物样品中的所述ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列的至少一部分进行测序,其中所述测序的部分包含对应于以下的位置:根据SEQ ID NO:11或其互补序列的位置600;根据SEQ ID NO:12或其互补序列的位置639;根据SEQ IDNO:13或其互补序列的位置562;根据SEQ ID NO:14或其互补序列的位置515;根据SEQ ID NO:15或其互补序列的位置579;根据SEQ ID NO:16或其互补序列的位置416;根据SEQ ID NO:17或其互补序列的位置639;或根据SEQ ID NO:18或其互补序列的位置600;

其中当所述生物样品中的所述ST6GALNAC5 mRNA分子的所述测序的部分包含:在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶时,则所述生物样品中的所述ST6GALNAC5 mRNA分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体mRNA分子。

73.根据权利要求66至70中任一项所述的方法,其中所述确定步骤包括对所述生物样品中的所述ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列的至少一部分进行测序,其中所述测序的部分包含对应于以下的位置:根据SEQ ID NO:27或其互补序列的位置600;根据SEQ ID NO:28或其互补序列的位置639;根据SEQ ID NO:29或其互补序列的位置562;根据SEQ ID NO:30或其互补序列的位置515;根据SEQ ID NO:31或其互补序列的位置579;根据SEQ ID NO:32或其互补序列的位置416;根据SEQ ID NO:33或其互补序列的位置639;或根据SEQ ID NO:34或其互补序列的位置600;

其中当所述生物样品中的所述ST6GALNAC5 cDNA分子的所述测序的部分包含:在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根

据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶时,则所述生物样品中的所述ST6GALNAC5 cDNA分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体cDNA分子。

74.根据权利要求66至70中任一项所述的方法,其中所述确定步骤包括:

a)使所述生物样品与引物接触,所述引物与所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列的一部分杂交,所述部分接近对应于根据SEQ ID NO:2的位置176,867的位置;

b)将所述引物延伸至少通过所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列的对应于根据SEQ ID NO:2的位置176,867的位置;以及

c)确定所述引物的所述延伸产物是否包含在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶。

75.根据权利要求66至70中任一项所述的方法,其中所述确定步骤包括:

a)使所述生物样品与引物接触,所述引物与所述ST6GALNAC5mRNA分子或其互补序列的核苷酸序列的一部分杂交,所述部分接近对应于以下的位置:根据SEQ ID NO:11的位置600、根据SEQ ID NO:12的位置639、根据SEQ ID NO:13的位置562、根据SEQ ID NO:14的位置515、根据SEQ ID NO:15的位置579、根据SEQ ID NO:16的位置416、根据SEQ ID NO:17的位置639或根据SEQ ID NO:18的位置600;

b)将所述引物延伸至少通过所述ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列的对应于以下的位置:根据SEQ ID NO:11的位置600、根据SEQ ID NO:12的位置639、根据SEQ ID NO:13的位置562、根据SEQ ID NO:14的位置515、根据SEQ ID NO:15的位置579、根据SEQ ID NO:16的位置416、根据SEQ ID NO:17的位置639或根据SEQ ID NO:18的位置600;以及

c)确定所述引物的所述延伸产物是否包含:在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶。

76.根据权利要求66至70中任一项所述的方法,其中所述确定步骤包括:

a)使所述生物样品与引物接触,所述引物与所述ST6GALNAC5cDNA分子或其互补序列的核苷酸序列的一部分杂交,所述部分接近对应于以下的位置:根据SEQ ID NO:27的位置600、根据SEQ ID NO:28的位置639、根据SEQ ID NO:29的位置562、根据SEQ ID NO:30的位置515、根据SEQ ID NO:31的位置579、根据SEQ ID NO:32的位置416、根据SEQ ID NO:33的位置639或根据SEQ ID NO:34的位置600;

b)将所述引物延伸至少通过所述ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列的对应于以下的位置:根据SEQ ID NO:27的位置600、根据SEQ ID NO:28的位置639、根据SEQ ID NO:29的位置562、根据SEQ ID NO:30的位置515、根据SEQ ID NO:31的位置579、根据SEQ ID NO:32的位置416、根据SEQ ID NO:33的位置639或根据SEQ ID NO:34的位置600;以及

c)确定所述引物的所述延伸产物是否包含:在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶。

77.根据权利要求71至76中任一项所述的方法,其中所述确定步骤包括对整个核酸分子进行测序。

78.根据权利要求66至70中任一项所述的方法,其中所述确定步骤包括:

a)对所述生物样品中的所述ST6GALNAC5基因组核酸分子或其互补序列的至少一部分进行扩增,其中所述部分包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;

b)用可检测标记来标记所述扩增的核酸分子;

c)使所述标记的核酸分子与包含改变特异性探针的支持物接触,其中所述改变特异性探针包含在严格条件下与所述扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;以及

d)检测所述可检测标记。

79.根据权利要求66至70中任一项所述的方法,其中所述确定步骤包括:

a)对所述生物样品中的所述ST6GALNAC5 mRNA分子或其互补序列的至少一部分进行扩增,其中所述部分包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;

b)用可检测标记来标记所述扩增的核酸分子;

c)使所述标记的核酸分子与包含改变特异性探针的支持物接触,其中所述改变特异性探针包含在严格条件下与所述扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;以及

d)检测所述可检测标记。

80.根据权利要求66至70中任一项所述的方法,其中所述确定步骤包括:

a)对所述生物样品中的所述ST6GALNAC5 cDNA分子或其互补序列的至少一部分进行扩增,其中所述部分包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;

b)用可检测标记来标记所述扩增的核酸分子;

c)使所述标记的核酸分子与包含改变特异性探针的支持物接触,其中所述改变特异性探针包含在严格条件下与所述扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;以及

d)检测所述可检测标记。

81.根据权利要求80所述的方法,其中所述样品中的所述核酸分子是mRNA,并且在所述扩增步骤之前将所述mRNA逆转录成cDNA。

82.根据权利要求66至70中任一项所述的方法,其中所述检测步骤包括:

使所述生物样品中的所述ST6GALNAC5基因组核酸分子或其互补序列与包含可检测标记的改变特异性探针接触,其中所述改变特异性探针包含在严格条件下与所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列杂交的核苷酸序列,所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;以及

检测所述可检测标记。

83.根据权利要求66至70中任一项所述的方法,其中所述检测步骤包括:

使所述生物样品中的所述ST6GALNAC5 mRNA分子或其互补序列与包含可检测标记的改变特异性探针接触,其中所述改变特异性探针包含在严格条件下与所述ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列杂交的核苷酸序列,所述ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;以及

检测所述可检测标记。

84.根据权利要求66至70中任一项所述的方法,其中所述检测步骤包括:

使所述生物样品中的所述ST6GALNAC5 cDNA分子或其互补序列与包含可检测标记的改变特异性探针接触,其中所述改变特异性探针包含在严格条件下与所述ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列杂交的核苷酸序列,所述ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;以及

检测所述可检测标记。

85.根据权利要求66至84中任一项所述的方法,其中所述受试者是ST6GALNAC5参考,并且以标准剂量量向所述受试者施用治疗或抑制认知障碍的治疗剂,并且施用ST6GALNAC5抑制剂。

86.根据权利要求66至84中任一项所述的方法,其中所述受试者对于ST6GALNAC5预测的功能丧失变体是杂合的,并且以同于或低于标准剂量量的量向所述受试者施用治疗或抑制认知障碍的治疗剂,并且施用ST6GALNAC5抑制剂。

87.一种治疗或抑制认知障碍的治疗剂,其用于治疗或预防被鉴定为具有以下各项的受试者的认知障碍:

编码α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)预测的功能丧失多肽的基因组核酸分子或其互补序列,其中所述基因组核酸分子具有包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶的核苷酸序列;

编码ST6GALNAC5预测的功能丧失多肽的mRNA分子或其互补序列,其中所述mRNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;或

编码ST6GALNAC5预测的功能丧失多肽的cDNA分子或其互补序列,其中所述cDNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或

在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

88.一种α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)抑制剂,其用于治疗或预防受试者的认知障碍,所述受试者:

a)对于ST6GALNAC5基因组核酸分子、ST6GALNAC5 mRNA分子或ST6GALNAC5 cDNA分子是参考;或

b)对于以下各项是杂合的:

i)编码ST6GALNAC5预测的功能丧失多肽的基因组核酸分子或其互补序列,其中所述基因组核酸分子具有包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶的核苷酸序列;

ii)编码ST6GALNAC5预测的功能丧失多肽的mRNA分子或其互补序列,其中所述mRNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;

在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;或

iii)编码ST6GALNAC5预测的功能丧失多肽的cDNA分子或其互补序列,其中所述cDNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;

在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

89.根据权利要求88所述的ST6GALNAC5抑制剂,其是抑制性核酸分子。

90.根据权利要求89所述的ST6GALNAC5抑制剂,其中所述抑制性核酸分子是与ST6GALNAC5核酸分子杂交的反义核酸分子、小干扰RNA(siRNA)或短发夹RNA(shRNA)。

91.根据权利要求88所述的ST6GALNAC5抑制剂,其包含与ST6GALNAC5基因组核酸分子内的指导RNA(gRNA)识别序列杂交的Cas蛋白和gRNA。

92.根据权利要求91所述的ST6GALNAC5抑制剂,其中所述Cas蛋白是Cas9或Cpf1。

93.根据权利要求91或权利要求92所述的ST6GALNAC5抑制剂,其中所述gRNA识别序列包括或接近根据SEQ ID NO:1的位置176,867。

94.根据权利要求91或权利要求92所述的ST6GALNAC5抑制剂,其中所述gRNA识别序列位于距对应于根据SEQ ID NO:1的位置176,867的位置约1000、约500、约400、约300、约200、约100、约50、约45、约40、约35、约30、约25、约20、约15、约10或约5个核苷酸处。

95.根据权利要求91或权利要求92所述的ST6GALNAC5抑制剂,其中原间隔序列邻近基

序(PAM)序列在所述gRNA识别序列下游约2至约6个核苷酸处。

96.根据权利要求91至95中任一项所述的ST6GALNAC5抑制剂,其中所述gRNA包含约17至约23个核苷酸。

97.根据权利要求91至96中任一项所述的ST6GALNAC5抑制剂,其中所述gRNA识别序列包含根据SEQ ID NO:43-62中任一个的核苷酸序列。

使用α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)抑制剂治疗认知障碍

[0001] 序列表的引用

[0002] 本申请包括以电子方式作为文本文件提交的序列表,其命名为18923808002SEQ,创建于2022年6月25日,大小为634千字节。所述序列表以引用的方式并入本文。

技术领域

[0003] 本公开一般涉及用α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)抑制剂治疗患有认知障碍的受试者,以及鉴定具有增加的发展出认知障碍的风险的受试者的方法。

背景技术

[0004] 认知障碍的特征可以是个体难以记忆、学习新事物、集中注意力或做出影响其日常生活的决定。认知障碍的范围从轻度到严重。患有轻度障碍的个体可能会开始注意到认知功能的变化,但仍然能够进行其日常活动。严重程度的障碍可能会导致丧失理解某事物的含义或重要性的能力以及说话或写作的能力,从而导致无法独立生活。认知障碍包括但不限于髓鞘完整性降低、神经变性、灰质/白质对比度(GWC)降低以及轻度认知障碍转变为痴呆。

[0005] GWC可用于一组分散的大脑区域来评估认知障碍。GWC是灰质/白质脑区边界之间模糊的衡量标准,并且被认为是组织完整性和髓鞘降解、白质中水含量增加或铁沉积局部变化的指标(Uribe等人,Front.Aging Neurosci.,2018,10,89)。较低的GWC与衰老和较低的认知指数(Lewis等人,Neuroimage.,2018,173,341-350),以及从轻度认知障碍转变为痴呆的比率增加(Jefferson等人,Brain Imaging Behav.,2015,9,141-8)相关。

[0006] α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)是一种催化大脑中GM1b生物合成神经节苷脂GD1α的基因(Okajima等人,J.Biol.Chem.,1999,274,30557-30562)。ST6GALNAC5基因先前被鉴定为介导乳腺癌脑转移的基因之一。

发明内容

[0007] 本公开提供了治疗患有认知障碍或处于发展出认知障碍的风险中的受试者的方法,所述方法包括向所述受试者施用ST6GALNAC5抑制剂。

[0008] 本公开还提供了治疗患有髓鞘完整性降低或处于发展出髓鞘完整性降低的风险中的受试者的方法,所述方法包括向所述受试者施用ST6GALNAC5抑制剂。

[0009] 本公开还提供了治疗患有神经变性或处于发展出神经变性的风险中的受试者的方法,所述方法包括向所述受试者施用ST6GALNAC5抑制剂。

[0010] 本公开还提供了治疗患有灰质/白质对比度(GWC)降低或处于发展出GWC降低的风险中的受试者的方法,所述方法包括向所述受试者施用ST6GALNAC5抑制剂。

[0011] 本公开还提供了治疗正在经历从轻度认知障碍转变为痴呆或处于从轻度认知障

碍转变为痴呆的风险中的受试者的方法,所述方法包括向所述受试者施用ST6GALNAC5抑制剂。

[0012] 本公开还提供了用治疗或抑制认知障碍的治疗剂治疗受试者的方法,其中所述受试者患有认知障碍或处于发展出认知障碍的风险中,所述方法包括以下步骤:通过以下方式确定受试者是否具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子:从受试者获得或已经获得生物样品;并且对生物样品进行或已经进行序列分析以确定受试者是否具有包含编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的基因型;以及i)以标准剂量量向作为ST6GALNAC5参考的受试者施用或继续施用治疗或抑制认知障碍的治疗剂,和/或向受试者施用ST6GALNAC5抑制剂;或ii)以同于或低于标准剂量量的量向对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的受试者施用或继续施用治疗或抑制认知障碍的治疗剂,和/或向受试者施用ST6GALNAC5抑制剂;其中具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的基因型的存在指示受试者具有降低的发展出认知障碍的风险。

[0013] 本公开还提供了鉴定具有增加的发展出认知障碍的风险的受试者的方法,所述方法包括:确定或已经确定在从受试者获得的生物样品中存在或不存在编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子;其中当受试者是ST6GALNAC5参考时,受试者具有增加的发展出认知障碍的风险;并且当受试者对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的或纯合的时,受试者具有降低的发展出认知障碍的风险。

[0014] 本公开还提供了检测受试者中的编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子或其互补序列的方法,所述方法包括测定从受试者获得的生物样品以确定生物样品中的核酸分子是否是:i)具有包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶的核苷酸序列的基因组核酸分子;ii)具有包含以下的核苷酸序列的mRNA分子:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;或iii)由生物样品中的mRNA分子产生的cDNA分子,其中所述cDNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

[0015] 本公开还提供了治疗、抑制或预防认知障碍的治疗剂,其用于治疗或预防被鉴定

为具有以下各项的受试者的认知障碍(或用于制备用于治疗被鉴定为具有以下各项的受试者的认知障碍的药物):i)编码ST6GALNAC5预测的功能丧失多肽的基因组核酸分子或其互补序列,其中所述基因组核酸分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;ii)具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列的mRNA分子,其中所述核苷酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;或iii)具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列的cDNA分子,其中所述核苷酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

[0016] 本公开还提供了用于治疗或预防受试者的认知障碍(或用于制备用于治疗或预防受试者的认知障碍的药物)的ST6GALNAC5抑制剂,所述受试者:a)对于ST6GALNAC5基因组核酸分子、ST6GALNAC5mRNA分子或ST6GALNAC5 cDNA分子是参考;或b)对于以下各项是杂合的:i)编码ST6GALNAC5预测的功能丧失多肽的基因组核酸分子或其互补序列,其中所述基因组核酸分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;ii)编码ST6GALNAC5预测的功能丧失多肽的mRNA分子或其互补序列,其中所述mRNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;或iii)编码ST6GALNAC5预测的功能丧失多肽的cDNA分子或其互补序列,其中所述cDNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置

处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

具体实施方式

[0017] 在整个说明书和权利要求书中使用与本公开的多个方面相关的各种术语。除非另外指示,否则此类术语将被赋予其在本领域中的普通含义。其他具体定义的术语应以与本文提供的定义一致的方式解释。

[0018] 除非另外明确说明,否则决不意图将本文陈述的任何方法或方面解释为要求以特定顺序执行其步骤。因此,在权利要求书或说明书中,当方法权利要求没有确切地说明步骤是限于特定顺序时,在任何方面决非意图推断顺序。这适用于任何可能的非表达解释基础,包括相对于步骤排列或操作流程的逻辑事项、从语法组织或标点中得到的普通含义或者在说明书中描述的方面的编号或类型。

[0019] 如本文所用,除非上下文另外明确指出,否则单数形式“一个(种)(a/an)”和“所述(the)”包括复数指代物。

[0020] 如本文所用,术语“约”意指所列举的数值是近似值并且小的变化不会显著影响所公开的实施方案的实践。在使用数值的情况下,除非上下文另外指明,否则术语“约”意指数值可变化±10%且仍在所公开的实施方案的范围内。

[0021] 如本文所用,根据需要,在特定实施方案中,术语“包含”可替换为“由……组成”或“基本上由……组成”。

[0022] 如本文所用,关于核酸分子或多肽的术语“分离的”意指核酸分子或多肽处于不同于其天然环境的条件下,诸如远离血液和/或动物组织。在一些实施方案中,分离的核酸分子或多肽基本上不含其他核酸分子或其他多肽,特别是动物来源的其他核酸分子或多肽。在一些实施方案中,核酸分子或多肽可呈高度纯化的形式,即大于95%纯或大于99%纯。当在此上下文中使用时,术语“分离的”不排除存在呈替代物理形式,诸如二聚体或替代地磷酸化或衍生化形式的相同核酸分子或多肽。

[0023] 如本文所用,术语“核酸”、“核酸分子”、“核酸序列”、“多核苷酸”或“寡核苷酸”可包括任何长度的核苷酸的聚合物形式,可包括DNA和/或RNA,并且可以是单链的、双链的或多链的。核酸的一条链还是指其互补序列。

[0024] 如本文所用,术语“受试者”包括任何动物,包括哺乳动物。哺乳动物包括但不限于农场动物(诸如例如,马、牛、猪)、伴侣动物(诸如例如,狗、猫)、实验室动物(诸如例如,小鼠、大鼠、兔)和非人灵长类动物(诸如例如,猿和猴)。在一些实施方案中,受试者是人。在一些实施方案中,受试者是在医生护理下的患者。

[0025] 根据本公开已经鉴定了与人发展出认知障碍的风险降低相关的ST6GALNAC5基因中的罕见功能丧失变体。例如,已观察到将ST6GALNAC5参考基因组核酸分子(参见SEQ ID NO:1)中的位置176,867处的胸腺嘧啶改变为胞嘧啶的遗传改变,表明具有此类改变的受试者发展出认知障碍的风险可能降低。据信,ST6GALNAC5基因或蛋白的变体与认知障碍不具有任何已知的关联。总而言之,本文所述的遗传分析令人惊讶地表明ST6GALNAC5基因,并且特别是ST6GALNAC5基因的变体与发展出认知障碍的风险降低相关。因此,可对作为ST6GALNAC5参考的受试者(其具有增加的发展出认知障碍,诸如髓鞘完整性降低、神经变性、GWC降低和轻度认知障碍转变为痴呆的风险)进行治疗,从而预防认知障碍、减轻其症

状,和/或抑制症状的发展。因此,本公开提供了利用受试者中此类变体的鉴定来鉴定或分层此类受试者中发展出认知障碍,诸如髓鞘完整性降低、神经变性、GWC降低和轻度认知障碍转变为痴呆的风险,或诊断受试者具有增加的发展出认知障碍,诸如髓鞘完整性降低、神经变性、GWC降低和轻度认知障碍转变为痴呆的风险,使得处于风险中的受试者或患有活动性疾病的受试者可得到相应治疗的方法。

[0026] 出于本公开的目的,任何特定的受试者都可归类为具有以下三种ST6GALNAC5基因型之一:i)ST6GALNAC5参考;ii)对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的;或iii)对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是纯合的。当受试者不具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的拷贝时,所述受试者是ST6GALNAC5参考。当受试者具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的单个拷贝时,所述受试者对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的。如本文所用,ST6GALNAC5错义变体核酸分子是编码具有部分功能丧失、完全功能丧失、预测的部分功能丧失或预测的完全功能丧失的ST6GALNAC5多肽的任何ST6GALNAC5核酸分子(诸如基因组核酸分子、mRNA分子或cDNA分子)。具有编码具有部分功能丧失(或预测的部分功能丧失)的ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的受试者对于ST6GALNAC5是亚等位基因的。编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子可以是编码ST6GALNAC5Val135Ala、Val45Ala、Val45Ala*或Val135Ala*多肽的任何核酸分子。当受试者具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的两个拷贝时,所述受试者对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是纯合的。

[0027] 对于基因分型或确定为ST6GALNAC5参考的受试者,此类受试者具有增加的发展出认知障碍,诸如髓鞘完整性降低、神经变性、GWC降低和轻度认知障碍转变为痴呆的风险。对于基因分型或确定为ST6GALNAC5参考或对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的受试者,可用ST6GALNAC5抑制剂治疗此类受试者。

[0028] 在本公开通篇描述的任何实施方案中,编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子可以是编码具有部分功能丧失、完全功能丧失、预测的部分功能丧失或预测的完全功能丧失的ST6GALNAC5多肽的任何ST6GALNAC5核酸分子(诸如例如基因组核酸分子、mRNA分子或cDNA分子)。例如,ST6GALNAC5错义变体核酸分子可以是编码ST6GALNAC5 Val135Ala、Val45Ala、Val45Ala*或Val135Ala*的任何核酸分子。在一些实施方案中,ST6GALNAC5错义变体核酸分子编码ST6GALNAC5 Val135Ala。在一些实施方案中,ST6GALNAC5错义变体核酸分子编码ST6GALNAC5 Val45Ala。在一些实施方案中,ST6GALNAC5错义变体核酸分子编码ST6GALNAC5 Val45Ala*。在一些实施方案中,ST6GALNAC5错义变体核酸分子编码ST6GALNAC5 Val135Ala*。

[0029] 在本公开通篇描述的任何实施方案中,ST6GALNAC5预测的功能丧失多肽可以是具有部分功能丧失、完全功能丧失、预测的部分功能丧失或预测的完全功能丧失的任何ST6GALNAC5多肽。在本公开通篇描述的任何实施方案中,ST6GALNAC5预测的功能丧失多肽可以是本文描述的任何ST6GALNAC5多肽,包括例如ST6GALNAC5Val135Ala、Val45Ala、Val45Ala*或Val135Ala*。在一些实施方案中,ST6GALNAC5预测的功能丧失多肽是

ST6GALNAC5 Val135Ala。在一些实施方案中,ST6GALNAC5预测的功能丧失多肽是ST6GALNAC5 Val45Ala。在一些实施方案中,ST6GALNAC5预测的功能丧失多肽是ST6GALNAC5 Val45Ala*。在一些实施方案中,ST6GALNAC5预测的功能丧失多肽是ST6GALNAC5 Val135Ala*。

[0030] 在本公开通篇描述的任何实施方案中,认知障碍是髓鞘完整性降低、神经变性、GWC降低或轻度认知障碍转变为痴呆。在本公开通篇描述的任何实施方案中,认知障碍是髓鞘完整性降低。在本公开通篇描述的任何实施方案中,认知障碍是神经变性。在本公开通篇描述的任何实施方案中,认知障碍是GWC降低。在本公开通篇描述的任何实施方案中,认知障碍是轻度认知障碍转变为痴呆。

[0031] 认知障碍的症状包括但不限于记忆丧失、经常问同一个问题或多次重复同一故事、不认识熟悉的人和地方、难以进行判断(诸如知道在紧急情况下该做什么)、情绪或行为变化、视力问题以及难以计划和执行任务。

[0032] 本公开提供了治疗患有认知障碍或处于发展出认知障碍的风险中的受试者的方法,所述方法包括向所述受试者施用ST6GALNAC5抑制剂。

[0033] 本公开还提供了治疗患有髓鞘完整性降低或处于发展出髓鞘完整性降低的风险中的受试者的方法,所述方法包括向所述受试者施用ST6GALNAC5抑制剂。

[0034] 本公开还提供了治疗患有神经变性或处于发展出神经变性的风险中的受试者的方法,所述方法包括向所述受试者施用ST6GALNAC5抑制剂。

[0035] 本公开还提供了治疗患有GWC降低或处于发展出GWC降低的风险中的受试者的方法,所述方法包括向所述受试者施用ST6GALNAC5抑制剂。

[0036] 本公开还提供了治疗患有轻度认知障碍转变为痴呆或处于从轻度认知障碍转变为痴呆的风险中的受试者的方法,所述方法包括向所述受试者施用ST6GALNAC5抑制剂。

[0037] 在一些实施方案中,ST6GALNAC5抑制剂包含抑制性核酸分子。在一些实施方案中,抑制性核酸分子包含反义分子、小干扰RNA(siRNA)分子或短发夹RNA(shRNA)分子。在一些实施方案中,抑制性核酸分子包含反义分子。在一些实施方案中,抑制性核酸分子包含siRNA分子。在一些实施方案中,抑制性核酸分子包含shRNA分子。此类抑制性核酸分子可设计成靶向ST6GALNAC5核酸分子(诸如mRNA分子)的任何区域。在一些实施方案中,抑制性核酸分子与ST6GALNAC5基因组核酸分子或mRNA分子内的序列杂交,并降低受试者细胞中ST6GALNAC5多肽的表达。在一些实施方案中,ST6GALNAC5抑制剂包含与ST6GALNAC5基因组核酸分子或mRNA分子杂交并降低受试者的细胞中ST6GALNAC5多肽的表达的反义分子。在一些实施方案中,ST6GALNAC5抑制剂包含与ST6GALNAC5基因组核酸分子或mRNA分子杂交并降低受试者的细胞中ST6GALNAC5多肽的表达的siRNA。在一些实施方案中,ST6GALNAC5抑制剂包含与ST6GALNAC5基因组核酸分子或mRNA分子杂交并降低受试者的细胞中ST6GALNAC5多肽的表达的shRNA。

[0038] 抑制性核酸分子可包括RNA、DNA、或RNA和DNA两者。抑制性核酸分子还可与异源核酸序列(诸如载体中的异源核酸序列)或异源标记连接或融合。例如,抑制性核酸分子可在包含抑制性核酸分子和异源核酸序列的载体内或作为包含抑制性核酸分子和异源核酸序列的外源供体序列。抑制性核酸分子还可与异源标记连接或融合。标记可以是直接可检测的(诸如例如荧光团)或间接可检测的(诸如例如半抗原、酶或荧光团淬灭剂)。此类标记可

通过光谱学、光化学、生物化学、免疫化学或化学手段检测。此类标记包括例如放射性标记、颜料、染料、色原、自旋标记和荧光标记。标记也可以是例如化学发光物质;含金属物质;或酶,其中发生依赖于酶的二次信号生成。术语“标记”也可指“标签”或半抗原,其可选择性地与缀合分子结合,使得缀合分子在随后与底物一起添加时用于生成可检测信号。例如,生物素可与辣根过氧化物(HRP)的亲和素或链霉亲和素缀合物一起用作标签以与标签结合,并使用量热底物(诸如例如四甲基联苯胺(TMB))或荧光底物进行检查以检测HRP的存在。可用作标签来促进纯化的示例性标记包括但不限于myc、HA、FLAG或3XFLAG、6XHis或聚组氨酸、谷胱甘肽-S-转移酶(GST)、麦芽糖结合蛋白、表位标签或免疫球蛋白的Fc部分。许多标记包括例如颗粒、荧光团、半抗原、酶及其量热、荧光和化学发光底物和其他标记。

[0039] 抑制性核酸分子可包括例如核苷酸或非天然或修饰的核苷酸,诸如核苷酸类似物或核苷酸替代物。此类核苷酸包括含有修饰的碱基、糖或磷酸酯基团的核苷酸,或者在其结构中掺入非天然部分的核苷酸。非天然核苷酸的实例包括但不限于双脱氧核苷酸、生物素酰化的、胺化的、脱氨基的、烷基化的、苄化的和荧光团标记的核苷酸。

[0040] 抑制性核酸分子还可包含一种或多种核苷酸类似物或取代。核苷酸类似物是含有对碱基、糖或磷酸酯部分的修饰的核苷酸。对碱基部分的修饰包括但不限于A、C、G和T/U以及不同的嘌呤或嘧啶碱基(诸如例如假尿苷、尿嘧啶-5-基、次黄嘌呤-9-基(I)和2-氨基腺嘌呤-9-基)的天然和合成修饰。修饰的碱基包括但不限于5-甲基胞嘧啶(5-me-C)、5-羟甲基胞嘧啶、黄嘌呤、次黄嘌呤、2-氨基腺嘌呤、腺嘌呤和鸟嘌呤的6-甲基和其他烷基衍生物、腺嘌呤和鸟嘌呤的2-丙基和其他烷基衍生物、2-硫代尿嘧啶、2-硫代胸腺嘧啶和2-硫代胞嘧啶、5-卤代尿嘧啶和胞嘧啶、5-丙炔基尿嘧啶和胞嘧啶、6-偶氮尿嘧啶、胞嘧啶和胸腺嘧啶、5-尿嘧啶(假尿嘧啶)、4-硫代尿嘧啶、8-卤代、8-氨基、8-硫代、8-硫代烷基、8-羟基和其他8-取代的腺嘌呤和鸟嘌呤、5-卤代(诸如例如5-溴)、5-三氟甲基和其他5-取代的尿嘧啶和胞嘧啶、7-甲基鸟嘌呤、7-甲基腺嘌呤、8-氮杂鸟嘌呤、8-氮杂腺嘌呤、7-脱氮鸟嘌呤、7-脱氮腺嘌呤、3-脱氮鸟嘌呤和3-脱氮腺嘌呤。

[0041] 核苷酸类似物还可包括对糖部分的修饰。对糖部分的修饰包括但不限于核糖和脱氧核糖的天然修饰以及合成修饰。糖修饰包括但不限于在2’位置处的以下修饰:OH;F;O-、S-或N-烷基;O-、S-或N-烯基;O-、S-或N-炔基;或O-烷基-O-烷基,其中烷基、烯基和炔基可以是取代或未取代的C_{1-10}烷基或C_{2-10}烯基和C_{2-10}炔基。示例性的2’糖修饰还包括但不限于-O[$(CH_2)_nO]_mCH_3$、-O$(CH_2)_nOCH_3$、-O$(CH_2)_nNH_2$、-O$(CH_2)_nCH_3$、-O$(CH_2)_n$-ONH_2和-O$(CH_2)_nON[(CH_2)_nCH_3)]_2$,其中n和m独立地为1至约10。2’位置处的其他修饰包括但不限于C_{1-10}烷基、取代的低级烷基、烷芳基、芳烷基、O-烷芳基或O-芳烷基、SH、SCH_3、OCN、Cl、Br、CN、CF_3、OCF_3、$SOCH_3$、SO_2CH_3、ONO_2、NO_2、N_3、NH_2、杂环烷基、杂环烷芳基、氨基烷基氨基、聚烷基氨基、取代的甲硅烷基、RNA切割基团、报告基团、嵌入剂、用于改善寡核苷酸的药代动力学特性的基团或用于改善寡核苷酸的药效动力学特性的基团,以及具有类似特性的其他取代基。还可在糖上的其他位置上做出类似的修饰,具体地在3’末端核苷酸上或在2’-5’连接的寡核苷酸中的糖的3’位置和5’末端核苷酸的5’位置。修饰的糖还可包括在桥环氧处含有修饰(诸如CH_2和S)的那些糖。核苷酸糖类似物还可具有替代呋喃戊糖的糖模拟物诸如环丁基部分。

[0042] 核苷酸类似物还可在磷酸酯部分处进行修饰。修饰的磷酸酯部分包括但不限于可被修饰以使得两个核苷酸之间的键联含有以下的修饰的磷酸酯部分:硫代磷酸酯、手性硫

代磷酸酯、二硫代磷酸酯、磷酸三酯、氨基烷基磷酸三酯、甲基和其他烷基膦酸酯(包括3'-亚烷基膦酸酯和手性膦酸酯)、次膦酸酯、氨基磷酸酯(包括3'-氨基氨基磷酸酯和氨基烷基氨基磷酸酯)、硫羰氨基磷酸酯、硫羰烷基膦酸酯、硫羰烷基磷酸三酯和硼烷磷酸酯。两个核苷酸之间的这些磷酸酯或修饰的磷酸酯键联可通过3'-5'键联或2'-5'键联,并且所述键联可含有反转的极性,诸如3'-5'至5'-3'或2'-5'至5'-2'。还包括各种盐、混合盐以及游离酸形式。核苷酸替代物还包括肽核酸(PNA)。

[0043] 在一些实施方案中,反义核酸分子是间隔体(gapmer),由此在5'和3'端的前一至七个核苷酸各自具有2'-甲氧基乙基(2'-MOE)修饰。在一些实施方案中,5'和3'端的前五个核苷酸各自具有2'-MOE修饰。在一些实施方案中,5'和3'端的前一至七个核苷酸是RNA核苷酸。在一些实施方案中,5'和3'端的前五个核苷酸是RNA核苷酸。在一些实施方案中,核苷酸之间的每个骨架键联是硫代磷酸酯键联。

[0044] 在一些实施方案中,siRNA分子具有末端修饰。在一些实施方案中,反义链的5'端被磷酸化。在一些实施方案中,使用不能水解的5'-磷酸酯类似物,诸如5'-(E)-乙烯基膦酸酯。

[0045] 在一些实施方案中,siRNA分子具有骨架修饰。在一些实施方案中,已证明连接连续核糖核苷的修饰的磷酸二酯基团会增强siRNA的稳定性和体内生物利用率。磷酸二酯键联的非酯类基团(-OH、=O)可被硫、硼或乙酸酯替换,得到硫代磷酸酯、硼酸磷酸酯和膦酰基乙酸酯键联。另外,用磷酸三酯取代磷酸二酯基团可促进siRNA的细胞摄取并通过消除它们的负电荷而保留在血清组分上。在一些实施方案中,siRNA分子具有糖修饰。在一些实施方案中,糖被去质子化(由外切和内切核酸酶催化的反应),由此2'-羟基可充当亲核试剂并攻击磷酸二酯键中相邻的磷。此类替代方案包括2'-O-甲基、2'-O-甲氧基乙基和2'-氟修饰。

[0046] 在一些实施方案中,siRNA分子具有碱基修饰。在一些实施方案中,碱基可被修饰的碱基,诸如假尿苷、5'-甲基胞苷、N6-甲基腺苷、肌苷和N7-甲基鸟苷取代。

[0047] 在一些实施方案中,siRNA分子与脂质缀合。脂质可缀合到siRNA的5'或3'末端,以通过允许它们与血清脂蛋白缔合来提高它们的体内生物利用率。代表性的脂质包括但不限于胆固醇和维生素E,以及脂肪酸,诸如棕榈酸酯和生育酚。

[0048] 在一些实施方案中,代表性siRNA具有下式:

[0049] 有义:mN*mN*/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/*mN*/32FN/

[0050] 反义:/52FN/*/i2FN/*mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN*N*N

[0051] 其中:"N"是碱基;"2F"是2'-F修饰;"m"是2'-O-甲基修饰,"I"是内部碱基;并且"*"是硫代磷酸酯骨架键联。

[0052] 本公开还提供了包含抑制性核酸分子中的任一种或多种的载体。在一些实施方案中,载体包含抑制性核酸分子中的任一种或多种和异源核酸。所述载体可为能够转运核酸分子的病毒或非病毒载体。在一些实施方案中,载体是质粒或粘粒(诸如例如可将额外的DNA区段连接到其中的环状双链DNA)。在一些实施方案中,载体是病毒载体,其中额外的DNA区段可连接到病毒基因组中。表达载体包括但不限于质粒、粘粒、逆转录病毒、腺病毒、腺相

关病毒(AAV)、植物病毒(诸如花椰菜花叶病毒和烟草花叶病毒)、酵母人工染色体(YAC)、Epstein-Barr(EBV)衍生的附加体以及本领域已知的其他表达载体。

[0053] 本公开还提供了包含抑制性核酸分子中的任一种或多种的组合物。在一些实施方案中,所述组合物是药物组合物。在一些实施方案中,组合物包含载剂和/或赋形剂。载剂的实例包括但不限于聚(乳酸)(PLA)微球、聚(D,L-乳酸-共乙醇酸)(PLGA)微球、脂质体、胶束、反胶束、脂质螺旋物和脂质微管。载剂可包括缓冲盐溶液,诸如PBS、HBSS等。

[0054] 在一些实施方案中,所述ST6GALNAC5抑制剂包含在(多个)识别序列处诱导一个或多个切口或双链断裂的核酸酶剂或与ST6GALNAC5基因组核酸分子内的识别序列结合的DNA结合蛋白。所述识别序列可位于所述ST6GALNAC5基因的编码区内或影响所述基因的表达的调控区内。所述DNA结合蛋白或核酸酶剂的识别序列可位于内含子、外显子、启动子、增强子、调控区或任何非蛋白质编码区中。识别序列可包含或接近ST6GALNAC5基因的起始密码子。例如,所述识别序列可位于距起始密码子约10、约20、约30、约40、约50、约100、约200、约300、约400、约500或约1,000个核苷酸处。作为另一实例,可使用两种或更多种核酸酶剂,每种核酸酶剂均靶向包括或接近起始密码子的核酸酶识别序列。作为另一实例,可使用两种核酸酶剂,一种靶向包括或接近起始密码子的核酸酶识别序列,且一种靶向包括或接近终止密码子的核酸酶识别序列,其中核酸酶剂的切割可导致两个核酸酶识别序列之间的编码区的缺失。将切口或双链断裂诱导到所需识别序列中的任何核酸酶剂均可用于本文公开的方法和组合物中。结合所需识别序列的任何DNA结合蛋白均可用于本文公开的方法和组合物中。

[0055] 用于本文的合适的核酸酶剂和DNA结合蛋白包括但不限于锌指蛋白或锌指核酸酶(ZFN)对、转录激活因子样效应物(TALE)蛋白或转录激活因子样效应物核酸酶(TALEN),或成簇规则散布的短回文重复序列(CRISPR)/CRISPR相关(Cas)系统。识别序列的长度可变化,并且包括例如对于锌指蛋白或ZFN对为约30-36bp、对于每个ZFN为约15-18bp、对于TALE蛋白或TALEN为约36bp和对于CRISPR/Cas指导RNA为约20bp的识别序列。

[0056] 在一些实施方案中,CRISPR/Cas系统可用于修饰细胞内的ST6GALNAC5基因组核酸分子。本文公开的方法和组合物可通过利用CRISPR复合物(包含与Cas蛋白复合的指导RNA(gRNA))而使用CRISPR-Cas系统,用于ST6GALNAC5核酸分子的位点定向切割。

[0057] Cas蛋白通常包含至少一个可与gRNA相互作用的RNA识别或结合结构域。Cas蛋白还可包含核酸酶结构域(诸如例如DNA酶或RNA酶结构域)、DNA结合结构域、解旋酶结构域、蛋白质-蛋白质相互作用结构域、二聚化结构域和其他结构域。合适的Cas蛋白包括例如野生型Cas9蛋白和野生型Cpf1蛋白(诸如例如FnCpf1)。Cas蛋白可具有完全切割活性以在ST6GALNAC5基因组核酸分子中产生双链断裂,或者其可以是在ST6GALNAC5基因组核酸分子中产生单链断裂的切口酶。Cas蛋白的另外的实例包括但不限于Cas1、Cas1B、Cas2、Cas3、Cas4、Cas5、Cas5e(CasD)、Cas6、Cas6e、Cas6f、Cas7、Cas8a1、Cas8a2、Cas8b、Cas8c、Cas9(Csn1或Csx12)、Cas10、Cas10d、CasF、CasG、CasH、Csy1、Csy2、Csy3、Cse1(CasA)、Cse2(CasB)、Cse3(CasE)、Cse4(CasC)、Csc1、Csc2、Csa5、Csn2、Csm2、Csm3、Csm4、Csm5、Csm6、Cmr1、Cmr3、Cmr4、Cmr5、Cmr6、Csb1、Csb2、Csb3、Csx17、Csx14、Csx10、Csx16、CsaX、Csx3、Csx1、Csx15、Csf1、Csf2、Csf3、Csf4和Cu1966以及其同源物或修饰型式。Cas蛋白也可作为融合蛋白可操作地连接至异源多肽。例如,Cas蛋白可与切割结构域、表观遗传修饰结构域、

转录激活结构域或转录抑制结构域融合。Cas蛋白可以任何形式提供。例如,Cas蛋白可以蛋白,诸如与gRNA复合的Cas蛋白的形式提供。替代地,Cas蛋白可以编码Cas蛋白的核酸分子,诸如RNA或DNA的形式提供。

[0058] 在一些实施方案中,ST6GALNAC5基因组核酸分子的靶向遗传修饰可通过使细胞与Cas蛋白和一种或多种gRNA接触来产生,所述一种或多种gRNA与所述ST6GALNAC5基因组核酸分子中的靶基因组基因座内的一个或多个gRNA识别序列杂交。例如,gRNA识别序列可位于SEQ ID NO:1的区域内。所述gRNA识别序列还可包括或接近对应于根据SEQ ID NO:1的位置176,867的位置。例如,gRNA识别序列可位于距对应于根据SEQ ID NO:1的位置176,867的位置约1000、约500、约400、约300、约200、约100、约50、约45、约40、约35、约30、约25、约20、约15、约10或约5个核苷酸处。所述gRNA识别序列可包括或接近ST6GALNAC5基因组核酸分子的起始密码子或ST6GALNAC5基因组核酸分子的终止密码子。例如,gRNA识别序列可位于距起始密码子或终止密码子的约10、约20、约30、约40、约50、约100、约200、约300、约400、约500、或约1,000个核苷酸处。

[0059] ST6GALNAC5基因组核酸分子中的靶基因组基因座内的gRNA识别序列位于原间隔序列邻近基序(PAM)序列附近,PAM序列是紧随Cas9核酸酶靶向的DNA序列之后的2-6个碱基对的DNA序列。经典PAM是序列5'-NGG-3',其中"N"是后接两个鸟嘌呤("G")核碱基的任何核碱基。gRNA可将Cas9转运至基因组中的任何位置用于基因编辑,但在除了Cas9识别PAM的位点之外的任何位点都不会发生编辑。另外,5'-NGA-3'可作为人体细胞的高效非经典PAM。一般地,PAM在gRNA靶向的DNA序列下游约2至约6个核苷酸处。PAM可侧接gRNA识别序列。在一些实施方案中,gRNA识别序列可在3'端被PAM侧接。在一些实施方案中,gRNA识别序列可在5'端被PAM侧接。例如,Cas蛋白的切割位点可在PAM序列上游或下游约1个至约10个碱基对、约2个至约5个碱基对,或3个碱基对处。在一些实施方案中(诸如当使用来自酿脓链球菌(S.pyogenes)的Cas9或密切相关的Cas9时),非互补链的PAM序列可为5'-NGG-3',其中N是任何DNA核苷酸并且紧邻靶DNA的非互补链的gRNA识别序列的3'。因而,互补链的PAM序列将是5'-CCN-3',其中N是任何DNA核苷酸并且紧邻靶DNA的互补链的gRNA识别序列的5'。

[0060] gRNA是与Cas蛋白结合并将Cas蛋白靶向ST6GALNAC5基因组核酸分子内的特定位置的RNA分子。示例性gRNA是有效引导Cas酶结合或切割ST6GALNAC5基因组核酸分子的gRNA,其中所述gRNA包含DNA靶向区段,其与ST6GALNAC5基因组核酸分子内的gRNA识别序列杂交,所述gRNA识别序列包括或接近对应于根据SEQ ID NO:1的位置176,867的位置。例如,可选择gRNA以使其与位于距对应于根据SEQ ID NO:1的位置176,867的位置约5、约10、约15、约20、约25、约30、约35、约40、约45、约50、约100、约200、约300、约400、约500或约1,000个核苷酸处的gRNA识别序列杂交。其他示例性gRNA包含DNA靶向区段,其与ST6GALNAC5基因组核酸分子内存在的gRNA识别序列杂交,所述gRNA识别序列包括或接近起始密码子或终止密码子。例如,可选择gRNA以使其与位于距起始密码子约5、约10、约15、约20、约25、约30、约35、约40、约45、约50、约100、约200、约300、约400、约500或约1,000个核苷酸处或位于距终止密码子约5、约10、约15、约20、约25、约30、约35、约40、约45、约50、约100、约200、约300、约400、约500或约1,000个核苷酸处的gRNA识别序列杂交。合适的gRNA可包含约17至约25个核苷酸、约17至约23个核苷酸、约18至约22个核苷酸或约19至约21个核苷酸。在一些实施方案中,gRNA可包含20个核苷酸。

[0061] 位于ST6GALNAC5参考基因内的合适gRNA识别序列的实例作为SEQ ID NO:43-62列示于表1中。

[0062] 表1:ST6GALNAC5内的指导RNA识别序列

[0063]

链	gRNA 识别序列	SEQ ID NO:
-	CTTGTGGCGAGTAATCATGA	43
+	ATGAATGACGCCCCCACACG	44
+	GGACGGATACCTCGGAGTGG	45
+	CGCGGCTATGGGCGTGACGT	46
-	GTGTGGGGGCGTCATTCATG	47
-	GCTGTACACTAGCAACAAGC	48
+	GGGGCCCCAGCAGCTACATG	49
-	CCTTGTGGTCCGCCACTCCG	50
+	CCTCGGAGTGGCGGACCACA	51
-	ACTCCGAGGTATCCGTCCAG	52
+	TGGGCAATCGCACCAGCCTG	53
+	AGCAGCTACATGCGGCGGGA	54
-	AGGTATCCGTCCAGTGGCCG	55
-	GTCACGCCCATAGCCGCGTG	56
-	CACGCCCATAGCCGCGTGTG	57
+	TCGCGCATTCCAGCATCCAG	58
-	ACGCCCATAGCCGCGTGTGG	59

[0064]

-	GCGGGCATGCTGTCACCTTG	60
+	CATCTGCTGCACAGTCGGCA	61
+	CAGCAGCAGGCGTCGGCCAC	62

[0065] Cas蛋白和gRNA形成复合物,并且Cas蛋白切割靶ST6GALNAC5基因组核酸分子。Cas蛋白可在gRNA的DNA靶向区段将与之结合的靶ST6GALNAC5基因组核酸分子中存在的核酸序列内或外的位点处切割核酸分子。例如,CRISPR复合物(包含与gRNA识别序列杂交并与Cas蛋白复合的gRNA)的形成可导致在gRNA的DNA靶向区段将结合的ST6GALNAC5基因组核酸分子中存在的核酸序列中或附近(诸如例如来自所述核酸序列的1个、2个、3个、4个、5个、6个、7个、8个、9个、10个、20个、50个或更多个碱基对内)的一条或两条链的切割。

[0066] 此类方法可产生,例如,其中SEQ ID NO:1的区域被破坏、起始密码子被破坏、终止密码子被破坏或编码序列被破坏或缺失的ST6GALNAC5基因组核酸分子。任选地,细胞可进一步与一种或多种另外的gRNA接触,所述另外的gRNA与ST6GALNAC5基因组核酸分子中靶基因组基因座内另外的gRNA识别序列杂交。通过使所述细胞与一种或多种另外的gRNA(诸如例如,与第二gRNA识别序列杂交的第二gRNA)接触,由Cas蛋白切割可产生两个或更多个双链断裂或者两个或更多个单链断裂。

[0067] 在一些实施方案中,ST6GALNAC5抑制剂包含小分子。在一些实施方案中,ST6GALNAC5抑制剂是杨梅素。

[0068] 在一些实施方案中,治疗的方法还包括检测从受试者获得的生物样品中存在或不存在编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子。如本公开通篇所用,“ST6GALNAC5错义变体核酸分子”是编码具有部分功能丧失、完全功能丧失、预测的部分功能丧失或预测的完全功能丧失的ST6GALNAC5多肽的任何ST6GALNAC5核酸分子(诸如例

如,基因组核酸分子、mRNA分子或cDNA分子)。

[0069] 本公开还提供了用治疗或抑制认知障碍的治疗剂治疗受试者的方法。在一些实施方案中,受试者患有认知障碍。在一些实施方案中,所述方法包括通过以下方式确定受试者是否具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子:从受试者获得或已经获得生物样品,并且对生物样品进行或已经进行序列分析以确定受试者是否具有包含ST6GALNAC5错义变体核酸分子的基因型。当受试者是ST6GALNAC5参考时,以标准剂量量向受试者施用或继续施用治疗或抑制认知障碍的治疗剂,和/或向受试者施用ST6GALNAC5抑制剂。当受试者对于ST6GALNAC5错义变体核酸分子是杂合的时,以同于或低于标准剂量量的量向受试者施用或继续施用治疗或抑制认知障碍的治疗剂,和/或向受试者施用ST6GALNAC5抑制剂。具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的基因型的存在指示受试者具有降低的发展出认知障碍的风险。在一些实施方案中,受试者是ST6GALNAC5参考。在一些实施方案中,受试者对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的。

[0070] 对于基因分型或确定为ST6GALNAC5参考或对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的受试者,可用ST6GALNAC5抑制剂治疗此类受试者,如本文所述。

[0071] 检测来自受试者的生物样品中存在或不存在编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子和/或确定受试者是否具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子可通过本文所述的任何方法进行。在一些实施方案中,这些方法可在体外进行。在一些实施方案中,这些方法可原位进行。在一些实施方案中,这些方法可在体内进行。在这些实施方案中的任一项中,编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子可存在于从受试者获得的细胞内。

[0072] 在一些实施方案中,当受试者是ST6GALNAC5参考时,还以标准剂量量向受试者施用治疗或抑制认知障碍的治疗剂。在一些实施方案中,当受试者对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的时,还以同于或低于标准剂量量的剂量量向受试者施用治疗或抑制认知障碍的治疗剂。

[0073] 在一些实施方案中,治疗方法还包括检测来自受试者的生物样品中ST6GALNAC5预测的功能丧失多肽的存在或不存在。在一些实施方案中,当受试者不具有ST6GALNAC5预测的功能丧失多肽时,还以标准剂量量向受试者施用治疗或抑制认知障碍的治疗剂。在一些实施方案中,当受试者具有ST6GALNAC5预测的功能丧失多肽时,还以同于或低于标准剂量量的剂量量向受试者施用治疗或抑制认知障碍的治疗剂。

[0074] 本公开还提供了用治疗或预防认知障碍的治疗剂治疗受试者的方法。在一些实施方案中,受试者患有认知障碍。在一些实施方案中,受试者处于发展出认知障碍的风险中。在一些实施方案中,方法包括通过以下方式确定受试者是否具有ST6GALNAC5预测的功能丧失多肽:从受试者获得或已经获得生物样品,并且对生物样品进行或已经进行测定以确定受试者是否具有ST6GALNAC5预测的功能丧失多肽。当受试者不具有ST6GALNAC5预测的功能丧失多肽时,以标准剂量量向受试者施用或继续施用治疗或抑制认知障碍的治疗剂,和/或向受试者施用ST6GALNAC5抑制剂。当受试者具有ST6GALNAC5预测的功能丧失多肽时,以同于或低于标准剂量量的量向受试者施用或继续施用治疗或抑制认知障碍的治疗剂,和/或

向受试者施用ST6GALNAC5抑制剂。ST6GALNAC5预测的功能丧失多肽的存在指示受试者具有降低的发展出认知障碍的风险。在一些实施方案中,受试者具有ST6GALNAC5预测的功能丧失多肽。在一些实施方案中,受试者不具有ST6GALNAC5预测的功能丧失多肽。

[0075] 检测来自受试者的生物样品中存在或不存在ST6GALNAC5预测的功能丧失多肽和/或确定受试者是否具有ST6GALNAC5预测的功能丧失多肽可通过本文所述的任何方法进行。在一些实施方案中,这些方法可在体外进行。在一些实施方案中,这些方法可原位进行。在一些实施方案中,这些方法可在体内进行。在这些实施方案中的任一项中,ST6GALNAC5预测的功能丧失多肽可存在于从受试者获得的细胞内。

[0076] 治疗或预防认知障碍的治疗剂的实例包括但不限于:胆碱酯酶抑制剂、维生素E和银杏。在一些实施方案中,治疗或抑制认知障碍的治疗剂是胆碱酯酶抑制剂。在一些实施方案中,治疗或抑制认知障碍的治疗剂是维生素E。在一些实施方案中,治疗或抑制认知障碍的治疗剂是银杏。在一些实施方案中,胆碱酯酶抑制剂是ARICEPT®或ARICEPT® ODT(多奈哌齐(donepezil))、COGNEX®(他克林(tacrine))、EXELON®或EXELON® Patch(利斯的明(rivastigmine))、RAZADYNE®(加兰他敏(galantamine))、NAMZARIC®(美金刚(memantine)/多奈哌齐)、MYTELASE®(安贝氯铵(ambenonium))或BLOXIVERZ®(新斯的明(neostigmine))。在一些实施方案中,胆碱酯酶抑制剂是多奈哌齐、他克林、利斯的明、加兰他敏、美金刚/多奈哌齐、安贝氯铵或新斯的明。在一些实施方案中,胆碱酯酶抑制剂是多奈哌齐。在一些实施方案中,胆碱酯酶抑制剂是他克林。在一些实施方案中,胆碱酯酶抑制剂是利斯的明。在一些实施方案中,胆碱酯酶抑制剂是加兰他敏。在一些实施方案中,胆碱酯酶抑制剂是美金刚/多奈哌齐。在一些实施方案中,胆碱酯酶抑制剂是安贝氯铵。在一些实施方案中,胆碱酯酶抑制剂是新斯的明。

[0077] 在一些实施方案中,与作为ST6GALNAC5参考的受试者(其可接受标准剂量量)相比,对于对编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的受试者,治疗或预防认知障碍的治疗剂的剂量可减少约10%、约20%、约30%、约40%、约50%、约60%、约70%、约80%或约90%(即,低于标准剂量量)。在一些实施方案中,治疗或预防认知障碍的治疗剂的剂量可减少约10%、约20%、约30%、约40%或约50%。另外,与作为ST6GALNAC5参考的受试者相比,在对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的受试者中,治疗或预防认知障碍的治疗剂的剂量可以较低频率施用。

[0078] 治疗或预防认知障碍的治疗剂和/或ST6GALNAC5抑制剂的施用可例如在一天、两天、三天、五天、一周、两周、三周、一个月、五周、六周、七周、八周、两个月或三个月后重复。重复施用可按相同的剂量或按不同的剂量。施用可重复一次、两次、三次、四次、五次、六次、七次、八次、九次、十次或更多次。例如,根据某些剂量方案,受试者可接受较长时间段的治疗诸如例如6个月、1年或更长时间。另外,治疗或预防认知障碍的治疗剂和/或ST6GALNAC5抑制剂可依序或同时施用。另外,治疗或预防认知障碍的治疗剂和/或ST6GALNAC5抑制剂可在单独的组合物中施用或者可在同一组合物中一起施用。

[0079] 治疗或预防认知障碍的治疗剂和/或ST6GALNAC5抑制剂的施用可通过任何合适的途径进行,包括但不限于肠胃外、静脉内、口服、皮下、动脉内、颅内、鞘内、腹膜内、局部、鼻

内或肌内。用于施用的药物组合物期望是无菌的且基本上等渗的,并且在GMP条件下制造。药物组合物可以单位剂型(即单次施用的剂量)提供。药物组合物可使用一种或多种生理上和药学上可接受的载剂、稀释剂、赋形剂或助剂来配制。制剂取决于所选的施用途径。术语"药学上可接受的"意指载剂、稀释剂、赋形剂或助剂与制剂的其他成分相容,且对其接受者基本上无害。

[0080] 如本文所用,术语"治疗(treat)"、"治疗(treating)"和"治疗(treatment)"以及"预防(prevent)"、"预防(preventing)"和"预防(prevention)"分别是指引发期望的生物反应,诸如治疗效果和预防效果。在一些实施方案中,在施用所述剂或包含所述剂的组合物后,治疗效果包括以下中的一者或多者:认知障碍的降低/减轻、认知障碍的严重程度的降低/减轻(诸如例如减轻或抑制认知障碍的发展)、症状和认知障碍相关效应的降低/减轻、延迟症状和认知障碍相关效应的发作、减轻认知障碍相关效应的症状的严重程度、减轻急性发作的严重程度、减少症状和认知障碍相关效应的数目、减少症状和认知障碍相关效应的潜伏期、症状和认知障碍相关效应的改善、减少继发性症状、减少继发性感染、预防认知障碍复发、降低复发发作的次数或频率、增加症状发作间隔的潜伏期、增加持续进展的时间、加快缓解、诱导缓解、加强缓解、加速恢复,或增加替代治疗剂的功效或降低对替代治疗剂的抗性,和/或增加受影响宿主动物的存活时间。预防效果可包括在治疗方案的施用后完全或部分避免/抑制或延迟认知障碍发展/进展(诸如例如完全或部分避免/抑制或延迟),以及增加受影响宿主动物的存活时间。认知障碍的治疗涵盖治疗已经被诊断为患有处于任何临床阶段或表现的任何形式的认知障碍的受试者,延迟认知障碍的症状或体征的发作或演变或加重或恶化,和/或预防和/或减轻认知障碍的严重程度。

[0081] 本公开还提供了鉴定具有增加的发展出认知障碍的风险的受试者的方法。在一些实施方案中,所述方法包括确定或已经确定在从受试者获得的生物样品中存在或不存在编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子(诸如基因组核酸分子、mRNA分子和/或cDNA分子)。当受试者缺乏编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子(即,受试者在基因型上被归类为ST6GALNAC5参考)时,则受试者具有增加的发展出认知障碍的风险。当受试者具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子(即,受试者对于ST6GALNAC5错义变体核酸分子是杂合或纯合的)时,则与作为ST6GALNAC5参考的受试者相比,所述受试者具有降低的发展出认知障碍的风险。

[0082] 与不具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的拷贝相比,具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的单个拷贝更能保护受试者免于发展出认知障碍。无意受限于任何特定理论或作用机制,据信编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的单个拷贝(即,对于ST6GALNAC5错义变体核酸分子是杂合的)能够保护受试者免于发展出认知障碍,并且还据信相对于具有单个拷贝的受试者,具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的两个拷贝(即,对于ST6GALNAC5错义变体核酸分子是纯合的)可能更能保护受试者免于发展出认知障碍。因此,在一些实施方案中,编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的单个拷贝可能不具完全保护性,而是可能部分或不完全地保护受试者免于发展出认知障碍。虽然不希望受任何特定理论约

束,但可能存在涉及发展出认知障碍的其他因子或分子,其仍存在于具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子的单个拷贝的受试者中,因此导致对发展出认知障碍的保护不太完全。

[0083] 检测来自受试者的生物样品中存在或不存在编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子和/或确定受试者是否具有编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子可通过本文所述的任何方法进行。在一些实施方案中,这些方法可在体外进行。在一些实施方案中,这些方法可原位进行。在一些实施方案中,这些方法可在体内进行。在这些实施方案中的任一项中,编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子可存在于从受试者获得的细胞内。

[0084] 在一些实施方案中,当受试者被鉴定为具有增加的发展出认知障碍的风险时,受试者进一步如本文所述用治疗或抑制认知障碍的治疗剂和/或ST6GALNAC5抑制剂治疗。例如,当受试者是ST6GALNAC5参考,并因此具有增加的发展出认知障碍的风险时,向受试者施用ST6GALNAC5抑制剂。在一些实施方案中,向此类受试者施用治疗或抑制认知障碍的治疗剂。在一些实施方案中,当受试者对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的时,以同于或低于标准剂量量的剂量量向受试者施用治疗或抑制认知障碍的治疗剂,和/或施用ST6GALNAC5抑制剂。在一些实施方案中,受试者是ST6GALNAC5参考。在一些实施方案中,受试者对于编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子是杂合的。

[0085] 本公开还提供了检测从受试者获得的生物样品中存在或不存在编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体基因组核酸分子,和/或从受试者获得的生物样品中存在或不存在编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体mRNA分子,和/或从受试者获得的生物样品中存在或不存在由mRNA分子产生的编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体cDNA分子的方法。应理解,群体内的基因序列和由此类基因编码的mRNA分子可因多态性诸如单核苷酸多态性而变化。本文提供的ST6GALNAC5变体基因组核酸分子、ST6GALNAC5变体mRNA分子和ST6GALNAC5变体cDNA分子的序列仅为示例性序列。ST6GALNAC5变体基因组核酸分子、变体mRNA分子和变体cDNA分子的其他序列也是可能的。

[0086] 生物样品可来源于来自受试者的任何细胞、组织或生物流体。生物样品可包括任何临床相关组织,诸如例如骨髓样品、肿瘤活检、细针抽吸物或体液样品,诸如血液、龈沟液、血浆、血清、淋巴、腹水、囊液或尿液。在一些实施方案中,生物样品包括口腔拭子。本文所公开的方法中使用的生物样品可基于测定形式、检测方法的性质以及用作样品的组织、细胞或提取物而变化。生物样品可根据所采用的测定进行不同处理。例如,当检测任何ST6GALNAC5变体核酸分子时,可采用被设计用于分离ST6GALNAC5变体核酸分子或使生物样品富集所述分子的初步处理。多种技术可用于此目的。当检测任何ST6GALNAC5变体mRNA分子的水平时,可使用不同的技术富集生物样品的mRNA分子。可使用各种方法来检测mRNA分子的存在或水平或特定变体基因组DNA基因座的存在。

[0087] 本公开还提供了在受试者中检测编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子或其互补序列的方法。所述方法包括测定从受试者获得的生物样品以确定生物样品中的核酸分子是否是编码ST6GALNAC5预测的功能丧失多肽的

ST6GALNAC5错义变体核酸分子。

[0088] 在一些实施方案中,编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子或其互补序列是具有包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶的核苷酸序列的基因组核酸分子。

[0089] 在一些实施方案中,编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子或其互补序列是具有包含以下的核苷酸序列的mRNA分子:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶。

[0090] 在一些实施方案中,编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子或其互补序列是由生物样品中的mRNA分子产生的cDNA分子,其具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

[0091] 在一些实施方案中,ST6GALNAC5错义变体核酸分子具有包含以下的核苷酸序列:i)在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶(对于基因组核酸分子);ii)在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶(对于mRNA分子);或iii)在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶(对于从mRNA分子获得的cDNA分子)。

[0092] 在一些实施方案中,生物样品包含细胞或细胞裂解物。此类方法还可包括,例如,从受试者获得包含ST6GALNAC5基因组核酸分子或mRNA分子的生物样品,并且如果是mRNA,则任选地将mRNA逆转录成cDNA。此类测定可包括,例如确定特定ST6GALNAC5核酸分子的这

些位置的身份。在一些实施方案中,方法是体外方法。

[0093] 在一些实施方案中,确定步骤、检测步骤或序列分析包括对生物样品中的ST6GALNAC5基因组核酸分子、ST6GALNAC5 mRNA分子或由mRNA分子产生的ST6GALNAC5 cDNA分子的核苷酸序列的至少一部分测序,其中测序的部分包含导致功能丧失(部分或完全)或预测会导致功能丧失(部分或完全)的一种或多种变异。

[0094] 在一些实施方案中,确定步骤、检测步骤或序列分析包括对以下各项的至少一部分进行测序:i)生物样品中的ST6GALNAC5基因组核酸分子的核苷酸序列,其中测序的部分包含对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置;ii)生物样品中的ST6GALNAC5 mRNA分子的核苷酸序列,其中测序的部分包含对应于以下的位置:根据SEQ ID NO:11或其互补序列的位置600;根据SEQ ID NO:12或其互补序列的位置639;根据SEQ ID NO:13或其互补序列的位置562;根据SEQ ID NO:14或其互补序列的位置515;根据SEQ ID NO:15或其互补序列的位置579;根据SEQ ID NO:16或其互补序列的位置416;根据SEQ ID NO:17或其互补序列的位置639;或根据SEQ ID NO:18或其互补序列的位置600;和/或iii)由生物样品中的mRNA产生的ST6GALNAC5 cDNA分子的核苷酸序列,其中测序的部分包含对应于以下的位置:根据SEQ ID NO:27或其互补序列的位置600;根据SEQ ID NO:28或其互补序列的位置639;根据SEQ ID NO:29或其互补序列的位置562;根据SEQ ID NO:30或其互补序列的位置515;根据SEQ ID NO:31或其互补序列的位置579;根据SEQ ID NO:32或其互补序列的位置416;根据SEQ ID NO:33或其互补序列的位置639;或根据SEQ ID NO:34或其互补序列的位置600。当生物样品中ST6GALNAC5核酸分子的测序的部分包含:在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶、在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶时,则生物样品中的ST6GALNAC5核酸分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体核酸分子。

[0095] 在一些实施方案中,确定步骤、检测步骤或序列分析包括对生物样品中ST6GALNAC5基因组核酸分子的核苷酸序列的至少一部分进行测序,其中测序的部分包含对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置。当生物样品中ST6GALNAC5核酸分子的测序的部分包含在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶时,则生物样品中的ST6GALNAC5核酸分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体基因组核酸分子。

[0096] 在一些实施方案中,确定步骤、检测步骤或序列分析包括对生物样品中

ST6GALNAC5 mRNA分子的核苷酸序列的至少一部分进行测序,其中测序的部分包含对应于以下的位置:根据SEQ ID NO:11或其互补序列的位置600;根据SEQ ID NO:12或其互补序列的位置639;根据SEQ ID NO:13或其互补序列的位置562;根据SEQ ID NO:14或其互补序列的位置515;根据SEQ ID NO:15或其互补序列的位置579;根据SEQ ID NO:16或其互补序列的位置416;根据SEQ ID NO:17或其互补序列的位置639;或根据SEQ ID NO:18或其互补序列的位置600。当生物样品中ST6GALNAC5 mRNA分子的测序的部分包含:在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶时,则生物样品中的ST6GALNAC5核酸分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体mRNA分子。

[0097] 在一些实施方案中,确定步骤、检测步骤或序列分析包括对由生物样品中的mRNA分子产生的ST6GALNAC5 cDNA分子的核苷酸序列的至少一部分进行测序,其中测序的部分包含对应于以下的位置:根据SEQ ID NO:27或其互补序列的位置600;根据SEQ ID NO:28或其互补序列的位置639;根据SEQ ID NO:29或其互补序列的位置562;根据SEQ ID NO:30或其互补序列的位置515;根据SEQ ID NO:31或其互补序列的位置579;根据SEQ ID NO:32或其互补序列的位置416;根据SEQ ID NO:33或其互补序列的位置639;或根据SEQ ID NO:34或其互补序列的位置600。当生物样品中ST6GALNAC5 cDNA分子的测序的部分包含:在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶时,则生物样品中的ST6GALNAC5核酸分子是编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体cDNA分子。

[0098] 在一些实施方案中,确定步骤、检测步骤或序列分析包括:a)使生物样品与引物接触,所述引物与以下的核苷酸序列的一部分杂交:i)ST6GALNAC5基因组核酸分子或其互补序列,所述部分接近对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置;ii)ST6GALNAC5 mRNA分子或其互补序列,所述部分接近对应于以下的位置:根据SEQ ID NO:11或其互补序列的位置600;根据SEQ ID NO:12或其互补序列的位置639;根据SEQ ID NO:13或其互补序列的位置562;根据SEQ ID NO:14或其互补序列的位置515;根据SEQ ID NO:15或其互补序列的位置579;根据SEQ ID NO:16或其互补序列的位置416;根据SEQ ID NO:17或其互补序列的位置639;或根据SEQ ID NO:18或其互补序列的位置600;和/或iii)ST6GALNAC5

[0099] cDNA分子或其互补序列,所述部分接近对应于以下的位置:根据SEQ ID NO:27或其互补序列的位置600;根据SEQ ID NO:28或其互补序列的位置639;根据SEQ ID NO:29或其互补序列的位置562;根据SEQ ID NO:30或其互补序列的位置515;根据SEQ ID NO:31或

其互补序列的位置579;根据SEQ ID NO:32或其互补序列的位置416;根据SEQ ID NO:33或其互补序列的位置639;或根据SEQ ID NO:34或其互补序列的位置600;b)将所述引物延伸至少通过以下的核苷酸序列的位置:i)ST6GALNAC5基因组核酸分子或其互补序列,对应于根据SEQ ID NO:2或其互补序列的位置176,867;ii)ST6GALNAC5mRNA分子或其互补序列,对应于:根据SEQ ID NO:11或其互补序列的位置600;根据SEQ ID NO:12或其互补序列的位置639;根据SEQ ID NO:13或其互补序列的位置562;根据SEQ ID NO:14或其互补序列的位置515;根据SEQ ID NO:15或其互补序列的位置579;根据SEQ ID NO:16或其互补序列的位置416;根据SEQ ID NO:17或其互补序列的位置639;或根据SEQ ID NO:18或其互补序列的位置600;和/或iii)ST6GALNAC5 cDNA分子或其互补序列,对应于:根据SEQ ID NO:27或其互补序列的位置600;根据SEQ ID NO:28或其互补序列的位置639;根据SEQ ID NO:29或其互补序列的位置562;根据SEQ ID NO:30或其互补序列的位置515;根据SEQ ID NO:31或其互补序列的位置579;根据SEQ ID NO:32或其互补序列的位置416;根据SEQ ID NO:33或其互补序列的位置639;或根据SEQ ID NO:34或其互补序列的位置600;以及c)确定所述引物的所述延伸产物是否包含:在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

[0100] 在一些实施方案中,确定步骤、检测步骤或序列分析包括:a)使生物样品与引物接触,所述引物与ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列的一部分杂交,所述部分接近对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置;b)将所述引物延伸至少通过ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列的对应于以下的位置:根据SEQ ID NO:2或其互补序列的位置176,867;以及c)确定所述引物的所述延伸产物是否包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶。

[0101] 在一些实施方案中,确定步骤、检测步骤或序列分析包括:a)使生物样品与引物接触,所述引物与ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列的一部分杂交,所述部分接近对应于以下的位置:根据SEQ ID NO:11或其互补序列的位置600;根据SEQ ID NO:12或其互补序列的位置639;根据SEQ ID NO:13或其互补序列的位置562;根据SEQ ID NO:14或其互补序列的位置515;根据SEQ ID NO:15或其互补序列的位置579;根据SEQ ID NO:16或

其互补序列的位置416;根据SEQ ID NO:17或其互补序列的位置639;或根据SEQ ID NO:18或其互补序列的位置600;b)将所述引物延伸至少通过ST6GALNAC5 mRNA分子的核苷酸序列的对应于以下的位置:根据SEQ ID NO:11或其互补序列的位置600;根据SEQ ID NO:12或其互补序列的位置639;根据SEQ ID NO:13或其互补序列的位置562;根据SEQ ID NO:14或其互补序列的位置515;根据SEQ ID NO:15或其互补序列的位置579;根据SEQ ID NO:16或其互补序列的位置416;根据SEQ ID NO:17或其互补序列的位置639;或根据SEQ ID NO:18或其互补序列的位置600;以及c)确定所述引物的所述延伸产物是否包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶。

[0102] 在一些实施方案中,确定步骤、检测步骤或序列分析包括:a)使生物样品与引物接触,所述引物与ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列的一部分杂交,所述部分接近对应于以下的位置:根据SEQ ID NO:27或其互补序列的位置600;根据SEQ ID NO:28或其互补序列的位置639;根据SEQ ID NO:29或其互补序列的位置562;根据SEQ ID NO:30或其互补序列的位置515;根据SEQ ID NO:31或其互补序列的位置579;根据SEQ ID NO:32或其互补序列的位置416;根据SEQ ID NO:33或其互补序列的位置639;或根据SEQ ID NO:34或其互补序列的位置600;b)将所述引物延伸至少通过ST6GALNAC5 cDNA分子的核苷酸序列的对应于以下的位置:根据SEQ ID NO:27或其互补序列的位置600;根据SEQ ID NO:28或其互补序列的位置639;根据SEQ ID NO:29或其互补序列的位置562;根据SEQ ID NO:30或其互补序列的位置515;根据SEQ ID NO:31或其互补序列的位置579;根据SEQ ID NO:32或其互补序列的位置416;根据SEQ ID NO:33或其互补序列的位置639;或根据SEQ ID NO:34或其互补序列的位置600;以及c)确定所述引物的所述延伸产物是否包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

[0103] 在一些实施方案中,对整个核酸分子进行测序。在一些实施方案中,仅分析ST6GALNAC5基因组核酸分子。在一些实施方案中,仅分析ST6GALNAC5 mRNA。在一些实施方案中,仅分析从ST6GALNAC5 mRNA获得的ST6GALNAC5 cDNA。

[0104] 在一些实施方案中,确定步骤、检测步骤或序列分析包括:a)对生物样品中的ST6GALNAC5核酸分子或其互补序列的至少一部分进行扩增,其中扩增的部分包含:在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;在对应于根据SEQ ID

NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;b)用可检测标记来标记扩增的核酸分子;c)使标记的核酸分子与包含改变特异性探针的支持物接触,其中改变特异性探针包含在严格条件下与扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含:在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;以及d)检测可检测标记。

[0105] 在一些实施方案中,确定步骤、检测步骤或序列分析包括:a)对生物样品中的ST6GALNAC5基因组核酸分子或其互补序列的至少一部分进行扩增,其中所述部分包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;b)用可检测标记来标记扩增的核酸分子;c)使标记的核酸分子与包含改变特异性探针的支持物接触,其中改变特异性探针包含在严格条件下与扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;以及d)检测可检测标记。

[0106] 在一些实施方案中,确定步骤、检测步骤或序列分析包括:a)对生物样品中的ST6GALNAC5 mRNA分子或其互补序列的至少一部分进行扩增,其中所述部分包含:在对应于

根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;b)用可检测标记来标记扩增的核酸分子;c)使标记的核酸分子与包含改变特异性探针的支持物接触,其中改变特异性探针包含在严格条件下与扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;以及d)检测可检测标记。

[0107] 在一些实施方案中,确定步骤、检测步骤或序列分析包括:a)对生物样品中的ST6GALNAC5 cDNA分子或其互补序列的至少一部分进行扩增,其中所述部分包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;b)用可检测标记来标记扩增的核酸分子;c)使标记的核酸分子与包含改变特异性探针的支持物接触,其中改变特异性探针包含在严格条件下与扩增的核酸分子的核酸序列杂交的核苷酸序列,所述扩增的核酸分子的核酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;以及d)检测可检测标记。

[0108] 在一些实施方案中,核酸分子是mRNA,并且确定步骤还包括在扩增步骤之前将mRNA逆转录成cDNA。

[0109] 在一些实施方案中,确定步骤、检测步骤或序列分析包括:使生物样品中的ST6GALNAC5核酸分子或其互补序列与包含可检测标记的改变特异性探针接触,其中所述改变特异性探针包含在严格条件下与ST6GALNAC5核酸分子或其互补序列的核苷酸序列杂交

的核苷酸序列,所述ST6GALNAC5核酸分子或其互补序列的核苷酸序列包含:在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;以及检测可检测标记。

[0110] 在一些实施方案中,确定步骤、检测步骤或序列分析包括:使生物样品中的ST6GALNAC5基因组核酸分子或其互补序列与包含可检测标记的改变特异性探针接触,其中改变特异性探针包含在严格条件下与ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列杂交的核苷酸序列,所述ST6GALNAC5基因组核酸分子或其互补序列的核苷酸序列包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;以及检测可检测标记。

[0111] 在一些实施方案中,确定步骤、检测步骤或序列分析包括:使生物样品中的ST6GALNAC5 mRNA分子或其互补序列与包含可检测标记的改变特异性探针接触,其中改变特异性探针包含在严格条件下与ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列杂交的核苷酸序列,所述ST6GALNAC5 mRNA分子或其互补序列的核苷酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;以及检测可检测标记。

[0112] 在一些实施方案中,确定步骤、检测步骤或序列分析包括:使由生物样品中的mRNA分子产生的ST6GALNAC5 cDNA分子或其互补序列与包含可检测标记的改变特异性探针接触,其中改变特异性探针包含在严格条件下与ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列杂交的核苷酸序列,所述ST6GALNAC5 cDNA分子或其互补序列的核苷酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515

的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶;以及检测可检测标记。

[0113] 在一些实施方案中,ST6GALNAC5核酸分子存在于从受试者获得的细胞内。

[0114] 改变特异性聚合酶链反应技术可用于检测核酸序列中的突变,诸如SNP。可使用改变特异性引物是因为当与模板存在失配时,DNA聚合酶将不延伸。

[0115] 在一些实施方案中,确定步骤、检测步骤或序列分析包括使生物样品与引物或探针接触,所述引物或探针诸如改变特异性引物或改变特异性探针,其在严格条件下与ST6GALNAC5变体基因组序列、变体mRNA序列或变体cDNA序列而不是对应的ST6GALNAC5参考序列特异性杂交,以及确定是否发生了杂交。

[0116] 在一些实施方案中,测定包括RNA测序(RNA-Seq)。在一些实施方案中,测定还包括诸如通过逆转录酶聚合酶链反应(RT-PCR)将mRNA逆转录成cDNA。

[0117] 在一些实施方案中,所述方法利用核苷酸长度足以与靶核苷酸序列结合并特异性地检测和/或鉴定包含ST6GALNAC5变体基因组核酸分子、变体mRNA分子或变体cDNA分子的多核苷酸的探针和引物。杂交条件或反应条件可由操作人员确定以实现此结果。核苷酸长度可为足以用于所选择的检测方法(包括本文描述或例示的任何测定)的任何长度。此类探针和引物可在高严格杂交条件下与靶核苷酸序列特异性杂交。探针和引物可具有与靶核苷酸序列内连续核苷酸的完全核苷酸序列同一性,但可通过常规方法设计不同于靶核苷酸序列并保留特异性检测和/或鉴定靶核苷酸序列的能力的探针。探针和引物可与靶核酸分子的核苷酸序列具有约80%、约85%、约90%、约91%、约92%、约93%、约94%、约95%、约96%、约97%、约98%、约99%或100%序列同一性或互补性。

[0118] 在一些实施方案中,为了确定生物样品内的ST6GALNAC5核酸分子(基因组核酸分子、mRNA分子或cDNA分子)或其互补序列是否包含有包含以下的核苷酸序列:在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶、在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶,可使用引物对使生物样品经历扩增方法,所述引物对包括来源于与以下相邻的5'侧接序列的第一引物:在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶、在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ

ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶,以及来源于与以下相邻的3'侧接序列的第二引物:在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶、在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶,以产生指示在编码以下的位置处存在SNP的扩增子:在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶、在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶。在一些实施方案中,扩增子的长度范围可从引物对加上一个核苷酸碱基对的组合长度至可通过DNA扩增方案产生的扩增子的任何长度。此距离的范围可从一个核苷酸碱基对至扩增反应的极限,或约两万个核苷酸碱基对。任选地,引物对侧接包括以下位置的区域,所述位置包含在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶、在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根

据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶,以及在包含以下的位置的每一侧上的至少1、2、3、4、5、6、7、8、9、10个或更多个核苷酸:在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶、在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶、在对应于根据SEQ IDNO:27的位置600的位置处的胞嘧啶、在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶、在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶、在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶、在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶、在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶、在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶或在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶。

[0119] 可由mRNA和/或cDNA序列产生类似的扩增子。PCR引物对可例如通过使用意图用于此目的的计算机程序衍生自已知的序列,所述计算机程序诸如Vector NTI版本10的PCR引物分析工具(Informax Inc.,Bethesda Md.);PrimerSelect(DNASTAR Inc.,Madison,Wis.);以及Primer3(版本0.4.0.COPYRGT.,1991,Whitehead Institute for Biomedical Research,Cambridge,Mass.)。另外,可视觉扫描序列并使用已知指南手动鉴定引物。

[0120] 核酸测序技术的说明性实例包括但不限于链终止子(Sanger)测序和染料终止子测序。其他方法涉及除了测序以外的核酸杂交方法,其包括使用针对纯化的DNA、扩增的DNA和固定细胞制品的标记的引物或探针(荧光原位杂交(FISH))。在一些方法中,可在检测之前或在检测的同时对靶核酸分子进行扩增。核酸扩增技术的例示性实例包括但不限于聚合酶链反应(PCR)、连接酶链反应(LCR)、链置换扩增反应(SDA)以及基于核酸序列的扩增反应(NASBA)。其他方法包括但不限于连接酶链反应、链置换扩增反应和嗜热SDA(tSDA)。

[0121] 在杂交技术中,可采用严格条件,使得探针或引物特异性地与其靶标杂交。在一些实施方案中,在严格条件下的多核苷酸引物或探针将与其靶序列杂交,其程度可检测地比与其他非靶序列的杂交大诸如至少2倍、至少3倍、至少4倍或更多倍(相对于背景),包括大超过10倍(相对于背景)。在一些实施方案中,在严格条件下的多核苷酸引物或探针将与其靶核苷酸序列杂交,其程度可检测地比与其他核苷酸序列的杂交大至少2倍。在一些实施方案中,在严格条件下的多核苷酸引物或探针将与其靶核苷酸序列杂交,其程度可检测地比与其他核苷酸序列的杂交大至少3倍。在一些实施方案中,在严格条件下的多核苷酸引物或探针将与其靶核苷酸序列杂交,其程度可检测地比与其他核苷酸序列的杂交大至少4倍。在

一些实施方案中,在严格条件下的多核苷酸引物或探针将与其靶核苷酸序列杂交,其程度可检测地比与其他核苷酸序列的杂交大超过10倍(相对于背景)。严格条件是序列依赖性的且在不同的环境中将是不同的。

[0122] 促进DNA杂交的适当严格性条件(例如6X氯化钠/柠檬酸钠(SSC),在约45℃下,接着在50℃下进行2X SSC的洗涤)是已知的且可见于Current Protocols in Molecular Biology,John Wiley&Sons,N.Y.(1989),6.3.1-6.3.6。通常,用于杂交和检测的严格条件将是其中如下所述的那些:在pH 7.0至8.3时的盐浓度低于约1.5M Na^+离子、通常约0.01至1.0M Na^+离子浓度(或其他盐),并且温度对于短探针(诸如例如10至50个核苷酸)是至少约30℃,且对于较长探针(诸如例如大于50个核苷酸)是至少约60℃。还可通过添加去稳定剂诸如甲酰胺来实现严格条件。任选地,洗涤缓冲液可包含约0.1%至约1%SDS。杂交的持续时间通常少于约24小时,通常为约4至约12小时。洗涤时间的持续时间将是至少足以达到平衡的时间长度。

[0123] 本公开还提供了检测ST6GALNAC5预测的功能丧失多肽的存在的方法,其包括对从受试者获得的生物样品进行测定以确定生物样品中的ST6GALNAC5多肽是否含有致使多肽具有功能丧失(部分或完全)或预测的功能丧失(部分或完全)的一种或多种变异。ST6GALNAC5预测的功能丧失多肽可以是本文所述的ST6GALNAC5预测的功能丧失多肽中的任一种。在一些实施方案中,所述方法检测ST6GALNAC5 Val135Ala、Val45Ala、Val45Ala*或Val135Ala*的存在。在一些实施方案中,所述方法检测ST6GALNAC5Val135Ala的存在。在一些实施方案中,所述方法检测ST6GALNAC5Val45Ala的存在。在一些实施方案中,所述方法检测ST6GALNAC5Val45Ala*的存在。在一些实施方案中,所述方法检测ST6GALNAC5Val135Ala*的存在。

[0124] 在一些实施方案中,所述方法包括对从受试者获得的生物样品进行测定以确定生物样品中的ST6GALNAC5多肽是否包含在对应于根据SEQ ID NO:39的位置135的位置处的丙氨酸、在对应于根据SEQ ID NO:40的位置45的位置处的丙氨酸、在对应于根据SEQ ID NO:41的位置45的位置处的丙氨酸或在对应于根据SEQ ID NO:42的位置135的位置处的丙氨酸。

[0125] 在一些实施方案中,检测步骤包括对包含对应于以下的位置的ST6GALNAC5多肽的至少一部分进行测序:根据SEQ ID NO:39的位置135、根据SEQ ID NO:40的位置45、根据SEQ ID NO:41的位置45,或根据SEQ ID NO:42,或SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37或SEQ ID NO:38的位置135。

[0126] 在一些实施方案中,检测步骤包括用于检测包含对应于以下的位置的ST6GALNAC5多肽的存在的免疫测定:根据SEQ ID NO:39的位置135、根据SEQ ID NO:40的位置45、根据SEQ ID NO:41的位置45,或根据SEQ ID NO:42,或SEQ ID NO:35、SEQ ID NO:36、SEQ ID NO:37或SEQ ID NO:38的位置135。

[0127] 在一些实施方案中,当受试者不具有ST6GALNAC5预测的功能丧失多肽时,受试者具有增加的发展出认知障碍或髓鞘完整性降低、神经变性、GWC降低和轻度认知障碍转变为痴呆中的任一种的风险。在一些实施方案中,当受试者具有ST6GALNAC5预测的功能丧失多肽时,受试者具有降低的发展出认知障碍或髓鞘完整性降低、神经变性、GWC降低和轻度认知障碍转变为痴呆中的任一种的风险。

[0128] 本公开还提供了与ST6GALNAC5错义变体基因组核酸分子、ST6GALNAC5错义变体mRNA分子和/或ST6GALNAC5错义变体cDNA分子(诸如本文公开的基因组变体核酸分子、mRNA变体分子和cDNA变体分子中的任一种)杂交的分离的核酸分子。在一些实施方案中,此类分离的核酸分子在严格条件下与ST6GALNAC5错义变体核酸分子杂交。此类核酸分子可例如用作探针、引物、改变特异性探针或改变特异性引物,如本文所述或例示。

[0129] 在一些实施方案中,分离的核酸分子与ST6GALNAC5错义核酸分子的一部分杂交,所述部分包括对应于以下的位置:根据SEQ ID NO:2的位置176,867、根据SEQ ID NO:11的位置600、根据SEQ ID NO:12的位置639、根据SEQ ID NO:13的位置562、根据SEQ ID NO:14的位置515、根据SEQ ID NO:15的位置579、根据SEQ ID NO:16的位置416、根据SEQ ID NO:17的位置639、根据SEQ ID NO:18的位置600、根据SEQ ID NO:27的位置600、根据SEQ ID NO:28的位置639、根据SEQ ID NO:29的位置562、根据SEQ ID NO:30的位置515、根据SEQ ID NO:31的位置579、根据SEQ ID NO:32的位置416、根据SEQ ID NO:33的位置639或根据SEQ ID NO:34的位置600。

[0130] 在一些实施方案中,此类分离的核酸分子包含至少约5个、至少约8个、至少约10个、至少约11个、至少约12个、至少约13个、至少约14个、至少约15个、至少约16个、至少约17个、至少约18个、至少约19个、至少约20个、至少约21个、至少约22个、至少约23个、至少约24个、至少约25个、至少约30个、至少约35个、至少约40个、至少约45个、至少约50个、至少约55个、至少约60个、至少约65个、至少约70个、至少约75个、至少约80个、至少约85个、至少约90个、至少约95个、至少约100个、至少约200个、至少约300个、至少约400个、至少约500个、至少约600个、至少约700个、至少约800个、至少约900个、至少约1000个、至少约2000个、至少约3000个、至少约4000个或至少约5000个核苷酸或由其组成。在一些实施方案中,此类分离的核酸分子包含至少约5个、至少约8个、至少约10个、至少约11个、至少约12个、至少约13个、至少约14个、至少约15个、至少约16个、至少约17个、至少约18个、至少约19个、至少约20个、至少约21个、至少约22个、至少约23个、至少约24个或至少约25个核苷酸或由其组成。在一些实施方案中,分离的核酸分子包含至少约18个核苷酸或由其组成。在一些实施方案中,分离的核酸分子包含至少约15个核苷酸或由其组成。在一些实施方案中,分离的核酸分子包含约10个至约35个、约10个至约30个、约10个至约25个、约12个至约30个、约12个至约28个、约12个至约24个、约15个至约30个、约15个至约25个、约18个至约30个、约18个至约25个、约18个至约24个或约18个至约22个核苷酸或由其组成。在一些实施方案中,分离的核酸分子包含约18个至约30个核苷酸或由其组成。在一些实施方案中,分离的核酸分子包含至少约15个核苷酸至至少约35个核苷酸或由其组成。

[0131] 在一些实施方案中,分离的核酸分子与同ST6GALNAC5错义变体基因组核酸分子、ST6GALNAC5错义变体mRNA分子和/或ST6GALNAC5错义变体cDNA分子具有至少约70%、至少约75%、至少约80%、至少约85%、至少约90%、至少约95%、至少约96%、至少约97%、至少约98%、至少约99%或100%同一性的核酸分子的至少约15个连续核苷酸杂交。在一些实施方案中,分离的核酸分子包含约15个至约100个核苷酸或约15个至约35个核苷酸或由其组成。在一些实施方案中,分离的核酸分子包含约15个至约100个核苷酸或由其组成。在一些实施方案中,分离的核酸分子包含约15个至约35个核苷酸或由其组成。

[0132] 在一些实施方案中,分离的改变特异性探针或改变特异性引物包含至少约15个核

苷酸,其中改变特异性探针或改变特异性引物包含与编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义核酸分子或其互补序列的一部分的核苷酸序列互补的核苷酸序列。在一些实施方案中,所述部分包含对应于以下的位置:根据SEQ ID NO:2或其互补序列的位置176,867;根据SEQ ID NO:11或其互补序列的位置600;根据SEQ ID NO:12或其互补序列的位置639;根据SEQ IDNO:13或其互补序列的位置562;根据SEQ ID NO:14或其互补序列的位置515;根据SEQ ID NO:15或其互补序列的位置579;根据SEQ ID NO:16或其互补序列的位置416;根据SEQ ID NO:17或其互补序列的位置639;根据SEQ ID NO:18或其互补序列的位置600;根据SEQ ID NO:27或其互补序列的位置600;根据SEQ ID NO:28或其互补序列的位置639;根据SEQ ID NO:29或其互补序列的位置562;根据SEQ ID NO:30或其互补序列的位置515;根据SEQ ID NO:31或其互补序列的位置579;根据SEQ ID NO:32或其互补序列的位置416;根据SEQ ID NO:33或其互补序列的位置639;或根据SEQ ID NO:34或其互补序列的位置600。在一些实施方案中,所述部分包含对应于以下的位置:根据SEQ ID NO:2或其互补序列的位置176,866-176,868;根据SEQ ID NO:11或其互补序列的位置599-601;根据SEQ ID NO:12或其互补序列的位置638-640;根据SEQ ID NO:13或其互补序列的位置561-563;根据SEQ ID NO:14或其互补序列的位置514-516;根据SEQ ID NO:15或其互补序列的位置578-580;根据SEQ ID NO:16或其互补序列的位置415-417;根据SEQ ID NO:17或其互补序列的位置638-640;根据SEQ ID NO:18或其互补序列的位置599-601;根据SEQ ID NO:27或其互补序列的位置599-601;根据SEQ ID NO:28或其互补序列的位置638-640;根据SEQ ID NO:29或其互补序列的位置561-563;根据SEQ ID NO:30或其互补序列的位置514-516;根据SEQ ID NO:31或其互补序列的位置578-580;根据SEQ ID NO:32或其互补序列的位置415-417;根据SEQ ID NO:33或其互补序列的位置638-640;或根据SEQ ID NO:34或其互补序列的位置599-601。

[0133] 在一些实施方案中,改变特异性探针和改变特异性引物包含DNA。在一些实施方案中,改变特异性探针和改变特异性引物包含RNA。

[0134] 在一些实施方案中,本文所述的探针和引物(包括改变特异性探针和改变特异性引物)具有与本文公开的任何核酸分子或其互补序列特异性杂交的核苷酸序列。在一些实施方案中,所述探针和引物在严格条件下与本文公开的任何核酸分子特异性地杂交。

[0135] 在一些实施方案中,引物(包括改变特异性引物)可用于第二代测序或高通量测序中。在一些情况下,可修饰引物,包括改变特异性引物。特别地,引物可包含在例如大规模平行签名测序(MPSS)、聚合酶克隆测序(Polony sequencing)和454焦磷酸测序的不同步骤中使用的各种修饰。可在所述过程的几个步骤中使用修饰的引物,包括在克隆步骤中使用生物素酰化的引物,并且在珠粒装载步骤和检测步骤中使用荧光标记的引物。通常使用双端测序(paired-end)标签文库进行聚合酶克隆测序,其中每个DNA模板分子的长度为约135bp。在珠粒装载步骤和乳液PCR中使用生物素酰化的引物。在检测步骤中使用荧光标记的简并九聚物寡核苷酸。衔接子可含有用于将DNA文库固定到链霉亲和素包被的珠粒上的5'-生物素标签。

[0136] 本文所述的探针和引物可用于检测本文所公开的ST6GALNAC5错义变体基因组核酸分子、ST6GALNAC5错义变体mRNA分子和/或ST6GALNAC5错义变体cDNA分子中的任一种内的核苷酸变异。本文所述的引物可用于扩增ST6GALNAC5错义变体基因组核酸分子、

ST6GALNAC5错义变体mRNA分子或ST6GALNAC5错义变体cDNA分子,或其片段。

[0137] 本公开还提供了包含上述引物中的任一种的引物对。例如,如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:1的位置176,867的位置处的胸腺嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考基因组核酸分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶(而不是胸腺嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体基因组核酸分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0138] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:3的位置600的位置处的尿嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考mRNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 mRNA分子中在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶(而不是尿嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体mRNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0139] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:4的位置639的位置处的尿嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考mRNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 mRNA分子中在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶(而不是尿嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体mRNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:12的位置639的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0140] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:5的位置562的位置处的尿嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考mRNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 mRNA分子中在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶(而不是尿嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体mRNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:13的位置562的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0141] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:6的位置515的位置处的尿嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考mRNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 mRNA分子中在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶(而不是尿嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体mRNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:14的位置515的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0142] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:7的位置579的位置处的尿嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考mRNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 mRNA分子中在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶(而不是尿嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体mRNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:15的位置579的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0143] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:8

的位置416的位置处的尿嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考mRNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 mRNA分子中在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶(而不是尿嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体mRNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:16的位置416的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0144] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:9的位置639的位置处的尿嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考mRNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 mRNA分子中在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶(而不是尿嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体mRNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:17的位置639的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0145] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:10的位置600的位置处的尿嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考mRNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 mRNA分子中在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶(而不是尿嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体mRNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:18的位置600的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0146] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:19的位置600的位置处的胸腺嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考cDNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 cDNA分子中在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶(而不是胸腺嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体cDNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0147] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:20的位置639的位置处的胸腺嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考cDNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 cDNA分子中在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶(而不是胸腺嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体cDNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:28的位置639的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0148] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:21的位置562的位置处的胸腺嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考cDNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 cDNA分子中在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶(而不是胸腺嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体cDNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:29的位置562的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0149] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:22

的位置515的位置处的胸腺嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考cDNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 cDNA分子中在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶(而不是胸腺嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体cDNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:30的位置515的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0150] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:23的位置579的位置处的胸腺嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考cDNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 cDNA分子中在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶(而不是胸腺嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体cDNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:31的位置579的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0151] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:24的位置416的位置处的胸腺嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考cDNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 cDNA分子中在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶(而不是胸腺嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体cDNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:32的位置416的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0152] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:25的位置639的位置处的胸腺嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考cDNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 cDNA分子中在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶(而不是胸腺嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体cDNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:33的位置639的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0153] 如果引物的3'-端之一与特定ST6GALNAC5核酸分子中在对应于根据SEQ ID NO:26的位置600的位置处的胸腺嘧啶(而不是胞嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5参考cDNA分子的存在。相反地,如果引物的3'-端之一与特定ST6GALNAC5 cDNA分子中在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶(而不是胸腺嘧啶)杂交,则扩增片段的存在将指示ST6GALNAC5错义变体cDNA分子的存在。在一些实施方案中,与在对应于根据SEQ ID NO:34的位置600的位置处的胞嘧啶互补的引物的核苷酸可在引物的3'端。

[0154] 在本公开的上下文中,"特异性杂交"意指探针或引物(诸如例如,改变特异性探针或改变特异性引物)不与编码ST6GALNAC5参考基因组核酸分子、ST6GALNAC5参考mRNA分子和/或ST6GALNAC5参考cDNA分子的核酸序列杂交。

[0155] 在本公开通篇描述的实施方案中的任一项中,探针(诸如例如,改变特异性探针)可包含标记。在一些实施方案中,所述标记是荧光标记、放射性标记或生物素。

[0156] 本公开还提供了包含本文所公开的探针中的任一种或多种所附接的基底的支持物。固体支持物是分子(诸如本文公开的探针中的任一种)可与其结合的固态基底或支持物。固体支持物的一种形式是阵列。固体支持物的另一种形式是阵列检测物。阵列检测物是多种不同的探针以阵列、网格或其他组织化模式与其偶联的固体支持物。固态基底的一种形式是微量滴定皿,诸如标准96孔类型。在一些实施方案中,可采用通常每孔含有一个阵列的多孔玻璃载片。在一些实施方案中,所述支持物是微阵列。

[0157] 本公开还提供了包含本文所述的ST6GALNAC5错义核酸分子中的任一种(基因组核酸分子、mRNA分子或cDNA分子),或其互补序列以及本文所述的改变特异性引物或改变特异性探针的任一种,或者由其组成的分子复合物。在一些实施方案中,分子复合物中的ST6GALNAC5错义核酸分子(基因组核酸分子、mRNA分子或cDNA分子),或其互补序列是单链的。在一些实施方案中,ST6GALNAC5错义核酸分子是本文所述的基因组核酸分子中的任一种。在一些实施方案中,ST6GALNAC5错义核酸分子是本文所述的mRNA分子中的任一种。在一些实施方案中,ST6GALNAC5错义核酸分子是本文所述的cDNA分子中的任一种。在一些实施方案中,分子复合物包含本文所述的ST6GALNAC5错义核酸分子中的任一种(基因组核酸分子、mRNA分子或cDNA分子),或其互补序列以及本文所述的改变特异性引物中的任一种,或者由其组成。在一些实施方案中,分子复合物包含本文所述的ST6GALNAC5错义核酸分子中的任一种(基因组核酸分子、mRNA分子或cDNA分子),或其互补序列以及本文所述的改变特异性探针中的任一种,或者由其组成。

[0158] 在一些实施方案中,分子复合物包含与编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5基因组核酸分子杂交的改变特异性引物或改变特异性探针,或者由其组成,其中改变特异性引物或改变特异性探针与在对应于以下的位置处的ST6GALNAC5基因组核酸分子杂交:根据SEQ ID NO:2或其互补序列的位置176,867。

[0159] 在一些实施方案中,分子复合物包含改变特异性引物或改变特异性探针或者由其组成,所述改变特异性引物或改变特异性探针与在对应于根据SEQ ID NO:2的位置176,866-176,868的位置处的GCG密码子杂交。

[0160] 在一些实施方案中,分子复合物包含含有SEQ ID NO:2的基因组核酸分子或者由其组成。

[0161] 在一些实施方案中,分子复合物包含与编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5 mRNA分子杂交的改变特异性引物或改变特异性探针,或者由其组成,其中改变特异性引物或改变特异性探针与ST6GALNAC5 mRNA分子在对应于以下的位置处杂交:根据SEQ ID NO:11或其互补序列的位置600;根据SEQ ID NO:12或其互补序列的位置639;根据SEQ ID NO:13或其互补序列的位置562;根据SEQ ID NO:14或其互补序列的位置515;根据SEQ IDNO:15或其互补序列的位置579;根据SEQ ID NO:16或其互补序列的位置416;根据SEQ ID NO:17或其互补序列的位置639;或根据SEQ ID NO:18或其互补序列的位置600。

[0162] 在一些实施方案中,分子复合物包含与以下杂交的改变特异性引物或改变特异性探针,或者由其组成:在对应于根据SEQ ID NO:11的位置599-601的位置处的GCG密码子、在对应于根据SEQ ID NO:12的位置638-640的位置处的GCG密码子、在对应于根据SEQ ID NO:13的位置561-563的位置处的GCG密码子、在对应于根据SEQ ID NO:14的位置514-516的位置处的GCG密码子、在对应于根据SEQ ID NO:15的位置578-580的位置处的GCG密码子、在对

应于根据SEQ ID NO:16的位置415-417的位置处的GCG密码子、在对应于根据SEQ ID NO:17的位置638-640的位置处的GCG密码子或在对应于根据SEQ ID NO:18的位置599-601的位置处的GCG密码子。

[0163] 在一些实施方案中,分子复合物包含mRNA分子或由mRNA分子组成,所述mRNA分子包含SEQ ID NO:11、SEQ ID NO:12、SEQ ID NO:13、SEQ ID NO:14、SEQ ID NO:15、SEQ ID NO:16、SEQ ID NO:17或SEQ ID NO:18。

[0164] 在一些实施方案中,分子复合物包含与编码ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5 cDNA分子杂交的改变特异性引物或改变特异性探针,或者由其组成,其中改变特异性引物或改变特异性探针与ST6GALNAC5 cDNA分子在对应于以下的位置处杂交:根据SEQ ID NO:27或其互补序列的位置600;根据SEQ ID NO:28或其互补序列的位置639;根据SEQ ID NO:29或其互补序列的位置562;根据SEQ ID NO:30或其互补序列的位置515;根据SEQ ID NO:31或其互补序列的位置579;根据SEQ ID NO:32或其互补序列的位置416;根据SEQ ID NO:33或其互补序列的位置639;或根据SEQ ID NO:34或其互补序列的位置600。

[0165] 在一些实施方案中,分子复合物包含与以下杂交的改变特异性引物或改变特异性探针,或者由其组成:在对应于根据SEQ ID NO:27的位置599-601的位置处的GCG密码子、在对应于根据SEQ ID NO:28的位置638-640的位置处的GCG密码子、在对应于根据SEQ ID NO:29的位置561-563的位置处的GCG密码子、在对应于根据SEQ ID NO:30的位置514-516的位置处的GCG密码子、在对应于根据SEQ ID NO:31的位置578-580的位置处的GCG密码子、在对应于根据SEQ ID NO:32的位置415-417的位置处的GCG密码子、在对应于根据SEQ ID NO:33的位置638-640的位置处的GCG密码子或在对应于根据SEQ ID NO:34的位置599-601的位置处的GCG密码子。

[0166] 在一些实施方案中,分子复合物包含cDNA分子或由cDNA分子组成,所述cDNA分子包含SEQ ID NO:27、SEQ ID NO:28、SEQ ID NO:29、SEQ ID NO:30、SEQ ID NO:31、SEQ ID NO:32、SEQ ID NO:33或SEQ ID NO:34。

[0167] 在一些实施方案中,分子复合物包含含有标记的改变特异性探针或改变特异性引物。在一些实施方案中,所述标记是荧光标记、放射性标记或生物素。在一些实施方案中,分子复合物还包含非人聚合酶。

[0168] ST6GALNAC5参考基因组核酸分子的核苷酸序列列示于SEQ ID NO:1中。参考SEQ ID NO:1,位置176,867是胸腺嘧啶。

[0169] 存在ST6GALNAC5错义变体基因组核酸分子,其中位置176,867处的胸腺嘧啶被胞嘧啶替换(rs756654226;GRCh38/hg38人类基因组组装中的chr1:77044346)。此ST6GALNAC5错义变体基因组核酸分子的核苷酸序列列示于SEQ ID NO:2中。

[0170] ST6GALNAC5参考mRNA分子的核苷酸序列列示于SEQ ID NO:3中。参考SEQ ID NO:3,位置600是尿嘧啶。另一种ST6GALNAC5参考mRNA分子的核苷酸序列列示于SEQ ID NO:4中。参考SEQ ID NO:4,位置639是尿嘧啶。另一种ST6GALNAC5参考mRNA分子的核苷酸序列列示于SEQ ID NO:5中。参考SEQ ID NO:5,位置562是尿嘧啶。另一种ST6GALNAC5参考mRNA分子的核苷酸序列列示于SEQ ID NO:6中。参考SEQ ID NO:6,位置515是尿嘧啶。另一种ST6GALNAC5参考mRNA分子的核苷酸序列列示于SEQ ID NO:7中。参考SEQ ID NO:7,位置579是尿嘧啶。另一种ST6GALNAC5参考mRNA分子的核苷酸序列列示于SEQ ID NO:8中。参考SEQ

ID NO:8,位置416是尿嘧啶。另一种ST6GALNAC5参考mRNA分子的核苷酸序列列示于SEQ ID NO:9中。参考SEQ ID NO:9,位置639是尿嘧啶。另一种ST6GALNAC5参考mRNA分子的核苷酸序列列示于SEQ ID NO:10中。参考SEQ ID NO:10,位置600是尿嘧啶。

[0171] 存在一种ST6GALNAC5错义变体mRNA分子,其中位置600处的尿嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体mRNA分子的核苷酸序列列示于SEQ ID NO:11中。

[0172] 存在另一种ST6GALNAC5错义变体mRNA分子,其中位置639处的尿嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体mRNA分子的核苷酸序列列示于SEQ ID NO:12中。

[0173] 存在另一种ST6GALNAC5错义变体mRNA分子,其中位置562处的尿嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体mRNA分子的核苷酸序列列示于SEQ ID NO:13中。

[0174] 存在另一种ST6GALNAC5错义变体mRNA分子,其中位置515处的尿嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体mRNA分子的核苷酸序列列示于SEQ ID NO:14中。

[0175] 存在另一种ST6GALNAC5错义变体mRNA分子,其中位置579处的尿嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体mRNA分子的核苷酸序列列示于SEQ ID NO:15中。

[0176] 存在另一种ST6GALNAC5错义变体mRNA分子,其中位置416处的尿嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体mRNA分子的核苷酸序列列示于SEQ ID NO:16中。

[0177] 存在另一种ST6GALNAC5错义变体mRNA分子,其中位置639处的尿嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体mRNA分子的核苷酸序列列示于SEQ ID NO:17中。

[0178] 存在另一种ST6GALNAC5错义变体mRNA分子,其中位置600处的尿嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体mRNA分子的核苷酸序列列示于SEQ ID NO:18中。

[0179] ST6GALNAC5参考cDNA分子的核苷酸序列列示于SEQ ID NO:19中。参考SEQ ID NO:19,位置600是胸腺嘧啶。另一种ST6GALNAC5参考cDNA分子的核苷酸序列列示于SEQ ID NO:20中。参考SEQ ID NO:20,位置639是胸腺嘧啶。另一种ST6GALNAC5参考cDNA分子的核苷酸序列列示于SEQ ID NO:21中。参考SEQ ID NO:21,位置562是胸腺嘧啶。另一种ST6GALNAC5参考cDNA分子的核苷酸序列列示于SEQ ID NO:22中。参考SEQ ID NO:22,位置515是胸腺嘧啶。另一种ST6GALNAC5参考cDNA分子的核苷酸序列列示于SEQ ID NO:23中。参考SEQ ID NO:23,位置579是胸腺嘧啶。另一种ST6GALNAC5参考cDNA分子的核苷酸序列列示于SEQ ID NO:24中。参考SEQ ID NO:24,位置416是胸腺嘧啶。另一种ST6GALNAC5参考cDNA分子的核苷酸序列列示于SEQ ID NO:25中。参考SEQ ID NO:25,位置639是胸腺嘧啶。另一种ST6GALNAC5参考cDNA分子的核苷酸序列列示于SEQ ID NO:26中。参考SEQ ID NO:26,位置600是胸腺嘧啶。

[0180] 存在一种ST6GALNAC5错义变体cDNA分子,其中位置600处的胸腺嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体cDNA分子的核苷酸序列列示于SEQ ID NO:27中。

[0181] 存在另一种ST6GALNAC5错义变体cDNA分子,其中位置639处的胸腺嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体cDNA分子的核苷酸序列列示于SEQ ID NO:28中。

[0182] 存在另一种ST6GALNAC5错义变体cDNA分子,其中位置562处的胸腺嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体cDNA分子的核苷酸序列列示于SEQ ID NO:29中。

[0183] 存在另一种ST6GALNAC5错义变体cDNA分子,其中位置515处的胸腺嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体cDNA分子的核苷酸序列列示于SEQ ID NO:30中。

[0184] 存在另一种ST6GALNAC5错义变体cDNA分子,其中位置579处的胸腺嘧啶被胞嘧啶

替换。此ST6GALNAC5错义变体cDNA分子的核苷酸序列列示于SEQ ID NO:31中。

[0185] 存在另一种ST6GALNAC5错义变体cDNA分子,其中位置416处的胸腺嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体cDNA分子的核苷酸序列列示于SEQ ID NO:32中。

[0186] 存在另一种ST6GALNAC5错义变体cDNA分子,其中位置639处的胸腺嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体cDNA分子的核苷酸序列列示于SEQ ID NO:33中。

[0187] 存在另一种ST6GALNAC5错义变体cDNA分子,其中位置600处的胸腺嘧啶被胞嘧啶替换。此ST6GALNAC5错义变体cDNA分子的核苷酸序列列示于SEQ ID NO:34中。

[0188] 基因组核酸分子、mRNA分子和cDNA分子可来自任何生物体。例如,基因组核酸分子、mRNA分子和cDNA分子可以是人的或来自另一生物体(诸如非人哺乳动物、啮齿动物、小鼠或大鼠)的直系同源物。应理解,群体内的基因序列可由于多态性(诸如单核苷酸多态性)而变化。本文提供的实例仅为示例性序列。其他序列也是可能的。

[0189] 本文还提供了可与所公开的核酸分子相互作用的功能性多核苷酸。功能性多核苷酸的实例包括但不限于反义分子、适体、核酶、三链体形成分子以及外部指导序列。功能性多核苷酸可充当靶分子所具有的特定活性的影响剂、抑制剂、调节剂和刺激剂,或者功能性多核苷酸可具有独立于任何其他分子的全新活性。

[0190] 本文公开的分离的核酸分子可包括RNA、DNA或RNA和DNA两者。所述分离的核酸分子还可连接或融合至异源核酸序列(诸如在载体中)或异源标记。例如,本文所公开的分离的核酸分子可在载体中或作为包含分离的核酸分子和异源核酸序列的外源供体序列。分离的核酸分子还可与异源标记连接或融合。标记可以是直接可检测的(诸如例如荧光团)或间接可检测的(诸如例如半抗原、酶或荧光团淬灭剂)。此类标记可通过光谱学、光化学、生物化学、免疫化学或化学手段检测。此类标记包括例如放射性标记、颜料、染料、色原、自旋标记和荧光标记。标记也可以是例如化学发光物质;含金属物质;或酶,其中发生依赖于酶的二次信号生成。术语"标记"也可指"标签"或半抗原,其可选择性地与缀合分子结合,使得缀合分子在随后与底物一起添加时用于生成可检测信号。例如,生物素可与辣根过氧化物(HRP)的亲和素或链霉亲和素缀合物一起用作标签以与标签结合,并使用量热底物(诸如例如四甲基联苯胺(TMB))或荧光底物进行检查以检测HRP的存在。可用作标签来促进纯化的示例性标记包括但不限于myc、HA、FLAG或3XFLAG、6XHis或聚组氨酸、谷胱甘肽-S-转移酶(GST)、麦芽糖结合蛋白、表位标签或免疫球蛋白的Fc部分。许多标记包括例如颗粒、荧光团、半抗原、酶及其量热、荧光和化学发光底物和其他标记。

[0191] 分离的核酸分子或其互补序列也可存在于宿主细胞内。在一些实施方案中,宿主细胞可包含载体,所述载体包含本文所述的任何核酸分子或其互补序列。在一些实施方案中,核酸分子可操作地连接至在宿主细胞中具有活性的启动子。在一些实施方案中,启动子是外源启动子。在一些实施方案中,启动子是诱导型启动子。在一些实施方案中,宿主细胞是细菌细胞、酵母细胞、昆虫细胞或哺乳动物细胞。在一些实施方案中,宿主细胞是细菌细胞。在一些实施方案中,宿主细胞是酵母细胞。在一些实施方案中,宿主细胞是昆虫细胞。在一些实施方案中,宿主细胞是哺乳动物细胞。

[0192] 所公开的核酸分子可包括例如核苷酸或非天然或修饰的核苷酸,诸如核苷酸类似物或核苷酸替代物。此类核苷酸包括含有修饰的碱基、糖或磷酸酯基团的核苷酸,或者在其结构中掺入非天然部分的核苷酸。非天然核苷酸的实例包括但不限于双脱氧核苷酸、生物

素酰化的、胺化的、脱氨基的、烷基化的、苄化的和荧光团标记的核苷酸。

[0193] 本文公开的核酸分子还可包含一种或多种核苷酸类似物或取代。核苷酸类似物是含有对碱基、糖或磷酸酯部分的修饰的核苷酸。对碱基部分的修饰包括但不限于A、C、G和T/U以及不同的嘌呤或嘧啶碱基(诸如例如假尿苷、尿嘧啶-5-基、次黄嘌呤-9-基(I)和2-氨基腺嘌呤-9-基)的天然和合成修饰。修饰的碱基包括但不限于5-甲基胞嘧啶(5-me-C)、5-羟甲基胞嘧啶、黄嘌呤、次黄嘌呤、2-氨基腺嘌呤、腺嘌呤和鸟嘌呤的6-甲基和其他烷基衍生物、腺嘌呤和鸟嘌呤的2-丙基和其他烷基衍生物、2-硫代尿嘧啶、2-硫代胸腺嘧啶和2-硫代胞嘧啶、5-卤代尿嘧啶和胞嘧啶、5-丙炔基尿嘧啶和胞嘧啶、6-偶氮尿嘧啶、胞嘧啶和胸腺嘧啶、5-尿嘧啶(假尿嘧啶)、4-硫代尿嘧啶、8-卤代、8-氨基、8-硫代、8-硫代烷基、8-羟基和其他8-取代的腺嘌呤和鸟嘌呤、5-卤代(诸如例如5-溴)、5-三氟甲基和其他5-取代的尿嘧啶和胞嘧啶、7-甲基鸟嘌呤、7-甲基腺嘌呤、8-氮杂鸟嘌呤、8-氮杂腺嘌呤、7-脱氮鸟嘌呤、7-脱氮腺嘌呤、3-脱氮鸟嘌呤和3-脱氮腺嘌呤。

[0194] 核苷酸类似物还可包括对糖部分的修饰。对糖部分的修饰包括但不限于核糖和脱氧核糖的天然修饰以及合成修饰。糖修饰包括但不限于在2'位置处的以下修饰:OH;F;O-、S-或N-烷基;O-、S-或N-烯基;O-、S-或N-炔基;或O-烷基-O-烷基,其中烷基、烯基和炔基可以是取代或未取代的C_{1-10}烷基或C_{2-10}烯基和C_{2-10}炔基。示例性的2'糖修饰还包括但不限于-O[$(CH_2)_nO]_mCH_3$、-O$(CH_2)_nOCH_3$、-O$(CH_2)_nNH_2$、-O$(CH_2)_nCH_3$、-O$(CH_2)_n$-ONH_2和-O$(CH_2)_n$O N[$(CH_2)_nCH_3)]_2$,其中n和m独立地为1至约10。2'位置处的其他修饰包括但不限于C_{1-10}烷基、取代的低级烷基、烷芳基、芳烷基、O-烷芳基或O-芳烷基、SH、SCH_3、OCN、Cl、Br、CN、CF_3、OCF_3、$SOCH_3$、SO_2CH_3、ONO_2、NO_2、N_3、NH_2、杂环烷基、杂环烷芳基、氨基烷基氨基、聚烷基氨基、取代的甲硅烷基、RNA切割基团、报告基团、嵌入剂、用于改善寡核苷酸的药代动力学特性的基团或用于改善寡核苷酸的药效动力学特性的基团,以及具有类似特性的其他取代基。还可在糖上的其他位置上做出类似的修饰,具体地在3'末端核苷酸上或在2'-5'连接的寡核苷酸中的糖的3'位置和5'末端核苷酸的5'位置。修饰的糖还可包括在桥环氧处含有修饰(诸如CH_2和S)的那些糖。核苷酸糖类似物还可具有替代呋喃戊糖的糖模拟物诸如环丁基部分。

[0195] 核苷酸类似物还可在磷酸酯部分处进行修饰。修饰的磷酸酯部分包括但不限于可被修饰以使得两个核苷酸之间的键联含有以下的修饰的磷酸酯部分:硫代磷酸酯、手性硫代磷酸酯、二硫代磷酸酯、磷酸三酯、氨基烷基磷酸三酯、甲基和其他烷基膦酸酯(包括3'-亚烷基膦酸酯和手性膦酸酯)、次膦酸酯、氨基磷酸酯(包括3'-氨基氨基磷酸酯和氨基烷基氨基磷酸酯)、硫羰氨基磷酸酯、硫羰烷基膦酸酯、硫羰烷基磷酸三酯和硼烷磷酸酯。两个核苷酸之间的这些磷酸酯或修饰的磷酸酯键联可通过3'-5'键联或2'-5'键联,并且所述键联可含有反转的极性,诸如3'-5'至5'-3'或2'-5'至5'-2'。还包括各种盐、混合盐以及游离酸形式。核苷酸替代物还包括肽核酸(PNA)。

[0196] 本公开还提供包含本文公开的核酸分子中的任一者或多者的载体。在一些实施方案中,载体包含本文公开的任何一种或多种核酸分子和异源核酸。所述载体可为能够转运核酸分子的病毒或非病毒载体。在一些实施方案中,载体是质粒或粘粒(诸如例如可将额外的DNA区段连接到其中的环状双链DNA)。在一些实施方案中,载体是病毒载体,其中额外的DNA区段可连接到病毒基因组中。表达载体包括但不限于质粒、粘粒、逆转录病毒、腺病毒、腺相关病毒(AAV)、植物病毒(诸如花椰菜花叶病毒和烟草花叶病毒)、酵母人工染色体

(YAC)、Epstein-Barr(EBV)衍生的附加体以及本领域已知的其他表达载体。

[0197] 用于哺乳动物宿主细胞表达的所需调控序列可包括例如指导哺乳动物细胞中的高水平多肽表达的病毒元件,诸如衍生自逆转录病毒LTR、巨细胞病毒(CMV)(诸如例如CMV启动子/增强子)、猿猴病毒40(SV40)(诸如例如SV40启动子/增强子)、腺病毒(诸如例如腺病毒主要晚期启动子(AdMLP))的启动子和/或增强子、多瘤病毒和哺乳动物强启动子(诸如例如天然免疫球蛋白和肌动蛋白启动子)。在细菌细胞或真菌细胞(诸如例如酵母细胞)中表达多肽的方法也是众所周知的。启动子可为例如组成型活性启动子、条件启动子、诱导型启动子、时间受限型启动子(诸如例如发育调控型启动子)或空间受限型启动子(诸如例如细胞特异性或组织特异性启动子)。

[0198] 核酸分子内的核苷酸序列或多肽内的氨基酸序列的特定伸长段之间的同一性百分比(或互补性百分比)可使用BLAST程序(基本局部比对搜索工具)和PowerBLAST程序(Altschul等人,J.Mol.Biol.,1990,215,403-410;Zhang和Madden,Genome Res.,1997,7,649-656)或通过使用Gap程序(Wisconsin序列分析包,用于Unix的版本8,Genetics Computer Group,University Research Park,Madison Wis.)使用默认设置(其使用Smith和Waterman的算法(Adv.Appl.Math.,1981,2,482-489))来常规确定。在本文中,如果对序列一致性百分比进行参考,则较高的序列一致性百分比相对于较低的序列一致性百分比是优选的。

[0199] 本公开还提供包含本文公开的分离的核酸分子、基因组核酸分子、mRNA分子和/或cDNA分子中的任一者或多者的组合物。在一些实施方案中,所述组合物是药物组合物。在一些实施方案中,组合物包含载剂和/或赋形剂。载剂的实例包括但不限于聚(乳酸)(PLA)微球、聚(D,L-乳酸-共乙醇酸)(PLGA)微球、脂质体、胶束、反胶束、脂质螺旋物和脂质微管。载剂可包括缓冲盐溶液,诸如PBS、HBSS等。

[0200] 如本文所用,当在对特定核苷酸或核苷酸序列或位置的编号的语境中使用时,短语“对应于”或其语法变型是指当将特定核苷酸或核苷酸序列与参考序列进行比较时对指定参考序列的编号(诸如例如SEQ ID NO:1、SEQ ID NO:3或SEQ ID NO:19)。换句话说,特定聚合物的残基(诸如例如核苷酸或氨基酸)编号或残基(诸如例如核苷酸或氨基酸)位置相对于参考序列来指定,而不是通过残基在特定核苷酸或核苷酸序列内的实际数值位置来指定。例如,特定核苷酸序列可通过引入缺口以优化两个序列之间的残基匹配来与参考序列比对。在这些情况下,虽然存在缺口,但是对特定核苷酸或核苷酸序列中的残基的编号是相对于其所比对的参考序列来进行的。

[0201] 例如,包含编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列的ST6GALNAC5错义核酸分子,其中所述核苷酸序列包含在对应于根据SEQ ID NO:2的位置176,867的位置处的胞嘧啶意味着如果将ST6GALNAC5基因组核酸分子的核苷酸序列与SEQ ID NO:2的序列比对,则ST6GALNAC5序列具有在对应于SEQ ID NO:2的位置176,867的位置处的胞嘧啶残基。这同样适用于包含编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列的ST6GALNAC5错义mRNA分子,其中所述核苷酸序列包含在对应于根据SEQ ID NO:11的位置600的位置处的胞嘧啶,以及包含编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列的ST6GALNAC5错义cDNA分子,其中所述核苷酸序列包含在对应于根据SEQ ID NO:27的位置600的位置处的胞嘧啶。换句话说,这些短语是指编码ST6GALNAC5多肽的核酸分子,其中基因组核酸分子具有包含

与SEQ ID NO:2的位置176,867处的胞嘧啶残基同源的胞嘧啶残基的核苷酸序列(或其中mRNA分子具有包含与SEQ ID NO:11的位置600处的胞嘧啶残基同源的胞嘧啶残基的核苷酸序列,或其中cDNA分子具有包含与SEQ IDNO:27的位置600处的胞嘧啶残基同源的胞嘧啶残基的核苷酸序列)。

[0202] 如本文所述,ST6GALNAC5错义基因组核酸分子内对应于根据SEQ ID NO:2的位置176,867的位置,例如,可通过在特定ST6GALNAC5核酸分子的核苷酸序列与SEQ ID NO:2的核苷酸序列之间进行序列比对来鉴定。存在多种计算算法可用于进行序列比对以鉴定对应于例如SEQ ID NO:2中的位置176,867的核苷酸位置。例如,通过使用NCBI BLAST算法(Altschul等人,Nucleic Acids Res.,1997,25,3389-3402)或CLUSTALW软件(Sievers和Higgins,Methods Mol.Biol.,2014,1079,105-116),可进行序列比对。然而,序列也可手动地进行比对。

[0203] ST6GALNAC5参考多肽的氨基酸序列列示于SEQ ID NO:35(同工型1)、SEQ ID NO:36(同工型2)、SEQ ID NO:37(同工型3)和SEQ ID NO:38(同工型4)中。参考SEQ ID NO:35(同工型1),ST6GALNAC5参考多肽的长度为336个氨基酸。参考SEQ ID NO:35,位置135是缬氨酸。参考SEQ ID NO:36(同工型2),ST6GALNAC5参考多肽的长度为146个氨基酸。参考SEQ ID NO:36,位置45是缬氨酸。参考SEQ ID NO:37(同工型3),ST6GALNAC5参考多肽的长度为246个氨基酸。参考SEQ ID NO:37,位置45是缬氨酸。参考SEQ ID NO:38(同工型4),ST6GALNAC5参考多肽的长度为236个氨基酸。参考SEQ ID NO:38,位置135是缬氨酸。

[0204] ST6GALNAC5预测的功能丧失多肽的氨基酸序列列示于SEQ ID NO:39(同工型1;Val135Ala)、SEQ ID NO:40(同工型2;Val45Ala)、SEQ ID NO:41(同工型3;Val45Ala*)和SEQ ID NO:42(同工型4;Val135Ala*)中。参考SEQ ID NO:39,位置135是丙氨酸。参考SEQ ID NO:40,位置45是丙氨酸。参考SEQ ID NO:41,位置45是丙氨酸。参考SEQ ID NO:42,位置135是丙氨酸。

[0205] 所附序列表中列出的核苷酸序列和氨基酸序列是使用核苷酸碱基的标准字母缩写和氨基酸的三字母代码示出的。所述核苷酸序列遵循从序列的5'端开始且前进(即在每一行中从左到右)至3'端的标准惯例。仅示出了每一个核苷酸序列的一条链,但应理解的是,互补链是通过对所展示链的任何参考而被包括在内的。氨基酸序列遵循从序列的氨基末端开始且前进(即在每一行中从左到右)至羧基末端的标准惯例。

[0206] 本公开还提供了治疗或预防认知障碍的治疗剂,其用于治疗受试者的认知障碍(或用于制备用于治疗受试者的认知障碍的药物),其中所述受试者具有编码本文所述的ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体基因组核酸分子、错义变体mRNA分子和/或错义变体cDNA分子中的任一种。治疗或预防认知障碍的治疗剂可以是本文所述的治疗或预防认知障碍的治疗剂中的任一种。

[0207] 在一些实施方案中,受试者被鉴定为具有编码ST6GALNAC5预测的功能丧失多肽的基因组核酸分子,其中所述基因组核酸分子具有包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶的核苷酸序列。

[0208] 在一些实施方案中,受试者被鉴定为具有编码ST6GALNAC5预测的功能丧失多肽的mRNA分子,其中所述mRNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位

置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶。

[0209] 在一些实施方案中,受试者被鉴定为具有编码ST6GALNAC5预测的功能丧失多肽的cDNA分子,其中所述cDNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

[0210] 在一些实施方案中,受试者被鉴定为具有:i)具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列的基因组核酸分子,其中所述核苷酸序列包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;ii)具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列的mRNA分子,其中所述核苷酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;或iii)具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列的cDNA分子,其中所述核苷酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

[0211] 在一些实施方案中,受试者被鉴定为具有一种基因组核酸分子,所述基因组核酸分子具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列,其中所述核苷酸序列包含对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶。

[0212] 在一些实施方案中,受试者被鉴定为具有一种mRNA分子,所述mRNA分子具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列,其中所述核苷酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或

其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶。

[0213] 在一些实施方案中,受试者被鉴定为具有一种cDNA分子,所述cDNA分子具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列,其中所述核苷酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

[0214] 在一些实施方案中,受试者被鉴定为具有ST6GALNAC5预测的功能丧失多肽,所述多肽包含:在对应于根据SEQ ID NO:39的位置135的位置处的丙氨酸、在对应于根据SEQ ID NO:40的位置45的位置处的丙氨酸、在对应于根据SEQ ID NO:41的位置45的位置处的丙氨酸或在对应于根据SEQ ID NO:42的位置135的位置处的丙氨酸。

[0215] 本公开还提供了ST6GALNAC5抑制剂,其用于治疗受试者的认知障碍(或用于制备用于治疗受试者的认知障碍的药物),其中所述受试者对于编码本文所述的ST6GALNAC5预测的功能丧失多肽的ST6GALNAC5错义变体基因组核酸分子、错义变体mRNA分子和/或错义变体cDNA分子中的任一种是杂合的,或者其中所述受试者对于ST6GALNAC5基因组核酸分子、mRNA分子或cDNA分子是参考。ST6GALNAC5抑制剂可以是本文所述的ST6GALNAC5抑制剂中的任一种。

[0216] 在一些实施方案中,受试者对于ST6GALNAC5基因组核酸分子、ST6GALNAC5 mRNA分子或ST6GALNAC5 cDNA分子是参考。在一些实施方案中,受试者对于ST6GALNAC5基因组核酸分子是参考。在一些实施方案中,受试者对于ST6GALNAC5 mRNA分子是参考。在一些实施方案中,受试者对于ST6GALNAC5 cDNA分子是参考。

[0217] 在一些实施方案中,受试者被鉴定为对于编码ST6GALNAC5预测的功能丧失多肽的基因组核酸分子是杂合的,其中所述基因组核酸分子具有包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶的核苷酸序列。

[0218] 在一些实施方案中,受试者被鉴定为对于编码ST6GALNAC5预测的功能丧失多肽的mRNA分子是杂合的,其中所述mRNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互

补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶。

[0219] 在一些实施方案中,受试者被鉴定为对于编码ST6GALNAC5预测的功能丧失多肽的cDNA分子是杂合的,其中所述cDNA分子具有包含以下的核苷酸序列:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

[0220] 在一些实施方案中,受试者被鉴定为对于以下各项是杂合的:i)具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列的基因组核酸分子,其中所述核苷酸序列包含在对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶;ii)具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列的mRNA分子,其中所述核苷酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶;或iii)具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列的cDNA分子,其中所述核苷酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

[0221] 在一些实施方案中,受试者被鉴定为对于一种基因组核酸分子是杂合的,所述基因组核酸分子具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列,其中所述核苷酸序列包含对应于根据SEQ ID NO:2或其互补序列的位置176,867的位置处的胞嘧啶。

[0222] 在一些实施方案中,受试者被鉴定为对于一种mRNA分子是杂合的,所述mRNA分子具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列,其中所述核苷酸序列包含:在对应于根据SEQ ID NO:11或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:12或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:13或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:14或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:15或其互补序列的位置579的位置处的胞嘧啶;在

对应于根据SEQ ID NO:16或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:17或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:18或其互补序列的位置600的位置处的胞嘧啶。

[0223] 在一些实施方案中,受试者被鉴定为对于一种cDNA分子是杂合的,所述cDNA分子具有编码ST6GALNAC5预测的功能丧失多肽的核苷酸序列,其中所述核苷酸序列包含:在对应于根据SEQ ID NO:27或其互补序列的位置600的位置处的胞嘧啶;在对应于根据SEQ ID NO:28或其互补序列的位置639的位置处的胞嘧啶;在对应于根据SEQ ID NO:29或其互补序列的位置562的位置处的胞嘧啶;在对应于根据SEQ ID NO:30或其互补序列的位置515的位置处的胞嘧啶;在对应于根据SEQ ID NO:31或其互补序列的位置579的位置处的胞嘧啶;在对应于根据SEQ ID NO:32或其互补序列的位置416的位置处的胞嘧啶;在对应于根据SEQ ID NO:33或其互补序列的位置639的位置处的胞嘧啶;或在对应于根据SEQ ID NO:34或其互补序列的位置600的位置处的胞嘧啶。

[0224] 以上或以下所引用的所有专利文献、网站、其他出版物、登录号等均出于所有目的以引用的方式整体并入,其程度犹如每个单独项目均具体地且单独地被指示为以引用的方式如此并入一样。如果序列的不同版本与不同时间的登录号相关,则意指与本申请的有效提交日期的登录号相关的版本。有效提交日期意指实际提交日期或参考登录号的优先权申请的提交日期(如果适用)中较早的日期。同样,如果出版物、网站等的不同版本在不同时间公布,则除非另有说明,否则意指在本申请的有效提交日期最近公布的版本。除非另有特别说明,否则本公开的任何特征、步骤、要素、实施方案或方面可与任何其他特征、步骤、要素、实施方案或方面组合使用。尽管出于清楚和理解的目的已通过说明和实例详细描述本公开,但将显而易见的是,可在所附权利要求的范围内实施某些变化和修改。

[0225] 提供以下实施例来更详细地描述实施方案。它们意图说明但不限制所要求保护的实施方案。以下实施例为本领域普通技术人员提供本文所述的化合物、组合物、制品、装置和/或方法如何制备和评价的公开和描述,并且意图仅仅是示例性的且不意图限制任何权利要求的范围。已经努力确保关于数字(诸如例如量、温度等)的准确性,但可考虑一些误差和偏差。除非另有说明,否则份数是重量份,温度是以℃计或处于环境温度,且压力处于或接近大气压。

[0226] 实施例

[0227] 实施例1:罕见变体与脑成像特质的关联

[0228] 如前所述,对454,787名UKB研究参与者的外显子组进行了测序,95.8%的目标碱基覆盖深度为20倍或更大(Szustakowski,Advancing Human Genetics Research and Drug Discovery through Exome Sequencing of the UK Biobank.bioRxiv,2021;和Van Hout等人,Nature,2020)。在18,659个基因的编码区的3900万个碱基对中发现了一千二百万个变体(数据未示出)。在已鉴定的变体中,有3,375,252个(每个个体中位数为10,260个)同义变体、7,689,495个(每个个体9,284个)错义变体和889,957个(每个个体212个)推定功能丧失(pLOF)变体(数据未示出),其中约一半是在此数据集中仅观察到一次(单例变体;数据未示出)。

[0229] 对UK Biobank的成像部分进行了分析,其中包括36,968个个体。以GWAS信号为条件,对来源于磁共振成像(MRI)的2,077个脑成像表型进行了全外显子组关联分析,以将罕

见变体关联与常见变体信号分开。总体而言,在P≤10^{-7}下发现了与52个基因的343个关联,并且在P≤10^{-10}的更保守的显著性阈值下发现了与七个基因的98个关联(数据未示出)。与GWC最强的特质增加关联是ST6GALNAC5中的有害错义变体(rs756654226,9个携带者;效应=1.7SD单位,95% CI 1.1至2.4,P=8.2x 10^{-8})。这与当前的证据相一致,即大脑中特定神经节苷脂的相对丰度随年龄而变化并且在常见神经系统病状,包括亨廷顿病(Huntington's disease)、阿尔茨海默氏病(Alzheimer's disease)、帕金森病(Parkinson's disease)、肌萎缩性侧索硬化症、中风、多发性硬化症和癫痫中会发生变化。

[0230] 除了本文所述的修改之外,所描述主题的各种修改对于本领域技术人员来说将从前述描述中显而易见。此类修改也意图落在随附权利要求书的范围内。本申请中引用的每篇参考文献(包括但不限于期刊文章、美国和非美国专利、专利申请公布、国际专利申请公布、基因库登录号等)均通过引用以其整体且出于所有目的并入本文中。

序列表

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G·阿贝卡西斯(Abecasis, Goncalo)
A·巴拉斯(Baras, Aris)

<120> 使用α-N-乙酰半乳糖胺α-2,6-唾液酸转移酶5(ST6GALNAC5)抑制剂治疗认知障碍

<130> 189238.08002 (3523) (11027WO01)

<160> 62

<170> PatentIn version 3.5

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<211> 200067

<212> DNA

<213> 智人(homo sapien)

<400> 1

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aaacuaugga caugauuucu gaagagcaca ucuccacuga cuuucauaaa gcaaaugucc 1680

aauauuuauu uauugagagu uuuuuagugc aaucugggcc aguauuuuua uagauuauga 1740
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gaauuagguu acucuguuca caaugcucau uguuagcaug caauugguau uugacuugga 1860
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<210> 6
<211> 1291
<212> RNA
<213> 智人(homo sapien)
<400> 6

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ccuccaggaa aaaguggccc cggacgcgcg agccugagga uucugcacaa aagaggugcc 180
caaaaugaag acccugaugc gccauggucu ggcagugugu uuagcgcuca ccaccaugug 240
caccagcuug uugcuagugu acagcagccu cggcggccag aaggagcggc ggagagcagc 300
acccagcagc gccccgggguccccgcggga ccgcggccac uggacggaua ccucggagug 360
gcggaccaca agccccugaa aaugcacugc agggacugug cccuggugac cagcucaggg 420
caucugcugc acagucggca aggcucccag auugaccaga cagagugugu cauccgcaug 480
aaugacgccc ccacacgcgg cuaugggcgu gacgugggca aucgcaccag ccugaggguc 540
aucgcgcauu ccagcaucca gaggauccuc cgcaaccgcc augaccugcu caacgugagc 600
cagggcaccg uguucaucuu cuggggcccc agcagcuaca ugcggcggga cggcaagggc 660
caggucuaca acaaccugca ucuccugagc caggugcugc cccggcugaa ggccuucaug 720
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cucugugaca ggaucaaugu uuauggcaug gugcccccag acuucugcag ggaucccaau 900
cacccuucag uaccuuauca uuauuaugaa ccuuuuggac cugaugaaug uacaauguac 960
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ucacuugcua uaaaucaucc ugagaauaaa ccuguguucu aaggaaugag caugccagac 1140
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caggaaaggg uagcagaaaa cagcuucacu ccucaggaag uaccauggac agacgccuac 1260
caggggugac aaagcagugc aguuggauug u 1291

<210> 7
<211> 2048
<212> RNA
<213> 智人(homo sapien)
<400> 7

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aaaaguggcc ccggacgcgc gagccugagg auucugcaca aaagaggugc ccaaaaugaa 180
gacccugaug cgccaugguc uggcagugug uuuagcgcuc accaccaugu gcaccagcuu 240
guugcuagug uacagcagcc ucggcggcca gaaggagcgg cccccgcagc agcagcagca 300
gcagcagcaa cagcagcagc aggcgucggc caccggcagc ucgcagccgg cggcggagag 360
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aguggcggac cacaagcccc ugaaaaugca cugcagggac ugugcccugg ugaccagcuc 480
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aaaaaaaa 2048

<210> 8
<211> 1041
<212> RNA
<213> 智人(homo sapien)
<400> 8

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ccgcagcagc agcagcagca gcagcaacag cagcagcagg cgucggccac cggcagcucg 180
cagccggcgg cggagagcag cacccagcag cgccccgggg uccccgcggg accgcggcca 240
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gcccugguga ccagcucagg gcaucugcug cacagucggc aaggcuccca gauugaccag 360
acagagugug ucauccgcau gaaugacgcc cccacacgcg gcuaugggcg ugacgugggc 420
aaucgcacca gccugagggu caucgcgcau uccagcaucc agaggauccu ccgcaaccgc 480
caugaccugc ucaacgugag ccagggcacc guguucaucu ucuggggccc cagcagcuac 540
augcggcggg acggcaaggg ccaggucuac aacaaccugc aucuccugag ccaggugcug 600
ccccggcuga aggccuucau gauuacucgc cacaagaugc ugcaguuuga ugagcucuuc 660
aagcaggaga cuggcaaaga caggaagaua uccaacacuu ggcucagcac uggcugguuu 720
acaaugacaa uugcacugga gcucugugac aggaucaaug uuuauggcau ggugccccca 780
gacuucugca gggaucccaa ucacccuuca guaccuuauc auuauuauga accuuuugga 840
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uuuaucacag agaaacgagu cuuuaagaac ugggcacgga cauucaauau ucacuuuuuu 960
caaccagacu ggaaaccaga aucacuugcu auaaaucauc cugagaauaa accuguguuc 1020
uaaggaauga gcaugccaga c 1041

<210> 9
<211> 3751
<212> RNA
<213> 智人(homo sapien)
<400> 9

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cugcugggca aaaaucagag ccgccuccgc cccauuaccc aucauggaaa cccuccagga 180
aaaaguggcc ccggacgcgc gagccugagg auucugcaca aaagaggugc ccaaaaugaa 240
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caugaaugac gcccccacac gcggcuaugg gcgugacgug ggcaaucgca ccagccugag 660
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ggagcucugu gacaggauca auguuuaugg cauggugccc ccagacuucu gcagggaucc 1020
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uccuuuuugg cagaaugaug ucacuguacc agacaaccuc uuagguuuca ucccaaaucu 2220
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aacaccauua aauauaaacu cugauauaaa a 3751

<210> 10
<211> 2055
<212> RNA
<213> 智人(homo sapien)
<400> 10

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caucauggaa acccuccagg aaaaaguggc cccggacgcg cgagccugag gauucugcac 180
aaaagaggug cccaaaauga agacccugau gcgccauggu cuggcagugu guuuagcgcu 240
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gcccccgcag cagcagcagc agcagcagca acagcagcag caggcgucgg ccaccggcag 360
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gggcaaucgc accagccuga gggucaucgc gcauuccagc auccagagga uccuccgcaa 660
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cuacaugcgg cgggacggca agggccaggu cuacaacaac cugcaucucc ugagccaggu 780
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aaaugcuaac aagag 2055

〈210〉 11
〈211〉 5547
〈212〉 RNA
〈213〉 智人(homo sapien)
〈400〉 11

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cgcgcgaucu gccgcggccg gcugcugggc aaaaaucaga gccgccuccg ccccauuacc 120
caucauggaa acccuccagg aaaaaguggc cccggacgcg cgagccugag gauucugcac 180
aaaagaggug cccaaaauga agacccugau gcgccauggu cuggcagugu guuuagcgcu 240
caccaccaug ugcaccagcu uguugcuagu guacagcagc cucggcggcc agaaggagcg 300
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augcaugugu ccuguuaguc uaauuagauu guaagcuccu ugagggcaga gacucuuucu 5460
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ugucuguugu ugaucaugac cucuuaa 5547

<210> 12
<211> 2090
<212> RNA
<213> 智人(homo sapien)
<400> 12

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cugcugggca aaaaucagag ccgccuccgc cccauuaccc aucauggaaa cccuccagga 180
aaaaguggcc ccggacgcgc gagccugagg auucugcaca aaagaggugc ccaaaaugaa 240
gacccugaug cgccaugguc uggcagugug uuuagcgcuc accaccaugu gcaccagcuu 300

guugcuagug uacagcagcc ucggcggcca gaaggagcgg ccccccgcagc agcagcagca 360
gcagcagcaa cagcagcagc aggcgucggc caccggcagc ucgcagccgg cggcggagag 420
cagcacccag cagcgccccg ggguccccgc gggaccgcgg ccacuggacg gauaccucgg 480
aguggcggac cacaagcccc ugaaaaugca cugcagggac ugugcccugg ugaccagcuc 540
agggcaucug cugcacaguc ggcaaggcuc ccagauugac cagacagagu gugucauccg 600
caugaaugac gcccccacac gcggcuaugg gcgugacgcg ggcaaucgca ccagccugag 660
ggucaucgcg cauuccagca uccagaggau ccuccgcaac cgccaugacc ugcucaacgu 720
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guaccucucc caugagcgag gacgcaaggg cagucaucac cgcuuuauca cagagaaacg 1140
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agaaucacuu gcuauaaauc auccugagaa uaaaccugug uucuaaggaa ugagcaugcc 1260
agacuguaau cccagguauu cacugcauca gacaccgaga cacugaacuu ccugagccac 1320
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uacaccacua ugacuaaaaa caguuuggau cucuuaguau ugccuuugaa acugcaacau 1560
aagcaacuca acaauauuag uugcauuccu uuauagacau accaugucaa agacguuuuu 1620
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ugauuuguaa aaagcaggcu gaaacuaugg acaugauuuc ugaagagcac aucuccacug 1740
acuuucauaa agcaaauguc caauauuuau uuauugagag uuuuuuagug caaucugggc 1800
caguauuuuu auagauuaug auuauguggu aauuuauccu uccuaacucu uuaauccuga 1860
augaugguug gaaauggccu agaauuaggu uacucuguuc acaaugcuca uuguuagcau 1920
gcaauuggua uuugacuugg aaguguugug uuguauuuuu ugaaccccua ggcuucagga 1980
aaacugcucu uuuguaaaaa gaauagcgau gacauuuucu aaugugcaga aauguuccaa 2040
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<210> 13
<211> 2017
<212> RNA
<213> 智人(homo sapien)
<400> 13

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caucauggaa acccuccagg aaaaaguggc cccggacgcg cgagccugag gauucugcac 180
aaaagaggug cccaaaauga agacccugau gcgccauggu cuggcagugu guuuagcgcu 240

caccaccaug ugcaccagcu uguugcuagu guacagcagc cucggcggcc agaaggagcg 300
gccccccgcag cagcagcagc agcucgcagc cggcggcgga gagcagcacc cagcagcgcc 360
ccggggcccc cgcgggaccg cggccacugg acggauaccu cggaguggcg gaccacaagc 420
cccugaaaau guacugcagg gacugugccc uggugaccag cucagggcau cugcugcaca 480
gucggcaagg cucccagauu gaccagacag agugugucgu ccgcaugaau gacgccccca 540
cacgcggcua ugggcgugac gcgggcaauc gcaccagccu gagggucauc gcgcauucca 600
gcauccagag gauccucccgc aaccgccaug accugcucaa cgugagccag ggcaccgugu 660
ucaucuucug gggccccagc agcuacaugc ggcgggacgg caagggccag gucuacaaca 720
accugcaucu ccugagccag gugcugcccc ggcugaaggc cuucaugauu acucgccaca 780
agaugcugca guuugaugag cucuucaagc aggagacugg caaagacaga uauccaacac 840
uuggcucagc acuggcuggu uuacaaugac aauugcacug gagcucugug acaggaucaa 900
uguuuauggc auggugcccc cagacuucug cagggauccc aaucacccuu caguaccuua 960
ucauuauuau gaaccuuuug gaccugauga auguacaaug uaccucuccc augagcgagg 1020
acgcaagggc agucaucacc gcuuuaucac agagaaacga gucuuuaaga acugggcacg 1080
gacauucaau auucacuuuu cucaaccaga cuggaaacca gaaucacuug cuauaaauca 1140
uccugagaau aaaccugugu ucuaaggaau gagcaugcca gacuguaauc ccagguauuc 1200
acugcaucag acaccgagac acugaacuuc cugagccacc agacaggaaa ggguagcaga 1260
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ucaauggugu uaccuuagga gcugaacauu caauucaguu acaccacuau gacuaaaaac 1440
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ugcauuccuu uauagacaua ccaugucaaa gacguuuuuc uaucaaguug uauucuuucc 1560
uguucuauaa ccuuugucau cuguuagacu cuguaugugu gauuuguaaa aagcaggcug 1620
aaacuaugga caugauuucu gaagagcaca ucuccacuga cuuucauaaa gcaaaugucc 1680
aauauuuauu uauugagagu uuuuuagugc aaucugggcc aguauuuuua uagauuauga 1740
uuauguggua auuuauccuu ccuaacucuu uaauccugaa ugaugguugg aaauggccua 1800
gaauuagguu acucuguuca caaugcucau uguuagcaug caauugguau uugacuugga 1860
aguguugugu uguauuuuuu gaaccccuag gcuucaggga aacugcucuu uuguaaaaag 1920
aauagcgaug acauuuucua augugcagaa auguuccaaa aggacaaaau ugaaaaccaa 1980
aaacuauguu auuaaaacaa aaaaaugcua acaagag 2017

<210> 14
<211> 1291
<212> RNA
<213> 智人(homo sapien)
<400> 14

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cgcggccggc ugcugggcaa aaaucagagc cgccuccgcc ccauuaccca ucauggaaac 120
ccuccaggaa aaaguggccc cggacgcgcg agccugagga uucugcacaa aagaggugcc 180
caaaaugaag acccugaugc gccauggucu ggcagugugu uuagcgcuca ccaccaugug 240

caccagcuug uugcuagugu acagcagccu cggcggccag aaggagcggc ggagagcagc 300
acccagcagc gccccggggu ccccgcggga ccgcggccac uggacggaua ccucggagug 360
gcggaccaca agccccugaa aaugcacugc agggacugug cccuggugac cagcucaggg 420
caucugcugc acagucggca aggcucccag auugaccaga cagagugugu cauccgcaug 480
aaugacgccc ccacacgcgg cuaugggcgu gacgcgggca aucgcaccag ccugaggguc 540
aucgcgcauu ccagcaucca gaggauccuc cgcaaccgcc augaccugcu caacgugagc 600
cagggcaccg uguucaucuu cuggggcccc agcagcuaca ugcggcggga cggcaagggc 660
caggucuaca acaaccugca ucuccugagc caggugcugc cccggcugaa ggccuucaug 720
auuacucgcc acaagaugcu gcaguuugau gagcucuuca agcaggagac uggcaaagac 780
aggaagauau ccaacacuug gcucagcacu ggcugguuua caaugacaau ugcacuggag 840
cucugugaca ggaucaaugu uuauggcaug gugcccccag acuucugcag ggaucccaau 900
cacccuucag uaccuuauca uuauuaugaa ccuuuuggac cugaugaaug uacaauguac 960
cucucccaug agcgaggacg caagggcagu caucaccgcu uuaucacaga gaaacgaguc 1020
uuuaagaacu gggcacggac auucaauauu cacuuuuuuc aaccagacug gaaaccagaa 1080
ucacuugcua uaaaucaucc ugagaauaaa ccuguguucu aaggaaugag caugccagac 1140
uguaauccca gguauucacu gcaucagaca ccgagacacu gaacuuccug agccaccaga 1200
caggaaaggg uagcagaaaa cagcuucacu ccucaggaag uaccauggac agacgccuac 1260
caggggugac aaagcagugc aguuggauug u 1291

<210> 15
<211> 2048
<212> RNA
<213> 智人(homo sapien)
<400> 15

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aaaaguggcc ccggacgcgc gagccugagg auucugcaca aaagaggugc ccaaaaugaa 180
gacccugaug cgccaugguc uggcagugug uuuagcgcuc accaccaugu gcaccagcuu 240
guugcuagug uacagcagcc ucggcggcca gaaggagcgg cccccgcagc agcagcagca 300
gcagcagcaa cagcagcagc aggcgucggc caccggcagc ucgcagccgg cggcggagag 360
cagcacccag cagcgccccg ggguccccgc gggaccgcgg ccacuggacg gauaccucgg 420
aguggcggac cacaagcccc ugaaaaugca cugcagggac ugugcccugg ugaccagcuc 480
agggcaucug cugcacaguc ggcaaggcuc ccagauugac cagacagagu gugucauccg 540
caugaaugac gcccccacac gcggcuaugg gcgugacgcg ggcaaucgca ccagccugag 600
ggucaucgcg cauuccagca uccagaggau ccuccgcaac cgccaugacc ugcucaacgu 660
gagccagggc accguguuca ucuucugggg ccccagcagc uacaugcggc gggacggcaa 720
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ggagcucugu gacaggauca auguuuaugg cauggugccc ccagacuucu gcagggaucc 960

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cagacaggaa aggguagcag aaaacagcuu cacuccucag gaaguaccau ggacagacgc 1320
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aagcaacuca acaauauuag uugcauuccu uuauagacau accaugucaa agacguuuuu 1560
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ugauuuguaa aaagcaggcu gaaacuaugg acaugauuuc ugaagagcac aucuccacug 1680
acuuucauaa agcaaauguc caauauuuau uuauugagag uuuuuuagug caaucugggc 1740
caguauuuuu auagauuaug auuauguggu aauuuauccu uccuaacucu uuaauccuga 1800
augaugguug gaaauggccu agaauuaggu uacucuguuc acaaugcuca uuguuagcau 1860
gcaauuggua uuugacuugg aaguguugug uuguauuuuu ugaaccccua ggcuucagga 1920
aaacugcucu uuuguaaaaa gaauagcgau gacauuuucu aaugugcaga aauguuccaa 1980
aaggacaaaa uugaaaacca aaaacuaugu uauuaaaaca aaaaaaugcu aaaaaaaaaa 2040
aaaaaaaa 2048

<210> 16
<211> 1041
<212> RNA
<213> 智人(homo sapien)
<400> 16

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ccgcagcagc agcagcagca gcagcaacag cagcagcagg cgucggccac cggcagcucg 180
cagccggcgg cggagagcag cacccagcag cgccccgggg uccccgcggg accgcggcca 240
cuggacggau accucggagu ggcggaccac aagccccuga aaaugcacug cagggacugu 300
gcccugguga ccagcucagg gcaucugcug cacagucggc aaggcuccca gauugaccag 360
acagagugug ucauccgcau gaaugacgcc cccacacgcg gcuaugggcg ugacgcgggc 420
aaucgcacca gccugagggu caucgcgcau uccagcaucc agaggauccu ccgcaaccgc 480
caugaccugc ucaacgugag ccagggcacc guguucaucu ucuggggccc cagcagcuac 540
augcggcggg acggcaaggg ccaggucuac aacaaccugc aucuccugag ccaggugcug 600
ccccggcuga aggccuucau gauuacucgc cacaagaugc ugcaguuuga ugagcucuuc 660
aagcaggaga cuggcaaaga caggaagaua uccaacacuu ggcucagcac uggcugguuu 720
acaaugacaa uugcacugga gcucugugac aggaucaaug uuuauggcau ggugccccca 780
gacuucugca gggaucccaa ucacccuuca guaccuuauc auuauuauga accuuuugga 840
ccugaugaau guacaaugua ccucucccau gagcgaggac gcaagggcag ucaucaccgc 900

uuuaucacag agaaacgagu cuuuaagaac ugggcacgga cauucaauau ucacuuuuuu 960
caaccagacu ggaaaccaga aucacuugcu auaaaucauc cugagaauaa accuguguuc 1020
uaaggaauga gcaugccaga c 1041

<210> 17
<211> 3751
<212> RNA
<213> 智人(homo sapien)
<400> 17

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cugcugggca aaaaucagag ccgccuccgc cccauuaccc aucauggaaa cccuccagga 180
aaaaguggcc ccggacgcgc gagccugagg auucugcaca aaagaggugc ccaaaaugaa 240
gacccugaug cgccaugguc uggcagugug uuuagcgcuc accaccaugu gcaccagcuu 300
guugcuagug uacagcagcc ucggcggcca gaaggagcgg cccccgcagc agcagcagca 360
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agggcaucug cugcacaguc ggcaaggcuc ccagauugac cagacagagu gugucauccg 600
caugaaugac gcccccacac gcggcuaugg gcgugacgcg ggcaaucgca ccagccugag 660
ggucaucgcg cauuccagca uccagaggau ccuccgcaac cgccaugacc ugcucaacgu 720
gagccagggc accguguuca ucuucugggg ccccagcagc uacaugcggc gggacggcaa 780
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ggagcucugu gacaggauca auguuuaugg cauggugccc ccagacuucu gcagggaucc 1020
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guaccucucc caugagcgag gacgcaaggg cagucaucac cgcuuuauca cagagaaacg 1140
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uacaccacua ugacuaaaaa caguuuggau cucuuaguau ugccuuugaa acugcaacau 1560
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caguauuuuu auagauuaug auuauguggu aauuuauccu uccuaacucu uuaauccuga 1860

augaugguug gaaauggccu agaauuaggu uacucuguuc acaaugcuca uuguuagcau 1920
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aaacugcucu uuuguaaaaa gaauagcgau gacauuuucu aaugugcaga aauguuccaa 2040
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uccuuuuugg cagaaugaug ucacuguacc agacaaccuc uuagguuuca ucccaaaucu 2220
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acaguagcag gaacuuuauu uuggggagcc uuaucuacau ucauauuacu uauaucacuc 2340
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ucucgaagca ucaccauccg ccucuucccc agccucucaa cacaaaaaca caggauacac 2460
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<213> 智人(homo sapien)
<400> 20

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〈211〉 2017
〈212〉 DNA
〈213〉 智人(homo sapien)
〈400〉 21

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<400> 22

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<211> 2048
<212> DNA
<213> 智人(homo sapien)
<400> 23

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<210> 24
<211> 1041
<212> DNA
<213> 智人(homo sapien)
<400> 24

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<210> 25
<211> 3751
<212> DNA
<213> 智人(homo sapien)
<400> 25

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<210> 26
<211> 2055
<212> DNA
<213> 智人(homo sapien)
<400> 26

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<210> 27
<211> 5547
<212> DNA
<213> 智人(homo sapien)
<400> 27

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〈210〉 28

<211> 2090
<212> DNA
<213> 智人(homo sapien)
<400> 28

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<210> 29
<211> 2017
<212> DNA
<213> 智人(homo sapien)
<400> 29

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<210> 30
<211> 1291
<212> DNA
<213> 智人(homo sapien)
<400> 30

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<210> 31
<211> 2048
<212> DNA
<213> 智人(homo sapien)
<400> 31

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<210> 32
<211> 1041
<212> DNA
<213> 智人(homo sapien)
<400> 32

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<210> 33
<211> 3751
<212> DNA
<213> 智人(homo sapien)
<400> 33

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cacatatctg tctatcttca gattttagat tttctttatt aaaaaaacca tcatggtagc 3420
tccatctcgt ttgtaacttc tccctctcaa atactcagaa gaatgaagag gccaaattca 3480
gaaaatcatg gcttcactgg gaaatgtttt aaatctacat gtagctagga tatgtgattt 3540
catatttttt aaaaatgtgg tgtaaataag tgtaaaaaat taccacttgc aaagtggttt 3600
tattacagcg ttaaagaaaa tgactagaat tctgatagat tgtgctgatg aacctggttg 3660

tgggcaattg aattcttgtc ttcccactac catttcatga ctgtcgtctg aacactattc 3720
aacaccatta aatataaact ctgatataaa a 3751

<210> 34
<211> 2055
<212> DNA
<213> 智人(homo sapien)
<400> 34

agttttaatt ctgtctctaa tctctgcaac agccgcgctt cccgggtccc gcggctcccg 60
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catcatggaa accctccagg aaaaagtggc cccggacgcg cgagcctgag gattctgcac 180
aaaagaggtg cccaaaatga agaccctgat gcgccatggt ctggcagtgt gtttagcgct 240
caccaccatg tgcaccagct tgttgctagt gtacagcagc ctcggcggcc agaaggagcg 300
gcccccgcag cagcagcagc agcagcagca acagcagcag caggcgtcgg ccaccggcag 360
ctcgcagccg gcggcggaga gcagcaccca gcagcgcccc ggggtccccg cgggaccgcg 420
gccactggac ggatacctcg gagtggcgga ccacaagccc ctgaaaatgc actgcaggga 480
ctgtgccctg gtgaccagct cagggcatct gctgcacagt cggcaaggct cccagattga 540
ccagacagag tgtgtcatcc gcatgaatga cgcccccaca cgcggctatg ggcgtgacgc 600
gggcaatcgc accagcctga gggtcatcgc gcattccagc atccagagga tcctccgcaa 660
ccgccatgac ctgctcaacg tgagccaggg caccgtgttc atcttctggg gccccagcag 720
ctacatgcgg cgggacggca agggccaggt ctacaacaac ctgcatctcc tgagccaggt 780
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taaggaatga gcatgccaga ctgtaatccc aggtattcac tgcatcagac accgagacac 1260
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taactcttta atcctgaatg atggttggaa atggcctaga attaggttac tctgttcaca 1860
atgctcattg ttagcatgca attggtattt gacttggaag tgttgtgttg tatttttga 1920

acccctaggc ttcaggaaaa ctgctctttt gtaaaaagaa tagcgatgac attttctaat 1980
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aaatgctaac aagag 2055

<210> 35
<211> 336
<212> PRT
<213> 智人(homo sapien)
<400> 35

Met Lys Thr Leu Met Arg His Gly Leu Ala Val Cys Leu Ala Leu Thr
1 5 10 15
Thr Met Cys Thr Ser Leu Leu Leu Val Tyr Ser Ser Leu Gly Gly Gln
20 25 30
Lys Glu Arg Pro Pro Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
35 40 45
Gln Ala Ser Ala Thr Gly Ser Ser Gln Pro Ala Ala Glu Ser Ser Thr
50 55 60
Gln Gln Arg Pro Gly Val Pro Ala Gly Pro Arg Pro Leu Asp Gly Tyr
65 70 75 80
Leu Gly Val Ala Asp His Lys Pro Leu Lys Met His Cys Arg Asp Cys
85 90 95
Ala Leu Val Thr Ser Ser Gly His Leu Leu His Ser Arg Gln Gly Ser
100 105 110
Gln Ile Asp Gln Thr Glu Cys Val Ile Arg Met Asn Asp Ala Pro Thr
115 120 125
Arg Gly Tyr Gly Arg Asp Val Gly Asn Arg Thr Ser Leu Arg Val Ile
130 135 140
Ala His Ser Ser Ile Gln Arg Ile Leu Arg Asn Arg His Asp Leu Leu
145 150 155 160
Asn Val Ser Gln Gly Thr Val Phe Ile Phe Trp Gly Pro Ser Ser Tyr
165 170 175
Met Arg Arg Asp Gly Lys Gly Gln Val Tyr Asn Asn Leu His Leu Leu
180 185 190
Ser Gln Val Leu Pro Arg Leu Lys Ala Phe Met Ile Thr Arg His Lys
195 200 205
Met Leu Gln Phe Asp Glu Leu Phe Lys Gln Glu Thr Gly Lys Asp Arg
210 215 220
Lys Ile Ser Asn Thr Trp Leu Ser Thr Gly Trp Phe Thr Met Thr Ile
225 230 235 240
Ala Leu Glu Leu Cys Asp Arg Ile Asn Val Tyr Gly Met Val Pro Pro

245 250 255
Asp Phe Cys Arg Asp Pro Asn His Pro Ser Val Pro Tyr His Tyr Tyr
260 265 270
Glu Pro Phe Gly Pro Asp Glu Cys Thr Met Tyr Leu Ser His Glu Arg
275 280 285
Gly Arg Lys Gly Ser His His Arg Phe Ile Thr Glu Lys Arg Val Phe
290 295 300
Lys Asn Trp Ala Arg Thr Phe Asn Ile His Phe Phe Gln Pro Asp Trp
305 310 315 320
Lys Pro Glu Ser Leu Ala Ile Asn His Pro Glu Asn Lys Pro Val Phe
325 330 335

<210> 36
<211> 146
<212> PRT
<213> 智人(homo sapien)
<400> 36
Met Tyr Cys Arg Asp Cys Ala Leu Val Thr Ser Ser Gly His Leu Leu
1 5 10 15
His Ser Arg Gln Gly Ser Gln Ile Asp Gln Thr Glu Cys Val Val Arg
20 25 30
Met Asn Asp Ala Pro Thr Arg Gly Tyr Gly Arg Asp Val Gly Asn Arg
35 40 45
Thr Ser Leu Arg Val Ile Ala His Ser Ser Ile Gln Arg Ile Leu Arg
50 55 60
Asn Arg His Asp Leu Leu Asn Val Ser Gln Gly Thr Val Phe Ile Phe
65 70 75 80
Trp Gly Pro Ser Ser Tyr Met Arg Arg Asp Gly Lys Gly Gln Val Tyr
85 90 95
Asn Asn Leu His Leu Leu Ser Gln Val Leu Pro Arg Leu Lys Ala Phe
100 105 110
Met Ile Thr Arg His Lys Met Leu Gln Phe Asp Glu Leu Phe Lys Gln
115 120 125
Glu Thr Gly Lys Asp Arg Tyr Pro Thr Leu Gly Ser Ala Leu Ala Gly
130 135 140
Leu Gln
145

<210> 37
<211> 246
<212> PRT

<213> 智人(homo sapien)

<400> 37

Met His Cys Arg Asp Cys Ala Leu Val Thr Ser Ser Gly His Leu Leu
1 5 10 15
His Ser Arg Gln Gly Ser Gln Ile Asp Gln Thr Glu Cys Val Ile Arg
20 25 30
Met Asn Asp Ala Pro Thr Arg Gly Tyr Gly Arg Asp Val Gly Asn Arg
35 40 45
Thr Ser Leu Arg Val Ile Ala His Ser Ser Ile Gln Arg Ile Leu Arg
50 55 60
Asn Arg His Asp Leu Leu Asn Val Ser Gln Gly Thr Val Phe Ile Phe
65 70 75 80
Trp Gly Pro Ser Ser Tyr Met Arg Arg Asp Gly Lys Gly Gln Val Tyr
85 90 95
Asn Asn Leu His Leu Leu Ser Gln Val Leu Pro Arg Leu Lys Ala Phe
100 105 110
Met Ile Thr Arg His Lys Met Leu Gln Phe Asp Glu Leu Phe Lys Gln
115 120 125
Glu Thr Gly Lys Asp Arg Lys Ile Ser Asn Thr Trp Leu Ser Thr Gly
130 135 140
Trp Phe Thr Met Thr Ile Ala Leu Glu Leu Cys Asp Arg Ile Asn Val
145 150 155 160
Tyr Gly Met Val Pro Pro Asp Phe Cys Arg Asp Pro Asn His Pro Ser
165 170 175
Val Pro Tyr His Tyr Tyr Glu Pro Phe Gly Pro Asp Glu Cys Thr Met
180 185 190
Tyr Leu Ser His Glu Arg Gly Arg Lys Gly Ser His His Arg Phe Ile
195 200 205
Thr Glu Lys Arg Val Phe Lys Asn Trp Ala Arg Thr Phe Asn Ile His
210 215 220
Phe Phe Gln Pro Asp Trp Lys Pro Glu Ser Leu Ala Ile Asn His Pro
225 230 235 240
Glu Asn Lys Pro Val Phe
245

<210> 38

<211> 236

<212> PRT

<213> 智人(homo sapien)

<400> 38

Met Lys Thr Leu Met Arg His Gly Leu Ala Val Cys Leu Ala Leu Thr
1 5 10 15
Thr Met Cys Thr Ser Leu Leu Leu Val Tyr Ser Ser Leu Gly Gly Gln
20 25 30
Lys Glu Arg Pro Pro Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
35 40 45
Gln Ala Ser Ala Thr Gly Ser Ser Gln Pro Ala Ala Glu Ser Ser Thr
50 55 60
Gln Gln Arg Pro Gly Val Pro Ala Gly Pro Arg Pro Leu Asp Gly Tyr
65 70 75 80
Leu Gly Val Ala Asp His Lys Pro Leu Lys Met His Cys Arg Asp Cys
85 90 95
Ala Leu Val Thr Ser Ser Gly His Leu Leu His Ser Arg Gln Gly Ser
100 105 110
Gln Ile Asp Gln Thr Glu Cys Val Ile Arg Met Asn Asp Ala Pro Thr
115 120 125
Arg Gly Tyr Gly Arg Asp Val Gly Asn Arg Thr Ser Leu Arg Val Ile
130 135 140
Ala His Ser Ser Ile Gln Arg Ile Leu Arg Asn Arg His Asp Leu Leu
145 150 155 160
Asn Val Ser Gln Gly Thr Val Phe Ile Phe Trp Gly Pro Ser Ser Tyr
165 170 175
Met Arg Arg Asp Gly Lys Gly Gln Val Tyr Asn Asn Leu His Leu Leu
180 185 190
Ser Gln Val Leu Pro Arg Leu Lys Ala Phe Met Ile Thr Arg His Lys
195 200 205
Met Leu Gln Phe Asp Glu Leu Phe Lys Gln Glu Thr Gly Lys Asp Arg
210 215 220
Tyr Pro Thr Leu Gly Ser Ala Leu Ala Gly Leu Gln
225 230 235

<210> 39
<211> 336
<212> PRT
<213> 智人(homo sapien)
<400> 39

Met Lys Thr Leu Met Arg His Gly Leu Ala Val Cys Leu Ala Leu Thr
1 5 10 15
Thr Met Cys Thr Ser Leu Leu Leu Val Tyr Ser Ser Leu Gly Gly Gln
20 25 30

Lys Glu Arg Pro Pro Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
35 40 45
Gln Ala Ser Ala Thr Gly Ser Ser Gln Pro Ala Ala Glu Ser Ser Thr
50 55 60
Gln Gln Arg Pro Gly Val Pro Ala Gly Pro Arg Pro Leu Asp Gly Tyr
65 70 75 80
Leu Gly Val Ala Asp His Lys Pro Leu Lys Met His Cys Arg Asp Cys
85 90 95
Ala Leu Val Thr Ser Ser Gly His Leu Leu His Ser Arg Gln Gly Ser
100 105 110
Gln Ile Asp Gln Thr Glu Cys Val Ile Arg Met Asn Asp Ala Pro Thr
115 120 125
Arg Gly Tyr Gly Arg Asp Ala Gly Asn Arg Thr Ser Leu Arg Val Ile
130 135 140
Ala His Ser Ser Ile Gln Arg Ile Leu Arg Asn Arg His Asp Leu Leu
145 150 155 160
Asn Val Ser Gln Gly Thr Val Phe Ile Phe Trp Gly Pro Ser Ser Tyr
165 170 175
Met Arg Arg Asp Gly Lys Gly Gln Val Tyr Asn Asn Leu His Leu Leu
180 185 190
Ser Gln Val Leu Pro Arg Leu Lys Ala Phe Met Ile Thr Arg His Lys
195 200 205
Met Leu Gln Phe Asp Glu Leu Phe Lys Gln Glu Thr Gly Lys Asp Arg
210 215 220
Lys Ile Ser Asn Thr Trp Leu Ser Thr Gly Trp Phe Thr Met Thr Ile
225 230 235 240
Ala Leu Glu Leu Cys Asp Arg Ile Asn Val Tyr Gly Met Val Pro Pro
245 250 255
Asp Phe Cys Arg Asp Pro Asn His Pro Ser Val Pro Tyr His Tyr Tyr
260 265 270
Glu Pro Phe Gly Pro Asp Glu Cys Thr Met Tyr Leu Ser His Glu Arg
275 280 285
Gly Arg Lys Gly Ser His His Arg Phe Ile Thr Glu Lys Arg Val Phe
290 295 300
Lys Asn Trp Ala Arg Thr Phe Asn Ile His Phe Phe Gln Pro Asp Trp
305 310 315 320
Lys Pro Glu Ser Leu Ala Ile Asn His Pro Glu Asn Lys Pro Val Phe
325 330 335

<210> 40

<211> 146
<212> PRT
<213> 智人(homo sapien)
<400> 40

Met Tyr Cys Arg Asp Cys Ala Leu Val Thr Ser Ser Gly His Leu Leu
1 5 10 15
His Ser Arg Gln Gly Ser Gln Ile Asp Gln Thr Glu Cys Val Val Arg
20 25 30
Met Asn Asp Ala Pro Thr Arg Gly Tyr Gly Arg Asp Ala Gly Asn Arg
35 40 45
Thr Ser Leu Arg Val Ile Ala His Ser Ser Ile Gln Arg Ile Leu Arg
50 55 60
Asn Arg His Asp Leu Leu Asn Val Ser Gln Gly Thr Val Phe Ile Phe
65 70 75 80
Trp Gly Pro Ser Ser Tyr Met Arg Arg Asp Gly Lys Gly Gln Val Tyr
85 90 95
Asn Asn Leu His Leu Leu Ser Gln Val Leu Pro Arg Leu Lys Ala Phe
100 105 110
Met Ile Thr Arg His Lys Met Leu Gln Phe Asp Glu Leu Phe Lys Gln
115 120 125
Glu Thr Gly Lys Asp Arg Tyr Pro Thr Leu Gly Ser Ala Leu Ala Gly
130 135 140
Leu Gln
145

<210> 41
<211> 246
<212> PRT
<213> 智人(homo sapien)
<400> 41

Met His Cys Arg Asp Cys Ala Leu Val Thr Ser Ser Gly His Leu Leu
1 5 10 15
His Ser Arg Gln Gly Ser Gln Ile Asp Gln Thr Glu Cys Val Ile Arg
20 25 30
Met Asn Asp Ala Pro Thr Arg Gly Tyr Gly Arg Asp Ala Gly Asn Arg
35 40 45
Thr Ser Leu Arg Val Ile Ala His Ser Ser Ile Gln Arg Ile Leu Arg
50 55 60
Asn Arg His Asp Leu Leu Asn Val Ser Gln Gly Thr Val Phe Ile Phe
65 70 75 80

Trp Gly Pro Ser Ser Tyr Met Arg Arg Asp Gly Lys Gly Gln Val Tyr
85 90 95
Asn Asn Leu His Leu Leu Ser Gln Val Leu Pro Arg Leu Lys Ala Phe
100 105 110
Met Ile Thr Arg His Lys Met Leu Gln Phe Asp Glu Leu Phe Lys Gln
115 120 125
Glu Thr Gly Lys Asp Arg Lys Ile Ser Asn Thr Trp Leu Ser Thr Gly
130 135 140
Trp Phe Thr Met Thr Ile Ala Leu Glu Leu Cys Asp Arg Ile Asn Val
145 150 155 160
Tyr Gly Met Val Pro Pro Asp Phe Cys Arg Asp Pro Asn His Pro Ser
165 170 175
Val Pro Tyr His Tyr Tyr Glu Pro Phe Gly Pro Asp Glu Cys Thr Met
180 185 190
Tyr Leu Ser His Glu Arg Gly Arg Lys Gly Ser His His Arg Phe Ile
195 200 205
Thr Glu Lys Arg Val Phe Lys Asn Trp Ala Arg Thr Phe Asn Ile His
210 215 220
Phe Phe Gln Pro Asp Trp Lys Pro Glu Ser Leu Ala Ile Asn His Pro
225 230 235 240
Glu Asn Lys Pro Val Phe
245

<210> 42
<211> 236
<212> PRT
<213> 智人(homo sapien)
<400> 42

Met Lys Thr Leu Met Arg His Gly Leu Ala Val Cys Leu Ala Leu Thr
1 5 10 15
Thr Met Cys Thr Ser Leu Leu Leu Val Tyr Ser Ser Leu Gly Gly Gln
20 25 30
Lys Glu Arg Pro Pro Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
35 40 45
Gln Ala Ser Ala Thr Gly Ser Ser Gln Pro Ala Ala Glu Ser Ser Thr
50 55 60
Gln Gln Arg Pro Gly Val Pro Ala Gly Pro Arg Pro Leu Asp Gly Tyr
65 70 75 80
Leu Gly Val Ala Asp His Lys Pro Leu Lys Met His Cys Arg Asp Cys
85 90 95

Ala Leu Val Thr Ser Ser Gly His Leu Leu His Ser Arg Gln Gly Ser
100 105 110
Gln Ile Asp Gln Thr Glu Cys Val Ile Arg Met Asn Asp Ala Pro Thr
115 120 125
Arg Gly Tyr Gly Arg Asp Ala Gly Asn Arg Thr Ser Leu Arg Val Ile
130 135 140
Ala His Ser Ser Ile Gln Arg Ile Leu Arg Asn Arg His Asp Leu Leu
145 150 155 160
Asn Val Ser Gln Gly Thr Val Phe Ile Phe Trp Gly Pro Ser Ser Tyr
165 170 175
Met Arg Arg Asp Gly Lys Gly Gln Val Tyr Asn Asn Leu His Leu Leu
180 185 190
Ser Gln Val Leu Pro Arg Leu Lys Ala Phe Met Ile Thr Arg His Lys
195 200 205
Met Leu Gln Phe Asp Glu Leu Phe Lys Gln Glu Thr Gly Lys Asp Arg
210 215 220
Tyr Pro Thr Leu Gly Ser Ala Leu Ala Gly Leu Gln
225 230 235

<210> 43
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 43
cttgtggcga gtaatcatga 20

<210> 44
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 44
atgaatgacg cccccacacg 20

<210> 45
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>

<223> 合成序列;gRNA识别序列
<400> 45
ggacggatac ctcggagtgg 20
<210> 46
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 46
cgcggctatg ggcgtgacgt 20
<210> 47
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 47
gtgtgggggc gtcattcatg 20
<210> 48
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 48
gctgtacact agcaacaagc 20
<210> 49
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 49
ggggccccag cagctacatg 20
<210> 50
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)

<220>
<223> 合成序列;gRNA识别序列
<400> 50
ccttgtggtc cgccactccg 20
<210> 51
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 51
cctcggagtg gcggaccaca 20
<210> 52
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 52
actccgaggt atccgtccag 20
<210> 53
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 53
tgggcaatcg caccagcctg 20
<210> 54
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 54
agcagctaca tgcggcggga 20
<210> 55
<211> 20
<212> DNA

<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 55
aggtatccgt ccagtggccg 20
<210> 56
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 56
gtcacgccca tagccgcgtg 20
<210> 57
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 57
cacgcccata gccgcgtgtg 20
<210> 58
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 58
tcgcgcattc cagcatccag 20
<210> 59
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 59
acgcccatag ccgcgtgtgg 20
<210> 60
<211> 20

<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 60
gcgggcatgc tgtcaccttg 20
<210> 61
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 61
catctgctgc acagtcggca 20
<210> 62
<211> 20
<212> DNA
<213> 人工序列(artificial sequence)
<220>
<223> 合成序列;gRNA识别序列
<400> 62
cagcagcagg cgtcggccac 20