

cassettes, vectors, and pharmaceutical compositions for the purpose of providing transgene expression in target tissues that is capable of endogenous regulation for treating disorders such as dose-sensitive intellectual ability disorders.

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SUMMARY

The present disclosure is based, in part, on the development of feedback-enabled synthetic genes, polynucleotide target cassettes, vectors, and pharmaceutical compositions for the purpose of providing transgene expression in target tissues that is capable of endogenous regulation for treating disorders such as dose-sensitive intellectual ability disorders such as Rett Syndrome (RTT). This disclosure was made in part with support from the Rett Syndrome Research Trust.

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Thus, one aspect of the disclosure relates to a synthetic gene comprising a polynucleotide comprising a coding region encoding MeCP2 and one or more regulatory regions, wherein the polynucleotide further comprises three or more nucleic acid segments each comprising a seed match identified as a binding site for an endogenous miRNA and a 5' flanking sequence and a 3' flanking sequence neighboring said seed match, wherein said one or more nucleic acid segments are inserted into a regulatory region of said polynucleotide such that expression of MeCP2 when said synthetic gene is delivered to a cell expressing the endogenous miRNA is reduced relative to expression of a MeCP2 when a synthetic gene that does not comprise the one or more nucleic acid segments is delivered to a cell expressing the endogenous miRNA, wherein (i) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p, or (ii) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p, and wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:2.

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A further aspect of the disclosure relates to vectors and pharmaceutical compositions comprising the synthetic gene of the present disclosure.

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Another example of the disclosure relates to a polynucleotide target cassette for providing dose dependent inhibitory feedback to the synthetic gene of the present disclosure.

An additional example relates to a method of preparing a synthetic gene, comprising the step of inserting the polynucleotide target cassette into a regulatory region of the synthetic gene.

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A further example of the disclosure relates to a method of making a synthetic gene comprising the steps of inserting one or more nucleic acid segments comprising a seed match and 5' and 3' flanking sequences into a regulatory region of the polynucleotide of the synthetic gene.

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In one aspect of the present disclosure refers to a polynucleotide target cassette for providing dose dependent inhibitory feedback to a synthetic gene, the synthetic gene comprising a polynucleotide comprising a coding region encoding MeCP2 and one or more regulatory regions, and the cassette comprising one or more nucleic acid segments comprising a seed match identified as a binding site for an endogenous miRNA and 5' and 3' flanking sequences neighboring said seed match, wherein (i) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p, miR-23-3p, miR-

218-5p, miR-27-3p, and let-7-5p, or (ii) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p; and wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:2.

Another example of the disclosure relates to a method of identifying one or more seed matches and flanking sequences to be inserted into a synthetic gene, comprising the steps of identifying a seed match and flanking sequences, and inserting said seed match and flanking sequences into a regulatory region of a synthetic gene.

5 An additional example of the disclosure relates to a method of delivering a synthetic gene to a subject, comprising administering to the subject the synthetic gene, vectors, or pharmaceutical composition of the present disclosure. A further example of the disclosure relates to a method of treating a disease associated with abnormal expression of an endogenous gene, comprising administering the synthetic genes, vectors, or pharmaceutical compositions of the present disclosure, thereby treating the disease. In another aspect, the present disclosure refers to a method of preparing a synthetic gene comprising a polynucleotide comprising a coding region encoding a protein or nucleic acid of interest and one or more regulatory regions, comprising the step of inserting the polynucleotide target cassette as disclosed herein into a regulatory region of the synthetic gene. In another aspect, the present disclosure refers to a method of making the synthetic gene as described herein, comprising the steps of: inserting three or more nucleic acid segments each comprising a seed match identified as a binding site for an endogenous miRNA and 5' and 3' flanking sequences neighboring said seed match into a regulatory region of the polynucleotide comprising a coding region encoding MeCP2 and one or more regulatory regions, wherein (i) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p; or (ii) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p. In yet another aspect, the present disclosure refers to a use of the synthetic gene, the vector, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a disease associated with abnormal expression of an endogenous *MECP2* gene or expression of a mutant protein encoded by an endogenous *MECP2* gene in a subject.

25 Another example of the disclosure relates to a method of treating a disease associated with abnormal expression of an endogenous gene or expression of a mutant protein encoded by an endogenous gene in a subject, comprising genetically knocking down the endogenous gene in a cell of the subject, and administering the synthetic genes, vectors, or pharmaceutical compositions of the present disclosure, thereby treating the disease.

30 These and other examples of the disclosure are set forth in more detail in the description of the disclosure below.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a feedback loop depicting how MeCP2-driven miRNAs may attenuate toxic overexpression of exogenous MeCP2.

FIG. 2 shows sample microarray data. The selected data quantifies miRNA expression levels in MeCP2-null versus wild-type (WT) tissue.

FIG. 3 shows miRNAs with increased mean expression levels in saline-treated WT mice (compared to saline-treated KO mice). miRNAs with statistically significant increases in expression (in at least 1 of the 3 tissue types) are indicated (*). False positive hits that are also upregulated in response to AAV9/*EGFP* (in any tissue) are indicated (**). n=2 screening replicates per biological sample; n=3 mice per treatment group.

FIG. 4 shows miRNAs with increased expression in either cervical cord (CC) or medulla in correlation with endogenous MeCP2 expression. Each data point is the average of 2 screening replicates for a single mouse. Groups that are significantly different from each other are boxed in red. n=3 mice per treatment group.

What is claimed is:

1. A synthetic gene comprising:

a polynucleotide comprising a coding region encoding MeCP2 and one or more regulatory regions;

the polynucleotide further comprising three or more nucleic acid segments each comprising a seed match identified as a binding site for an endogenous miRNA and a 5' flanking sequence and a 3' flanking sequence neighboring said seed match;

wherein said one or more nucleic acid segments are inserted into a regulatory region of said polynucleotide such that expression of MeCP2 when said synthetic gene is delivered to a cell expressing the endogenous miRNA is reduced relative to expression of a MeCP2 when a synthetic gene that does not comprise the one or more nucleic acid segments is delivered to a cell expressing the endogenous miRNA, wherein (i) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p, or (ii) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p, and

wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:2.

2. The synthetic gene of claim 1, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p.

3. The synthetic gene of claim 1, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.

4. The synthetic gene of any one of claims 1-3, wherein the seed match is 5 to 10 nucleotides in length.

5. The synthetic gene of any one of claims 1-3, wherein the seed match is 6 to 8 nucleotides in length.

6. The synthetic gene of any one of claims 1-3, wherein the 5' and 3' flanking sequences neighboring the seed match are each 9 to 13 nucleotides in length.

7. The synthetic gene of any one of claims 1-3, wherein the 5' and 3' flanking sequences neighboring the seed match are each 11 nucleotides in length.
8. The synthetic gene of any one of claims 1-3, wherein the polynucleotide comprises at least three seed matches and the seed matches are separated by 7 to 40 nucleotides.
9. The synthetic gene of claim 8, wherein the at least three seed matches are separated by 20 to 25 nucleotides.
10. The synthetic gene of claim 8, wherein the at least three seed matches are separated by 22 nucleotides.
11. The synthetic gene of any one of claims 1-10 wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:1.
12. The synthetic gene of any one of claims 1-10 wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:2.
13. A vector comprising the synthetic gene of any one of claims 1-12.
14. The vector of claim 13, wherein the vector is a plasmid, a viral vector, an expression cassette, a transformed cell or a nanoparticle.
15. A pharmaceutical composition comprising the synthetic gene of any one of claims 1-11 or the vector of claim 13 or 14 and a pharmaceutically acceptable carrier.
16. A polynucleotide target cassette for providing dose dependent inhibitory feedback to a synthetic gene, the synthetic gene comprising a polynucleotide comprising a coding region encoding MeCP2 and one or more regulatory regions, and the cassette comprising one or more nucleic acid segments comprising a seed match identified as a binding site for an endogenous miRNA and 5' and 3' flanking sequences neighboring said seed match, wherein (i) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-

26-5p, miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p, or (ii) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p; and wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:2.

17. The polynucleotide target cassette of claim 16, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p.

18. The polynucleotide target cassette of claim 16, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.

19. The polynucleotide target cassette of any one of claims 16-18, wherein the seed match is 5 to 10 nucleotides in length.

20. The polynucleotide target cassette of any one of claims 16-18, wherein the seed match is 6 to 8 nucleotides in length.

21. The polynucleotide target cassette of any one of claims 16-20, wherein the 5' and 3' flanking sequences neighboring the seed match are each 9 to 13 nucleotides in length.

22. The polynucleotide target cassette of any one of claims 16-20, wherein the 5' and 3' flanking sequences neighboring the seed match are each 11 nucleotides in length.

23. The polynucleotide target cassette of any one of claims 16-22, wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:1.

24. The polynucleotide target cassette of any one of claims 16-22, wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:2.

25. A method of preparing a synthetic gene comprising a polynucleotide comprising a coding region encoding a protein or nucleic acid of interest and one or more regulatory

regions, comprising the step of inserting the polynucleotide target cassette of any one of claims 16-24 into a regulatory region of the synthetic gene.

26. A method of making the synthetic gene of any one of claims 1-12, comprising the steps of:

inserting three or more nucleic acid segments each comprising a seed match identified as a binding site for an endogenous miRNA and 5' and 3' flanking sequences neighboring said seed match into a regulatory region of the polynucleotide comprising a coding region encoding MeCP2 and one or more regulatory regions,

wherein (i) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p; or (ii) the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.

27. The method of claim 25 or 26, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-9-5p, miR-26-5p, miR-23-3p, miR-218-5p, miR-27-3p, and let-7-5p.

28. The method of claim 25 or 26, wherein the seed matches and 5' and 3' flanking sequences bind to the miRNAs miR-690, miR-451a, and let-7-5p.

29. The method of claim 26, further comprising the steps of:

screening for miRNAs with increased expression when the protein or nucleic acid of interest is expressed in a cell relative to when the protein or nucleic acid of interest is not expressed; identifying a seed match and 5' and 3' flanking sequences for three or more miRNAs having increased expression; and

preparing the nucleic acid segment comprising said seed match and 5' and 3' flanking sequences to be inserted into a regulatory region of said polynucleotide.

30. The method of any one of claims 26-29, wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:1.

31. The method of any one of claims 26-29, wherein the seed matches and flanking 3' and 5' sequences neighboring the seed matches comprise the nucleotide sequence set forth in SEQ ID NO:2.

32. Use of the synthetic gene of any one of claims 1-12, the vector of claim 13 or 14, or the pharmaceutical composition of claim 15 in the manufacture of a medicament for treating a disease associated with abnormal expression of an endogenous *MECP2* gene or expression of a mutant protein encoded by an endogenous *MECP2* gene in a subject.

33. The use of claim 32, wherein the disease is Rett syndrome.