(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
01 November 2018 (01.11.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/197863 A1

(51) International Patent Classification:
C12Q 1/6883 (2018.01)

(21) International Application Number:
PCT/GB2018/051075

(22) International Filing Date:
24 April 2018 (24.04.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
1706669.7 27 April 2017 (27.04.2017) GB
1720776.2 13 December 2017 (13.12.2017) GB

(71) Applicant: **THE UNIVERSITY OF LIVERPOOL** [GB/GB]; Foundation Building, 765 Brownlow Hill, Liverpool L69 7ZX (GB).

(72) Inventors: **HAWCUTT, Daniel**; c/o Alder Hey Children's Hospital, Eaton Road, Liverpool Merseyside L12 2AP (GB). **PIRMOHAMED, Munir**; c/o Institute of Translational Medicine, Block A Waterhouse Building, 1-5 Brownlow Street, Liverpool Merseyside L69 3GL (GB). **FRANCIS, Ben**; c/o Department of Biostatistics, Block F Waterhouse Building, 1-5 Brownlow Street, Liverpool Merseyside L69 3GL (GB). **JORGENSEN, Andrea**; c/o Department of Biostatistics, Block F Waterhouse Building, 1-5 Brownlow Street, Liverpoool Merseyside L69 3GL (GB).

(74) Agent: **HGF LIMITED**; Document Handling - HGF (Manchester), 1 City Walk, Leeds, Yorkshire LS11 9DX (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: BIOMARKER OF CORTICOSTEROID INDUCED ADRENAL SUPPRESSION

(57) Abstract: The present invention relates to a method of determining an increased risk of corticosteroid induced adrenal suppression in a subject based on the presence of at least one mutation in the PDGFD gene. The invention further relates to methods of selecting a treatment for a subject, methods of treatment of a subject and methods of assessing the suitability of a subject for corticosteroid treatment on the basis of the determination.

WO 2018/197863 A1

BIOMARKER OF CORTICOSTEROID INDUCED ADRENAL SUPPRESSION

FIELD OF THE INVENTION

The present invention relates to a method of determining an increased risk of corticosteroid induced adrenal suppression in a subject, methods of selecting a treatment for a subject, methods of treatment of a subject, and methods of assessing the suitability of a subject for corticosteroid treatment.

BACKGROUND

Corticosteroids are an effective treatment for many conditions. There are numerous different corticosteroid compounds, administered through a range of routes including oral, topical, inhaled, intranasal and intravenous. Inhaled corticosteroids (ICS) are the recommended treatment for adult and paediatric asthma, as well as chronic obstructive pulmonary disorder (COPD) [1-4]. ICS are used frequently, with prescriptions for them costing more than £700M in the UK in 2015 [5].

Asthma is a very common, chronic condition affecting around 5.4 million people in the UK alone. Inhaled corticosteroids are used for treating all but the mildest cases of the disease [1, 2]. There are several different treatments available for those with more severe disease, including increasing the dose of inhaled corticosteroids, or using additional medications.

COPD is also a chronic condition, but more debilitating and complex than asthma, which is also typically treated with inhaled corticosteroids in combination treatments. COPD includes emphysema and bronchitis, and affects around 3 million people in the UK.

However, treatment with corticosteroids has unwanted side effects. The adverse effects of ICS in particular are well described [6]. Corticosteroids can cause, amongst other problems, adrenal suppression. Adrenal suppression means that the adrenal gland does not produce and secrete enough steroid hormones including cortisol, but may also include impaired production of aldosterone (a mineralocorticoid), which regulates sodium conservation, potassium secretion, and water retention. The presentation of adrenal suppression can range from asymptomatic biochemical changes to florid adrenal crisis and death [7-10]. Symptoms can include severe abdominal pains, vomiting, profound muscle weakness and fatigue, extremely low blood pressure, weight loss, kidney failure, lethargy, growth restriction, and changes in mood and personality. It can be very difficult to diagnose clinically, and diagnosis is especially difficult in children. Unfortunately, there is no real explanation for the variability in patient responses to corticosteroid treatment, nor any explanation for why certain patients experience adrenal suppression.

A number of diagnostic tests to assess adrenal function do exist. The Low Dose Short Synacthen® test (LDSST) correlates well with results of an insulin tolerance test (ITT) in adults and is more sensitive for the diagnosis of adrenal insufficiency than the standard dose test [11-13]. However, use of such tests requires that the patient has already been using corticosteroids for a sufficient period of time to be exhibiting symptoms and to therefore be suspected of having adrenal suppression, by this point the patient may have very poor adrenal function. Furthermore, the test is painful and laborious to conduct which is unreasonable for every patient having a common condition such as asthma, especially children.

Despite the damaging nature of corticosteroid induced adrenal suppression, there is currently no test available to predict whether adrenal suppression will occur in a patient in response to corticosteroid treatment. Therefore corticosteroids are still routinely prescribed without knowing or assessing whether the patient is at high or low risk of an adverse reaction which could result in hospitalisation.

It is an aim of one or more of the aspects and embodiments of the invention described herein to address at least one or more of the problems in the art.

SUMMARY OF THE INVENTION

According to a first aspect of the present invention, there is provided a method of identifying an increased risk of corticosteroid induced adrenal suppression in a subject, comprising determining the presence of at least one mutation in the *PDGFD* gene by assaying a sample from the subject, wherein the presence of a mutation is indicative of an increased risk of corticosteroid induced adrenal suppression in the subject.

According to a second aspect of the present invention, there is provided a method of selecting a treatment for a subject having a disease, comprising determining the presence of at least one mutation in the *PDGFD* gene by assaying a sample from the subject, and selecting a conservative corticosteroid treatment in the presence of a mutation in the *PDGFD* gene.

According to a third aspect of the present invention, there is provided a method of selecting a treatment for a subject having a disease, comprising determining the presence of at least one mutation in the *PDGFD* gene by assaying a sample from the subject, and selecting a treatment comprising corticosteroids in the absence of a mutation in the *PDGFD* gene.

According to a fourth aspect of the present invention, there is provided a method of treating a subject having a disease, comprising determining the presence of at least one mutation in

the *PDGFD* gene by assaying a sample from the subject, and treating the subject with a conservative corticosteroid treatment in the presence of a mutation in the *PDGFD* gene.

According to a fifth aspect of the present invention, there is provided a method of treating a subject having a disease, comprising determining the presence of at least one mutation in the *PDGFD* gene by assaying a sample from the subject, and treating the subject with corticosteroids in the absence of a mutation in the *PDGFD* gene.

According to a sixth aspect of the present invention, there is provided a method of assessing the suitability of a subject for corticosteroid treatment, comprising determining the presence of at least one mutation in the *PDGFD* gene by assaying a sample from the subject, wherein the presence of a mutation is indicative that the subject is less suitable for corticosteroid treatment.

According to a seventh aspect of the present invention, there is provided a method of assessing the suitability of a subject for corticosteroid treatment comprising determining the presence of at least one mutation in the *PDGFD* gene by assaying a sample from the subject, wherein the absence of a mutation is indicative that the subject is suitable for corticosteroid treatment.

According to an eighth aspect of the present invention, there is provided use of at least one mutation in the *PDGFD* gene for *in vitro* determination of an increased risk of corticosteroid induced adrenal suppression in a subject.

According to a ninth aspect of the present invention, there is provided a method of determining the presence of a mutation in a subject suspected of having an increased risk of corticosteroid induced adrenal suppression, the method comprising:

(a) providing a sample from the subject; and
(b) determining the presence of at least one mutation in the *PDGFD* gene.

According to a tenth aspect of the present invention, there is provided a corticosteroid for use in the prevention or treatment of a disease in a subject identified as having an increased risk of corticosteroid induced adrenal suppression, wherein the subject is determined to have at least one mutation in the *PDGFD* gene, and the corticosteroid is for use in a conservative treatment.

The methods of each of aspects may, for the sake of brevity, be referred to herein as “methods of the invention”.

In the context of any of the second to seventh, and tenth aspect of the invention, the terms “treatment” and “treating” refer to the total amount of corticosteroid provided to the subject,

the dose of corticosteroid provided to the subject and/or the frequency at which the subject is monitored.

In the context of the second, fourth, or tenth aspects of the invention, the term 'conservative corticosteroid treatment' refers to a corticosteroid treatment which minimises the negative effect of adrenal suppression in a subject with at least one mutation in the *PDGFD* gene. Conservative corticosteroid treatment may comprise reduced corticosteroids, a low dose of corticosteroids, alternative treatments, and/or increased monitoring of the subject, for example. Any such conservative corticosteroid treatments may be used in combination.

In a suitable embodiment, the conservative corticosteroid treatment comprises a low dose of corticosteroids provided to the subject with at least one mutation in the *PDGFD* gene.

The term a 'low dose' of corticosteroid treatment refers to a dose of corticosteroids provided to a subject that is lower than the typical dose of corticosteroid treatment provided to a subject with the same disease. A low dose may be achieved by provision of a smaller dose of the corticosteroid treatment, reduction of the frequency at which the corticosteroid doses are provided, use of alternative treatments, or supplementation of corticosteroid treatment with alternative treatments.

In a suitable embodiment, the conservative corticosteroid treatment comprises reduced corticosteroids provided to the subject with at least one mutation in the *PDGFD* gene.

The term "reduced" corticosteroid treatment refers to the total amount of the corticosteroid treatment administered to a subject being lower than the typical amount of corticosteroid treatment administered to a subject with the same disease. Indeed, a reduced corticosteroid treatment may include no corticosteroids. A reduced corticosteroid treatment may also include treatment with a less potent corticosteroid. A reduced corticosteroid treatment may also include less frequent treatment. It shall be appreciated that treatment with reduced corticosteroids may also include treatment with alternative treatments, or supplementation with alternative treatments.

In a suitable embodiment, the conservative corticosteroid treatment comprises increased monitoring of the subject with at least one mutation in the *PDGFD* gene.

The term 'increased frequency of monitoring' or 'increased monitoring' of a subject refers to more regular testing, assessment and/or surveillance of a subject for adrenal suppression. By way of example, monitoring the subject may include assessing adrenal activity. Methods suitable for assessing adrenal suppression are discussed elsewhere in this specification. Increased frequency of monitoring may be especially useful in subjects which have at least one mutation in the *PDGFD* gene but which are still treated/must still be treated with a

standard dose of corticosteroids. It will be appreciated that a conservative corticosteroid treatment with increased monitoring may in combination utilise treatment with a standard dose of corticosteroids.

A method of the first and sixth aspects, and optionally a method of the eighth and ninth aspects may further comprise the step of selecting a treatment comprising a conservative corticosteroid treatment as defined above, and/or the step of treating the subject with a conservative corticosteroid treatment.

A method of the second aspect may further comprise the step of treating the subject with a conservative corticosteroid treatment as defined above.

A method of the seventh aspect may further comprise the step of selecting a treatment comprising corticosteroids, and/or the step of treating the subject with corticosteroids.

A method of the third aspect may further comprise the step of treating the subject with corticosteroids.

Examples of suitable corticosteroid treatments are described elsewhere herein.

In a method of the first, eighth and ninth aspects, suitably the absence of a mutation in the *PDGFD* gene is indicative of a decreased risk of corticosteroid induced adrenal suppression in the subject. In such an embodiment, the method may further comprise the step of selecting a treatment comprising corticosteroids, and/or the step of treating the subject with corticosteroids.

In a method or use of any of the aspects, the feature 'determining the presence of at least one mutation in the *PDGFD* gene' may refer to assaying for the presence of a *PDGFD* gene comprising a mutation in a sample, or equally may refer to assaying for the presence of a *PDGFD* gene without a mutation i.e. assaying for the presence of a reference or wild type *PDGFD* gene in a sample.

In a method or use of any of the aspects, suitably the presence of a mutation in the *PDGFD* gene indicates that the subject is at an increased risk of corticosteroid induced adrenal suppression and therefore is less suitable for corticosteroid treatment.

In a method or use of any of the aspects, suitably the absence of a mutation in the *PDGFD* gene indicates that the subject is at a decreased risk of corticosteroid induced adrenal suppression and therefore is suitable for corticosteroid treatment.

In a method or use of any of the aspects, the presence or absence of a mutation in the *PDGFD*, and/or the presence or absence of a reference/wild type *PDGFD* gene may be

determined by the use of target molecules indicative of the expression of a mutation in the *PDGFD* gene, or by the use of target molecules indicative of the expression of the reference/wild type *PDGFD* gene respectively.

Suitably, the method of any of the aspects is an *in vitro* method.

The following pages will provide more details of suitable embodiments of these, and other, aspects of the invention. Except for where the context requires otherwise embodiments described with reference to one aspect of the invention may also be applied to other aspects of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors have discovered that mutations in the *PDGFD* gene are strong indicators that a subject will experience adrenal suppression if treated with corticosteroids. Surprisingly, the inventors have found that mutations in the *PDGFD* gene can increase the risk of developing adrenal suppression nearly 6 fold. Therefore, mutations in the *PDGFD* may provide a useful indication as to whether a subject is at risk of developing adrenal suppression.

Accordingly, the methods of the invention can be used to identify those subjects that are at an increased risk of corticosteroid induced adrenal suppression before they are given any corticosteroid treatment.

Therefore, physicians can test subjects, to predict their reaction to corticosteroid treatment and adjust the treatment and/or monitoring schedule accordingly. The methods of the invention therefore provide a step towards personalised medicine. This is a significant development in the field of respiratory diseases such as asthma and COPD, in which physicians have typically relied on the provision of the same regimented corticosteroid treatment to all patients without regard to their individual needs.

This invention therefore helps to improve the efficiency of healthcare and avoids unnecessary suffering of patients by allowing physicians to act predictively rather than reactively to corticosteroid induced adrenal suppression. Furthermore the methods of the invention save on hospitalisations and healthcare resources expended on those subjects having an adverse adrenal reaction to blanket corticosteroid treatments.

The methods may rely on genetic testing of samples from subjects to determine if a mutation is present in the *PDGFD* gene. This assaying is easy to conduct and can be done with less or no invasive or painful testing on the subject, this is especially useful when dealing with children who need to be tested. Large numbers of children have asthma and therefore testing for any adverse adrenal suppression in less invasive, pain-free manner aids in avoiding uncomfortable and time consuming adrenal tests such as the currently available LDSST.

For the avoidance of doubt, and in order to clarify the way in which the present disclosure is to be interpreted, certain terms used in accordance with the present invention will now be defined further.

Adrenal Suppression

Corticosteroid induced adrenal suppression is a reduction in normal adrenal capacity caused by treatment with corticosteroids. The methods of the invention determine if this negative side-effect will be experienced by a subject when treated with corticosteroids.

Adrenal suppression can be determined by assessing adrenal activity, for example, by the low-dose short Synacthen® test (LDSST test) [14, 23, 24]. This test evaluates the ability of the adrenal cortex to produce cortisol after stimulation by synthetic ACTH (tetracosactide; Synacthen ® available from Alliance, Chippenham, UK).

Adrenal suppression is defined as a peak cortisol level of <500nmol/L for adults and <500nmol/L for children using the LDSST test. Suitably, adrenal suppression may be defined as a peak cortisol level of between 500-350nmol/L or lower for children using the LDSST test. Suitably, in the worst cases, adrenal suppression is defined as a peak cortisol level of <350nmol/L for children using the LDSST test

Normal adrenal activity is defined as a peak cortisol level of >500nmol/L for adults and at least >350nmol/L for children using the LDSST test. Suitably, normal adrenal activity is defined as a peak cortisol level of >500nmol/L for adults and >500nmol/L for children using the LDSST test.

Increased risk of adrenal suppression is defined as an increase in the likelihood of developing adrenal suppression. A subject with increased risk of adrenal suppression may have an approximately 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, 5-fold, 5.5-fold, 6-fold,

6.5-fold, 7-fold, 7.5-fold, 8-fold or more increased likelihood of developing adrenal suppression.

In one embodiment, a subject with an increased risk of adrenal suppression has an approximately 4 to 6-fold increased likelihood of developing adrenal suppression.

In one embodiment, a child with an increased risk of adrenal suppression has an approximately 6-fold increased likelihood of developing adrenal suppression.

In one embodiment, an adult with an increased risk of adrenal suppression has an approximately 4-fold increased likelihood of developing adrenal suppression.

Suitably the risk of developing corticosteroid induced adrenal suppression is increased by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 70%, 75% or more if a mutation in the *PDGFD* gene is present. Suitably, the risk of developing corticosteroid induced adrenal suppression is increased by between 40%-70% if a mutation in the *PDGFD* gene is present.

Suitably, the risk of developing corticosteroid induced adrenal suppression is between 50%-70% if a mutation in the *PDGFD* gene is present. Suitably, the risk of developing corticosteroid induced adrenal suppression is between 60%-70% if a mutation in the *PDGFD* gene is present.

In one embodiment, the risk of developing corticosteroid induced adrenal suppression is between 60%-65% if a mutation in the *PDGFD* gene is present.

Suitably the mutations in the *PDGFD* gene associated with the risks defined above are described below.

Mutation in the PDGFD Gene

The methods and uses of the invention involve determining if there is a mutation present in the *PDGFD* gene as a biomarker for increased risk of corticosteroid induced adrenal suppression by assaying a sample from a subject.

The *PDGFD* gene encodes platelet derived growth factor D protein. The gene encoding this protein is located on chromosome 11 and is composed of 3801 base pairs, encoding a 370

amino acid protein. The transcript has 7 exons and 6 introns. The reference or 'wild type' *PDGFD* gene consists of the sequence shown in SEQ ID NO.1

Suitably, the or each mutation in the *PDGFD* gene may be present in the non-coding and/or coding region of the *PDGFD* gene.

Suitably, therefore, the or each mutation in the *PDGFD* gene may be present in an intron or an exon. Suitably the mutation is present in an intron.

Suitably, the or each mutation in the *PDGFD* gene is present in an intronic region. Suitably, therefore, the methods and use of the invention comprise the step of assaying a sample for the presence of at least one mutation in an intronic region of the *PDGFD* gene.

Suitably, the presence of any mutation in the PDGFD gene may be determined by the methods and use of the present invention. The mutation may be any type of mutation such as a missense, nonsense, deletion, substitution, insertion, duplication, frameshift, or repeat expansion.

Suitably the or each mutation is a substitution.

Suitably, the or each mutation is an A/G substitution.

Suitably the or each mutation is a substitution at a single nucleotide in the *PDGFD* gene. Suitably, therefore, the or each mutation in the *PDGFD* gene is a polymorphism. Suitably, therefore, the or each mutation in the *PDGFD* gene is a single nucleotide polymorphism (SNP). Suitably, therefore, the methods and use of the invention comprise the step of assaying a sample for the presence of at least one SNP in an intronic region of the *PDGFD* gene.

Suitably, the *PDGFD* gene may comprise more than one mutation. Suitably, therefore the *PDGFD* gene may comprise more than one SNP.

Suitably, the subject may be homozygous or heterozygous for the or each SNP.

Suitably, the subject is homozygous for the or each SNP.

Suitably, the or each SNP is selected from the following SNPs: rs591118, rs7116655, rs361283, rs361284, rs590216, rs603781, rs589796, rs2515080, rs684212, rs517401, rs671851, rs2515083, rs620426, rs619954, rs574494, rs619114, rs618648, rs5794293, rs623031. Suitably, therefore, the methods and use of the invention comprise the step of assaying a sample for the presence of one or more of the SNPs listed.

Suitably, the or each SNP is selected from the following SNPs: rs591118 and rs7116655. Suitably, therefore, the methods and use of the invention comprise the step of assaying a sample for the presence of SNPs rs591118 and/or rs7116655 in the *PDGFD* gene.

Suitably the *PDGFD* gene comprises one mutation. Suitably the one mutation is a SNP. Suitably, therefore, the methods and use of the invention comprise the step of assaying a sample for the presence of a SNP in the *PDGFD* gene.

In one embodiment, the mutation in the *PDGFD* gene is SNP rs591118. In such an embodiment, the methods and use of the invention comprise the step of assaying a sample for the presence of SNP rs591118 in the *PDGFD* gene.

Suitably, the SNP rs591118 is an A/G substitution mutation.

In one embodiment, the mutation in the *PDGFD* gene is an A/G substitution at SNP rs591118. In such an embodiment, the methods and use of the invention comprise the step of assaying a sample for the presence of an A/G substitution at SNP rs591118 in the *PDGFD* gene.

Suitably, the subject is homozygous for SNP rs591118. Suitably, the subject is homozygous for an A/G substitution at SNP rs591118. Suitably, the subject may comprise the genotype GA, AG, or GG at SNP rs591118. In one embodiment, the subject comprises the genotype GG at SNP rs591118.

Suitably, therefore, the methods and use of the invention comprise the step of assaying a sample for the presence of genotype GA, AG, or GG at SNP rs591118 in the *PDGFD* gene. In one embodiment, the methods and use of the invention comprise the step of assaying a sample for the presence of genotype GG at SNP rs591118 in the *PDGFD* gene.

Suitably, the SNP rs591117 is at position 104095993 of chromosome 11.

Concomitant Mutations

Other mutations can be used as further biomarkers for an increased risk of corticosteroid induced adrenal suppression in combination with the at least one mutation in the *PDGFD* gene as defined above.

Suitably, the at least one mutation in the *PDGFD* gene may be part of a biomarker panel for determining if a subject has an increased risk of corticosteroid induced adrenal suppression.

Suitably, the methods of the invention may therefore comprise a step of determining the presence of at least one mutation in the *PDGFD* gene in combination with at least one further biomarker indicative of an increased risk of corticosteroid induced adrenal suppression by assaying a sample from the subject.

Suitably, the methods of the invention may therefore comprise an additional step of determining the presence of at least one further biomarker indicative of an increased risk of corticosteroid induced adrenal suppression by assaying a sample from the subject.

The methods of the invention may further involve determining if a subject has further biomarkers indicative of an increased risk of corticosteroid induced adrenal suppression. Suitably, the methods of the invention may assay for the presence of one or more further biomarkers indicative of an increased risk of corticosteroid induced adrenal suppression.

Suitably, the or each further biomarker is any mutation significantly associated with an increased risk of corticosteroid induced adrenal suppression.

Suitably the or each further biomarker is a mutation selected from those with a significance of $p < 1.00E-06$ for an increased risk of corticosteroid induced adrenal suppression when measured using GWAS.

Suitably the or each further biomarker is a mutation selected from those with a significance of $p < 0.05$ for an increased risk of corticosteroid induced adrenal suppression when measured using candidate gene analysis.

The or each further biomarker may be one or more mutations in one or more different genes that are not the *PDGFD* gene.

Suitably, the or each further biomarker is a mutation present in genes on any chromosome.

Suitably, the or each further biomarker is a mutation present in genes on chromosomes 1, 8, 11, 15, 17, 2, 4, 6, 12, 19, 5, 7, 9, 13, 14, and 20.

Suitably, the or each further biomarker is a mutation present in genes on chromosomes in genes on chromosomes 1, 8, 11, 15, and 17.

Suitably, the or each further biomarker is a mutation present in genes selected from: GJA8, TRPA1, KRT8P9, PSMD3, CSF3, MED24, LRP1B, GBA3, HMGN3, PDE7B, SCGN, ANKS1B, ELSPBP1, LINC00607, IL31RA, STK32A, FBXL7, KCNQ5, MYCT1, XR_242309.1, CAV1, LINGO2, PRSS3, UBE2R2, UBAP2, NM_015397.3, DCAF12, HSPA12A, NOS1, CLDN10, NR_039974.1, IGH, NPAS3, XR_243228.1, SLC2A10, MACROD2, XKR7, and BCL2L13.

Suitably, the or each further biomarker is a mutation present in genes selected from: GJA8, TRPA1, KRT8P9, PSMD3, CSF3, and MED24.

Suitably, the or each further biomarker is a SNP. Suitably, the or each further SNP biomarker is present one or more of the genes listed above.

Known further SNP biomarkers indicative of variability in response to corticosteroid treatments which may be used in the methods of the present invention are: CHRH1 [19], TBX21 [20, 21] and STIP1 [22].

Suitably, the or each further biomarker is a SNP selected from any of the following SNPs: rs201161441, rs6657114, rs6671502, rs75470088, rs111566682, rs9912981, rs3859188, rs71355433, rs7222556, rs9916279, rs8080546, rs11654706, rs11078932, rs58212353, rs2012, rs2827, rs11555254, rs2302778, rs7503939, rs17850739, rs72834789, rs142320277, rs111863753, rs13220233, rs149647891, rs5875060, rs191087489, rs143638033, rs142161979, rs137939366, rs146292085, rs111404331, rs55782611, rs200531273, rs146414687, rs192277931, rs140981584, rs116994288, rs117162246, rs141159828, rs72711408, rs72711411, rs188183827, rs182806557, rs12553078, rs181360551, rs140635353, rs148024340, rs12551409, rs147719472, rs200001967, rs144482947, rs151129137, rs149572349, rs192660407, rs77562913, rs76830467, rs75992652, rs17617937, rs34401373, rs35097914, rs34406980, rs71444623, rs9658258, rs113071293, rs111406705, rs137908418, rs74053481, rs201541519, rs56406213, rs200209719, rs117420762, rs73269069, rs73269081, rs184548348, rs112736204, rs73269083, rs80075471, rs149352662, rs189673743, rs140179402.

Suitably, the or each additional biomarker is a SNP selected from any of the following SNPs: rs201161441, rs6657114, rs6671502, rs75470088, rs111566682, rs9912981, rs3859188, rs71355433, rs7222556, rs9916279, rs8080546, rs11654706, rs11078932, rs58212353, rs2012, rs2827, rs11555254, rs2302778, rs7503939, rs17850739, rs72834789.

Sample

The methods and use of the present invention assay a sample that provides biological information regarding the presence of at least one mutation in the *PDGFD* gene and optionally additional mutations within the subject.

Suitably the sample is a biological sample.

Suitably the sample is any biological sample containing target molecules from which mutations in the *PDGFD* gene can be detected. Suitably target molecules are biological molecules.

Suitable target molecules may be selected from: proteins, or precursors, or variants produced on translation of the transcripts produced when the gene is expressed; and nucleic acids (for example DNA or RNA) encoding said proteins. In the event that a protein undergoes modification between first translation and its mature form, either or both of the precursor and the mature protein may be used as suitable target molecules.

Suitably, the target molecules are selected from the PDGFD protein or nucleic acids encoding the PDGFD protein. In a suitable embodiment the nucleic acid is DNA, suitably DNA comprising the *PDGFD* gene, suitably DNA encoding the PDGFD protein.

Genomic DNA, and particularly the genomic DNA of the PDGFD gene, represents an example of a suitable target molecule that may be indicative of the presence of at least one mutation in the *PDGFD* gene.

A suitable target molecule representative of gene expression may comprise an mRNA transcript of the relevant biomarker gene, such as the *PDGFD* gene, translatable to yield the PDGFD protein.

In one embodiment, the target molecule is gDNA comprising the *PDGFD* gene. Therefore, in one embodiment, the sample is any biological sample containing gDNA comprising the *PDGFD* gene.

Suitably, the sample may be a tissue sample. By way of example, the sample may be a biopsy sample.

Although the collection of tissue samples, such as biopsy samples, is generally more invasive than the collection of body fluid samples, it may still represent a commonly used procedure in many clinical contexts.

Suitably, the sample may be a body fluid sample. The sample may be selected from the group consisting of: blood or saliva. A blood sample may be collected by intravenous withdrawal. A saliva sample may be collected by an oral swab.

In one embodiment, the sample is blood.

In one embodiment, the sample is whole blood treated with EDTA.

Subject

The methods and uses of the present invention comprise assaying a sample from a subject for the presence of at least one mutation in the *PDGFD* gene, and optionally further biomarkers.

The subject may also be referred to herein as a patient.

Suitably the subject is human.

Suitably the subject is an adult or child, and suitably is male or female.

Suitably, the subject is suffering from a disease or suspected of suffering from a disease and therefore requires treatment.

Suitably, the subject may be suspected of having an increased risk of corticosteroid induced adrenal suppression.

Suitably the disease is typically treated with corticosteroids. Therefore the subject may be defined as a subject intended for treatment with a corticosteroid, or a subject having a corticosteroid-treatable disease.

In one embodiment, the methods of the present invention may comprise a method of selecting a treatment for, or a method of treating, a subject having a corticosteroid-treatable disease.

Suitably, the subject has a disease selected from: a respiratory disease, an oncology disease, a dermatology disease, an endocrinology disease, a gastroenterology disease, a haematology disease, an immunology disease, and an ophthalmology disease. Suitably, the subject has a corticosteroid-treatable disease selected from this list.

Suitably, the subject has a disease selected from: Asthma, COPD, Allergic rhinitis, Atopic dermatitis, Hives, Angioedema, Anaphylaxis, Food allergies, Drug allergies, Nasal polyps,

Hypersensitivity pneumonitis, Sarcoidosis, Eosinophilic pneumonia, Interstitial lung disease, Pemphigus vulgaris, Contact dermatitis, Congenital adrenal hyperplasia, Ulcerative colitis, Crohn's disease, Autoimmune hepatitis, Lymphoma, Leukaemia, Haemolytic anaemia, Idiopathic thrombocytopenic purpura, Rheumatoid arthritis, Systemic lupus erythematosus, Polymyalgia rheumatic, Polymyositis, Dermatomyositis, Polyarteritis, Vasculitis, Uveitis, Keratoconjunctivitis, Multiple sclerosis, Organ transplantation, Nephrotic syndrome, Chronic hepatitis (flare ups), Cerebral edema, IgG4-related disease, Duchenne Muscular Dystrophy and Prostate cancer. In one embodiment, the methods of the present invention may comprise a method of selecting a treatment for, or a method of treating, a subject having a disease selected from this list.

Suitably, the subject has a respiratory disease. Suitably, the subject has a corticosteroid-treatable respiratory disease.

In one embodiment, the methods of the present invention may comprise a method of selecting a treatment for, or a method of treating, a subject having a corticosteroid-treatable respiratory disease.

In one embodiment, the subject has a respiratory disease selected from asthma and COPD.

In one embodiment, the methods of the present invention may comprise a method of selecting a treatment for, or a method of treating, a subject having asthma or COPD.

Assaying

The methods of the invention involve assaying samples from a subject in order to determine the presence of mutations in the *PDGFD* gene, and optionally further biomarkers, and what these mutations are.

Generally, the assays used to detect the mutations may be any assay known to those skilled in the art suitable for such detection.

Suitably, the assays used to detect the mutations may detect target molecules indicative of the presence of mutations in the *PDGFD* gene and optionally further biomarkers. The assays may allow comparison of such target molecules with corresponding reference molecules.

Alternatively, the assays used to detect mutations may detect target molecules indicative of the presence of the reference or 'wild type' *PDGFD* gene and optionally further biomarkers.

Suitable target molecules are as defined above.

The reference sequence of the *PDGFD* gene is shown at SEQ ID NO. 1.

The assaying of the methods of the invention may involve the formation of complexes between naturally occurring target molecules in a sample and non-natural agents. The non-natural agents may comprise binding partners capable of binding to the target molecule, and may further incorporate indicators.

Suitably, binding partners are capable of binding to the target molecules indicative of the presence of mutations in the *PDGFD* gene, and optionally further biomarkers. Binding partners may be selected from the group comprising: complementary nucleic acids; aptamers; antibodies or antibody fragments.

In the context of the present invention, a binding partner specific to a target molecule indicative of the presence or expression of a biomarker should be taken as requiring that the binding partners should be capable of binding to at least one such target molecule in a manner that can be distinguished from non-specific binding to molecules that are not target molecules. A suitable distinction may, for example, be based on distinguishable differences in the magnitude of such binding. An individual binding partner may be able to bind to a target molecule, or molecules, indicative of the presence or expression of a single biomarker. Alternatively an individual binding partner may be able to bind to target molecules indicative of the presence or expression of two, three or more biomarkers.

Suitably, indicators are capable of indicating when said binding occurs. Indicators may be reagents, reporter moieties or other suitable means that allow their detection when complexed with the target molecules from the sample. Suitable examples of indicators include: fluorophores; chromogenic substrates; and chromogenic enzymes.

Assaying for the presence of a mutation in the methods of the invention may involve detection of these complexes formed between the target molecule and the binding partner, and optionally the indicator, such as a synthetic oligonucleotide, labelled antibody, or the like.

By way of example, in a suitable embodiment, the assay used to determine the presence of the target biological molecule may comprise an amplification step in which a target biological molecule in the sample is used as a template for the generation of further molecules for analysis. The polymerase chain reaction (PCR) represents a suitable technique by which target nucleic acids may be amplified in order to facilitate their sequencing.

Various suitable assay types will be known to those skilled in the art, including, but not limited to: enzyme linked immunosorbant assays (ELISA), including variants such as sandwich ELISAs; radioimmuno assays (RIA); immunocytochemistry labelling; immunohistochemistry labelling of a tissue sample; fluorescence activated cell sorting

(FACS); chemiluminescence; reverse transcription PCR (rt PCR), and multiplex assays such as Luminex or proteomic MRM.

In certain embodiments where the binding partner is an antibody, or antibody fragment, the detection of the target molecule may utilise an immunological method. The immunological method may be an enzyme-linked immunosorbent assay (ELISA). In other embodiments, an immunological method may utilise a lateral flow device.

In certain embodiments, the presence of the target molecule may be detected by direct assessment of binding of the target molecules and binding partners. Suitable assays may, for example, involve labelling only the native target molecule present in the sample. Suitable examples in accordance with this embodiment of the invention may utilise detectors such as electro-impedance spectroscopy (EIS) to directly assess binding of binding partners (such as antibodies) to target molecules (such as the biomarker proteins).

In certain embodiments, the presence of the target molecule is detected using detectors such as biosensors selected from impedance spectroscopy, surface plasmon resonance, quantum dots, and nanoparticle technology.

In other embodiments, the presence of the target molecule is detected using indicators as described above.

In the embodiments where it is desired to make use of indicators wherein the indicators may be directly attached to the binding partners. Examples of such embodiments include those utilising labelled antibodies. In other embodiments of the methods of the invention the indicators may be attached to reporter molecules that interact with the binding partners. Examples of such embodiments include those utilising antibodies indirectly attached to an indicator by means of biotin/avidin complex.

In one embodiment, the assaying of the methods of the present invention comprises comparing a *PDGFD* gene from a sample with a reference *PDGFD* gene (SEQ ID NO.1) to identify mutations present therein. Suitably, the methods comprise comparing DNA encoding the *PDGFD* gene from a sample with DNA encoding the reference *PDGFD* gene (SEQ ID NO.1) to identify mutations present therein. Suitably, the same comparisons are conducted for any further biomarkers present in a sample.

Suitably, the assaying of the methods of the present invention therefore comprises a step of extracting the target biological molecule from the sample. In the case of DNA, the methods

may comprise a step of DNA extraction from the sample. Such extraction may performed by any suitable means in the art, for example using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germany). A further purification step may follow such an extraction step.

Comparing target biological molecules from a sample with corresponding reference biological molecules to identify mutations therein may be conducted by sequencing. Suitable sequencing techniques are known to those in the art, this includes Next Generation Deep Sequencing which exploits massively parallel sequencing of single DNA molecules sequestered on beads or (as in Ion Torrent technology) individual lipid droplets within an emulsion. Such techniques can be used for whole genome sequencing or targeted to specific DNA regions.

Suitably whole genome sequencing is used to assay the sample in the methods of the present invention. In one embodiment, the Illumina Infinium™ Super HD assay is used.

In suitable embodiments amplified and/or isolated nucleic acid molecules comprising one or more mutations in the *PDGFD* gene may be sequenced in order to determine the presence or absence of such mutations. Suitably, amplified and/or isolated nucleic acid molecules comprising one or more further biomarkers may also be sequenced in order to determine the presence or absence of the biomarkers.

Suitably, the assaying of the methods of the present invention therefore comprises a step of sequencing the target biological molecule in a sample. In one embodiment, the assaying of the methods of the present invention therefore comprises a step of sequencing DNA in a sample. Methods suitable for sequencing DNA are known to those skilled in the art. Merely by way of example, such methods involve microarray sequencing or whole genome sequencing.

Microarray sequencing typically comprises hybridisation of a target molecule (for example a short DNA fragment) to a probe. The probe (which may be a single stranded complementary DNA molecule) may be attached to, for example, a silica bead or a glass plate. Suitable types of microarrays will be known to those skilled in the art. By way of example these may include Infinium® OmniExpressExome-8 v1.4 BeadChip, Illumina ®, Genome-Wide Human SNP Array, Affymetrix®, Genome-Wide Human SNP Array 6.0, Affymetrix®.

Whole genome sequencing typically comprises separating the target biological molecule into fragments for sequencing. Suitably, in the assaying of the methods of the present invention, the target molecule of DNA is split into fragments, suitably into fragments spread across the *PDGFD* gene.

Suitably primers are designed for sequencing the *PDGFD* gene fragments, suitably this may be done using the known *PDGFD* gene sequence available on GenBank, for example. Suitable oligonucleotide primers that may be used to amplify *PDGFD* encoding nucleic acid molecules comprising a mutation can be designed based on amplification of exonic sequences from within introns. These should ideally be barcoded to allow production of patient specific libraries that can be read with a next generation sequencer to give high depth for each barcode but at the same time allow multiplexing of patient samples. Commercial panels to sequence the *PDGFD* gene in this way are available from ThermoFisher as part of their Oncomine™ platform system. In the same way, suitable primers for sequencing the genes containing one or more further biomarkers may be designed.

In one embodiment, each fragment of each sample is sequenced multiple times. Suitably deep sequencing or ultra-deep sequencing is used. In one embodiment, each fragment in each sample is sequenced between 2 and 20 times, between 5 and 15 times, or between 7 and 13 times. In one embodiment, each fragment in each sample is sequenced 10 times.

Suitably, the assaying of the methods of the present invention may further comprise a step of confirming that mutations are present in the *PDGFD* gene, and optionally confirming the presence of one or more further biomarkers. Suitably, this step comprises confirming that the mutations detected by sequencing are present in the *PDGFD* gene, and optionally in the further biomarker genes. Confirmation may be performed by nested PCR or other suitable techniques in the art.

In one embodiment, replication cohorts are used to confirm the presence of the identified mutations in the *PDGFD* gene. The samples from replication cohorts may be genotyped using method shown in Hawcutt *et al* [25].

In one embodiment, mutations found to be present in the *PDGFD* gene are confirmed using nested PCR. Suitably, the nested PCR is performed using 'short product' primers and 'long product' primers. Suitably, the same method is used to confirm the presence of further biomarker mutations.

Optionally, the assaying of the methods of the present invention may further comprise internal controls. Suitably, the assaying of the methods of the invention further comprises the step of genotyping quality control. Genotyping quality control may comprise a Gender check,

assessment of genotype call rate, assessment of pairwise identity, and assessment of principal component analysis (PCA).

In one embodiment, there is provided a kit for use in the methods of the invention comprising binding partners capable of binding to a mutation in the *PDGFD* gene, and indicators capable of indicating when said binding occurs.

In another embodiment, there is provided an assay device comprising a loading area for receipt of a sample from a subject; binding partners capable of binding to a mutation in the *PDGFD* gene; and a detector capable of detecting when said binding occurs.

Suitably, the presence of any indication or detection means that there is a mutation in the *PDGFD* gene.

Suitably, the absence of any indication or detection means that there is no mutation in the *PDGFD* gene. Suitably, this may indicate the presence of the reference *PDGFD* gene.

In the kits or devices, the binding partners may be capable of binding to a target molecule indicative of the expression of a mutation in the *PDGFD* gene, and optionally to a target molecule indicative of the expression of one or more further biomarkers.

Alternatively, in the kits or devices, the binding partners may be capable of binding to a target molecule indicative of the expression of the reference *PDGFD* gene, and optionally to a target molecule indicative of the expression of one or more further biomarkers.

Suitably, wherein the reference *PDGFD* gene is defined by SEQ ID NO.1

Suitably, the kit and assay device may further comprise binding partners capable of binding to one or more further biomarkers. Suitably, in which case, the presence of any relevant indication or detection means that the or each further biomarker is present and the absence of any relevant indication or detection means that there are no further biomarkers present.

Generally the assaying will be practiced *in vitro*. The assaying may be practiced using a kit, or an assay device in accordance with the above statements. The kit and assay device of the present invention are suitable for carrying out any assaying step or method described above.

In a suitable embodiment, a kit or device of the invention may comprise binding partners such as oligonucleotides capable of amplifying some, or all, of the *PDGFD* gene. Merely by way of example, suitable oligonucleotides may be capable of amplifying the full coding sequence and intron/exon boundaries of *PDGFD*.

Suitable kits or assay devices may include oligonucleotides capable of amplifying each of the recited mutations in the *PDGFD* gene as described hereinabove, and optionally each of the further biomarkers if present. In suitable embodiments the oligonucleotides are capable of amplifying the entire *PDGFD* encoding region comprising the recited mutation(s), and optionally the further biomarkers. In alternative embodiments the oligonucleotides are capable of amplifying a portion of the *PDGFD*-encoding nucleic acid comprising the recited mutation(s), and optionally the further biomarkers. In a suitable embodiment the oligonucleotides are capable of specifically amplifying a portion of the *PDGFD*-encoding nucleic acid comprising the recited mutation(s), and optionally the further biomarkers.

In certain embodiments the assay device may have a reader. In alternative embodiments the device may be readerless. In a device having a reader, the presence or absence of one or more biomarkers may be visible on a reader, such as a digital reader. In one embodiment, the digital reader may be visible using reflectance technology or fluorescence technology.

An assay device may further comprise indicators capable of indicating binding of the one or more of the binding partners to a mutation in the *PDGFD* gene and optionally further biomarkers, or to target molecules indicative of the expression of a mutation in the *PDGFD* gene and optionally further biomarkers. Suitable indicators are referred to above. As set out above the indicators may be directly attached to the binding partners, or may be attached to reporter molecules that interact with the binding partners. Alternatively, a device according to the invention may further comprise means for directly assessing binding of binding partners to target molecules. For example, an assay device of the invention may comprise means for conducting EIS. As referred to above, EIS is suitable for assessing the binding of antibody binding partners to biomarker protein target molecules.

In certain embodiments the results provided by a method of the invention, suitably by an assay device of the invention, could be incorporated into an algorithm, for example, a mathematic equation on a computing device, such as a mobile computing device. The result of the algorithm would indicate identification of appropriate treatment for subjects.

An assay device will generally comprise a reaction area in which binding partners provided in the device are able to bind specifically to a mutation in the *PDGFD* gene, and optionally the further biomarkers, or target molecules indicative of the expression of a mutation in the *PDGFD* gene, and optionally the further biomarkers, present in the sample. A device in accordance with the present invention will generally comprise a detector in which binding of the binding partners to a mutation in the *PDGFD* gene, and optionally the further biomarkers,

or target molecules indicative of the expression of a mutation in the *PDGFD* gene, and optionally the further biomarkers, present in the sample is detected. Such detection may be carried out by means of an indicator. In suitable embodiments the assay device comprises means for moving a sample from the loading area to the reaction area.

Additionally, or alternatively, an assay device may comprise means for moving a sample from the reaction area to the detector. In suitable embodiments the means for moving the sample comprises a pump. In other suitable embodiments, the means for moving the sample comprises capillary flow means.

The assay device may comprise a lateral flow device, a spectroscope, a sequencer, a PCR machine, for example. In one embodiment, the assay device comprises a sequencer. The sequencer is suitably operable to conduct the assaying steps described hereinabove. Suitably the sequencer is a whole genome sequencer.

Treatment

The methods and uses of the present invention identify those subjects that are suitable for corticosteroid treatment and those that are less suitable for corticosteroid treatment on the basis of whether the subject has an increased risk of corticosteroid induced adrenal suppression.

Subjects that are less suitable for corticosteroid treatment may be provided with a conservative corticosteroid treatment as defined above.

Suitably, corticosteroid treatment comprises treatment with any natural or synthetic steroid hormone.

Suitably, corticosteroid treatment comprises treatment with any molecule acting on the glucocorticoid receptor.

Suitably, corticosteroid treatment may comprise a combination of two or more corticosteroids.

Suitably, the corticosteroids may be selected from glucocorticoids or mineralocorticoids.

Suitably, the corticosteroids may be selected from group A, B, C, D1, or D2 corticosteroids.

Suitably, corticosteroid treatment from group A may comprise treatment with one or more of the following corticosteroids: hydrocortisone, methylprednisolone, prednisolone, prednisone, and triamcinolone

Suitably, corticosteroid treatment from group B may comprise treatment with one or more of the following corticosteroids: amcinonide, budesonide, desonide, fluocinolone acetonide, fluocinonide, halcinonide, and triamcinolone acetonide.

Suitably, corticosteroid treatment from group C may comprise treatment with one or more of the following corticosteroids: beclometasone, betamethasone, dexamethasone, fluocortolone, halometasone, and mometasone.

Suitably, corticosteroid treatment from group D1 may comprise treatment with one or more of the following corticosteroids: alclometasone dipropionate, betamethasone dipropionate, betamethasone valerate, clobetasol propionate, clobetasone butyrate, fluprednidene acetate, and mometasone furoate.

Suitably, corticosteroid treatment from group D2 may comprise treatment with one or more of the following corticosteroids: ciclesonide, cortisone acetate, hydrocortisone aceponate, hydrocortisone acetate, hydrocortisone buteprate, hydrocortisone butyrate, hydrocortisone valerate, prednicarbate, and tixocortol pivalate.

Suitably, corticosteroid treatment may be provided by the following routes: topical, inhaled, oral, intranasal, or systemic. A corticosteroid which may be provided by a topical, inhaled, oral, intranasal or systemic route may be also referred to as a topical, inhaled, oral, intranasal or systemic corticosteroid, respectively.

In one embodiment, the corticosteroid treatment is an inhaled corticosteroid treatment. Therefore, in one embodiment, the methods and use of the present invention determine if the subject has an increased risk of inhaled corticosteroid induced adrenal suppression.

Suitably, the inhaled corticosteroid treatment may be selected from: Flunisolide, Fluticasone, Mometasone, Fluticasone Furoate, Ciclesonide, Fluticasone Propionate, Triamcinolone Acetonide, Beclomethasone Dipropionate, Salmeterol Xinafoate, and Budesonide.

Suitably, the inhaled corticosteroid treatment may be selected from one or more of Budesonide, Fluticasone, Ciclesonide, Beclomethasone Dipropionate and Mometasone. Suitably, the inhaled corticosteroid treatment may be used in combination with a long lasting β2-agonist.

Suitably, the corticosteroid treatment may be provided in doses of between 10µg to 150mg.

It will be appreciated that such a typical amount of a corticosteroid treatment may be provided to a subject without a mutation in the *PDGFD* gene. It will also be appreciated that the typical amount may depend upon the type of corticosteroid treatment, type of disease

(for example whether the subject has asthma and/or COPD), and/or subject specific factors (for example the subject's body weight and/or subject's age). A typical amount of corticosteroid treatment will be known to those skilled in the art. Typical amounts may be found in reference literature, for example, in the Davis's Drug Guide.

What is meant by a 'low dose' of corticosteroids or 'reduced' corticosteroid treatment is defined above.

Suitably, the methods and use of the present invention may comprise the step of selecting a low dose of corticosteroids as described above, for example treatment comprising below 2mg, below 1mg, below 0.7mg, below 0.5mg, below 0.4mg, below 0.3 mg, below 0.2mg, below 0.1mg, below 50µg, below 40µg, below 30µg, below 20µg, below 10µg or below 5µg of corticosteroids in the presence of a mutation in the *PDGFD* gene.

In one embodiment, the methods and use of the present invention may comprise the step of selecting a low dose of corticosteroids comprising below 1mg of corticosteroids in the presence of a mutation in the PDGFD gene.

By way of example, if a subject is without a mutation in the *PDGFD* gene, the subject may be treated with 200µg of beclomethasone diproprionate. However, a subject with a mutation in the *PDGFD* gene may be treated with 10mg of Montelukast. Suitably corticosteroid treatment is administered once or twice per day.

Suitably, the methods and use of the present invention may comprise the step of selecting a reduced corticosteroid treatment, such as less frequent administration of corticosteroids or less potent corticosteroids in the presence of a mutation in the *PDGFD* gene. By way of example, a less potent corticosteroid may be selected from the group consisting of Flunisolide and/or Triamcinolone Acetonide.

Less frequent administration of corticosteroids may comprise administration of corticosteroids once per day, or once every other day, once every 3 days, once per week, once per fortnight, or once per month.

In one embodiment, the methods and use of the present invention may comprise the step of selecting reduced corticosteroid treatment comprising administration of corticosteroids once per day in the presence of a mutation in the *PDGFD* gene.

Suitable alternatives treatments to corticosteroids are treatment with leukotriene-modifying drugs, long or short acting sympathomimetics (e.g. beta2-agonists), anticholinergics (e.g. Ipratropium bromide), xanthene derivatives (e.g. theophylline), biological therapies (e.g.

omalizumab), breathing exercises, acupuncture, massage therapy, herbal medications, and dietary supplements, for example.

Non-steroidal treatment may include treatment with sodium cromoglicate, or nedocromil sodium.

Bronchodilator treatment may include treatment with long or short acting beta-2 agonists (for example salbutamol, albuterol, terbutaline, seretide, formoterol or vilanterol).

Leukotriene-modifying drugs may include treatment with montelukast, zafirlukast or ziluton.

Therefore, in one embodiment, the methods and use of the present invention may comprise the step of selecting an alternative treatment to corticosteroids such as those listed above, for example treatment with leukotriene-modifying drugs in the presence of a mutation in the *PDGFD* gene.

In one embodiment, the methods and use of the present invention may comprise the step of selecting a non-steroidal treatment in the presence of a mutation in the *PDGFD* gene.

The term “frequency of monitoring” as used herein refers to the time interval between assessing a subject’s adrenal activity. Suitably, the subject’s adrenal activity may be assessed prior to the onset of symptoms associated with adrenal suppression.

Suitably, subjects treated with corticosteroids are monitored once every six to twelve months. It will be appreciated that it may be desirable to monitor paediatric subjects more often than adult patients. Thus, paediatric subjects treated with corticosteroids are, for example, monitored once every three to six months. Suitably, this monitoring is sporadic.

It will be appreciated that in subjects less suitable for corticosteroid treatment it may be desirable to monitor the subjects more often. Suitably, subjects which are less suitable for corticosteroid treatment may be monitored approximately once every six months, once every five months, once every four months, once every three months, or more often.

It will be appreciated that in subjects less suitable for corticosteroid treatment it may be desirable to monitor the subjects more regularly. Suitably, subjects less suitable for corticosteroid treatment are monitored at regular time intervals.

Therefore, in one embodiment, the methods and use of the present invention may comprise the step of increasing monitoring of a subject, for example monitoring a subject once every three months in the presence of a mutation in the *PDGFD* gene.

In such an embodiment, the subject may be treated with reduced corticosteroids and/or a low dose of corticosteroids. Alternatively, in such an embodiment, the subject may be treated with a standard dose of corticosteroids.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the invention are further described hereinafter with reference to the accompanying drawings and tables, in which:

Figure 1 shows a principal component analysis of the PASS cohort of patients with reference datasets;
Figure 2 shows log transformed QQ plots of GWAS of the four phenotypes of patients tested (A) Peak cortisol 350 (B) Peak cortisol 500 (C) Peak continuous (D) Base continuous;
Figure 3 shows log transformed Manhattan plots of GWAS of (A) Peak cortisol 350 (B) Peak cortisol 500 (C) Peak continuous (D) Base continuous;
Figure 4 shows a locus zoom of the log transformed Manhattan plot for chromosome 11 of the *PDGFD* gene of (A) Peak cortisol 350 (B) Peak cortisol 500;
Figure 5 shows the results of meta-analysis of the PASS cohort and the PASS replication cohort (A) and the PASS, PASS replication and PASIC replication cohorts (B);
Figure 6 shows box whisker plots of peak cortisol against rs591118 genotype for each cohort tested;
Table 1 and 1A shows the demographics of the PASS cohort of patients and the replication cohorts;
Table 2 shows the genes identified in the PASS cohort that contain SNPs with a significance of p<1.00E-06;
Table 3 and 3A shows pathways associated with the phenotypes of the PASS cohort,
*Pathways with FDR corrected p-value < 0.05.

Throughout the description and claims of this specification, the words “comprise” and “contain” and variations of them mean “including but not limited to”, and they are not intended to (and do not) exclude other moieties, additives, components, integers or steps. Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith. All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

The invention is not restricted to the details of any foregoing embodiments. The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed. The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

EXAMPLES

1. METHODS AND MATERIALS

1.1 Pharmacogenetics of Adrenal Suppression with Inhaled Steroids (PASS) Study

PASS received full ethical approval from Liverpool Paediatric Research Ethics Committee. Participants were recruited from November 2008 to September 2011 (primary analysis cohort) and October 2011 to December 2012 (internal replication cohort) from 25 sites across the UK. Full eligibility criteria have been published previously [9], and included treatment with ICS for greater than 6 months; diagnosis of asthma; under care of a paediatrician experienced in the treatment of asthma; clinical concern about adrenal suppression sufficient to warrant a LDSST; informed written consent (and assent if participant judged competent by study team); age 5-18 years. Exclusion criteria included: Type 1 or Type 2 diabetes; competent older participant declining assent. Study participants were recruited either prospectively (if LDSST not yet undertaken) or retrospectively (if LDSST already undertaken). The target recruitment for the primary analysis cohort set in the ethics approval was 500. This We specified two benchmarks for the power analyses: we calculate the estimated power for (a) OR=3 and a rare variant (minor allele frequency=5%) and adrenal suppression; (b) OR=2 for a common variant (minor allele frequency=20%). The

assumed type 1 error is 5% and this liberal type 1 error rate is allowed because a confirmatory prospective study will be used to help eliminate false positives arising from the initial analyses, and because functional analyses will be used in combination with the results from the association analysis. Assuming first of all a prevalence of impaired adrenal response (as confirmed by the short synacthen® test) of 17% the power for scenario a) is calculated as 77% and the power for scenario b) is calculated as 75%. If the prevalence is 40% however the power increases to 91% for both scenarios. A prevalence of 20% would ensure power of at least 80% in both scenarios.

1.2 Pharmacogenomics of Adrenal Suppression in COPD (PASIC) Study

PASIC received full ethical approval from North West 2 Research Ethics Committee (Liverpool Central). Participants whose data were analysed for this study were all those recruited from February 2010 to June 2015 (external replication cohort) from two sites in the UK. Full eligibility criteria include COPD diagnosis by formal spirometry; age 18 to 80; informed consent; high dose ICS for more than 12 months (defined as >500mcg fluticasone or equivalent). Exclusion Criteria included: anaphylaxis to Synacthen®; adrenal disease; long term oral prednisolone or hydrocortisone or taken oral corticosteroid in the last 4 weeks; intranasal steroid use; significant other illnesses (including pulmonary fibrosis or bronchiectasis); a current diagnosis of asthma; active malignancy; active tuberculosis; daytime oxygen therapy for more than one hour per day; pregnant.

1.3 LDSST procedure

For all prospectively recruited patients (PASS and PASIC), the following LDSST procedure was undertaken: All tests were commenced before 11:00am and as close to 7:00am as possible. Participants were not fasted, but any corticosteroid medicine was withheld on the morning of the LDSST until test completion. For patients in the PASS cohort treated with alternate day oral corticosteroid, the LDSST was performed on the day a dose was due to be given, withholding it until completion of the LDSST.

An indwelling venous catheter was sited following the application of local anaesthetic cream. A blood sample was collected (time 0). One hundred and twenty five micrograms (0.5ml) Synacthen® (Alliance, Chippenham, UK) 0.25mg/ml solution was added to 500ml 0.9%Nacl (final concentration of 250nanograms/ml) and agitated. For PASS, five hundred nanograms/1.73m2 was administered as a bolus injection directly into the cannula and samples were collected 15, 25 and 35 minutes following Synacthen® administration [14]. A

Synacthen® dose calculator was used at all sites to ensure consistency of dosing at different study locations. For PASIC, 1 microgram of Synacthen® was used [23, 24], with samples collected at 0, 30 and 60 minutes. after injection with low dose Synacthen®. For PASS and PASIC, all participating hospitals were required to be experienced in undertaking a LDSST, and have guidelines for management of anaphylaxis.

1.4 DNA Storage and Extraction

For all cohorts, patient samples for DNA were collected as whole blood ethylenediaminetetraacetic acid (EDTA) samples or salivary samples. DNA collection and extraction for saliva samples has been described previously [25]. EDTA blood samples were stored at -80°C and following defrosting, genomic DNA was extracted using the Chemagen whole-blood DNA extraction kit on the Chemagic Magnetic Separation Module I according to the manufacturer's protocol (PerkinElmer® chemagen Technologie GmbH, Baesweiler, Germany; www.chemagen.com).

1.5 Genotyping

DNA samples from the PASS primary analysis cohort patients were shipped to Edinburgh-Genomics (The Roslin Institute, University of Edinburgh) for genome-wide genotyping on the Illumina® Human OmniExpressExome-8v1 BeadChip using the Infinium HD Super assay (Illumina®, Inc.). A total of 951,117 SNPs were genotyped. Genotyping of samples from the PASS replication cohort and the PASIC replication cohort was undertaken for a single *PDGFD* (rs591118), as described previously [25].

1.6 Genotyping quality control (QC) and Imputation

Patients within the primary analysis cohort were excluded from association analyses if any of the following criteria were met: a) gender as determined by the “Sex Check” function within PLINK [26] differed from that reported in the clinical data, b) the genotype call-rate was <90%, c) the pairwise identity by descent (IBD) statistic of relatedness was >0.1875 (patient with lowest call rate of the pair excluded) or d) Principle component analysis (PCA) (using SNPRelate [27] in R v3.01) demonstrated that the individual did not cluster with the HapMap CEU (Utah residents with European ancestry) population.

SNPs genotyped in the primary analysis cohort were excluded if: a) minor allele frequency (MAF) was less than 0.01, b) there was deviation from Hardy-Weinberg Equilibrium (HWE),

taken as p<0.0001 or c) genotyping success rate was <95%. All QC analysis was undertaken using PLINK v1.07 [26] unless otherwise stated. The same SNP quality control criteria was also applied on both replication cohorts.

The primary analysis cohort genotypes were merged with the Wellcome Trust Case Control Consortium (WTCCC) dataset prior to SNP phasing using SHAPEIT [28] and imputation using IMPUTE2 [29, 30] was undertaken using the 1000 genome phase 3 reference panel (March 2012 release).

1.7 Statistical Analysis

For the primary analysis cohort, tests for association with cortisol levels were undertaken in two ways. Firstly, a genome-wide case-control analysis was undertaken with cases defined as those with peak cortisol level from the LDSST test being below 500nmol/L, and controls defined as those with peak level above 500nmol/L. The case-control analysis was then repeated, with cases this time defined as those with peak levels below 350nmol/L and controls defined as those with peak levels above 350nmol/L. In both analyses, the controls were augmented by utilising SNP array data from the WTCCC2 cohort (n=2,501) as population controls. To test for association between each SNP in turn and case-control status, a logistic regression model assuming an additive genetic model was fitted in SNPtest available from [http://www.nature.com/ng/journal/v39/n7/full/ng2088.html]. To adjust for population substructure we included the first two principal components as covariates in this genome-wide analysis. Previously in Hawcutt *et al.* (2015) [9], cumulative dose in the past 6 was identified as significantly associated with impaired peak cortisol. However, due to the inclusion of WTCCC2 in the control group this meant that cumulative dose could not be adjusted for in the genome-wide analysis of binary outcomes.

Secondary analyses were also undertaken within the primary analysis cohort to test for association with peak cortisol levels and baseline cortisol levels, both as continuous phenotypes. Baseline cortisol level data were logarithmically transformed to achieve normality. Tests for association between each SNP in turn and phenotype were undertaken by fitting linear regression models in SNPtest, again with two principle components included as covariates. A covariate to adjust for total corticosteroid dose and age was also included in the regression models for peak and baseline phenotypes respectively. These covariates were chosen based on the findings of Hawcutt *et al.* (2015) [9]. In all analyses, as an additive genetic model was assumed, all odds ratios quoted in the results section represent that for heterozygotes versus wild-type homozygotes.

For SNPs that were selected for replication, meta-analysis was performed in meta-analysis helper (METAL) [31]. Two meta-analyses were undertaken: the first for entirely paediatric cohorts PASS primary analysis and replication cohorts and the second for all three cohorts combined. This was to enable us to investigate any differences in the phenotypes between the paediatric and elderly populations.

1.8 Selection of gene(s) for replication

Any SNPs located within genes identified in the primary analysis cohort, with a p-value less than $1x10^{-6}$ were considered for replication. Commonly, the threshold of $1x10^{-05}$ is used in genome-wide analysis as a threshold of "nominal significance", for this study we considered the lower threshold of $1x10^{-06}$ to reduce the risk of identifying false positive results whilst taking into consideration the relatively modest size of the PASS cohort.

Due to the modest size of our replication cohorts, we reduced the number of SNPs taken forward for replication by carefully reviewing each SNP's genetic region as follows: a) LocusZoom [http://locuszoom.org/] were reviewed for linkage disequilibrium structure and proximity to genes; and b) a literature search (January 2016) was undertaken to identify any existing data on relationships between these genes and the hypothalamic-pituitary axis, steroid receptors, or any other biological systems that might plausibly be linked to altered cortisol secretion.

Statistical analyses of SNPs selected for replication were conducted in R [https://www.r-project.org/] repeating the same methodology as that used for genome-wide analysis. That is logistic regression with binary phenotypes and linear regression with continuous phenotypes; adjusting for the same covariates as the genome-wide analysis, except for principal components, which are not possible to calculate from single genotypes.

1.9 Pathway Analysis

We used MAGENTA available from (https://www.ncbi.nlm.nih.gov/pubmed/21085203) to scrutinise the results from all phenotypes to identify any enrichment of functional and biological pathways [33].

SNPs were annotated to genes if they fell within a 5kb boundary surrounding the gene. The pathways and ontology terms were from: Biocarta, KEGG, Ingenuity, Panther, Reactome and Gene Ontology (GO) databases. Pathways were not limited to gene set size. A nominal

p-value for GSEA was calculated through permutation via random resampling of 10,000 gene sets of identical size. Both of MAGENTA thresholds for gene-level association signals were utilised (75th and 95th percentile) this was to ensure that weaker gene-level association signals could still be investigated. The FDR threshold of <0.01 was chosen across all pathway analyses so as to consider whether there were shared pathways across the phenotypes.

2. RESULTS

2.1 Primary analysis cohort – GWAS

The primary analysis cohort included 499 children and young people, but 92 failed genotype QC meaning that 407 were included in the GWAS. The details of these patients are shown in Table 1 (1: Mean ± SD). Of the 92 individuals failing QC, 19 failed the sample call-rate criteria, 5 failed the gender identity check, 9 failed IBD and 59 individuals were excluded as population outliers after principal component analysis (shown in Figure 1). 654, 246 SNPs passed the predefined genotyping QC criteria.

Table 1

		PASS discovery cohort	PASS replication cohort	PASIC replication cohort
Number recruited		499	81	78
Gender				Male: 42 Female: 37
Age[1]				67 (45-82)
Total daily corticosteroid dose (mg/kg beclomethasone equivalent)[1]				1578 (200-4000)
Inhaled corticosteroid exposure (%)				100
Rescue corticosteroid use (%)				100
Regular oral steroid (%)				0
Diagnoses requiring corticosteroid treatment n (%)	Asthma COPD Eczema Hayfever Other			 1 1
Duration of corticosteroid treatment (years)[1]				5 (0-20)
Biochemical Adrenal Insufficiency (%)				350: 5% 500: 22%

Figure 2 displays the QQ plots for the four phenotypes. No genomic inflation is evident in the GWAS of the Peak <500nmol/L, Peak <350nmol/L or Peak continuous phenotypes, as indicated by respective lambda values of 0.99, 1.03 and 0.99. However, there is genomic inflation for the Baseline phenotype, as indicated by a lambda value of 1.22. Due to the rigorous quality control procedures applied to the dataset prior to genome-wide analysis, the common causes of genomic inflation, such as, undetected sample duplications, unknown familial relationships, a poorly calibrated test statistic, systematic technical bias or gross population stratification have been eliminated. Therefore, as the cause of genomic inflation is unknown, in our analysis of the Baseline phenotype we adjusted the p-values for genomic inflation as described in [https://www.ncbi.nlm.nih.gov/pubmed/11315092].

Figure 3 displays the Manhattan plots for the four phenotypes. A total of 4 SNPs were initially identified as notionally statistically significant ($p<1x10^{-6}$) for the peak cortisol < 500nmol/L phenotype (Figure 3A), 51 for the peak cortisol < 350nmol/L phenotype (Figure 3B), 23 for the continuous peak cortisol phenotype (Figure 3C) and 16 for the baseline cortisol phenotype (Figure 3D).

The SNPs giving a p-value of $<1x10^{-6}$ in the analyses of association with each phenotype in turn are shown in Table 2, together with its genetic region and information on whether the SNP was genotyped or imputed. In total, there were sixty SNPs identified in 19 genes across the phenotypes (Table 2). *PDGFD* was the only gene to be found to be associated with more than one phenotype (both peak 500 and 350nmol/L cutoff outcomes).

Table 2

Phenotype	Gene (Chromosome)	SNP(s) identified[1]	Lowest p-value in PASS discovery cohort
Peak 500	*GJA8 (1)*	rs201161441, **rs6657114**, rs6671502	1.69E-22
	TRPA1 (8)	rs75470088	5.04E-07
	PDGFD (11)	rs7116655	8.14E-07
Peak 350	*PDGFD (11)*	rs361283, rs361284, **rs590216, rs603781**, rs591118, rs589796, rs2515080, rs684212, rs517401, rs671851, rs2515083, rs620426, rs619954, rs574494, rs619114, rs618648, rs5794293, rs623031	5.80E-08
	KRT8P9 (15)	rs111566682	2.32E-07
	PSMD3 (17)	rs9912981, rs3859188, rs71355433, rs7222556, **rs9916279**, rs8080546, rs11654706, rs11078932,	4.37E-07

		rs58212353, rs2012	
	CSF3 (17)	rs2827	4.48E-07
	MED24 (17)	**rs11555254,** rs2302778, rs7503939, **rs17850739,** rs72834789	4.02E-07
Peak Continuous	*LRP1B (2)*	rs142320277	5.23E-08
	GBA3 (4)	rs111863753	7.75E-07
	HMGN3 (6)	rs13220233	2.88E-07
	PDE7B (6)	rs149647891	6.43E-07
	SCGN (6)	rs5875060	7.62E-08
	ANKS1B (12)	rs191087489, rs143638033, rs142161979	8.98E-09
	ELSPBP1 (19)	rs137939366	3.05E-07
Baseline Continuous	*NOS1 (12)*	rs12815584, rs77562913, rs76830467, rs75992652, rs34406980, rs150941488	3.00E-07
	IGH (14)	rs201541519	3.02E-07
	SLC2A10 (20)	rs117420762	3.12E-07
	BCL2L13 (22)	rs149352662, rs189673743, rs140179402	2.00E-8

The LocusZoom plots for genes other than *PDGFD* and *TRPA1* were not-supportive in terms of linkage disequilibrium structure. However, the LocusZoom of the regions on chromosome 11 where *PDGFD* is located (Figure 4 A&B) suggested a true signal, with a narrow column of SNPs in increasing linkage disequilibrium with increasing p-value. A similar linkage disequilibrium structure was observed in the Locus Zoom plot of the *TRPA1* gene located in chromosome 8.

Publications for each gene were reviewed to examine possible biological plausibility. There were no relevant publications noted for six of the eight genes. TRPA1 has been associated with Familial Episodic Pain Syndrome [34], and TRPA1 expression (after painful stimuli) has been linked increased HPA axis responses in mice [35]. Pain is well recognised as a stimulus to the HPA axis, as part of its normal function. There was also literature relating to PDGFD. PDGF receptors are required in the development of steroid-producing cells in multiple organs, including the testis, ovary, and adrenal cortex [36]. In addition, expression of PDGFD has been negatively correlated with cortisol secretion in adrenocortical adenomas [37]. This was the only publication from any of the genes related to decreased cortisol secretion.

PDGFD therefore had the most convincing association from the primary analysis cohort, and some supportive literature suggesting biological plausibility.

Therefore it was selected as the only gene to be investigated in the replication cohorts. The *PDGFD* SNP selected to be genotyped in the replication cohorts was the one giving the lowest p-value for association, rs591118. This gave a p-value of $5.8x10^{-08}$ in the analysis of association with the peak <350 phenotype (odds ratio 7.32 (95% CI: 3.17-16.87).

2.2 Replication

On analysing association between rs591118 and case-control status with the peak cut-off of 500 phenotype, a p-value of 0.04 (odds ratio 2.12 [95% CI: 1.03-4.37]) and with the peak cut-off of 350 phenotype, a p-value of 0.02 (odds ratio 3.86 [95% CI: 1.19-12.50]) were achieved in the PASS internal replication cohort.

In the PASIC replication cohort the association results between rs591118 and case-control status with the peak cut-off of 500 phenotype gave a p-value of 0.03 (odds ratio 2.41 [95% CI: 1.10-5.28]) and with the peak cut-off of 350 phenotype, gave a p-value of 0.72 (odds ratio 1.27 [95% CI: 0.34-4.82]).

2.3 Meta-analysis

Due to the definition of adrenal suppression differing between paediatric and elderly cohorts, the meta-analysis performed combined the two paediatric cohorts' odds ratios from the peak <350 phenotype with and without the PASIC cohort's odds ratio from the peak.

Meta-analysing either the two PASS cohorts (discovery and replication), or the three cohorts together (PASS discovery and replication, and PASIC replication) led to genome-wide significance of the association. On meta-analysing the two PASS cohorts, the meta-analysis produced a p-value of $4.3x10^{-09}$ (odds ratio 5.89 [95% CI: 2.97-11.68]), shown in Figure 5A. On meta-analysing all three cohorts together, the meta-analysis produced a p-value of $3.5x10^{-10}$(odds ratio 4.05 [95% CI: 2.00-8.21]), shown in Figure 5B.

2.4 Number needed to test

For children with asthma, using ICS: Considering rs59118 genotype within the PASS discovery and replication cohorts, using the <350nmol/L definition of adrenal suppression, the frequency of adrenal suppression in WT group is 0.002 (0.2 per 100); Het group is 0.006

(0.6 per 1000); MT group is 0.038 (3.8 per 100). The minor allele frequency of rs59118 in the paediatric discovery cohort was 0.47, with a homozygous MT proportion of 0.14. Using alternative treatments, we avoid 0.6 events (peak cortisol <350nmol/L) per 100 paediatric patients in Het group and 3.8 events per 100 patients in MT group. This translates to needing to stop treating 167 ([1/0.6]*100) from Het group and 27 ([1/3.8]*100) from MT group. The numbers needed to test (NNT) to avoid a case of adrenal suppression (peak cortisol <350nmol/L) would therefore be 356 (167/0.47) for Het rs59118 patients (95% CI 179-709) and 193 (27/0.14) for MT rs59118 patients (95% CI 122-315).

For adults with COPD: For the rs59118 genotype within the PASIC replication cohort, using the <500nmol/L definition of adrenal suppression, the frequency of adrenal suppression in WT group is 0.11 (11 per 100); Het group is 0.21 (21 per 100); MT group is 0.41 (41 per 100). The minor allele frequency of rs59118 in the adult COPD replication (PASIC) cohort was 0.44, with a homozygous MT proportion of 0.22. Using alternative treatments, we avoid 21 events (peak cortisol <500nmol/L) per 100 patients in Het group and 41 events per 100 patients in MT group. This translates to needing to stop treating 5 ([1/21]*100) from Het group and 3 ([1/41]*100) from MT group. The NNT to avoid a case of adrenal suppression in the adult COPD (PASIC) population would therefore be 12 (5/0.44) for Het rs59118 patients (95% CI 7-23) and 14 (3/0.22) for MT rs59118 patients (95% CI 9-23).

2.5 Pathway Analysis

The pathway analysis of the GWAS with clinical covariates resulted in twelve pathways and gene ontology terms significantly enriched (FDR < 10%, Table 3). No terms were found in common between the analyses with a cut-off of FDR < 10%.

Table 3

Phenotype	Database	Gene Set	#Gene	GSEA p-value	FDR Corrected (Percentile Cut-off)
Peak	KEGG	Selenoamino Acid Metabolism	26	$2x10^{-4}$	$1x10^{-2}$ (95)
	KEGG	Phosphatidylinositol Signalling System	76	$1x10^{-3}$	$9x10^{-2}$ (75)
	Panther Molecular Function	Histone	86	$2x10^{-4}$	$4x10^{-2}$ (95)
Peak 350	Ingenuity	VEGF Signalling	16	$4.1x10^{-3}$	$5.2x10^{-2}$ (75)
	Ingenuity	PI3K AKT Signalling	29	$1.9x10^{-3}$	$6.5x10^{-2}$ (75)
	Panther Biological Process	mRNA transcription	296	$2.0x10^{-4}$	$4.9x10^{-2}$ (75)
	Ingenuity	JAK STAT Signalling	10	$4.6x10^{-3}$	$5.6x10^{-2}$ (75)
Baseline	Panther Molecular Function	Transferase	96	$5.0x10^{-4}$	$4.9x10^{-2}$ (75)
	Panther Molecular Function	Surfactant	11	$1.2x10^{-3}$	$5.5x10^{-2}$ (75)
	Panther Biological Process	Phosphate Metabolism	91	$5.5x10^{-5}$	$8.4x10^{-3}$ (75)
	Panther Biological Process	Extracellular Transport and Import	89	$4.0x10^{-4}$	$9.8x10^{-2}$ (95)
	Panther	Cytoskeletal regulation by Rho GTPase	29	$1.2x10^{-3}$	$8.2x10^{-2}$ (75)

For the peak cortisol phenotype GWAS, the pathway analysis resulted in three pathways and gene ontology terms significantly enriched; selenoamino acid metabolism, phosphatidylinositol signalling system and histone. The pathway analysis of the peak cortisol 500 cut-off phenotype GWAS resulted in no pathways or gene ontology terms significantly enriched (FDR < 10%). For the peak cortisol 350 cut-off phenotype GWAS, four pathways and terms were significantly enriched; vascular endothelial growth factor (VEGF) signalling, phosphoinositide 3-kinase (PI3K) serine/threonine kinase (AKT) signalling, micro RNA transcription and the Janus kinase (JAK) and two Signal Transducer and Activator of Transcription (STAT) signalling. For the base cortisol phenotype GWAS, the pathway analysis resulted in five pathways and gene ontology terms significantly enriched; transferase, surfactant, phosphate metabolism, extracellular transport and import and cytoskeletal regulation by Rho GTPases.

2.6 Expression analysis of rs591118 in tissue databases

No associations were found between any of the SNPs and gene expression of the forty-four tissues included in the GTEx database [32].

Further data was obtained, and further analysis was conducted on the data described above. These results are reported below:

2.7 Peak Cortisol

Figure 6 shows box whisker plots of peak cortisol against rs591118 genotype for each cohort tested.

The peak cortisol responses were generally lower in individuals who had the GG genotype at rs591118 (see figure 6). The proportion of patients with a peak cortisol response of either <350nmol/L (children) or <500nmol/L (adults) according to genotypes were 5.9% (AA), 9.9% (AG) and 23.3% (GG)

2.8 Pathway Analysis

Sixteen pathways were included in a further pathway analysis that contained the *PDGFD* gene. We used MAGENTA to scrutinise the results from all phenotypes to identify any enrichment of functional and biological pathways [33]. SNPs were annotated to genes if they fell within a 5kb boundary surrounding a gene (including all promotor and intronic regions) [50]. The pathways and ontology terms used in MAGENTA are from Biocarta, KEGG, Ingenuity, Panther, Reactome and Gene Ontology (GO) databases. We refined the databases down to the pathway and ontology terms that included the genes that had been identified through annotation of the SNPs selected for replication.

Pathways were not limited to gene set size. A nominal p-value for Gene Set Enrichment Analysis (GSEA) was calculated through permutation via random resampling of 10,000 gene sets of identical size. Both MAGENTA thresholds for gene-level association signals were utilised (75th and 95th percentile), to ensure that weaker gene-level association signals

could still be investigated. The FDR threshold of 5% was chosen across all pathway analyses to indicate a significant pathway.

There were two pathways identified that were associated with a FDR corrected p-value of less than 0.05 (see Table 3A); these were Focal Adhesion and Regulation of Actin Cytoskeleton both from the KEGG database.

Table 3A

	Database	Gene Set	#Gene	GSEA p-value	FDR Corrected (Percentile Cut-off)
Peak 350	GOTERM	Golgi membrane	350	0.12	0.23 (75)
	GOTERM	Extracellular Region	1863	0.06	0.32 (75)
	GOTERM	Growth Factor Activity	161	0.30	0.35 (75)
	GOTERM	Regulation of Peptidyl-tyrosine Phosphorylation	10	0.36	0.38 (75)
	GOTERM	Positive Regulation of Cell Division	37	0.75	0.67 (75)
	Panther Biological Process	Cell Proliferation and Differentiation	1028	0.24	0.70 (95)
	Panther Biological Process	Other Developmental Process	104	0.39	0.55 (95)
	Panther Biological Process	Ligand-mediated Signaling	421	0.90	0.90 (95)
	Panther Molecular Function	Growth Factor	118	0.53	0.53 (75)
	Panther	Angiogenesis	80	0.08	0.08 (95)
	Ingenuity	PDGF Signaling	23	0.27	0.27 (75)
	KEGG	KEGG Focal Adhesion	201	$2.4x10^{-4}$	$6.0x10^{-4}$ (95)*
	KEGG	KEGG Gap Junction	90	0.07	0.09 (95)
	KEGG	KEGG Regulation of Actin Cytoskeleton	216	0.01	0.01 (95)*
	KEGG	KEGG Prostate Cancer	89	0.10	0.27 (95)
	KEGG	KEGG Melanoma	71	0.49	0.43 (95)

We also used IPA® The Ingenuity® Pathway Analysis software (QIAGEN Redwood City), to evaluate the biological interactions and pathways known to encompass PDGFD. Within the

canonical PDGF signalling pathway involving PDGFD, several cytoplasmic signalling proteins are recruited, leading to the activation of a number of key signalling cascades including the mobilization of cytosolic calcium and mitogen activated protein kinase (MAPK) pathway.

2.9 Primary analysis cohort – GWAS

The primary analysis cohort included 499 children and young people, but 92 failed genotype QC meaning that 407 were included in the GWAS as stated above. Further details of these patients were obtained and/or calculated, and are shown in Table 1A (1: Mean ± SD):

Table 1A

		PASS discovery cohort	PASS replication cohort	PASIC replication cohort
Number recruited		499	81	78
Female Gender		42%	46%	47%
Age[1]		11.6 ± 3.3	14.6 ± 4.9	65.8±15.0
Total daily corticosteroid dose (mg/kg beclomethasone equivalent)[1]		6915 ± 17014	10638 ± 30127	12878±7163
Inhaled corticosteroid exposure (%)		100%	100%	100%
Rescue corticosteroid use (%)		53%	53%	100%
Regular oral corticosteroid (%)		10%	9%	0%
Diagnoses requiring corticosteroid treatment n (%)	Asthma COPD Eczema Hayfever Other			 1 1
Duration of corticosteroid treatment (years)[1]				5 (0-20)
Biochemical Adrenal Insufficiency (%)		350:7% 500: 35%	350:8% 500:40%	350: 5% 500: 22%

3.0 DISCUSSION

This study has identified and replicated a polymorphism (rs591118) in the Platelet Derived Growth Factor D (*PDGFD*) gene as being associated with development of adrenal suppression in children with asthma and adults with COPD using corticosteroids. This is the

first study to examine the pharmacogenomic associations of corticosteroid induced adrenal suppression.

Using a genome-wide approach in children with asthma treated with predominantly inhaled corticosteroids, we identified that common variants in the Platelet Derived Growth Factor D (*PDGFD*) gene were associated with the development of adrenal suppression. Using the top hit in the GWAS (rs591118), this finding was replicated in another group of paediatric patients with asthma, with the meta-analytic p-value being genome-wide significant (figure 5A). We also replicated the finding in an adult group of patients with COPD, with meta-analysis of all 3 cohorts again showing genome-wide significance (figure 5B). The replication in the adult cohort is especially remarkable given that these patients were suffering from a different complex disease, had multiple co-morbidities and were on multiple medications, further reinforcing the strength of the polymorphism rs591118 in indicating development of adrenal suppression.

rs591118 is an A/G substitution in an intronic region of the *PDGFD* gene. A search of the GWAS catalogue (14 August 2017) showed that this particular SNP has not been reported to be associated with any disease or trait. The only associations with other variants within *PDGFD* gene at a p-value of $<5 \times 10^{-8}$ (i.e. genome-wide significant) have been reported with unrelated coronary artery disease and myocardial infarction [51] [52].

PDGFD is 4th member of the platelet derived growth factor family, originally described in 2001 [38, 39]. Unlike PDGFA and PDGFB, the C and D ligands are activated post-secretion by cleavage of their N-terminal CUB domains [40]. PDGFs have been shown to direct the migration, differentiation and function of a variety of specialized mesenchymal and migratory cell types [40], and PDGFB (but not PDGFD) has been associated with alterations in airway remodelling in asthma [41, 42].

For childhood asthma, corticosteroid efficacy in childhood asthma has been investigated, identifying approximately 20 genetic polymorphisms affecting a child's response to this treatment [43], while there is no similar research we are aware of in adult COPD. PDGFD has not previously been associated with corticosteroid efficacy in these studies.

There have also been extensive investigations of the genetic associations underpinning the development and severity of asthma, with numerous genetic polymorphisms identified [44-46], but PDGFD has not been identified by these techniques.

We are the first to consider whether *PDGFD* genotype may affect asthma or COPD severity, and hence corticosteroid dose used in the patients.

The minor allele frequency for the SNP rs591118 in the *PDGFD* gene is 0.44. Current asthma prevalence is 11% in childhood, meaning that approximately 1.5% of all children will have both asthma and be homozygous for the rs59118 polymorphism. This means that up to 165,000 children in the UK and 1.1 million children in the USA are at increased risk of adrenal suppression from corticosteroid therapy from corticosteroid asthma medication. Estimates of COPD frequency in the UK suggest that 1.2 million adults have been diagnosed with this condition, and in the USA there are 11 million cases, meaning there are approximately 232,000 (UK) and 2,130,000 (USA) individuals homozygous for rs59118 and with a diagnosis of COPD, at increased risk of corticosteroid induced adrenal suppression.

Asthma is a very common, chronic condition, with inhaled corticosteroids used for all but the mildest of disease [1, 2]. There are several different treatments available for those with more severe disease, including increasing the dose of inhaled corticosteroids, or using additional medications. This polymorphism in PDGFD identifies children who are at least at 3x greater risk of corticosteroid induced adrenal suppression. Children with asthma who are homozygous for the polymorphism in PDGFD identified herein and who are using ICS are nearly 6x more likely to develop adrenal suppression, while the adults with COPD who are homozygous for the PDGFD polymorphism and are using high dose ICS are 4x more likely to develop adrenal suppression. This gives a calculated population attributable risk of developing corticosteroid induced adrenal suppression due to the presently identified polymorphism of 61%.

There is little difference in the frequency of adrenal suppression across the dose range of inhaled corticosteroids [9], only increasing with oral doses. Using the SNP rs591118 identified herein as a marker will make it possible to identify those individuals at high risk of adrenal suppression, and modify their treatment. It might therefore be possible to alter the management of mild childhood asthma by substituting montelukast for inhaled corticosteroids in high risk individuals who are escalated from beta-2-agonist only treatment. However, the greater benefits of this discovery are likely to be either from clinicians focussing their screening on a targeted population at highest risk, or incorporation of this test into genetic asthma panels that will assess risks and benefits of a range of asthma medications. The test for adrenal suppression, the LDSST, is invasive and painful for children, and the current decision to test often relies on clinical suspicion. By understanding the patient's individual risk better, clinicians will be able to target this test more appropriately.

As with childhood asthma, there are few treatment alternatives for adults with COPD apart from inhaled corticosteroids for those with more than mild disease, with maximal effect achieved when used in combination with long acting beta-2-agonists [47]. Again, we would

predict that the key improvement in patient care from this discovery will be in stratification of COPD patients by risk of corticosteroid induced adrenal suppression, to allow targeted testing.

A strength of these data is that replication of the gene in question was achieved in a population that differ in age, underlying disease and co-morbidities. The PASIC cohort specifically excluded those with asthma, instead recruiting those with COPD. However, there are many different medical conditions treated with corticosteroids, and it will be necessary to investigate these conditions to see if this effect is also replicated there.

Identification of the correct threshold for diagnosis of adrenal suppression in children is difficult, as there is little normative data. For paediatric populations, a peak of 350nmol/L has been shown to be the most sensitive and specific at determining adrenal insufficiency [14], although 500nmol/L remains widespread in clinical practice. For adults, 500nmol/L is the accepted threshold. In a detailed review of symptomatic cases of adrenal insufficiency during inhaled corticosteroid use in childhood asthma [14], there were no published cases in patients with peak cortisol concentrations >350nmol/L in either standard dose or low dose tests. We therefore believe that we have selected the most appropriate threshold for each cohort in this study.

The inventors have surprisingly shown that *PDGFD* polymorphism rs59118 is associated with increased risk of adrenal suppression in children and adults who use corticosteroids to treat asthma and COPD respectively. This marks an important step towards personalised medicine for respiratory conditions requiring corticosteroid treatment.

References

1. National Guideline, C. Medications: Expert panel report 3: guidelines for the diagnosis and management of asthma. 7/19/2011]; Available from: http://www.guideline.gov.
2. James, D.R. and M.D. Lyttle, British guideline on the management of asthma: SIGN Clinical Guideline 141, 2014. Archives of disease in childhood-Education & practice edition, 2016: p. edpract-2015-310145.
3. Restrepo, R.D., A stepwise approach to management of stable COPD with inhaled pharmacotherapy: a review. Respiratory care, 2009. **54**(8): p. 1058-1081.
4. Excellence, N.I.f.H.a.C. Chronic obstructive pulmonary disease in over 16s: diagnosis and management. 2010 [cited 2016 21st September]; Available from: https://www.nice.org.uk/guidance/CG101/chapter/1-Guidance#managing-stable-copd.

5. Statistics, N. Prescriptions Dispensed in the Community, Statistics for England - 2005-2015. 2016 [cited 2016 21st September]; Available from: http://digital.nhs.uk/searchcatalogue?productid=20895&q=title%3a%22Prescriptions+Dispensed+in+the+Community%22&sort=Relevance&size=10&page=1#top.
6. Lipworth, B.J., Systemic adverse effects of inhaled corticosteroid therapy: a systematic review and meta-analysis. Archives of Internal Medicine, 1999. **159**(9): p. 941-955.
7. Paton, J., et al., Adrenal responses to low dose synthetic ACTH (Synacthen) in children receiving high dose inhaled fluticasone. Archives of Disease in Childhood, 2006. **91**(10): p. 808-813.
8. Todd, G.R.G., et al., Survey of adrenal crisis associated with inhaled corticosteroids in the United Kingdom. Archives of Disease in Childhood, 2002. **87**(6): p. 457-461.
9. Hawcutt, D.B., et al., Adrenal responses to a low-dose short synacthen test in children with asthma. Clinical endocrinology, 2015. **82**(5): p. 648-656.
10. Zhang, L.J., et al., Dose Response of Inhaled Corticosteroids in Children With Persistent Asthma: A Systematic Review. Pediatrics, 2011. **127**(1): p. 129-138.
11. Rasmuson, S., T. Olsson, and E. Hagg, A low dose ACTH test to assess the function of the hypothalamic-pituitary-adrenal axis. Clinical Endocrinology, 1996. **44**(2): p. 151-156.
12. Lipworth, B.J., Systemic adverse effects of inhaled corticosteroid therapy - A systematic review and meta-analysis. Archives of Internal Medicine, 1999. **159**(9): p. 941-955.
13. Kazlauskaite, R., et al., Corticotropin Tests for Hypothalamic-Pituitary- Adrenal Insufficiency: A Metaanalysis. J Clin Endocrinol Metab, 2008. **93**(11): p. 4245-4253.
14. Blair, J., et al., Early morning salivary cortisol and cortisone, and adrenal responses to a simplified low-dose short Synacthen test in children with asthma. Clinical endocrinology, 2014. **80**(3): p. 376-383.
15. Priftis, K.N., et al., Endocrine-Immune Interactions in Adrenal Function of Asthmatic Children on Inhaled Corticosteroids. Neuroimmunomodulation, 2009. **16**(5): p. 333-339.
16. Clark, P.M., et al., Defining the normal cortisol response to the short Synacthen test: implications for the investigation of hypothalamic-pituitary disorders. Clinical Endocrinology, 1998. **49**(3): p. 287-292.
17. Romeo, R.D., Pubertal maturation and programming of hypothalamic-pituitary-adrenal reactivity. Frontiers in Neuroendocrinology, 2010. **31**(2): p. 232-240.
18. Shenoy, S.D., P.G. Swift, and D. Cody, Growth impairment and adrenal suppression on low-dose inhaled beclomethasone. J Paediatr Child Health, 2006. **42**(3): p. 143-4.

19. Tantisira, K.G., et al., Corticosteroid pharmacogenetics: association of sequence variants in CRHR1 with improved lung function in asthmatics treated with inhaled corticosteroids. Human Molecular Genetics, 2004. **13**(13): p. 1353-1359.
20. Tantisira, K.G., et al., TBX21: A functional variant predicts improvement in asthma with the use of inhaled corticosteroids. Proceedings of the National Academy of Sciences of the United States of America, 2004. **101**(52): p. 18099-18104.
21. Raby, B.A., et al., T-bet polymorphisms are associated with asthma and airway hyperresponsiveness. American Journal of Respiratory and Critical Care Medicine, 2006. **173**(1): p. 64-70.
22. Hawkins, G.A., et al., The glucocorticoid receptor heterocomplex gene STIP1 is associated with improved lung function in asthmatic subjects treated with inhaled corticosteroids. Journal of Allergy and Clinical Immunology, 2009. **123**(6): p. 1376-1383.
23. Magnotti, M. and M. Shimshi, Diagnosing Adrenal Insufficiency: Which Test is Best—The 1-µg or the 250-µg Cosyntropin Stimulation Test? Endocrine Practice, 2008. **14**(2): p. 233-238.
24. Lekkakou, L., et al., Effects of gender and age on hypothalamic–pituitary–adrenal reactivity after pharmacological challenge with low-dose 1-µg ACTH test: a prospective study in healthy adults. Clinical endocrinology, 2013. **79**(5): p. 683-688.
25. Hawcutt, D., et al., Pharmacogenetics of warfarin in a paediatric population: time in therapeutic range, initial and stable dosing and adverse effects. The pharmacogenomics journal, 2014.
26. Purcell, S., et al., PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet, 2007. **81**(3): p. 559-75.
27. Zheng, X., et al., A high-performance computing toolset for relatedness and principal component analysis of SNP data. Bioinformatics, 2012. **28**(24): p. 3326-8.
28. Delaneau, O., J. Marchini, and J.F. Zagury, A linear complexity phasing method for thousands of genomes. Nat Methods, 2012. **9**(2): p. 179-81.
29. Howie, B., J. Marchini, and M. Stephens, Genotype imputation with thousands of genomes. G3 (Bethesda), 2011. **1**(6): p. 457-70.
30. Howie, B.N., P. Donnelly, and J. Marchini, A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet, 2009. **5**(6): p. e1000529.
31. Willer, C.J., Y. Li, and G.R. Abecasis, METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics, 2010. **26**(17): p. 2190-2191.
32. The Genotype-Tissue Expression (GTEx) project. Nat Genet, 2013. **45**(6): p. 580-5.

33. Wang, K., M. Li, and H. Hakonarson, Analysing biological pathways in genome-wide association studies. Nat Rev Genet, 2010. **11**(12): p. 843-54.
34. Kremeyer, B., et al., A Gain-of-Function Mutation in TRPA1 Causes Familial Episodic Pain Syndrome. Neuron, 2010. **66**(5): p. 671-680.
35. Pierce, A.N., et al., Neonatal vaginal irritation results in long-term visceral and somatic hypersensitivity and increased hypothalamic-pituitary-adrenal axis output in female mice. Pain, 2015. **156**(10): p. 2021-2031.
36. Schmahl, J., K. Rizzolo, and P. Soriano, The PDGF signaling pathway controls multiple steroid-producing lineages. Genes & development, 2008. **22**(23): p. 3255-3267.
37. Wilmot Roussel, H., et al., Identification of gene expression profiles associated with cortisol secretion in adrenocortical adenomas. The Journal of Clinical Endocrinology & Metabolism, 2013. **98**(6): p. E1109-E1121.
38. Bergsten, E., et al., PDGF-D is a specific, protease-activated ligand for the PDGF β-receptor. Nature cell biology, 2001. **3**(5): p. 512-516.
39. LaRochelle, W.J., et al., PDGF-D, a new protease-activated growth factor. Nature cell biology, 2001. **3**(5): p. 517-521.
40. Hoch, R.V. and P. Soriano, Roles of PDGF in animal development. Development, 2003. **130**(20): p. 4769-4784.
41. Lewis, C.C., et al., Airway fibroblasts exhibit a synthetic phenotype in severe asthma. Journal of allergy and clinical immunology, 2005. **115**(3): p. 534-540.
42. Ito, I., et al., Platelet-derived growth factor and transforming growth factor-β modulate the expression of matrix metalloproteinases and migratory function of human airway smooth muscle cells. Clinical & Experimental Allergy, 2009. **39**(9): p. 1370-1380.
43. Davis, J.S., S.T. Weiss, and K.G. Tantisira, Asthma Pharmacogenomics: 2015 Update. Current Allergy and Asthma Reports, 2015. **15**(7): p. 12.
44. Sharma, S., et al., Glucocorticoid Genes and the Developmental Origins of Asthma Susceptibility and Treatment Response. American Journal of Respiratory Cell and Molecular Biology, 2014. **52**(5): p. 543-553.
45. Nieuwenhuis, M., et al., Combining genomewide association study and lung eQTL analysis provides evidence for novel genes associated with asthma. Allergy, 2016.
46. Dijk, F.N., et al., Genetics of onset of asthma. Current Opinion in Allergy and Clinical Immunology, 2013. **13**(2): p. 193-202.
47. G.I.f.C.O.L.D.G. Global Strategy for the Diagnosis, Management and Prevention of COPD. 2017 [cited 2017 30th January]; Available from: http://goldcopd.org.

48. McCormack, M., et al., HLA-A* 3101 and carbamazepine-induced hypersensitivity reactions in Europeans. New England Journal of Medicine, 2011. **364**(12): p. 1134-1143.

49. Gonzalbez, J., et al., Establishment of reference values for standard dose short synacthen test (250 microgram), low dose short synacthen test (1 microgram) and insulin tolerance test for assessment of the hypothalamo-pituitary-adrenal axis in normal subjects. Clinical endocrinology, 2000. **53**(2): p. 199-204.

50. Aslibekyan S, Almeida M, Tintle N. Pathway analysis approaches for rare and common variants: Insights from GAW18. Genetic epidemiology 2014; 38(0 1): S86-S91.

51. Nikpay M, Goel A, Won H-H, et al. A comprehensive 1000 Genomes-based genome-wide association meta-analysis of coronary artery disease. Nature genetics 2015; 47(10): 1121.

52. Consortium CADG. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. Nature genetics 2011; 43(4): 339-44.

CLAIMS

1. A method of identifying an increased risk of corticosteroid induced adrenal suppression in a subject, comprising determining the presence of at least one mutation in the *PDGFD* gene by assaying a sample from the subject, wherein the presence of a mutation is indicative of an increased risk of corticosteroid induced adrenal suppression in the subject.

2. A method of determining the presence of a mutation in a subject suspected of having an increased risk of corticosteroid induced adrenal suppression, the method comprising:
   (a) providing a sample from the subject; and
   (b) determining the presence of at least one mutation in the *PDGFD* gene.

3. A method of selecting a treatment for a subject having a disease, comprising determining the presence of at least one mutation in the *PDGFD* gene by assaying a sample from the subject, and selecting a treatment comprising corticosteroids in the absence of a mutation in the *PDGFD* gene.

4. A method of selecting a treatment for a subject having a disease, comprising determining the presence of at least one mutation in the *PDGFD* gene by assaying a sample from the subject, and selecting a conservative corticosteroid treatment in the presence of a mutation in the *PDGFD* gene.

5. A method of assessing the suitability of a subject for corticosteroid treatment, comprising determining the presence of at least one mutation in the *PDGFD* gene by assaying a sample from the subject, wherein the presence of a mutation is indicative that the subject is less suitable for corticosteroid treatment.

6. A method of assessing the suitability of a subject for corticosteroid treatment comprising determining the presence of at least one mutation in the *PDGFD* gene by assaying a sample from the subject, wherein the absence of a mutation is indicative that the subject is suitable for corticosteroid treatment.

7. A method according to claims 1 to 6, wherein the at least one mutation in the *PDGFD* gene is present in a non-coding region of the *PDGFD* gene.

8. A method according claims 1 to 7, wherein the at least one mutation in the *PDGFD* gene is present in an intron of the *PDGFD* gene.

9. A method according to any preceding claim wherein the at least one mutation is selected from the group consisting of substitution, missense, nonsense, deletion, insertion, duplication, frameshift, and repeat expansion.

10. A method according to claim 9, wherein the mutation is a substitution mutation.

11. A method according to claim 9 or 10, wherein the mutation is an A/G substitution mutation.

12. A method according to any preceding claim, wherein the mutation is a SNP.

13. A method according to claim 12, wherein the SNP is selected from the group consisting of rs591118, rs7116655, rs361283, rs361284, rs590216, rs603781, rs589796, rs2515080, rs684212, rs517401, rs671851, rs2515083, rs620426, rs619954, rs574494, rs619114, rs618648, rs5794293, and rs623031.

14. A method according to claim 13, wherein the SNP is selected from the group consisting of rs591118 and rs7116655.

15. A method according to any preceding claim wherein the mutation is SNP rs591118.

16. A method according to any preceding claim wherein the mutation is an A/G substitution at SNP rs591118.

17. A method according to any preceding claim wherein the method further comprises determining the presence of at least one further biomarker indicative of an increased risk of corticosteroid induced adrenal suppression.

18. A method according to claim 17, wherein the at least one further biomarker is a mutation present in genes selected from: GJA8, TRPA1, KRT8P9, PSMD3, CSF3, MED24, LRP1B, GBA3, HMGN3, PDE7B, SCGN, ANKS1B, ELSPBP1, LINC00607, IL31RA, STK32A, FBXL7, KCNQ5, MYCT1, XR_242309.1, CAV1, LINGO2, PRSS3, UBE2R2, UBAP2, NM_015397.3, DCAF12, HSPA12A, NOS1, CLDN10, NR_039974.1, IGH, NPAS3, XR_243228.1, SLC2A10, MACROD2, XKR7, and BCL2L13.

19. A method according to claim 18, wherein the at least one further biomarker is a mutation present in genes selected from: GJA8, TRPA1, KRT8P9, PSMD3, CSF3, and MED24.

20. A method according to claims 17-19, wherein the at least one further biomarker is a SNP.

21. A method according to claim 20, wherein the SNP is selected from the group consisting of: rs201161441, rs6657114, rs6671502, rs75470088, rs111566682, rs9912981, rs3859188, rs71355433, rs7222556, rs9916279, rs8080546, rs11654706, rs11078932, rs58212353, rs2012, rs2827, rs11555254, rs2302778, rs7503939, rs17850739, rs72834789, rs142320277, rs111863753, rs13220233, rs149647891, rs5875060, rs191087489, rs143638033, rs142161979, rs137939366, rs146292085, rs111404331, rs55782611, rs200531273, rs146414687, rs192277931, rs140981584, rs116994288, rs117162246, rs141159828, rs72711408, rs72711411, rs188183827, rs182806557, rs12553078, rs181360551, rs140635353, rs148024340, rs12551409, rs147719472, rs200001967, rs144482947, rs151129137, rs149572349, rs192660407, rs77562913, rs76830467, rs75992652, rs17617937, rs34401373, rs35097914, rs34406980, rs71444623, rs9658258, rs113071293, rs111406705, rs137908418, rs74053481, rs201541519, rs56406213, rs200209719, rs117420762, rs73269069, rs73269081, rs184548348, rs112736204, rs73269083, rs80075471, rs149352662, rs189673743, and rs140179402.

22. A method according to any preceding claim, wherein the subject is a human.

23. A method according to any preceding claim, wherein the subject has a disease selected from a group consisting of a respiratory disease, an oncology disease, a dermatology disease, an endocrinology disease, a gastroenterology disease, a haematology disease, an immunology disease, and an ophthalmology disease.

24. A method according to claim 23, wherein the disease is a respiratory disease.

25. A method according to any preceding claim, wherein the subject has a corticosteroid-treatable respiratory disease.

26. A method according to claims 23 to 25, wherein the respiratory disease is selected from the group consisting of asthma and COPD.

27. A method according to any preceding claim wherein the sample is a biological sample.

28. A method according to claim 27, wherein the biological sample contains a target molecule from which mutations in the *PDGFD* gene can be detected.

29. A method according to claim 28, wherein the target molecule is selected from the group consisting of nucleic acids, proteins, and precursors or variants thereof.

30. A method according to claim 29, wherein the nucleic acids are selected from the group consisting of DNA and RNA.

31. A method according to claims 27 to 30, wherein the biological sample is a tissue sample or a body fluid sample.

32. A method according to claim 31, wherein the body fluid sample is selected from the group consisting of blood or saliva.

33. A method according to claim 32, wherein the blood is whole blood treated with EDTA.

34. A method according to claims 1-33, wherein the corticosteroid is selected from a group consisting of group A corticosteroids, group B corticosteroids, group C corticosteroids, group D1 corticosteroids, and group D2 corticosteroids.

35. A method according to claims 1-34, wherein the corticosteroid is selected from the group consisting of a topical, inhaled, oral, intranasal, or systemic corticosteroid.

36. A method according to claim 1-29, wherein the corticosteroid is an inhaled corticosteroid.

37. A method according to claim 36, wherein the inhaled corticosteroid is selected from the group consisting of Flunisolide, Fluticasone, Mometasone, Fluticasone Furoate, Ciclesonide, Fluticasone Propionate, Triamcinolone Acetonide, Beclomethasone Dipropionate, Salmeterol Xinafoate, and Budesonide.

38. A method according to claims 1 or 5, wherein the method further comprises a step of selecting a conservative corticosteroid treatment in the presence of a mutation in the *PDGFD* gene.

39. A method according to claims 4 or 38, wherein the conservative corticosteroid treatment comprises reduced corticosteroids, a low dose of corticosteroids, alternative treatments, and/or increased monitoring of the subject.

40. A method according to claim 39, wherein a low dose of corticosteroids comprises below 2mg, below 1mg, below 0.7mg, below 0.5mg, below 0.4mg, below 0.3 mg, below 0.2mg, below 0.1mg, below 50µg, below 40µg, below 30µg, below 20µg, or below 10µg of corticosteroids.

41. A method according to claims 39 or 40 wherein reduced corticosteroids comprises less frequent administration of corticosteroids, or less potent corticosteroids.

42. A method according to claim 41, wherein less frequent administration of corticosteroids comprises administration of corticosteroids once per day, or once every other day, once every 3 days, once per week, once per fortnight, or once per month.

43. A method according to claims 39-42, wherein alternative treatment comprises treatment with leukotriene-modifying drugs, long or short acting sympathomimetics (e.g. beta2-agonists), anticholinergics (e.g. Ipratropium bromide), xanthene derivatives (e.g. theophylline), biological therapies (e.g. omalizumab), breathing exercises, acupuncture, massage therapy, herbal medications, or dietary supplements.

44. A method according to claims 39-43, wherein increased monitoring of the subject comprises monitoring the subject approximately once every six months, once every five months, once every four months, once every three months, or more often.

45. A method according to claims 39-44, wherein increased monitoring of the subject comprises monitoring the subject at regular intervals.

46. Use of at least one mutation in the *PDGFD* gene for *in vitro* determination of an increased risk of corticosteroid induced adrenal suppression in a subject.

47. The use according to claim 46, comprising any of the features according to claims 7-45.

48. A corticosteroid for use in the prevention or treatment of a disease in a subject identified as having an increased risk of corticosteroid induced adrenal suppression, wherein the subject is determined to have at least one mutation in the *PDGFD* gene, and the corticosteroid is for use in a conservative treatment.

49. A corticosteroid for use according to claim 48, comprising any of the features according to claims 7-45.

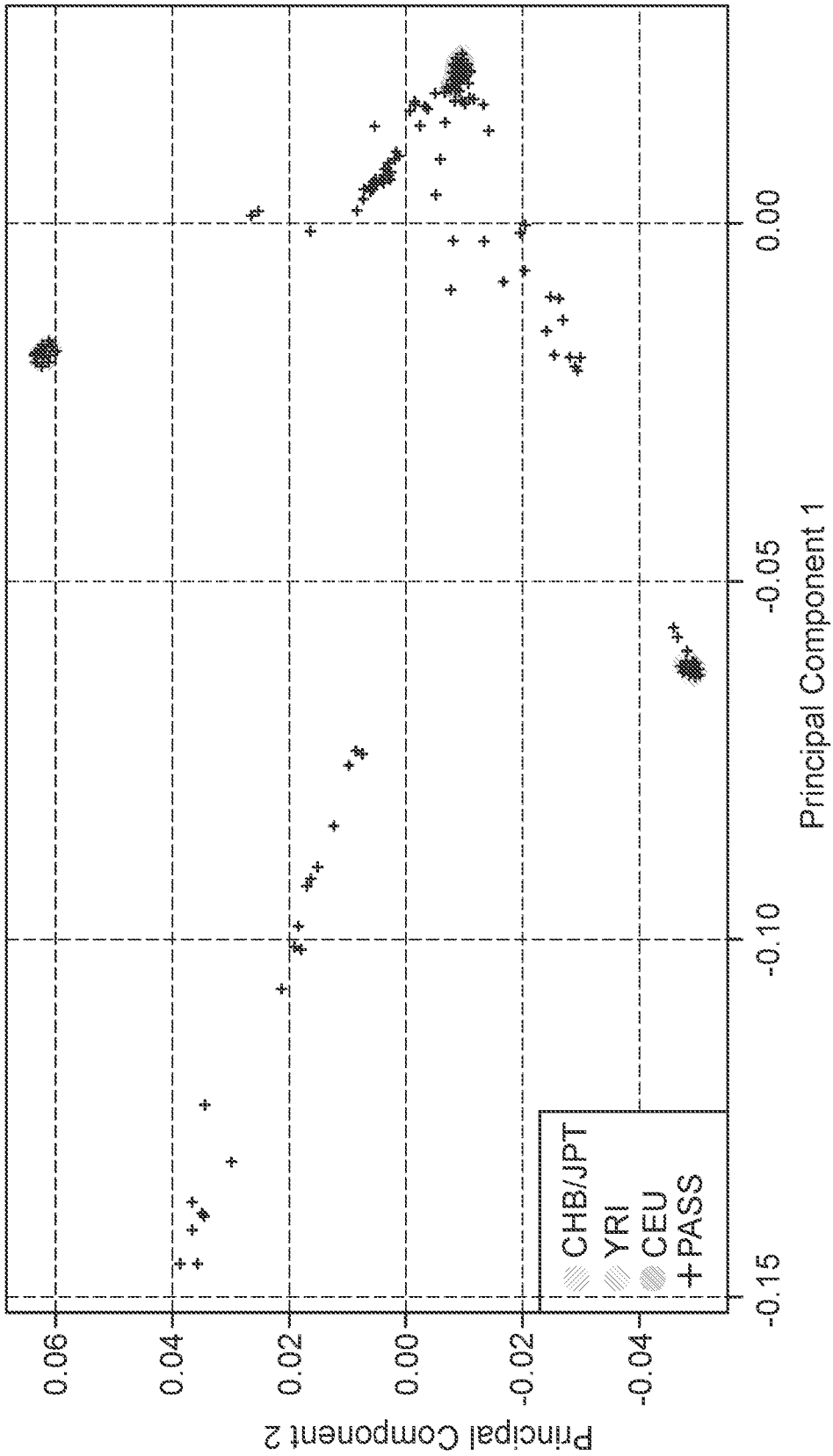

Principal Component 2
0.06
0.04
0.02
0.00
-0.02
-0.04
Principal Component 1
0.00
-0.05
-0.10
-0.15
CHB/JPT
YRI
CEU
+PASS

Fig. 1

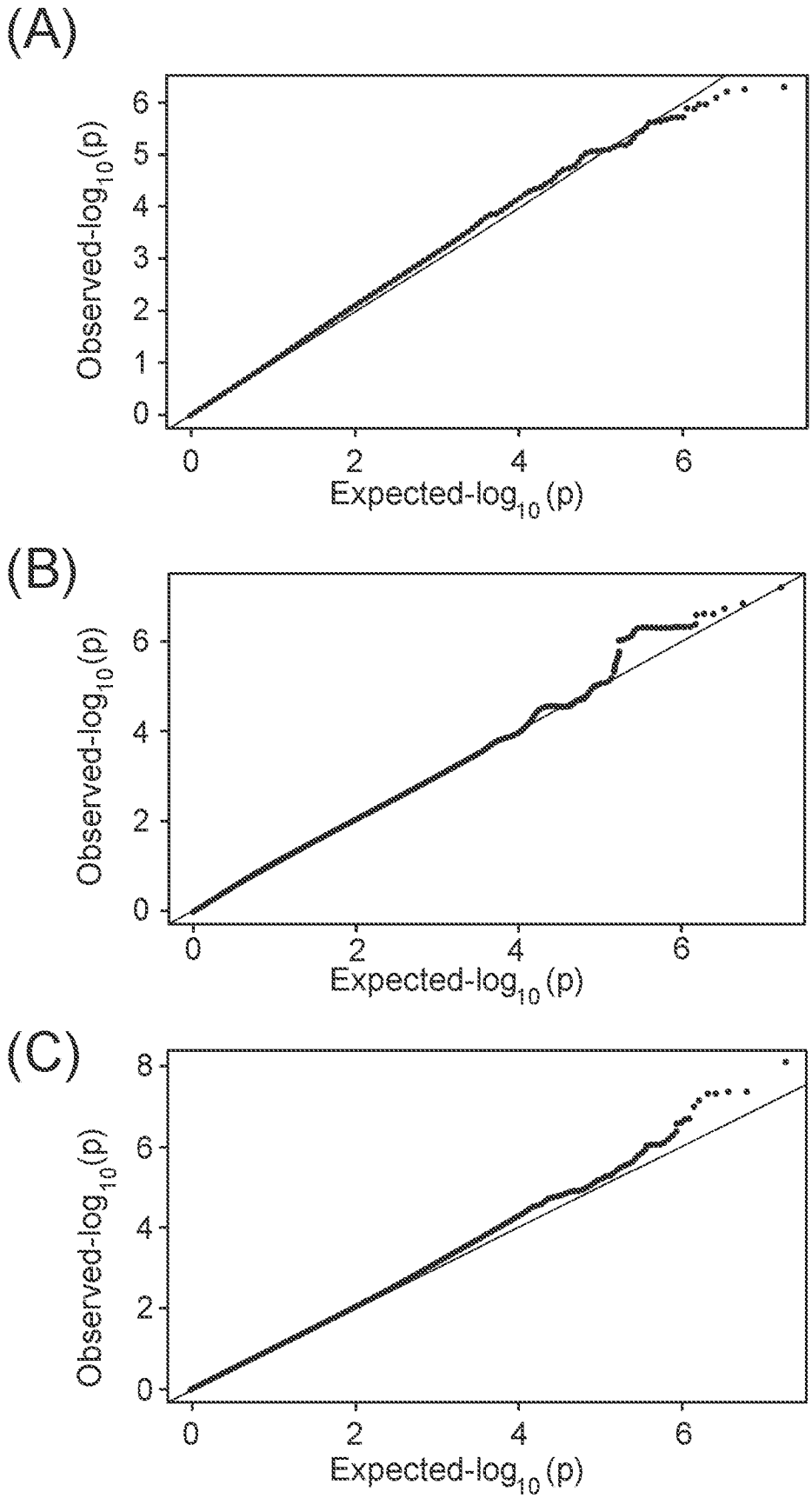

(A)
Observed-log10(p)
Expected-log10(p)
(B)
Observed-log10(p)
Expected-log10(p)
(C)
Observed-log10(p)
Expected-log10(p)

Fig. 2

(D)

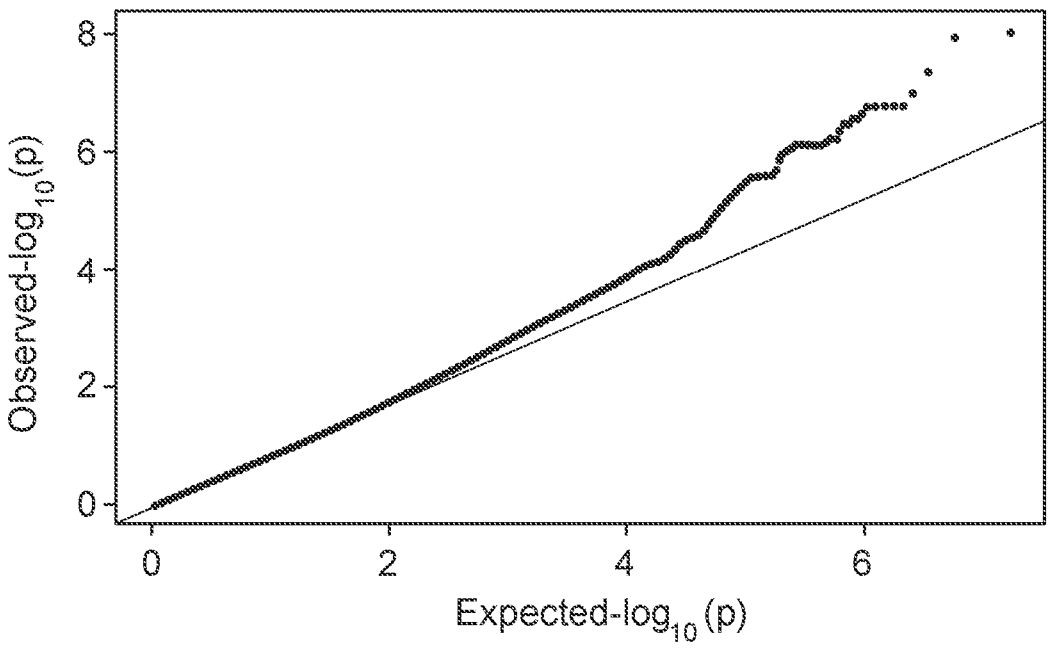


Fig. 2 Continued

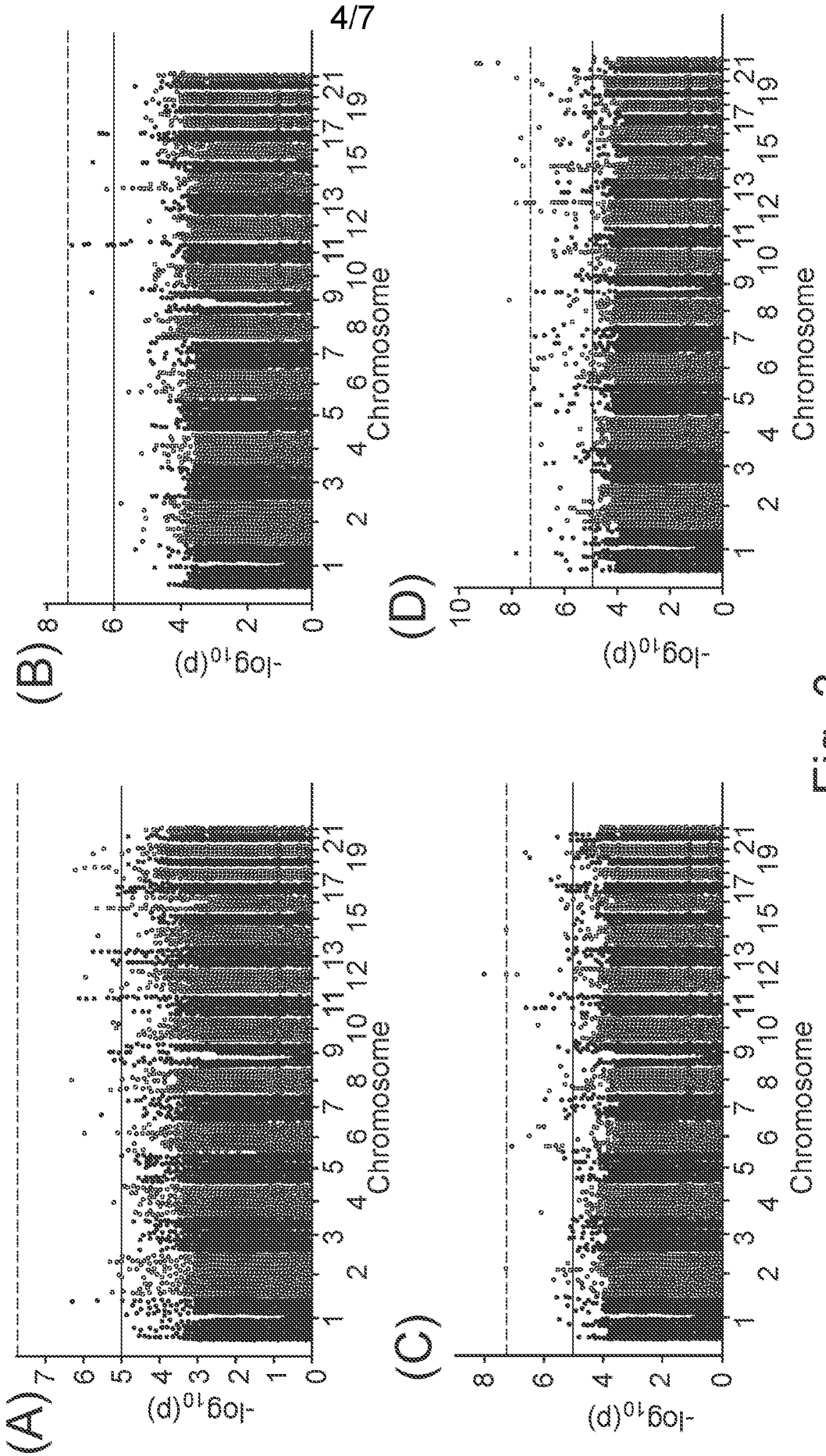


Fig. 3

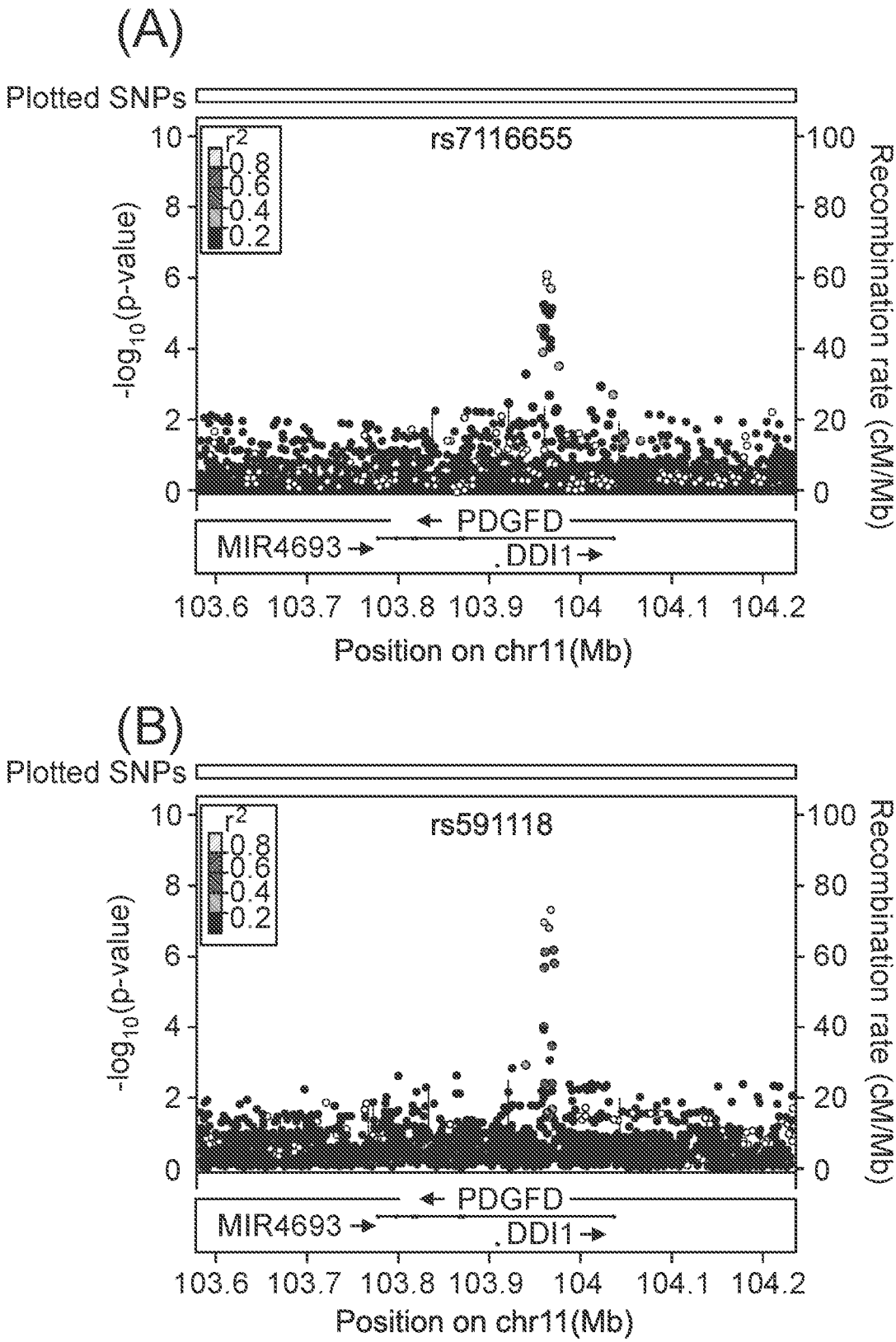

(A)
Plotted SNPs
rs7116655
r2
0.8
0.6
0.4
0.2
-log10(p-value)
Recombination rate (cM/Mb)
PDGFD
MIR4693
DDI1
103.6 103.7 103.8 103.9 104 104.1 104.2
Position on chr11(Mb)
(B)
Plotted SNPs
rs591118
r2
0.8
0.6
0.4
0.2
-log10(p-value)
Recombination rate (cM/Mb)
PDGFD
MIR4693
DDI1
103.6 103.7 103.8 103.9 104 104.1 104.2
Position on chr11(Mb)

Fig. 4

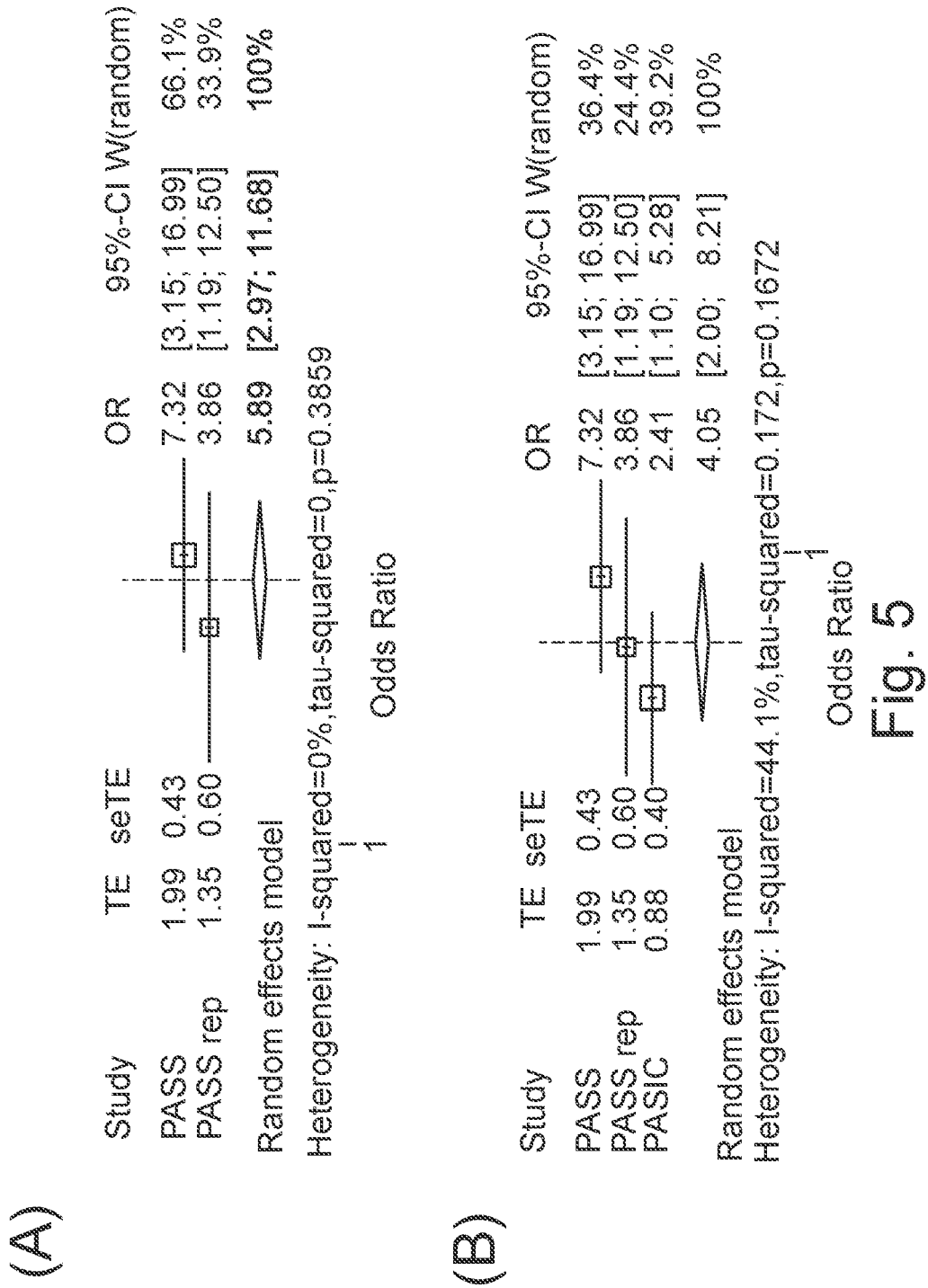

(A)
Study TE seTE OR 95%-CI W(random)
PASS 1.99 0.43 7.32 [3.15; 16.99] 66.1%
PASS rep 1.35 0.60 3.86 [1.19; 12.50] 33.9%
Random effects model 5.89 [2.97; 11.68] 100%
Heterogeneity: I-squared=0%,tau-squared=0,p=0.3859
1
Odds Ratio
(B)
Study TE seTE OR 95%-CI W(random)
PASS 1.99 0.43 7.32 [3.15; 16.99] 36.4%
PASS rep 1.35 0.60 3.86 [1.19; 12.50] 24.4%
PASIC 0.88 0.40 2.41 [1.10; 5.28] 39.2%
Random effects model 4.05 [2.00; 8.21] 100%
Heterogeneity: I-squared=44.1%,tau-squared=0.172,p=0.1672
1
Odds Ratio

Fig. 5

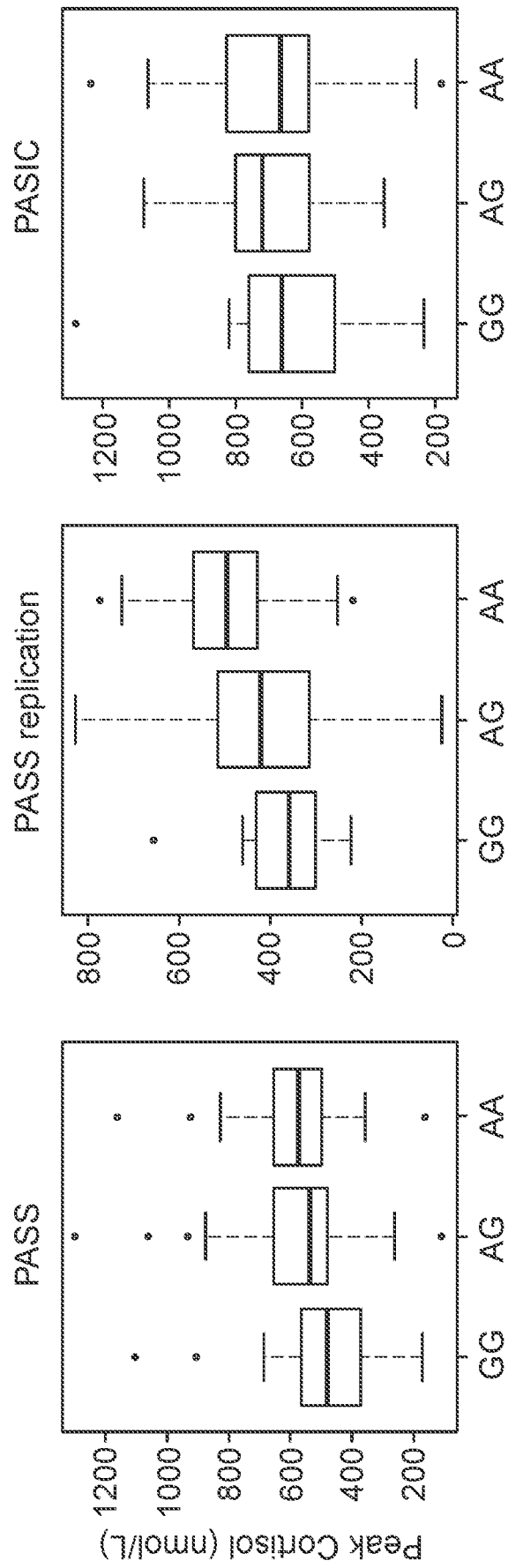

PASS
PASS replication
PASIC
Peak Cortisol (nmol/L)
1200
1000
800
600
400
200
0
GG
AG
AA
rs591118 Genotype

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2018/051075

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/6883
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Daniel B Hawcutt ET AL: "Susceptibility to corticosteroid-induced adrenal suppression: a genome-wide association study", The Lancet Respiratory Medicine, 15 March 2018 (2018-03-15), pages 442-450, XP055495934, England DOI: 10.1016/S2213-2600(18)30058-4 Retrieved from the Internet: URL:https://www.thelancet.com/action/showPdf?pii=S2213-2600(18)30058-4 [retrieved on 2018-07-30] table 2 abstract the whole document ----- -/--	1-49

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 August 2018	08/08/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Helliot, Bertrand

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	MICHAEL CHO ET AL: "Adrenal insufficiency and ICS: genetics takes a breath", THE LANCET, vol. 6, 15 March 2018 (2018-03-15), pages 407-408, XP055495955, columns 1-2	1-49
A	MAREK L. KOWALSKI ET AL: "Adrenal suppression by inhaled corticosteroids in patients with asthma: A systematic review and quantitative analysis", ALLERGY AND ASTHMA PROCEEDINGS., vol. 37, no. 1, 1 January 2016 (2016-01-01), pages 9-17, XP055495935, US ISSN: 1088-5412, DOI: 10.2500/aap.2016.37.3912 abstract the whole document	1-49
A	Vishnu Sannarangappa ET AL: "Send Orders for Reprints to reprints@benthamscience.ae Inhaled Corticosteroids and Secondary Adrenal Insufficiency", Open Respiratory Medicine Journal, 1 January 2014 (2014-01-01), XP055495949, Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4319207/pdf/TORMJ-8-93.pdf [retrieved on 2018-07-30] abstract the whole document	1-49
A	DLUHY ET AL: "Clinical relevance of inhaled corticosteroids and HPA axis suppression", JOURNAL OF ALLERGY AND CLINICAL IMMUNO, ELSEVIER, AMSTERDAM, NL, vol. 101, no. 4, 1 April 1998 (1998-04-01), pages S447-S450, XP005178467, ISSN: 0091-6749, DOI: 10.1016/S0091-6749(98)70157-5 abstract the whole document	1-49

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DAHL ET AL: "Systemic side effects of inhaled corticosteroids in patients with asthma", RESPIRATORY MEDI, BAILLIERE TINDALL, LONDON, GB, vol. 100, no. 8, 1 August 2006 (2006-08-01), pages 1307-1317, XP005533798, ISSN: 0954-6111, DOI: 10.1016/J.RMED.2005.11.020 abstract the whole document -----	1-49