METHODS FOR DIAGNOSING AND ASSESSING TREATMENT FOR CUSHING'S SYNDROME

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/163,130, filed on May 18, 2015, the contents of which are hereby incorporated in the entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] Glucocorticoids (GCs) are a class of steroid hormones that bind to and activate the glucocorticoid receptor (GR), which is present in almost every vertebrate cell. The GR is pleiotropic and regulates a variety of important pathways in the vertebrate organism, for example, metabolism, immunity, and development. As such, detection of GR activity or regulation can be used to diagnose a variety of different vertebrate diseases, or assess a clinical or biochemical response to treatments that modulate GR activity.

[0003] For example, detection of GR activity or regulation can be used for detection of various forms of Cushing's syndrome. As another example, glucocorticoid receptor antagonists (GRAs) can be administered to a patient to treat a number of different diseases and conditions, and detection of a change in GR activity in response to administration of the GRA can indicate or assess a clinical or biochemical response to the treatment. However, current methods and compositions for assessing GR activity suffer from one or more of the following insufficiencies: high cost, low sensitivity, low specificity, high false positive rate, or high false negative rate. Therefore, there remains a need for improved methods and compositions for detection of GR activity.

BRIEF SUMMARY

[0003a] In one aspect of the present disclosure, there is provided an in vitro method for assessing a biochemical response to a glucocorticoid receptor antagonist (GRA) in a human subject, the method comprising:

a) measuring a first amount, or activity of 51 kDa FK506 binding protein (FKBP5 protein) or a first expression level of a gene encoding FKBP5 protein in a first sample obtained from the subject, wherein:

i) the first sample comprises primary cells; and

ii) the first sample is obtained before the GRA is administered to the subject;

b) measuring a second amount or activity of FKBP5 protein or a second expression level of a gene encoding FKBP5 protein in a second sample obtained from the subject, wherein:

i) the second sample comprises primary cells; and

ii) the second sample is obtained after the GRA is administered to the subject; and

c) comparing the first and second amounts, activities, or expression levels,

wherein a reduction in the amount or activity of FKBP5 protein or a reduction in the expression level of the gene encoding FKBP5 protein in the second sample indicates the biochemical response to the GRA.

[0003b] In another aspect of the present disclosure, there is provided an in vitro method for diagnosing Cushing's syndrome in a human subject, the method comprising:

a) measuring an amount, or activity of 51 kDa FK506 binding protein (FKBP5 protein) or an expression level of a gene encoding FKBP5 protein in a sample obtained from the subject, wherein the sample comprises primary cells; and

b) identifying the subject as likely to be suffering from Cushing's syndrome when the amount, activity or expression level is high relative to a control.

[0003c] In yet another aspect of the present disclosure, there is provided an in vitro method for assessing a biochemical response in a human subject to administering to the subject a medical or surgical therapy for treatment of hypercortisolemia, the method comprising:

a) measuring a first amount, or activity of 51 kDa FK506 binding protein (FKBP5 protein) or a first expression level of a gene encoding FKBP5 protein in a first sample obtained from the subject, wherein:

i) the first sample comprises primary cells; and

ii) the first sample is obtained before administering the medical or surgical therapy for treatment of hypercortisolemia to the subject;

b) measuring a second amount or activity of FKBP5 protein or a second expression level of a gene encoding FKBP5 protein in a second sample obtained from the subject, wherein:

i) the second sample comprises primary cells; and

ii) the second sample is obtained after the medical or surgical therapy for treatment of hypercortisolemia is administered to the subject; and

c) comparing the first and second amounts, activities, or expression levels,

wherein a reduction in the amount or activity of FKBP5 protein or a reduction in the expression level of the gene encoding FKBP5 protein in the second sample indicates the biochemical response to the medical or surgical therapy for treatment of hypercortisolemia.

[0004] The present disclosure provides a method for assessing a clinical or biochemical response to a glucocorticoid receptor antagonist (GRA) in a human subject, the method comprising: a) measuring a first amount, or activity of 51 kDa FK506 binding protein

(FKBP5 protein) or a first expression level of a gene encoding FKBP5 protein in a first sample from the subject, wherein: i) the first sample comprises primary cells; and ii) the first sample is or was obtained before administering the GRA to the subject; b) optionally administering the GRA to the subject; c) measuring a second amount or activity of FKBP5 protein or a second expression level of a gene encoding FKBP5 protein in a second sample from the subject, wherein: i) the second sample comprises primary cells; and ii) the second sample is or was obtained after administering the GRA to the subject; and d) comparing the first and second amounts, activities, or expression levels, wherein a reduction in the amount or activity of FKBP5 protein or a reduction in the expression level of the gene encoding FKBP5 protein in the second sample as compared to the first sample indicates the clinical or biochemical response to the GRA. In some cases, the absence of a reduction indicates a lack of a clinical response or a lack of biochemical response to the GRA.

[0005] The present disclosure also provides a method for assessing a clinical or biochemical response to a GRA in a human subject, the method comprising: a) measuring a first amount, or activity of 51 kDa FK506 binding protein (FKBP5 protein) or a first expression level of a gene encoding FKBP5 protein in a first sample from the subject, wherein: i) the first sample comprises primary cells; and ii) the first sample is or was obtained before administering the GRA to the subject; b) optionally administering the GRA to the subject; c) measuring a second amount or activity of FKBP5 protein or a second expression level of a gene encoding FKBP5 protein in a second sample from the subject, wherein: i) the second sample comprises primary cells; and ii) the second sample is or was obtained after administering the GRA to the subject; d) comparing the first and second amounts, activities, or expression levels to obtain an FKBP5 difference value; e) comparing the difference value to a threshold difference value derived from a cohort of at least 20 or 30 or 50 test individuals; and f) identifying the subject as having or not having the clinical or biochemical response to the GRA based on a comparison of the difference value and threshold difference value. In some cases, the threshold difference value is a threshold reduction value and a presence of a reduction in FKBP5 amount or activity between the first and second sample that is greater than a threshold reduction value indicates a clinical or biochemical response to the GRA. In some cases, the threshold difference value is a threshold reduction value and a presence of a reduction in FKBP5 amount or activity between the first and second sample that is similar to (*e.g.*, within about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, or 70% of) a threshold reduction value indicates a clinical or biochemical response to

administering an increased amount of GRA to the subject in the absence of a detected reduction in the amount or activity of FKBP5 protein or a detected reduction in the expression level of the gene encoding FKBP5 protein in the second sample.

[0009] The present disclosure also provides a method for diagnosing Cushing's syndrome in a human subject, the method comprising: a) measuring an amount, or activity of 51 kDa FK506 binding protein (FKBP5 protein) or an expression level of a gene encoding FKBP5 protein in a sample obtained or provided from the subject, wherein the sample comprises primary cells; and b) identifying the subject as likely to be suffering from Cushing's syndrome when the amount, activity or expression level is high relative to a control. In some cases, the control comprises a value derived from at least 100 or at least 200 test individuals. In some cases, the test individuals are subjects that are known to not exhibit Cushing's syndrome. In some cases, the test individuals are subjects that are known to have normal cortisol levels or are known to lack hypercortisolemia. In some cases, the control comprises a value derived from at least 20 or 30 or 50 test individuals. In some cases, the test individuals are subjects diagnosed with Cushing's syndrome and undergoing therapy with a GRA. In some cases, the test individuals are subjects diagnosed with Cushing's syndrome, undergoing therapy with a GRA, wherein at least one symptom of the Cushing's syndrome is mitigated or eliminated by the GRA therapy.

[0010] The present disclosure also provides a method for assessing a clinical or biochemical response to a GRA in a human subject, the method comprising: a) measuring an amount, or activity of 51 kDa FK506 binding protein (FKBP5 protein) or an expression level of a gene encoding FKBP5 protein in a sample from the subject, wherein: i) the sample comprises primary cells; and ii) the sample is or was obtained after administering the GRA to the subject; d) comparing the amount, activities, or expression level of FKBP5 to a control value derived from a cohort of at least 100 or at least 200 test individuals; and f) identifying the subject as having or not having the clinical or biochemical response to the GRA based on a comparison of the FKBP5 amount, activity or expression level to the control value. In some cases, the at least 100 or at least 200 test individuals are normal subjects that are not otherwise in need of a GRA. In some cases, the at least 100 or at least 200 test individuals do not have Cushing's syndrome. In some cases, the at least 100 or at least 200 test individuals do not have elevated levels of cortisol. In some cases, the at least 100 or at least 200 test individuals do not have, or do not exhibit symptoms of hypercortisolemia.

[0011] In some embodiments, the measuring comprises quantitating an amount of mRNA encoding FKBP5 protein in the primary cells of the sample. In some embodiments, the measuring comprises quantitating the amount of FKBP5 protein in the primary cells of the sample. In some embodiments, the measuring comprises quantitating the amount of FKBP5 protein activity in the primary cells of the sample. In some embodiments, the quantitating the amount of FKBP5 protein activity in the primary cells of the sample comprises quantitating FKBP5 protein peptidyl-prolyl-cis-trans isomerase activity in the primary cells of the sample. In some embodiments, the quantitating the amount of FKBP5 protein activity in the primary cells of the sample comprises quantitating the amount of FKBP5 protein bound to GR in the primary cells of the sample. In some embodiments, the sample obtained from the subject comprises whole blood, or a fraction thereof. In some embodiments, the subject has undergone transsphenoidal surgery before the sample is obtained from the subject. In some embodiments, the sample is obtained from the subject less than eleven days after the subject is treated with transsphenoidal surgery. In some embodiments, the sample is obtained from the subject less than two, four, or six weeks after the subject is treated with transsphenoidal surgery. In some embodiments, the method comprises administering a treatment for Cushing's syndrome when the amount or activity of FKBP5 protein or the expression level of the gene encoding FKBP5 protein is high relative to a control. In some cases, the administering the treatment for Cushing's syndrome comprises administering to the subject a glucocorticoid receptor antagonist (GRA)

[0012] The present disclosure also provides a method for assessing a clinical or biochemical response in a human subject to administering to the subject a medical or surgical therapy for treatment of hypercortisolemia, the method comprising: a) measuring a first amount, or activity of 51 kDa FK506 binding protein (FKBP5 protein) or a first expression level of a gene encoding FKBP5 protein in a first sample from the subject, wherein: i) the first sample comprises primary cells; and ii) the first sample is or was obtained before administering the medical or surgical therapy for treatment of hypercortisolemia to the subject; b) optionally administering the medical or surgical therapy for treatment of hypercortisolemia to the subject; c) measuring a second amount or activity of FKBP5 protein or a second expression level of a gene encoding FKBP5 protein in a second sample from the subject, wherein: i) the second sample comprises primary cells; and ii) the second sample is or was obtained after administering the medical or surgical therapy for treatment of hypercortisolemia to the subject; and d) comparing the first and second amounts, activities, or

expression levels, wherein a reduction in the amount or activity of FKBP5 protein or a reduction in the expression level of the gene encoding FKBP5 protein in the second sample indicates the clinical response or the biochemical response to the medical or surgical therapy for treatment of hypercortisolemia. For example, a reduction in the amount or activity of FKBP5 protein or a reduction in the expression level of the gene encoding FKBP5 protein in the second sample can indicate that the medical or surgical therapy is successful in treating the hypercortisolism.

[0013] The present disclosure also provides a method for assessing a clinical or biochemical response to administering to the subject a medical or surgical therapy for treatment of hypercortisolemia, the method comprising: a) measuring a first amount, or activity of 51 kDa FK506 binding protein (FKBP5 protein) or a first expression level of a gene encoding FKBP5 protein in a first sample from the subject, wherein: i) the first sample comprises primary cells; and ii) the first sample is or was obtained before administering the GRA to the subject; b) optionally administering the medical or surgical therapy for treatment of hypercortisolemia to the subject; c) measuring a second amount or activity of FKBP5 protein or a second expression level of a gene encoding FKBP5 protein in a second sample from the subject, wherein: i) the second sample comprises primary cells; and ii) the second sample is or was obtained after administering the GRA to the subject; d) comparing the first and second amounts, activities, or expression levels to obtain an FKBP5 difference value; e) comparing the difference value to a threshold difference value derived from a cohort of at least 20 or 30 or 50 test individuals; and f) identifying the subject as having or not having the clinical or biochemical response to the GRA based on a comparison of the difference value and threshold difference value. In some cases, the threshold difference value is a threshold reduction value and a presence of a reduction in FKBP5 amount or activity between the first and second sample that is greater than, or similar to, a threshold reduction value indicates a clinical or biochemical response to the GRA. In some cases, the at least 20 or 30 or 50 test individuals are subjects known to have or suspected of having Cushing's syndrome.

[0014] In some embodiments, the medical or surgical therapy for treatment of hypercortisolemia is selected from the group consisting of: inhibition of steroidogenesis, administration of an ACTH modulator, GRA administration, : transsphenoidal surgery, repeat transsphenoidal surgery, unilateral adrenalectomy, bilateral adrenalectomy, radiotherapy, resection of a non-pituitary ACTH-secreting tumor, treatment with a peptide receptor radionuclide therapy, and combinations thereof. In some embodiments, the inhibition of

WHAT IS CLAIMED IS:

1. An in vitro method for assessing a biochemical response to a glucocorticoid receptor antagonist (GRA) in a human subject, the method comprising:

a) measuring a first amount, or activity of 51 kDa FK506 binding protein (FKBP5 protein) or a first expression level of a gene encoding FKBP5 protein in a first sample obtained from the subject, wherein:

i) the first sample comprises primary cells; and

ii) the first sample is obtained before the GRA is administered to the subject;

b) measuring a second amount or activity of FKBP5 protein or a second expression level of a gene encoding FKBP5 protein in a second sample obtained from the subject, wherein:

i) the second sample comprises primary cells; and

ii) the second sample is obtained after the GRA is administered to the subject; and

c) comparing the first and second amounts, activities, or expression levels, wherein a reduction in the amount or activity of FKBP5 protein or a reduction in the expression level of the gene encoding FKBP5 protein in the second sample indicates the biochemical response to the GRA.

2. The in vitro method of claim 1, wherein one or more of i) the first sample is obtained prior to administering multiple doses of GRA to the subject, and the second sample is obtained after administering the multiple doses of GRA to the subject; ii) the measuring of a) and/or b) comprises quantitating an amount of mRNA encoding FKBP5 protein in the sample; iii) the measuring of a) and/or b) comprises quantitating the amount of FKBP5 protein in the sample; or iv) the measuring comprises quantitating the amount of FKBP5 protein activity in the sample.

3. The in vitro method of claim 1, wherein the quantitating the amount of FKBP5 protein activity in the sample comprises measuring FKBP5 protein peptidyl-prolyl-cis-trans isomerase activity in the sample.

4. The in vitro method of claim 1, wherein the quantitating the amount of FKBP5 protein activity in the sample comprises measuring the amount of FKBP5 protein bound to glucocorticoid receptor (GR) in the sample.

5. The in vitro method of claim 1 or 2, wherein the GRA administered to the subject comprises mifepristone.

6. The in vitro method of claim 1 or 2, wherein the GRA administered to the subject is not mifepristone.

7. The in vitro method of claim 6, wherein the GRA administered to the subject comprises a heteroaryl-ketone GRA.

8. The in vitro method of any of claims 1 to 7, wherein the first or second samples comprise whole blood, or a fraction thereof, or wherein the first or second samples comprise nasal epithelial scraping samples, or wherein the first and second samples comprise whole blood, or a fraction thereof.

9. The in vitro method of any of claims 1 to 8, wherein the patient a) is in need of administration of the glucocorticoid receptor antagonist (GRA); b) has elevated levels of cortisol; or c) has cancer and the first and second samples comprise tumor cells.

10. The in vitro method of claim 1, wherein the first and second samples comprise whole blood, or a fraction thereof.

11. The in vitro method of any of claims 1 to 10, wherein the method comprises administering an increased amount of GRA to the subject in the absence of a detected reduction in the

amount or activity of FKBP5 protein or a detected reduction in the expression level of the gene encoding FKBP5 protein in the second sample.

12. An in vitro method for diagnosing Cushing's syndrome in a human subject, the method comprising:

a) measuring an amount, or activity of 51 kDa FK506 binding protein (FKBP5 protein) or an expression level of a gene encoding FKBP5 protein in a sample obtained from the subject, wherein the sample comprises primary cells; and

b) identifying the subject as likely to be suffering from Cushing's syndrome when the amount, activity or expression level is high relative to a control.

13. The in vitro method of claim 12, wherein the measuring comprises quantitating an amount of mRNA encoding FKBP5 protein in the primary cells of the sample.

14. The in vitro method of claim 12, wherein the measuring comprises a) quantitating the amount of FKBP5 protein in the primary cells of the sample, or b) quantitating the FKBP5 protein activity in the primary cells of the sample.

15. The in vitro method of claim 12, 13, or 14, wherein the quantitating the FKBP5 protein activity in the primary cells of the sample comprises quantitating FKBP5 protein peptidyl-prolyl-cis-trans isomerase activity in the primary cells of the sample.

16. The in vitro method of claim 12, 13, or 14, wherein the quantitating the FKBP5 protein activity in the primary cells of the sample comprises quantitating the amount of FKBP5 protein bound to GR in the primary cells of the sample.

17. The in vitro method of any of claims 12, 13, or 14, wherein the sample obtained from the subject comprises whole blood, or a fraction thereof, and optionally wherein the subject has undergone transsphenoidal surgery before the sample is obtained from the subject.

18. The in vitro method of any of claims 12 to 17, wherein the method comprises administering a treatment for Cushing's syndrome when the amount or activity of FKBP5 protein or the expression level of the gene encoding FKBP5 protein is high relative to a control and optionally,

wherein the administering the treatment for Cushing's syndrome comprises administering to the subject a glucocorticoid receptor antagonist (GRA).

19. An in vitro method for assessing a biochemical response in a human subject to administering to the subject a medical or surgical therapy for treatment of hypercortisolemia, the method comprising:

a) measuring a first amount, or activity of 51 kDa FK506 binding protein (FKBP5 protein) or a first expression level of a gene encoding FKBP5 protein in a first sample obtained from the subject, wherein:

i) the first sample comprises primary cells; and

ii) the first sample is obtained before administering the medical or surgical therapy for treatment of hypercortisolemia to the subject;

b) measuring a second amount or activity of FKBP5 protein or a second expression level of a gene encoding FKBP5 protein in a second sample obtained from the subject, wherein:

i) the second sample comprises primary cells; and

ii) the second sample is obtained after the medical or surgical therapy for treatment of hypercortisolemia is administered to the subject; and

c) comparing the first and second amounts, activities, or expression levels,

wherein a reduction in the amount or activity of FKBP5 protein or a reduction in the expression level of the gene encoding FKBP5 protein in the second sample indicates the biochemical response to the medical or surgical therapy for treatment of hypercortisolemia.

20. The in vitro method of claim 19, wherein the medical or surgical therapy for treatment of hypercortisolemia is selected from the group consisting of: inhibition of steroidogenesis, administration of an ACTH modulator, GRA administration, transsphenoidal surgery, repeat transsphenoidal surgery, unilateral adrenalectomy, bilateral adrenalectomy, radiotherapy, resection of a non-pituitary ACTH-secreting tumor, treatment with a peptide receptor radionuclide therapy, and combinations thereof, and optionally, a)

wherein the inhibition of steroidogenesis comprises administration of ketoconazole, levoketoconazole, metyrapone, LCI699, mitotane, aminoglutethimide, etomidate, or a combination thereof; b) wherein the administration of an ACTH modulator comprises administration of a dopamine agonist, somatostatin, a somatostatin analog, retinoic acid, R-roscovitine, or a combination thereof; c) wherein the dopamine agonist is selected from the group consisting of bromocriptine and cabergoline; and d) wherein the method comprises administering an additional medical or surgical therapy for treatment of hypercortisolemia in an absence of a detected reduction in the amount or activity of FKBP5 protein or reduction in the expression level of the gene encoding FKBP5 protein in the second sample.