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(54) Title: METHODS OF SELECTIVELY TREATING ASTHMA USING IL-13 ANTAGONISTS

(57) **Abstract**: The disclosure is directed to novel predictive methods and personalized therapies for treating asthma. Specifically, this disclosure relates to methods of treating a patient having asthma by selectively administering an IL-13 antagonist, on the basis of that patient being genetically predisposed to have a favorable response to treatment with the IL-13 antagonist. Also disclosed herein are transmittable forms of information, diagnostic methods, and kits useful in predicting the likelihood that a patient having asthma will respond to treatment with an IL-13 antagonist.

METHODS OF SELECTIVELY TREATING ASTHMA USING IL-13 ANTAGONISTS

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 19, 2015, is named PAT056217-WO-PCT_SL.txt and is 40,804 bytes in size.

TECHNICAL FIELD

The disclosure is directed to predictive methods, personalized therapies, transmittable forms of information and methods for treating patients having asthma.

BACKGROUND OF THE DISCLOSURE

Asthma presents a major global health burden. Despite existing therapies, there is still significant unmet medical need in asthma, with an estimated 300 million people affected worldwide. The World Health Organization estimates that 15 million disability–adjusted life years are lost annually due to asthma, representing 1% of the total global burden. Annual worldwide deaths have been estimated at 250,000. Uncontrolled asthma has a prevalence of greater than 6 million patients worldwide.

Interleukin 13 (IL-13) is a cytokine produced by type 2 helper T cells (Th2), mast cells, eosinophils, and basophils (Kelly-Welch A, (2005), Sci STKE; 293:pcm 8) that promotes the production of inflammatory cytokines, up-regulates MHC class II and CD23 expression on monocytes, induces anti-CD40-dependent IgE class switch, and induces IgG and IgM synthesis in B cells (Joshi BH, (2006), Vitam Horm; 74:479-504). IL-13 has been shown to play a major role in the several biological processes, including airway hyper-responsiveness, allergic inflammation, tissue eosinophilia, parasite elimination, mast cell hyperplasia, IgE antibody synthesis, goblet cell metaplasia, tissue remodeling, and fibrosis (Belperio JA, (2002) Am J Respir Cell Mol Biol; 27(4): 419-427; Brombacher F (2000) Bioessays; 22: 646-656; Wynn TA, (2004), Immunol Rev; 201:156-67; Kolodsick JE, (2004), J Immunol;172: 4068-4076). In particular, IL-13 has been shown to be a central mediator of allergic asthma in an animal model (Wills-Karp M, (1998), Science; 282: 2258-2261). This observation has been complemented by data showing that anti-IL-13 Ab inhibits asthma progression in mice (Yang G, (2005), J Pharmacol Exp Ther; 313(1): 8-15). IL-13 engages two related receptors on responsive cells, IL-13Rα1 and IL-13Rα2 (Wills-Karp M, (2008), Sci Signal; 1(51) pe55). IL-13Rα1 forms a complex with the IL-4Rα receptor subunit, which signals through the JAK/STAT pathway to phosphorylate STAT6, which acts as a transcription factor promoting the expression of eotaxin and other products involved in Th2-dependent inflammation. The second IL-13Rα2 receptor also binds IL-13, but does not appear to produce signals involved in allergy. Thus, the IL-13Rα1/IL-4Rα receptor complex provides a key common point in both IL-13 and IL-4 signaling pathways. See also Ingram and Kraft (2012) J Allergy Clin Immunol 130(4) : 829-842.

WO05007699, WO07036745, WO12049278, and WO08106116 refer to anti-IL-13 antibodies and/or IL-13 antagonists for treatment of asthma.

There has been an ongoing search for biomarkers that define different asthma phenotypes (Wenzel SE (2012), Nat Med; 18(5): 716-725). WO12083132 refers to a method of identifying an asthma patient or a respiratory disorder patient who is likely to be responsive to treatment with a TH2 Pathway Inhibitor encompassing using an Eosinophilic Inflammation Diagnostic Assay.

Slager RE, (2012), J Allergy Clin Immunol; 130(2): 516-22, refer to a series of single nucleotide polymorphisms (SNPs) in the IL-4Rα receptor associated with a reduced risk of asthma exacerbations in patients treated with an IL-4 inhibitor. WO11156000 refers to use of methods and kits for determining the major allele in certain SNPs in the IL-4Rα receptor as an indication of likely response to IL-4/IL13 (IL-4 and IL-13) antagonist treatment, such as treatment with mutant human Il-4 protein.

BRIEF SUMMARY OF THE DISCLOSURE

There exists a need for identification of single nucleotide polymorphisms (SNP’s) as being predictive of whether a patient having asthma will respond to a treatment with an IL-13 antagonist, as a pharmacogenomic biomarker approach to diagnosis and treatment of asthma. Provided herein are predictive methods and personalized therapies for patients having asthma that maximize the benefit and minimize the risk of IL-13 antagonism in these populations by identifying the patients likely to respond favorably, prior to treatment with an IL-13 antagonist. The inventive methods described herein are related to the discovery that patients with particular responsive genotypes had substantial reductions in the frequency of asthma exacerbations upon treatment antibody 01951/G12 (SEQ ID No. 14 and 16), a human IgG1/κ anti-Il-13 monoclonal antibody further described in WO2007/045477. The responsive genotypes in the patients are specific responsive alleles in SNPs of the IL-4Rα receptor gene and are provided in Table 1.

Table 1

AIR marker	SNP	5’ Nucleotide sequence	SEQ ID NO:	RefSNP alleles
1	rs1110470	GAAGGTTGGCAGGCCAGGGACAACA[C/**T**]CGTCTGCCAAGCCATGGCAGTAGAC	22	C/T (REV)
2	rs3024530	TAAGGTATTTTTGTTATAGCAGCCT[**A**/G]TATGGACTAAGCTGACTTGTAACGT	23	A/G (FWD)
3	rs1805010	CTGTGTCTGCAGAGCCCACACGTGT[**A**/G]TCCCTGAGAACAACGGAGGCGCGGG	24	A/G (FWD)
4	rs2239347	ACCCCAGGTCCCATATGTCCAGAGA[G/**T**]TGTCCCTCCAATGGGAATGTGAGGA	25	G/T (REV)
5	rs1805011	AGGGATGACTTCCAGGAGGGAAGGG[A/**C**]GGGCATTGTGGCCCGGCTAACAGAG	26	A/C (FWD)
6	rs1801275	GTCTCGGCCCCCACCAGTGGCTATC[A/**G**]GGAGTTTGTACATGCGGTGGAGCAG	27	A/G (FWD)
7	rs8832	GCAACAGAGGACATGAAAAATTGCT[A/**G**]TGACTAAAGCAGGGACAATTTGCTG	28	A/G (FWD)
8	rs1029489	CTTGTATGGGGAACCCAAACCCAGA[**C**/T]GGCAAGTTTCTTAACCTCTTGCATC	29	C/T (REV)
9	rs4787956	GCTTATGTCATCCTGACACCTACGC[**A**/G]GATGTCGGCTCGAATCCACTTTGCC	30	A/G (FWD)

Table 1 sets forth the SNP nucleotide sequences for the IL-4Rα receptor as designated by the corresponding rs number. The SNP sequences are also provided in the dbSNP database as referenced in further detail below. The alternative alleles are shown in brackets. The inventive responsive allele is shown in Table 1 in bold type, and respectively designated as anti-Il-13 response marker (“AIR marker” hereinafter). Accordingly, the designation AIR marker is in reference to the responsive allele only and excludes the non-responsive allele. In this regard, it is further recognized that a patient can be homozygous or heterozygous with respect to a particular

AIR marker. Thus, for example, a patient that is determined to be homozygous for AIR marker 3, has an AA genotype for the rs1805010 SNP, whereas the patient that is heterozygous for AIR marker 3 has an AG genotype for this SNP. In the inventive methods of the invention, a patient is positive for a particular AIR marker, and therefore for the responsive allele, where the patient is homozygous or heterozygous for the responsive allele. A patient that is negative for a particular AIR marker is homozygous for the non-responsive allele. For example, a patient that is negative for AIR marker 3 has a GG for the rs1805010 SNP.

Various aspects of the invention provide a method of selectively treating a patient having asthma, comprising identifying a patient having at least one AIR marker selected from the group consisting of AIR marker- 1, 2, 3, 4, 5, 6, 7, 8, and 9, and thereafter administering a therapeutically effective amount of an IL-13 antagonist to the patient.

In one aspect, the present invention provides a method of selectively treating a patient having asthma, comprising

i) identifying a patient having at least one AIR marker selected from the group consisting of AIR marker- 1, 2, 3, 4, 5, 6, 7, 8, and 9,

ii) determining whether the patient is homozygous or heterozygous for said AIR marker, and

iii) selectively administering a therapeutically effective amount of an IL-13 antagonist to the patient that is

   a. homozygous for one of AIR marker 3 and AIR marker 7 and heterozygous at the other;

   b. homozygous for both AIR marker 3 and AIR marker 7; or

   c. homozygous for AIR marker 3.

In another aspect, the present invention provides a method of selectively treating a patient having asthma, comprising

i) assaying a biological sample from the patient for the presence or absence of at least one AIR marker selected from said group consisting of AIR marker- 1, 2, 3, 4, 5, 6, 7, 8, and 9.

ii) detecting the presence of at least one AIR marker selected from said group in said sample and thereby determining that the patient is positive for said AIR marker, and

iii) determining whether the patient is homozygous or heterozygous for said AIR marker, and selectively administering a therapeutically effective amount of an IL-13 antagonist to the patient that is

a. homozygous for one of AIR marker 3 and AIR marker 7 and heterozygous at the other;

b. homozygous for both AIR marker 3 and AIR marker 7; or

c. homozygous for AIR marker 3.

In a further aspect, the present invention provides a method of predicting the likelihood that a patient having asthma will respond to treatment with an IL-13 antagonist, comprising the steps of:

a) assaying a biological sample from the patient for the presence or absence of at least one AIR marker, and

b) determining whether said AIR marker is present in homozygous or heterozygous form, wherein

the presence of the at least one AIR marker in homozygous form is indicative of an increased likelihood that the patient will respond to treatment with the IL-13 antagonist, wherein said at least one AIR marker is selected from the group consisting of:

i) AIR marker 3 and AIR marker 7 each present in homozygous form,

ii) AIR marker 3 present in homozygous form and AIR marker 7 in heterozygous form,

  a. AIR marker 7 present in homozygous form and AIR marker 3 in heterozygous form, and

  b. AIR marker 3 in homozygous form.

In one embodiment, the identification comprises assaying a biological sample from the patient for the presence of at least one AIR marker selected from said group.

In another embodiment, the invention provides a method of selectively treating a patient having asthma, comprising:

i) assaying a biological sample from the patient for the presence or absence of at least one AIR marker selected from said group consisting of AIR marker- 1, 2, 3, 4, 5, 6, 7, 8, and 9;

ii) detecting the presence of at least one AIR marker selected from said group in said sample and thereby determining that the patient is positive for said AIR marker, and

iii) selectively administering a therapeutically effective amount of an IL-13 antagonist to the patient that is positive.

In another embodiment, the inventive methods further comprise determining whether said AIR marker is present in homozygous or heterozygous form wherein the presence of the at least one AIR marker in homozygous form is determinative that the patient is positive for said AIR marker.

In another embodiment, the AIR marker is selected from the group consisting of Air marker 3 and Air marker 10.

In another embodiment, the inventive selective treatment methods further comprise determining whether the patient is homozygous or heterozygous for said AIR marker, and selectively administering a therapeutically effective amount of an IL-13 antagonist to the patient that is homozygous for one of AIR marker 3 and Air marker 10 and heterozygous for the other; homozygous for both AIR markers 3 and 10; or homozygous for AIR marker 3.

In other embodiments, the invention is directed to methods for predicting the likelihood that a patient having asthma will respond to treatment with an IL-13 antagonist. In one such embodiment, the methods comprise assaying a biological sample from the patient for the presence or absence of at least one AIR marker selected from the group consisting of AIR marker- 1, 2, 3, 4, 5, 6, 7, 8, and 9, wherein:

a) the presence of the at least one AIR marker is indicative of an increased likelihood that the patient will respond to treatment with the IL-13 antagonist; and

b) the absence of the at least one AIR marker is indicative of a decreased likelihood that the patient will respond to treatment with the IL-13 antagonist.

In another such embodiment, the methods comprise the step of assaying a biological sample from the patient for the presence or absence of at least one AIR marker selected from the group consisting of AIR marker- 1, 2, 3, 4, 5, 6, 7, 8, and 9 in homozygous form, wherein:

a) the presence of the at least one AIR marker in homozygous form is indicative of an increased likelihood that the patient will respond to treatment with the IL-13 antagonist; and

b) the absence of the at least one AIR marker in homozygous form is indicative of a decreased likelihood that the patient will respond to treatment with the IL-13 antagonist.

In another such embodiment, the methods comprise the steps of:

a) assaying a biological sample from the patient for the presence or absence of at least one AIR marker, and

b) determining whether said AIR marker is present in homozygous or heterozygous form, wherein

the presence of the at least one AIR marker in homozygous form is indicative of an increased likelihood that the patient will respond to treatment with the IL-13 antagonist, wherein said at least one AIR marker is selected from the group consisting of:

i) AIR marker 3 and 7 each present in homozygous form;

ii) AIR marker 3 present in homozygous form and AIR marker 7 in heterozygous form;

iii) AIR marker 7 present in homozygous form and AIR marker 3 in heterozygous form; and

iv) AIR marker 3 in homozygous form.

In another embodiment, the step of assaying comprises assaying the biological sample for a nucleic acid product of the at least one AIR marker, or a polypeptide product of the at least one AIR marker. In another embodiment, the step of assaying comprises assaying the biological sample for a genomic sequence of the at least one AIR marker.

In another embodiment, the biological sample is selected from the group consisting of blood, serum, feces, plasma, urine, tear, saliva, and a tissue sample.

In another embodiment, the step of assaying comprises a technique selected from the group consisting of Northern blot analysis, polymerase chain reaction (PCR), reverse transcription-polymerase chain reaction (RT-PCR), TaqMan-based assays, direct sequencing, dynamic allele-specific hybridization, high-density oligonucleotide SNP arrays, restriction fragment length polymorphism (RFLP) assays, primer extension assays, oligonucleotide ligase assays, analysis of single strand conformation polymorphism, temperature gradient gel electrophoresis (TGGE), denaturing high performance liquid chromatography, high-resolution melting analysis, DNA mismatch-binding protein assays, SNPLex®, capillary electrophoresis, Southern Blot, immunoassays, immunohistochemistry, ELISA, flow cytometry, Western blot, HPLC, and mass spectrometry.

In other embodiments, the invention is directed to methods for producing a transmittable form of information for predicting the responsiveness of a patient having asthma to treatment with an IL-13 antagonist, comprising determining an increased likelihood of the patient responding to treatment with the IL-13 antagonist according to the inventive methods set forth above; and recording the result of the determining step on a tangible or intangible media form for use in transmission.

In another embodiment, the IL-13 antagonist utilized in the inventive method competes with antibody 01951/G12 (SEQ ID No. 14 and 16) for binding to IL-13 under conditions that promote the competition.

In another embodiment, the IL-13 antagonist is a polypeptide or a fragment thereof, an antibody or an antigen binding fragment thereof, a Fab, an ScFv.

In another embodiment, the IL-13 antagonist is an antibody or a fragment thereof that binds to an epitope of IL-13 comprising residues FCPHKV (SEQ ID NO: 67) set forth as residues 103 to 107 of SEQID NO: 1.

In another embodiment, the IL-13 antagonist is antibody 01951/G12 (SEQ ID No. 14 and 16).

In another embodiment, the IL-13 antagonist is an antibody administered at a dose of about 50-1000 mg i.v. every four weeks (q4wk). In another embodiment, the IL-13 antagonist is an antibody administered at a dose of about 75 mg, or 750 mg i.v. every four weeks.

In another embodiment, the IL-13 antagonist has a K_D of about 100-200 pM. In other embodiments the antagonist has a higher affinity for IL-13 and exhibits a K_D of less than 100 pM. In a particular embodiment the IL-13 antagonist is an antibody that has a K_D of about 140 pM.

In another embodiment, the IL-13 antagonist has an *in vivo* half-life of about 15 -30 days, or about 21 days.

In another embodiment, the IL-13 antagonist is an antibody selected from the group consisting of:

i. an antibody comprising one or more of the CDRs selected from the list consisting of: (a) the V_H CDR1s shown in SEQ ID NOs: 2 or 5 (b) the V_H CDR2s shown in SEQ ID NOs: 3 or 6, (c) the V_H CDR3s shown in SEQ ID NOs: 4 or 7 (d) the V_L CDR1s shown in SEQ ID NOs: 8 or 11, (e) the V_L CDR2s shown in SEQ ID NOs: 9 or 12, (f) the V_L CDR3s shown in SEQ ID NOs: 10 or 13;

ii. an antibody comprising a heavy chain variable region CDR1 of SEQ ID NO: 2; a heavy chain variable region CDR2 of SEQ ID NO: 3; a heavy chain variable region CDR3 of SEQ ID NO: 4; a light chain variable region CDR1 of SEQ ID NO: 8; a light chain variable region CDR2 of SEQ ID NO: 9; and a light chain variable region CDR3 of SEQ ID NO: 10;

iii. an antibody comprising a heavy chain variable region CDR1 of SEQ ID NO: 5; a heavy chain variable region CDR2 of SEQ ID NO: 6; a heavy chain variable region CDR3 of SEQ ID NO: 7; a light chain variable region CDR1 of SEQ ID NO: 11; a light chain variable region CDR2 of SEQ ID NO: 12; and a light chain variable region CDR3 of SEQ ID NO: 13,

iv. an antibody comprising a heavy chain variable region as recited in SEQ ID NO: 14 and a light chain variable region as recited in SEQ ID NO: 16,

v. an antibody comprising a heavy chain as recited in SEQ ID NO: 20 and a light chain as recited in SEQ ID NO: 18.

In another embodiment, the IL-13 antagonist is a human antibody.

In a particular embodiment of the invention, the IL-13 antagonist is an antibody that prevents IL-13 binding to IL-13 Rα1. In another, the IL-13 antagonist prevents IL-13 binding to IL-13 Rα1, but allows binding to IL1-13 Rα2 also known as the decoy receptor.

In another embodiment, the patient has moderate asthma, and in another, severe asthma.

Additional methods, uses, and kits are provided in the following description and appended claims. Further features, advantages and aspects of the present disclosure will become apparent to those skilled in the art from the following description and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts substitution analysis for binding of ANTIBODY 01951/G12 to seleted IL-13 residues. Figure discloses SEQ ID NO: 33.

Figure 2 depicts asthma exacerbation risk by genotype class for ANTIBODY 01951/G12-treated patients.

Figure 3 depicts exacerbation risk by genotype class for placebo-treated patients in the ANTIBODY 01951/G12 study.

Figure 4 depicts identification of two linkage disequilibrium blocks among IL4-Rα SNPs.

DETAILED DESCRIPTION OF THE DISCLOSURE

It is envisioned that testing subjects for the presence of at least one of the aforementioned response alleles (AIR markers) will be useful in a variety of pharmaceutical products and methods that involve identifying asthma patients, including severe to moderate asthma patients who are more likely to respond to IL-13 antagonsim and in helping physicians decide whether to prescribe IL-13 antagonists (e.g. antibody 01951/G12) to those patients or whether to prescribe an alternative pharmaceutical agent.

Accordingly, in one aspect the invention provides methods of treating a patient having asthma by administering to the patient a therapeutically effective amount of an IL-13 antagonist, e.g., an IL-13 antibody, such as antibody 01951/G12, based on certain aspects of the patient's genotypic profile. In a related aspect, the invention further provides methods of identifying a patient having asthma who is more likely to respond to treatment of with an IL-13 antagonist, e.g., an IL-13 antibody, such as antibody 01951/G12, based on certain aspects of the patient's genotypic profile. In a further related aspect, the invention provides methods of determining the likelihood that a patient having asthma will respond to treatment with an IL-13 antagonist, e.g., an IL-13 antibody, such as antibody 01951/G12, based on certain aspects of the patient's genotypic profile. In another related aspect, the invention provides various methods of selectively treating a patient having asthma.

The inventive methods described herein encompass utilizing at least one AIR marker selected from a group consisting of nine specified AIR markers set forth herein. The term "at least one AIR marker" contemplates that one, two, three, four, 5, six, seven, eight, or nine AiR markers can be combined and utilized in the methods of the invention. Furthermore, each AIR marker member of such combinations can be in heterozygous or homozygous form. Particular embodiments of the invention set forth specific combinations of nine AIR markers (AIR markers- 1 to 9) and further specify desired zygosities with respect to a particular AIR marker.

The term "comprising" encompasses "including" as well as "consisting," e.g. a composition "comprising" X may consist exclusively of X or may include something additional, e.g., X + Y.

The term "about" in relation to a numerical value x means +/-10% unless the cotext dictates otherwise.

The term "assaying" is used to refer to the act of identifying, screening, probing, testing measuring or determining, which act may be performed by any conventional means. For example, a sample may be assayed for the presence of a particular genetic or protein marker by using an ELISA assay, a Northern blot, imaging, serotyping, cellular typing, gene sequencing, phenotyping, haplotyping, immunohistochemistry, western blot, mass spectrometry, etc. The term "detecting" (and the like) means the act of extracting particular information from a given source, which may be direct or indirect. In some embodiments of the predictive methods disclosed herein, the presence of a given thing (e.g., allele, level of protein, etc.) is detected in a biological sample indirectly, e.g., by querying a database. The terms “assaying” and “determining” contemplate a transformation of matter, e.g., a transformation of a biological sample, e.g., a blood sample or other tissue sample, from one state to another by means of subjecting that sample to physical testing.

The term “obtaining” means to procure, e.g., to acquire possession of in any way, e.g., by physical intervention (e.g., biopsy, blood draw) or non-physical intervention (e.g, transmittal of information via a server), etc.

The phrase “assaying a biological sample ...” and the like, is used to mean that a sample may be tested (either directly or indirectly) for either the presence of a given AIR marker. It will be understood that, in a situation where the presence of a substance denotes one probability and the absence of a substance denotes a different probabiltity, then either the presence or the absence of such substance may be used to guide a therapeutic decision. For example, one may determine if a patient has AIR marker by determining the actual existence of particular response allele in the patient or by determining the absence of the particular response allele in the patient. In both such cases, one has determined whether the patient has the presence of the AIR marker. The disclosed methods involve, *inter alia*, determining whether a particular individual has an AIR marker. This determination is undertaken by identifying whether the patient has one or more of the AIR markers disclosed in Table 1 set forth above. Each of these determinations (i.e., presence or absence), on its own, provides the allelic status of the patient and thus each of these deteriminations equally provide an indication of whether a particular individual would or would not respond more favorably to IL-13 antagonism. To provide an indication of increased responsiveness for an asthma patient, a biological sample need only be assayed for one or more AIR marker set forth in Table 1.

“IL-13 antagonist” as used herein refers to a molecule that antagonizes (e.g., reduces, inhibits, decreases, delays, eliminates) IL-13 function, expression and/or signalling by blocking the binding of IL-13 to the IL-13 receptor complex. In a particular embodiment of the invention, the IL-13 antagonist prevents IL-13 binding to IL-13 Rα1. In another, the IL-13 antagonist prevents binding to IL-13 Rα1, but allows binding to IL1-13 Rα2 (also known as the decoy receptor). See Ingram and Kraft (2012) J Allergy Clin Immunol 130(4): 829-842.

The binding reaction may be shown by standard methods (qualitative or quantitative assays) including, for example, a binding assay, competition assay or a bioassay for determining the inhibition of IL-13 binding to its receptor or any kind of binding assays, with reference to a negative control test in which an antibody of unrelated specificity, but ideally of the same isotype, e.g., an anti-CD25 antibody, is used. Such methods incude those set forth below in the EXAMPLES.

The term "antibody" as referred to herein includes whole antibodies and any antigen-binding portion or single chains thereof. A naturally occurring "antibody" is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed hypervariable regions or complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

The term "antigen-binding portion" of an antibody as used herein, refers to fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., IL-13). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include a Fab fragment, a monovalent fragment consisting of the V_L, V_H, CL and CH1 domains; a F(ab)2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the V_H and CH1 domains; a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; a dAb fragment (Ward et al., 1989 Nature 341:544-546), which consists of a V_H domain; and an isolated CDR. Exemplary antigen binding sites include the CDRs set forth in SEQ ID NOs:1-6 and 11-13 (**Table 2**), preferably the heavy chain CDR3. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see, e.g., Bird et al., 1988 Science 242:423-426; and Huston et al., 1988 Proc. Natl. Acad. Sci. 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antibody". Single chain antibodies and antigen-binding portions are obtained using conventional techniques known to those of skill in the art.

An "isolated antibody", as used herein, refers to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds IL-13is substantially free of antibodies that specifically bind antigens other than IL-13). The term "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. A “human antibody” need not be produced by a human, human tissue or human cell. The human antibodies of the disclosure may include amino acid residues not encoded by human sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro*, by N-nucleotide addition at junctions *in vivo* during recombination of antibody genes, or by somatic mutation *in vivo*). In some embodiments of the disclosed methods, the IL-13 antagonist is a human antibody, an isolated antibody, and/or a monoclonal antibody.

The term "K_D" is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (i.e. K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A method for determining the K_D of an antibody is by using surface plasmon resonance, or using a biosensor system such as a Biacore® system.

The term "affinity" refers to the strength of interaction between antibody and antigen at single antigenic sites. Within each antigenic site, the variable region of the antibody arm interacts through weak non-covalent forces with antigen at numerous sites; the more interactions, the stronger the affinity. Standard assays to evaluate the binding affinity of the antibodies toward IL-13 of various species are known in the art, including for example, ELISAs, western blots and RIAs. The binding kinetics (e.g., binding affinity) of the antibodies also can be assessed by standard assays known in the art, such as by Biacore analysis.

An antibody that "inhibits" one or more of these IL-13 functional properties (e.g., biochemical, immunochemical, cellular, physiological or other biological activities, or the like) as determined according to methodologies known to the art and described herein, will be understood to relate to a statistically significant decrease in the particular activity relative to that seen in the absence of the antibody (or when a control antibody of irrelevant specificity is present). An antibody that inhibits IL-13 activity affects a statistically significant decrease, e.g., by at least about 10% of the measured parameter, by at least 50%, 80% or 90%, and in certain embodiments of the disclosed methods, the IL-13 antibody used may inhibit greater than 95%, 98% or 99% of IL-13 functional activity.

The term "derivative", unless otherwise indicated, is used to define amino acid sequence variants, and covalent modifications (e.g., pegylation, deamidation, hydroxylation, phosphorylation, methylation, etc.) of an IL-13 antagonist (e.g., IL-13 antibody or antigen-binding portion thereof) , e.g., of a specified sequence (e.g., a variable domain). A “functional derivative” includes a molecule having a qualitative biological activity in common with the disclosed IL-13 antagonists. A functional derivative includes fragments and peptide analogs of an IL-13 antagonist as disclosed herein. Fragments comprise regions within the sequence of a polypeptide according to the present disclosure, e.g., of a specified sequence. Functional derivatives of the IL-13 antagonists disclosed herein preferably comprise V_H and/or V_L domains that have at least about 65%, 75%, 85%, 95%, 96%, 97%, 98%, or even 99% overall sequence identity with the V_H and/or V_L sequences of the IL-13 binding molecules disclosed herein (e.g., the V_H and/or V_L sequences of Table 2), and substantially retain the ability to bind human IL-13.

The phrase “substantially identical” means that the relevant amino acid or nucleotide sequence (e.g., V_H or V_L domain) will be identical to or have insubstantial differences (e.g., through conserved amino acid substitutions) in comparison to a particular reference sequence. Insubstantial differences include minor amino acid changes, such as 1 or 2 substitutions (e.g., conservative substitutions, such as swapping a serine for a threonine, or substitutions at positions not involved in antibody activity, structural integrity, complement fixation, etc.) in a 5 amino acid sequence of a specified region (e.g., V_H or V_L domain). In the case of antibodies, the second antibody has the same specificity and has at least 50% of the affinity of the same. Sequences substantially identical (e.g., at least about 85% sequence identity) to the sequences disclosed herein are also part of this disclosure. In some embodiments, the sequence identity of a derivative anti-IL-13 antibody (can be about 90% or greater, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher relative to the disclosed sequences.

”Identity” with respect to a native polypeptide and its functional derivative is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues of a corresponding native polypeptide, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent identity, and not considering any conservative substitutions as part of the sequence identity. Neither N- or C-terminal extensions nor insertions shall be construed as reducing identity. Methods and computer programs for the alignment are well known. The percent identity can be determined by standard alignment algorithms, for example, the Basic Local Alignment Search Tool (BLAST) described by Altshul et al. ((1990) J. Mol. Biol., 215: 403 410); the algorithm of Needleman et al. ((1970) J. Mol. Biol., 48: 444 453); or the algorithm of Meyers et al. ((1988) Comput. Appl. Biosci., 4: 11 17). A set of parameters may be the Blosum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The percent identity between two amino acid or nucleotide sequences can also be determined using the algorithm of E. Meyers and W. Miller ((1989) CABIOS, 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

"Amino acid(s)" refer to all naturally occurring L-α-amino acids, e.g., and include D-amino acids. The phrase "amino acid sequence variant" refers to molecules with some differences in their amino acid sequences as compared to the sequences according to the present disclosure. Amino acid sequence variants of an IL-13 antagonist polypeptide according to the present disclosure, e.g., of a specified sequence, still have the ability to bind the human IL-13 or. Amino acid sequence variants include substitutional variants (those that have at least one amino acid residue removed and a different amino acid inserted in its place at the same position in a polypeptide according to the present disclosure), insertional variants (those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in a polypeptide according to the present disclosure) and deletional variants (those with one or more amino acids removed in a polypeptide according to the present disclosure).

The term "pharmaceutically acceptable" means a nontoxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s).

The term "administering" in relation to a compound, e.g., an IL-13 binding molecule or another agent, is used to refer to delivery of that compound to a patient by any route.

As used herein, a "therapeutically effective amount" refers to an amount of an IL-13 antagonist, (e.g., anti-IL-13 antibody or antigen-binding portion thereof) that is effective, upon single or multiple dose administration to a patient (such as a human) for treating, preventing, preventing the onset of, curing, delaying, reducing the severity of, ameliorating at least one symptom of a disorder or recurring disorder, or prolonging the survival of the patient beyond that expected in the absence of such treatment. When applied to an individual active ingredient (e.g., an IL-13 antagonist, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

The term "treatment" or "treat" refer to both prophylactic or preventative treatment (as the case may be) as well as curative or disease modifying treatment, including treatment of a patient at risk of contracting the disease or suspected to have contracted the disease as well as patients who are ill or have been diagnosed as suffering from a disease or medical condition, and includes suppression of clinical relapse or exacerbation. The treatment may be administered to a patient having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong the survival of a patient beyond that expected in the absence of such treatment.

The phrase “responds to treatment” is used to mean that a patient, upon being delivered a particular treatment, e.g., an IL-13 antagonist, shows a clinically meaningful benefit from said treatment. In the case of asthma, including severe to moderate asthma, such criteria include reduction in exacerbations. The phrase “respond to treatment” is meant to be construed comparatively, rather than as an absolute response. For example, an asthma patient having an AIR marker is predicted to have more benefit from treatment with an IL-13 antagonist than a patient who does not have the AIR marker. These carriers of AIR markers respond more favorably to treatment with the IL-13 antagonist, and “respond to treatment” with an IL-13 antagonist. In certain embodiments of the invention, the patient that responds to treatment with an IL-13 antagonist according to the methods disclosed herin, has determinably reduced exacerbation of asthma for at least 24 weeks, at least 24-52 weeks, at least 52 weeks, or longer. In particular embodiments of the invention, the reduction in exacerbations is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% in the patient that responds to treatment with an IL-13 antagonist according to the methods disclosed herein.

The phrase “receiving data” is used to mean obtaining possession of information by any available means, e.g., orally, electronically (e.g., by electronic mail, encoded on diskette or other media), written, etc.

As used herein, “selecting” and “selected” in reference to a patient is used to mean that a particular patient is specifically chosen from a larger group of patients on the basis of (due to) the particular patient having a predetermined criteria, e.g., the patient has an AIR marker. Similarly, “selectively treating” refers to providing treatment to a patient having a particular disease, where that patient is specifically chosen from a larger group of patients on the basis of the particular patient having a predetermined criteria, e.g., an asthma patient specifically chosen for treatment due to the patient having a AIR marker. Similarly, “selectively administering” refers to administering a drug to a patient that is specifically chosen from a larger group of patients on the basis of (due to) the particular patient having a predetermined criteria, e.g., a particular genetic or other biological marker. By selecting, selectively treating and selectively administering, it is meant that a patient is delivered a personalized therapy based on the patient's particular biology, rather than being delivered a standard treatment regimen based solely on the patient having a particular disease. Selecting, in reference to a method of treatment as used herein, does not refer to fortuitous treatment of a patient that has an AIR marker, but rather refers to the deliberate choice to administer an IL-13 antagonist to a patient based on the patient having an AIR marker. Thus, selective treatment differs from standard treatment, which delivers a particular drug to all patients, regardless of their allelic status.

As used herein, "predicting" indicates that the methods described herein provide information to enable a health care provider to determine the likelihood that an individual having asthma will respond to or will respond more favorably to treatment with an IL-13 antagonist. It does not refer to the ability to predict response with 100% accuracy. Instead, the skilled artisan will understand that it refers to an increased probability.

As used herein, "likelihood" and "likely" is a measurement of how probable an event is to occur. It may be used interchangably with "probability". Likelihood refers to a probability that is more than speculation, but less than certainty. Thus, an event is likely if a reasonable person using common sense, training or experience concludes that, given the circumstances, an event is probable. In some embodiments, once likelihood has been ascertained, the patient may be treated (or treatment continued, or treatment proceed with a dosage increase) with the IL-13 antagonist or the patient may not be treated (or treatment discontinued, or treatment proceed with a lowered dose) with the IL-13 antagonist.

The phrase "increased likelihood" refers to an increase in the probability that an event will occur. For example, some methods herein allow prediction of whether a patient will display an increased likelihood of responding to treatment with an IL-13 antagonist or an increased likelihood of responding better to treatment with an IL-13 antagonist in comparison to a patient having asthma who does not have an AIR.

As used herein "SNP" refers to "single nucleotide polymorphism". A single nucleotide polymorphism is a DNA sequence variation occurring when a single nucleotide in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in an individual. Most SNPs have only two alleles, and one is usually more common in the population. A SNP may be present in an exon or an intron of a gene, an upstream or downstream untranslated region of a gene, or in a purely genomic location (i.e., non-transcribed). When a SNP occurs in the coding region of a gene, the SNP may be silent (i.e., a synonymous polymorphism) due to the redundancy of the genetic code, or the SNP may result in a change in the sequence of the encoded polypeptide (i.e., a non-synonymous polymorphism). In the instant disclosure, SNPs are identified by their Single Nucleotide Polymorphism Database (dbSNP) rs number, e.g. rs1805010. The dbSNP is a free public archive for genetic variation within and across different species developed and hosted by the National Center for Biotechnology Information (NCBI) in collaboration with the National Human Genome Research Institute (NHGRI).

A polymorphic site, such as a SNP, is usually preceded by and followed by conserved sequences in the genome of the population of interest and thus the location of a polymorphic site can often be made in reference to a consensus nucleic acid sequence (e.g., of thirty to sixty nucleotides) that bracket the polymorphic site, which in the case of a SNP is commonly referred to as the "SNP context sequence". Context sequences for the SNPs disclosed herein may be found in the NCBI SNP database available at: www.ncbi.nlm.nih.gov/snp. Alternatively, the location of the polymorphic site may be identified by its location in a reference sequence (e.g., GeneBank deposit) relative to the start of the gene, mRNA transcript, BAC clone or even relative to the initiation codon (ATG) for protein translation. The skilled artisan understands that the location of a particular polymorphic site may not occur at precisely the same position in a reference or context sequence in each individual in a population of interest due to the presence of one or more insertions or deletions in that individual’s genome as compared to the consensus or reference sequence. It is routine for the skilled artisan to design robust, specific and accurate assays for detecting the alternative alleles at a polymorphic site in any given individual, when the skilled artisan is provided with the identity of the alternative alleles at the polymorphic site to be detected and one or both of a reference sequence or context sequence in which the polymorphic site occurs. Thus, the skilled artisan will understand that specifying the location of any polymorphic site described herein by reference to a particular position in a reference or context sequence (or with respect to an initiation codon in such a sequence) is merely for convenience and that any specifically enumerated nucleotide position literally includes whatever nucleotide position the same polymorphic site is actually located at in the same locus in any individual being tested for the genetic marker of the invention using any of the genotyping methods described herein or other genotyping methods known in the art.

In addition to SNPs, genetic polymorphisms include translocations, insertions, substitutions, deletions, etc., that occur in gene enhancers, exons, introns, promoters, 5' UTR, 3'UTR, etc.

As used herein "rs1110470" refers to a C/T (reverse strand) SNP located within an intron of the human *IL-4RA* gene (IL-4Rα ; IL-4 receptor alpha) (GeneBank Accession No. NM_000418.3). The rs1110470 polymorphic site is located at chromosomal postion 27336427 (build 138; assembly GRCh37.p10), which is position 27276427 of Contig NT_010393.16.

As used herein "rs3024530" refers to an A/G (forward strand) SNP located within an intron of *IL-4RA* gene (GeneBank Accession No. NM_000418.3). The rs3024530 polymorphic site is located at chromosomal postion 27350687 (build 138; assembly GRCh37.p10), which is position 27290687 of Contig NT_010393.16.

As used herein "rs1805010" refers to an A/G (forward strand) SNP located within an exon of *IL-4RA* gene (GeneBank Accession No. NM_000418.3), and encoding an Ile to Val change. The rs1805010 polymorphic site is located at chromosomal postion 27356203 (build 138; assembly GRCh37.p10), which is position 27296203 of Contig NT_010393.16.

As used herein " rs2239347" refers to an G/T (reverse strand) SNP located within an intron of *IL-4RA* gene (GeneBank Accession No. NM_000418.3). The rs2239347 polymorphic site is located at chromosomal postion 27359021 (build 138; assembly GRCh37.p10), which is position 27299021 of Contig NT_010393.16.

As used herein "rs1805011" refers to an A/C (forward strand) SNP located within an exon of *IL-4RA* gene (GeneBank Accession No. NM_000418.3), and encoding an Glu to Ala change. The rs1805011 polymorphic site is located at chromosomal postion 27373872 (build 138; assembly GRCh37.p10), which is position 27313872 of Contig NT_010393.16.

As used herein "rs1801275" refers to an A/G (forward strand) SNP located within an exon of *IL-4RA* gene (GeneBank Accession No. NM_000418.3), and encoding an Gln to Arg change. The rs1801275 polymorphic site is located at chromosomal postion 27374400 (build 138; assembly GRCh37.p10), which is position 27314400 of Contig NT_010393.16.

As used herein "rs8832" refers to an A/G (forward strand) SNP located within the 3' UTR (untranslated) region of *IL-4RA* gene (GeneBank Accession No. NM_000418.3). The rs8832 polymorphic site is located at chromosomal postion 27375787 (build 138; assembly GRCh37.p10), which is position 27315787 of Contig NT_010393.16.

As used herein " rs1029489" refers to an C/T (reverse strand) SNP located 3' proximal of *IL-4RA* gene (GeneBank Accession No. NM_000418.3). The rs1029489 polymorphic site is located at chromosomal postion 27376217 (build 138; assembly GRCh37.p10), which is position 27316217 of Contig NT_010393.16.

As used herein "rs4787956" refers to an A/G (forward strand) SNP located 3' proximal of *IL-4RA* gene (GeneBank Accession No. NM_000418.3). The rs4787956 polymorphic site is located at chromosomal postion 27378249 (build 138; assembly GRCh37.p10), which is position 27318249 of Contig NT_010393.16.

As recognized by the skilled artisan, nucleic acid samples containing a particular SNP may be complementary double stranded molecules and thus reference to a particular site on the sense strand efers as well to the corresponding site on the complementary antisense strand. Similarly, reference to a particular genotype obtained for a SNP on both copies of one strand of a chromosome is equivalent to the complementary genotype obtained for the same SNP on both copies of the other strand.

As used herein, "genomic sequence" refers to a DNA sequence present in a genome, and includes a region within an allele, an allele itself, or a larger DNA sequence of a chromsome containing an allele of interest.

Products of the AIR markers of the invention can include nucleic acid products and polypeptide products. "Polypeptide product" refers to a polypeptide including the amino acid encoded by an AIR marker and fragments thereof. "Nucleic acid product" refers to any DNA (e.g., genomic, cDNA, etc.) or RNA (e.g., pre-mRNA, mRNA, miRNA, etc.) products of an AIR markers and fragments thereof.

An "equivalent genetic marker" refers to a genetic marker that is correlated to an allele of interest, e.g., it displays linkage disequilibrium (LD) or is in genetic linkage with the allele of interest. Equivalent genetic markers may be used to determine if a patient has an AIR marker, rather than directly interrogating a biological sample from the patient for the allele *per se*. Various programs exist to help determine LD for particular SNPs, e.g, HaploBlock (available at bioinfo.cs.technion.ac.il/haploblock/), HapMap, WGA Viewer.

The term “probe” refers to any composition of matter that is useful for specifically detecting another substance, e.g., a substance related to an AIR marker. A probe can be an oligonucleotide (including a conjugated oligonucleotide) that specifically hybridizes to a genomic sequence of an AIR marker, or a nucleic acid product of an AIR marker. A conjugated oligonucleotide refers to an oligonucleotide covalently bound to chromophore or molecules containing a ligand (e.g., an antigen), which is highly specific to a receptor molecule (e.g., an antibody specific to the antigen). The probe can also be a PCR primer, e.g., together with another primer, for amplifying a particular region within an AIR marker. Further, the probe can be an antibody that specifically binds to polypeptide products of these alleles. Further, the probe can be any composition of matter capable of detecting (e.g., binding or hybridizing) an equivalent genetic marker of an AIR marker. In preferred embodiments, the probe specifically hybridizes to a nucleic acid sequence (preferably genomic DNA) or specifically binds to a polypeptide sequence of an allele of interest.

The phrase “specifically hybridizes” is used to refer to hybrization under stringent hybridization conditions. Stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. One example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by at least one wash in 0.2X SSC, 0.1% SDS at 50°C. A second example of stringent hybridization conditions is hybridization in 6X SSC at about 45°C, followed by at least one wash in 0.2X SSC, 0.1% SDS at 55°C. Another example of stringent hybridization conditions is hybridization in 6X SSC at about 45°C, followed by at least one wash in 0.2X SSC, 0.1% SDS at 60°C. A further example of stringent hybridization conditions is hybridization in 6X SSC at about 45°C, followed by at least one wash in 0.2X SSC, 0.1% SDS at 65°C. High stringent conditions include hybridization in 0.5 M sodium phosphate, 7% SDS at 65°C, followed by at least one wash at 0.2X SSC, 1% SDS at 65°C.

The phrase “a region of a nucleic acid” is used to indicate a smaller sequence within a larger sequence of nucleic acids. For example, a gene is a region of a chromosome, an exon is a region of a gene, etc.

The term “specifically binds” in the context of polypeptides is used to mean that a probe binds a given polypeptide target (e.g., a polypeptide product an AIR marker) rather than randomly binding undesireable polypeptides. However, “specifically binds” does not exclude some cross reactivity with undesireable polypeptides, as long as that cross reactivity does not interfere with the capability of the probe to provide a a useful measure of the presence of the given polypeptide target.

The term “capable” is used to mean that ability to achieve a given result, e.g., a probe that is capable of detecting the presence of a particular substance means that the probe may be used to detect the particular substance.

An “oliogonucelotide” refers to a short sequence of nucleotides, e.g., 2-100 bases.

The term "biological sample" as used herein refers to a sample from a patient, which may be used for the purpose of identification, diagnosis, prediction, or monitoring. Preferred samples include synovial fluid, blood, blood-derived product (such as buffy coat, serum, and plasma), lymph, urine, tear, saliva, hair bulb cells, cerebrospinal fluid, buccal swabs, feces, synovial fluid, synovial cells, sputum, or tissue samples (e.g., cartilage samples). In addition, one of skill in the art would realize that some samples would be more readily analyzed following a fractionation or purification procedure, for example, isolation of DNA from whole blood.

The term "IL-13" includes wild-type IL-13 from various species (e.g., human, mouse, and monkey), polymorphic variants of IL-13, and functional equivalents of IL-13. Functional equivalents of IL-13 according to the present disclosure preferably have at least about 85%, 95%, 96%, 97%, 98%, or even 99% overall sequence identity with the wild-type IL-13 (e.g., human IL-13). More particularly, IL-13 refers to the polypeptide sequence set forth as in the following paragraph.

The IL-13 polypeptide has the below sequence. The N-terminal 34 amino acid residues (in italics) is a signal peptide. The mature cytokine thus has 112 amino acid residues. Anti-IL-13 antibodies will bind to an epitope on the mature polypeptide.

Interleukin 13 amino acid sequence:

1 *MHPLLNPLLL ALGLMALLLT TVIALTCLGG FASP*GPVPPS TALRELIEEL VNITQNQKAP
61 LCNGSMVWSI NLTAGMYCAA LESLINVSGC SAIEKTQRML SGFCPHKVSA GQFSSLHVRD
121 TKIEVAQFVK DLLLHLKKLF REGRFN (SEQ ID No. 1)

As also further set forth in detail in the EXAMPLES, in particular embodiments of the inventive methods, the IL-13 antagonist utilized in the methods, including antibody 01951/G12 binds an epitope that includes the residues: FCPHKV (SEQ ID NO: 67) (underlined as residues 103-107 of SEQ ID NO.1).

IL-13 Antagonists

In principle any anti-IL-13 antagonists, including antibodies, which inhibit or neutralize the activity of IL-13 may be used in the invention, so long as the anti-IL-13 antagonist or antibody determinably and selectively reduces exacerbations in an asthma patient that is positive for at least one AIR marker selected from said group consisting of AIR marker- 1, 2, 3, 4, 5, 6, 7, 8, and 9 as set forth in the methods of the invention. Such antibodies can be selected among those known in the art, see for example in WO2005/007699, US6468528, WO03007685, WO03034984, US20030143199, US2004028650, US20040242841, US2004023337, US20040248260, US20050054055, US20050065327, WO2006/124451, WO2006/003407, WO2005/062967, WO2006/085938, WO2006/055638, WO2007/036745, WO2007/080174 or WO2007/085815 Such antibodies are known in the art, see for example in WO2005/007699, US6468528, WO03007685, WO03034984, US20030143199, US2004028650, US20040242841, US2004023337, US20040248260, US20050054055, US20050065327, WO2006/124451, WO2006/003407, WO2005/062967, WO2006/085938, WO2006/055638, WO2007/036745, WO2007/080174 or WO2007/085815, WO2012/049278, and WO2008/106116.

In one embodiment, the antibodies used in the methods of the invention comprise one or more of the following CDRs. The CDRs listed in table 2a and 3a were determined according to the Kabat definition (E. Kabat et al, 1991, Sequences of Proteins of immunological Interest, 5th edition, public health Service, HIH, Bethesda, MD:

Table 2

Antibody	HCDR1	SEQ ID No. HCDR1	HCDR2	SEQ ID No. HCDR2	HCDR3	SEQ ID No. HCDR3
01951/G12	GFTFSSYG	2	IWYDGSN	3	ARLWFGDLD	4

Table 2a

Antibody	HCDR1	SEQ ID No. HCDR1	HCDR2	SEQ ID No. HCDR2	HCDR3	SEQ ID No. HCDR3
01951/G12	SYGMH	5	IIWYDGSNKYYADSVKG	6	LWFGDLDAFDI	7

Table 3

Antibody	LCDR1	SEQ ID No. LCDR1	LCDR2	SEQ ID No. LCDR2	LCDR3	SEQ ID No. LCDR3
01951/G12	QSVSSY	8	DA	9	QQRSSWPPV	10

Table 3a

Antibody	LCDR1	SEQ ID No. LCDR1	LCDR2	SEQ ID No. LCDR2	LCDR3	SEQ ID No. LCDR3
01951/G12	RAGQSVSSYLV	11	DASNRAT	12	QQRSSWPPVYT	13

The full IgG1 antibody light and heavy chain constant regions are also shown below, incorporating, as an example, the variable regions of antibody 01951/G12 (emboldened).

01951/G12 Antibody sequence

(i) HC variable region

The HC variable amino acid sequence for 01951/G12 is shown in SEQ ID NO: 14 and is encoded by the nucleotide sequence shown in SEQ ID NO: 15

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E  V  Q  L  V  E  S  G  G  G  V  V  Q  P  G  R  S  L  R  L
gaagtgcagctggtggagtctgggggaggcgtggtccagcctgggaggtccctgagactc    60

S  C  A  A  S  G  F  T  F  S  S  Y  G  M  H  W  V  R  Q  A
tcctgtgcagcgtctggattcaccttcagtagctatggcatgcactgggtccgccaggct   120

P  G  K  G  L  E  W  V  A  I  I  W  Y  D  G  S  N  K  Y  Y
ccaggcaaggggctggagtgggtggcaattatatggtatgatggaagtaataaatactat   180

A  D  S  V  K  G  R  F  T  I  S  R  D  N  S  K  N  T  L  Y
gcggactccgtgaagggccgattcaccatctccagagacaattccaagaacacgctgtat   240

L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y  C  A  R  L  W
ctgcaaatgaacagcctgagagccgaggacacggctgtgtattactgtgcgaggctatgg   300

F  G  D  L  D  A  F  D  I  W  G  Q  G  T  M  V  T (SEQ ID NO :14)
ttcggggacttagatgcttttgatatctggggccaagggacaatggtcacc 351 (SEQ ID NO :15)
```

(ii) LC variable region

The LC variable amino acid sequence for 01951/G12 is shown in SEQ ID NO: 16 and is encoded by the nucleotide sequence shown in SEQ ID NO: 17

```
E  I  V  L  T  Q  S  P  A  T  L  S  L  S  P  G  E  R  A  I
gaaattgtgttgacgcagtctccagccaccctgtctttgtctccaggggaaagagccatc  60

L  S  C  R  A  G  Q  S  V  S  S  Y  L  V  W  Y  Q  Q  K  P
ctctcctgcagggccggtcagagtgttagcagttacttagtctggtaccaacagaaacct  120

G  Q  A  P  R  L  L  I  Y  D  A  S  N  R  A  T  G  I  P  A
ggccaggctcccaggctcctcatctatgatgcatccaacagggccactggcatcccagcc  180

R  F  S  G  S  G  S  G  T  D  F  T  L  T  I  S  S  L  E  P
aggttcagtggcagtgggtctgggacagacttcactctcaccatcagcagcctagagcct  240

E  D  F  A  V  Y  Y  C  Q  Q  R  S  S  W  P  P  V  Y  T  F
gaagattttgcagtttattactgtcagcagcgcagcagctggcctccggtgtacactttt  300

G  Q  G  T (SEQ ID NO :16)
ggccaggggacc  312 (SEQ ID NO :17)
```

Full Antibody IgG1 Light Chain Sequence Incorporating The Variable Region Of Antibody 01951/G12 (Emboldened)

The LC amino acid sequence is shown in SEQ ID NO: 18 and is encoded by the nucleotide sequence of SEQ ID NO: 19

M S V L T Q V L A L L L L W L T G
1 ATGAGTGTGC TCACTCAGGT CCTGGCGTTG CTGCTGCTGT GGCTTACAGG

T R C **E I V L T Q S P A T L S L S**
51 TACGCGTTGT **GAAATTGTGT TGACGCAGTC TCCAGCCACC CTGTCTTTGT**

P G E R A I L S C R A G Q S V S
101 **CTCCAGGGGA AAGAGCCATC CTCTCCTGCA GGGCCGGTCA GAGTGTTAGC**

S Y L V W Y Q Q K P G Q A P R L L
151 **AGTTACTTAG TCTGGTACCA ACAGAAACCT GGCCAGGCTC CCAGGCTCCT**

I Y D A S N R A T G I P A R F S G
201 **CATCTATGAT GCATCCAACA GGGCCACTGG CATCCCAGCC AGGTTCAGTG**

S G S G T D F T L T I S S L E P
251 **GCAGTGGGTC TGGGACAGAC TTCACTCTCA CCATCAGCAG CCTAGAGCCT**

E D F A V Y Y C Q Q R S S W P P V
301 **GAAGATTTTG CAGTTTATTA CTGTCAGCAG CGCAGCAGCT GGCCTCCGGT**

Y T F G Q G T K L E I K R T V A A
351 **GTACACTTTT GGCCAGGGGA CC**AAGCTTGA AATCAAACGA ACTGTGGCTG

P S V F I F P P S D E Q L K S G
401 CACCATCTGT CTTCATCTTC CCGCCATCTG ATGAGCAGTT GAAATCTGGA

T A S V V C L L N N F Y P R E A K
451 ACTGCCTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA GAGAGGCCAA

V Q W K V D N A L Q S G N S Q E S
501 AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCCCAGGAGA

V T E Q D S K D S T Y S L S S T
551 GTGTCACAGA GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGCAGCACC

L T L S K A D Y E K H K V Y A C E
601 CTGACGCTGA GCAAAGCAGA CTACGAGAAA CACAAAGTCT ACGCCTGCGA

V T H Q G L S S P V T K S F N R G
651 AGTCACCCAT CAGGGCCTGA GCTCGCCCGT CACAAAGAGC TTCAACAGGG

E C * (SEQ ID NO:18)
701 GAGAGTGTTA G (SEQ ID NO:19)

Full Antibody IgG1 Heavy Chain Sequence Incorporating The Variable Region Of Antibody 01951/G12 (Emboldened)

The HC amino acid sequence is shown in SEQ ID NO: 20 and is encoded by the nucleotide sequence of SEQ ID NO: 21

M A W V W T L P F L M A A A Q S V
1 ATGGCTTGGG TGTGGACCTT GCCATTCCTG ATGGCAGCTG CCCAAAGTGT

Q A **E V Q L V E S G G G V V Q P G**
51 CCAGGCA**GAA GTGCAGCTGG TGGAGTCTGG GGGAGGCGTG GTCCAGCCTG**

R S L R L S C A A S G F T F S S
101 **GGAGGTCCCT GAGACTCTCC TGTGCAGCGT CTGGATTCAC CTTCAGTAGC**

Y G M H W V R Q A P G K G L E W V
151 **TATGGCATGC ACTGGGTCCG CCAGGCTCCA GGCAAGGGGC TGGAGTGGGT**

A I I W Y D G S N K Y Y A D S V K
201 **GGCAATTATA TGGTATGATG GAAGTAATAA ATACTATGCG GACTCCGTGA**

G R F T I S R D N S K N T L Y L
251 **AGGGCCGATT CACCATCTCC AGAGACAATT CCAAGAACAC GCTGTATCTG**

Q M N S L R A E D T A V Y Y C A R
301 CAAA**TGAACA GCCTGAGAGC CGAGGACACG GCTGTGTATT ACTGTGCGAG**

L W F G D L D A F D I W G Q G T M
351 **GCTATGGTTC GGGGACTTAG ATGCTTTTGA TATCTGGGGC CAAGGGACAA**

V T V S S A S T K G P S V F P L
401 **TGGTCACC**GT CTCCTCAGCC TCCACCAAGG GCCCATCGGT CTTCCCCCTG

A P S S K S T S G G T A A L G C L
451 GCACCCTCCT CCAAGAGCAC CTCTGGGGGC ACAGCGGCCC TGGGCTGCCT

V K D Y F P E P V T V S W N S G A
501 GGTCAAGGAC TACTTCCCCG AACCGGTGAC GGTGTCGTGG AACTCAGGCG

L T S G V H T F P A V L Q S S G
551 CCCTGACCAG CGGCGTGCAC ACCTTCCCGG CTGTCCTACA GTCCTCAGGA

L Y S L S S V V T V P S S S L G T
601 CTCTACTCCC TCAGCAGCGT CGTGACCGTG CCCTCCAGCA GCTTGGGCAC

Q T Y I C N V N H K P S N T K V D
651 CCAGACCTAC ATCTGCAACG TGAATCACAA GCCCAGCAAC ACCAAGGTGG

K R V E P K S C D K T H T C P P
701 ACAAGAGAGT TGAGCCCAAA TCTTGTGACA AAACTCACAC ATGCCCACCG

C P A P E L L G G P S V F L F P P
751 TGCCCAGCAC CTGAACTCCT GGGGGGACCG TCAGTCTTCC TCTTCCCCCC

K P K D T L M I S R T P E V T C V
801 AAAACCCAAG GACACCCTCA TGATCTCCCG GACCCCTGAG GTCACATGCG

V V D V S H E D P E V K F N W Y
851 TGGTGGTGGA CGTGAGCCAC GAAGACCCTG AGGTCAAGTT CAACTGGTAC

V D G V E V H N A K T K P R E E Q
901 GTGGACGGCG TGGAGGTGCA TAATGCCAAG ACAAAGCCGC GGGAGGAGCA

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          Y  N  S   T  Y  R  V   V  S  V   L  T  V   L  H  Q  D
  951    GTACAACAGC ACGTACCGTG TGGTCAGCGT CCTCACCGTC CTGCACCAGG

           W  L  N   G  K  E   Y  K  C  K   V  S  N   K  A  L
 1001    ACTGGCTGAA TGGCAAGGAG TACAAGTGCA AGGTCTCCAA CAAAGCCCTC

         P  A  P  I   E  K  T   I  S  K   A  K  G  Q   P  R  E
 1051    CCAGCCCCCA TCGAGAAAAC CATCTCCAAA GCCAAAGGGC AGCCCCGAGA

          P  Q  V   Y  T  L  P   P  S  R   E  E  M   T  K  N  Q
 1101    ACCACAGGTG TACACCCTGC CCCCATCCCG GGAGGAGATG ACCAAGAACC

           V  S  L   T  C  L   V  K  G  F   Y  P  S   D  I  A
 1151    AGGTCAGCCT GACCTGCCTG GTCAAAGGCT TCTATCCCAG CGACATCGCC

         V  E  W  E   S  N  G   Q  P  E   N  N  Y  K   T  T  P
 1201    GTGGAGTGGG AGAGCAATGG GCAGCCGGAG AACAACTACA AGACCACGCC

          P  V  L   D  S  D  G   S  F  F   L  Y  S   K  L  T  V
 1251    TCCCGTGCTG GACTCCGACG GCTCCTTCTT CCTCTATAGC AAGCTCACCG

           D  K  S   R  W  Q   Q  G  N  V   F  S  C   S  V  M
 1301    TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG

         H  E  A  L   H  N  H   Y  T  Q   K  S  L  S   L  S  P
 1351    CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCCCC

          G  K  * (SEQ ID NO:20)
 1401    GGGTAAATGA (SEQ ID NO:21)
```

In various embodiments, the invention encompasses disclosed pharmaceutical compositions, regimens, processes, uses, methods and kits utilzing an IL-13 antagonist (e.g., an anti-IL-13 antibody or antigen-binding portion thereof) set forth herein. In particular embodiments the kits comprise nucleic acid probes for detecting an AIR marker set forth herein. In further embodiments, the kits further comprise instructions comprising diagnosis or treatment of asthma.

In a particular embodiment, the invention provides a kit for determining the presence of at least one AIR marker selected from the group consisting of AIR marker 1, 2, 3, 4, 5, 6, 7, 8, or 9 in a sample obtained from an asthma patient, the kit comprising: a container containing one or more probes that specifically hybridize under stringent conditions to a nucleic acid comprising the responsive allele in one or more SNPs selected from the group set forth in Table 1.

In another embodiment, the kit comprises instructional materials indicating that the presence of at least AIR marker in a nucleic acid sample from the patient indicates that the patient is a candidate for treatment with an IL-13 antagonist as set forth herein.

In another embodiment, the kit comprises instructional materials indicating that the presence of at least 2 AIR markers in a nucleic acid sample from the patient indicates that the patient is a candidate for treatment with an IL-13 antagonist as set forth herein.

In another embodiment, the kit comprises instructional materials indicating that the presence of at least 3,4,5,6,7,8, or 9 AIR markers in a nucleic acid sample from the patient indicates that the patient is a candidate for treatment with an IL-13 antagonist as set forth herein.

In another embodiment, the kit comprisesa a container containing one or more probes that specifically hybridize under stringent conditions to a nucleic acid comprising the responsive allele in one or more SNPs selected from the group set forth in Table 1. In another, the nucleic acid comprises two or more SNPs selected from the group set forth in Table 1. In another, the nucleic acid comprises 2, 3, 3, 4, 5, 6, 7, 8, or 9 or more SNPs selected from the group set forth in Table 1.

In another embodiment, the one or more probes specifically hybridize under stringent conditions to a nucleic acid comprising the responsive allele in SNP rs 8832, and/or rs 1050 .

In another, the one or more probes and/or primers comprise probes and/or primers for use in nucleic acid amplification reaction.

Techniques for Assaying, Diagnostic Methods and Methods of Producing a Transmittable Form of Information

The disclosed methods are useful for the treatment, prevention, or amelioration of asthma diseases, as well as predicting the likelihood of an asthma patient's response to treatment with an IL-13 antagonist. These methods employ, *inter alia*, determining whether a patient has an AIR marker in a sample from the patient.

A biological sample from the patient may be assayed for the presence of an AIR marker by any applicable conventional means, which will be selected depending on whether the particular marker falls within an exon, an intron, a non-coding portion of mRNA or a non-conding genomic sequence.

Numerous biological samples may be used to identify the presence of alleles or proteins, the level of expression of genes or proteins, and the activity of a protein, e.g., blood, synovial fluid, buffy coat, serum, plasma, lymph, feces, urine, tear, saliva, cerebrospinal fluid, buccal swabs, sputum, or tissue. Various sources within a biological sample may be used in the disclosed methods, e.g., one may assay genomic DNA obtained from a biological sample to detect an AIR marker, or one may assay products of a an AIR marker, e.g., nucleic acid products (e.g., DNA, pre-mRNA, mRNA, micro RNAs, etc.) and polypeptide products (e.g., expressed proteins) obtained from a biological sample.

The inventive discovery encompasses the determination that the various AIR markers of Table 1 are useful for predicting certain patient's response to treatment by IL-13 antagonism. Of the SNPs in Table 1, most are found in genomic DNA and introns, such that a patient's allelic status may be determined by interrogating, e.g., pre-mRNA or genomic DNA. However, the presence of the AIR markers corresponding to exonic locations may be determined by assaying genomic DNA, RNA and/or protein sequence. Accordingly, a skilled artisan will understand that one may identify whether a subject has a given AIR marker by assaying a nucleic acid product of an AIR marker, a polypeptide product of an AIR marker, or an equivalent genetic marker of an AIR marker, as appropriate. In preferred embodiments, a genomic sequence of an AIR marker is analyzed to determine whether a subject has an AIR marker.

Numerous methods and devices are available to identify the presence of an AIR marker or polymorphism that results in a decreased level of expression, level of the coded protein or level of the protein's activity. DNA (genomic and cDNA) for SNP detection can be prepared from a biological sample by methods well known in the art, e.g., phenol/chloroform extraction, PUREGENE DNA® purification system from GentAS Systems (Qiagen, CA). Detection of a DNA sequence may include examining the nucleotide(s) located at either the sense or the anti-sense strand within that region. The presence of polymorphisms in a patient may be detected from DNA (genomic or cDNA) obtained from PCR using sequence-specific probes, e.g., hydrolysis probes from Taqman, Beacons, Scorpions; or hybridization probes that detect the marker or polymorphism. For the detection of the polymorphism, sequence specific probes may be designed such that they specifically hybridize to the genomic DNA for the alleles of interest or, in some cases, an RNA of interest. Primers and probes for polymorphic sites (e.g., SNP) may be designed based on context sequences found in the NCBI SNP database available at: www.ncbi.nlm.nih.gov/snp. These probes may be labeled for direct detection or contacted by a second, detectable molecule that specifically binds to the probe. The PCR products also can be detected by DNA-binding agents. Said PCR products can then be subsequently sequenced by any DNA sequencing method available in the art. Alternatively the presence of allele can be detected by sequencing using any sequencing methods such as, but not limited to, Sanger-based sequencing, pyrosequencing or next generation sequencing (Shendure J. and Ji, H., Nature Biotechnology (1998), Vol. 26, Nr 10, pages 1135-1145). Optimised allelic discrimination assays for SNPs may be purchased from Applied Biosystems (Foster City, California, USA).

Various techniques can be applied to interrogate a particular polymorphism (e.g., SNP), including, e.g., hybridization-based methods, such as dynamic allele-specific hybridization (DASH) genotyping, polymorphic site (e.g., SNP) detection through molecular beacons (Abravaya K., et al. (2003) Clin Chem Lab Med. 41:468-474), Luminex xMAP technology®, Illumina Golden Gate® technology and commercially available high-density oligonucleotide SNP arrays (e.g., the Affymetrix Human SNP 5.0 GeneChip® performs a genome-wide assay that can genotype over 500,000 human SNPs), BeadChip® kits from Illumina, e.g, Human660W-Quad and Human 1.2M-Duo); enzyme-based methods, such as restriction fragment length polymorphism (RFLP), PCR-based methods (e.g., Tetra-primer ARMS-PCR), Invader assays (Olivier M. (2005) Mutat Res. 573(1-2):103-10), various primer extension assays (incorporated into detection formats, e.g., MALDI-TOF Mass spectrometry, electrophoresis, blotting, and ELISA-like methods), TaqMan® assays, and oligonucleotide ligase assays; and other post-amplification methods, e.g., analysis of single strand conformation polymorphism (Costabile et al. (2006) Hum. Mutat. 27(12):1163-73), temperaure gradient gel electrophoresis (TGGE), denaturing high performance liquid chromatography, high-resolution melting analysis, DNA mismatch-binding protein assays (e.g., MutS protein from *Thermus aquaticus* binds different single nucleotide mismatches with different affinities and can be used in capillary electrophoresis to differentiate all six sets of mismatches), SNPLex® (proprietary SNP detecting system available from Applied Biosystems), capillary electrophoresis, mass spectrometry, and various sequencing methods, e.g., pyrosequencing and next generation sequencing, etc. Commercial kits for SNP genotyping include, e.g., Fluidigm Dynamic Array® IFCs (Fluidigm), TaqMan® SNP Genotyping Assay (Applied Biosystems), MassARRAY® iPLEX Gold (Sequenom), Type-it Fast® SNP Probe PCR Kit (Quiagen), etc.

In some embodiments, the presence of a polymorphic site (e.g., SNP) in a patient is detected using a hybridization assay. In a hybridization assay, the presence of the genetic marker is determined based on the ability of the nucleic acid from the sample to hybridize to a complementary nucleic acid molecule, e.g., an oligonucleotide probe. A variety of hybridization assays are available. In some, hybridization of a probe to the sequence of interest is detected directly by visualizing a bound probe, e.g., a Northern or Southern assay. In these assays, DNA (Southern) or RNA (Northern) is isolated. The DNA or RNA is then cleaved with a series of restriction enzymes that cleave infrequently in the genome and not near any of the markers being assayed. The DNA or RNA is then separated, e.g., on an agarose gel, and transferred to a membrane. A labeled probe or probes, e.g., by incorporating a radionucleotide or binding agent (e.g., SYBR® Green), is allowed to contact the membrane under low-, medium- or high-stringency conditions. Unbound probe is removed and the presence of binding is detected by visualizing the labeled probe. In some embodiments, arrays, e.g., the MassARRAY® system (Sequenom, San Diego, California, USA) may be used to genotype a subject.

Traditional genotyping methods may also be modified for use in genotyping. Such traditional methods include, e.g., DNA amplification techniques such as PCR and variants thereof, direct sequencing, SSO hybridization coupled with the Luminex xMAP® technology, SSP typing, and SBT.

Sequence-Specific Oligonucleotide (SSO) typing uses PCR target amplification, hybridization of PCR products to a panel of immobilized sequence-specific oligonucleotides on the beads, detection of probe-bound amplified product by color formation followed by data analysis. Those skilled in the art would understand that the described Sequence-Specific

Oligonucleotide (SSO) hybridization may be performed using various commercially available kits, such as those provided by One Lambda, Inc. (Canoga Park, CA) or Lifecodes HLA Typing Kits (Tepnel Life Sciences Corp.) coupled with Luminex® technology (Luminex, Corporation, TX). LABType® SSO is a reverse SSO (rSSO) DNA typing solution that uses sequence–specific oligonucleotide (SSO) probes and color-coded microspheres to identify HLA alleles. The target DNA is amplified by polymerase chain reactions (PCR) and then hybridized with the bead probe array. The assay takes place in a single well of a 96-well PCR plate; thus, 96 samples can be processed at one time.

Sequence Specific Primers (SSP) typing is a PCR based technique which uses sequence specific primers for DNA based typing. The SSP method is based on the principle that only primers with completely matched sequences to the target sequences result in amplified products under controlled PCR conditions. Allele sequence-specific primer pairs are designed to selectively amplify target sequences which are specific to a single allele or group of alleles. PCR products can be visualized on agarose gel. Control primer pairs that matches non-allelic sequences present in all samples act as an internal PCR control to verify the efficiency of the PCR amplification. Those skilled in the art would understand that low, medium and high resolution genotyping with the described sequence-specific primer typing may be performed using various commercially available kits, such as the Olerup SSP™ kits (Olerup, PA) or (Invitrogen) or Allset and ™Gold DQA1 Low resolution SSP (Invitrogen).

Sequence Based Typing (SBT) is based on PCR target amplification, followed by sequencing of the PCR products and data analysis.

In some cases, RNA, e.g., mature mRNA, pre-mRNA, can also be used to determine the presence of particular polymorphisms (see Table 1). Analysis of the sequence of mRNA transcribed from a given gene can be performed using any known method in the art including, but not limited, to Northern blot analysis, nuclease protection assays (NPA), *in situ* hybridization, reverse transcription-polymerase chain reaction (RT-PCR), RT-PCR ELISA, TaqMan-based quantitative RT-PCR (probe-based quantitative RT-PCR) and SYBR green-based quantitative RT-PCR. In one example, detection of mRNA levels involves contacting the isolated mRNA with an oligonucleotide that can hybridize to mRNA encoded by an AIR marker.

The nucleic acid probe can typically be, for example, a full-length cDNA, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, or 100 nucleotides in length and sufficient to specifically hybridize under stringent conditions to the mRNA. Hybridization of an mRNA with the probe indicates that the marker in question is being expressed. In one format, the RNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated RNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. Amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers. PCR products can be detected by any suitable method including, but not limited to, gel electrophoresis and staining with a DNA-specific stain or hybridization to a labeled probe.

The level of expression of a gene may be determined by measuring RNA (or reverse transcribed cDNA) levels using various techniques, e.g., a PCR-based assay, reverse-transcriptase PCR (RT-PCR) assay, Northern blot, etc. Quantitative RT-PCR with standardized mixtures of competitive templates can also be utilized.

In some cases, the presence of a polymorphism in a patient can be determined by analyzing polypeptide products of the AIR markers (see Table 1). Detection of polypeptide products can be performed using any known method in the art including, but not limited, to immunocytochemical staining, ELISA, flow cytometry, Western blot, spectrophotometry, HPLC, and mass spectrometry.

The use of immobilized antibodies specific for the proteins or polypeptides is also contemplated by the present disclosure. The antibodies can be immobilized onto a variety of solid supports, such as magnetic or chromatographic matrix particles, the surface of an assay place (such as microtiter wells), pieces of a solid substrate material (such as plastic, nylon, paper), and the like. An assay strip can be prepared by coating the antibody or a plurality of antibodies in an array on solid support. This strip can then be dipped into the test sample and then processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot.

In a two-step assay, immobilized polypeptide products of an AIR marker or ERAP1 protein may be incubated with an unlabeled antibody. The unlabeled antibody complex, if present, is then bound to a second, labeled antibody that is specific for the unlabeled antibody. The sample is washed and assayed for the presence of the label. The choice of marker used to label the antibodies will vary depending upon the application. However, the choice of the marker is readily determinable to one skilled in the art. The antibodies may be labeled with a radioactive atom, an enzyme, a chromophoric or fluorescent moiety, or a colorimetric tag. The choice of tagging label also will depend on the detection limitations desired. Enzyme assays (ELISAs) typically allow detection of a colored product formed by interaction of the enzyme-tagged complex with an enzyme substrate. Some examples of radioactive atoms include ^{32}P, ^{125}I, ^{3}H, and ^{14}P. Some examples of enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, and glucose-6-phosphate dehydrogenase. Some examples of chromophoric moieties include fluorescein and rhodamine. The antibodies may be conjugated to these labels by methods known in the art. For example, enzymes and chromophoric molecules may be conjugated to the antibodies by means of coupling agents, such as dialdehydes, carbodiimides, dimaleimides, and the like. Alternatively, conjugation may occur through a ligand-receptor pair. Some suitable ligand-receptor pairs include, for example, biotin-avidin or -streptavidin, and antibody-antigen.

In one aspect, the present disclosure contemplates the use of a sandwich technique for detecting polypeptide products in biological samples. The technique requires two antibodies capable of binding the protein of interest: e.g., one immobilized onto a solid support and one free in solution, but labeled with some easily detectable chemical compound. Examples of chemical labels that may be used for the second antibody include but are not limited to radioisotopes, fluorescent compounds, and enzymes or other molecules which generate colored or electrochemically active products when exposed to a reactant or enzyme substrate. When samples containing polypeptide products are placed in this system, the polypeptide products binds to both the immobilized antibody and the labeled antibody. The result is a "sandwich" immune complex on the support's surface. The complexed protein is detected by washing away nonbound sample components and excess labeled antibody, and measuring the amount of labeled antibody complexed to protein on the support's surface. The sandwich immunoassay is highly specific and very sensitive, provided that labels with good limits of detection are used.

Preferably, the presence of polypeptide products in a sample is detected by radioimmunoassays or enzyme-linked immunoassays, competitive binding enzyme-linked immunoassays, dot blot, Western blot, chromatography, preferably high performance liquid chromatography (HPLC), or other assays known in the art. Specific immunological binding of the antibody to the protein or polypeptide can be detected directly or indirectly.

Dot blotting is routinely practiced by the skilled artisan to detect a desired protein using an antibody as a probe (Promega Protocols and Applications Guide, Second Edition, 1991, Page 263, Promega Corporation). Samples are applied to a membrane using a dot blot apparatus. A labeled probe is incubated with the membrane, and the presence of the protein is detected.

Western blot analysis is well known to the skilled artisan (Sambrook et al., Molecular Cloning, A Laboratory Manual, 1989, Vol. 3, Chapter 18, Cold Spring Harbor Laboratory). In Western blot, the sample is separated by SDS-PAGE. The gel is transferred to a membrane. The membrane is incubated with labeled antibody for detection of the desired protein.

The assays described above involve steps such as but not limited to, immunoblotting, immunodiffusion, immunoelectrophoresis, or immunoprecipitation. In some embodiments, an automatic analyzer is used to determine the presence of an AIR marker.

In performing any of the methods described herein that require determining the presence of an AIR marker or polymorphism, such determination may be made by consulting a data repository that contains sufficient information on the patient's genetic composition to determine whether the patient has the marker of interest. Preferably, the data repository lists the genotype present (or absent) in the individual. The data repository could include the individual's patient records, a medical data card, a file (e. g., a flat ASCII file) accessible by a computer or other electronic or non-electronic media on which appropriate information or genetic data can be stored. As used herein, a medical data card is a portable storage device such as a magnetic data card, a smart card, which has an on-board processing unit and which is sold by vendors such as

Siemens of Munich Germany, or a flash-memory card. If the data repository is a file accessible by a computer; such files may be located on various media, including: a server, a client, a hard disk, a CD, a DVD, a personal digital assistant such as a smart phone, Palm Pilot, a tape recorder, a zip disk, the computer's internal ROM (read-only-memory) or the internet or worldwide web. Other media for the storage of files accessible by a computer will be obvious to one skilled in the art.

Typically, once the presence of an AIR marker or polymorphism is determined, physicians or genetic counselors or patients or other researchers may be informed of the result. Specifically the result can be cast in a transmittable form of information that can be communicated or transmitted to other researchers or physicians or genetic counselors or patients. Such a form can vary and can be tangible or intangible. The result in the individual tested can be embodied in descriptive statements, diagrams, photographs, charts, images or any other visual forms. For example, images of gel electrophoresis of PCR products can be used in explaining the results. Diagrams showing where a variant occurs in an individual's allele are also useful in indicating the testing results. Statements regarding the presence of an AIR marker or polymorphism are also useful in indicating the testing results. These statements and visual forms can be recorded on a tangible media such as papers, computer readable media such as floppy disks, compact disks, etc., or on an intangible media, e.g., an electronic media in the form of email or website on internet or intranet. In addition, the result can also be recorded in a sound form and transmitted through any suitable media, e.g., analog or digital cable lines, fiber optic cables, etc., via telephone, facsimile, wireless mobile phone, internet phone and the like. All such forms (tangible and intangible) would constitute a “transmittable form of information”. Thus, the information and data on a test result can be produced anywhere in the world and transmitted to a different location. For example, when a genotyping assay is conducted offshore, the information and data on a test result may be generated and cast in a transmittable form as described above. The test result in a transmittable form thus can be imported into the U.S. Accordingly, the present disclosure also encompasses a method for producing a transmittable form of information containing the presence or absence of an AIR marker or polymorphism in an individual. This form of information is useful for predicting the responsiveness of a patient having asthma, and for selectively treating a patient based upon that information.

Methods of Treatment and Uses of IL-13Antagonists

The disclosed methods allow clinicians to provide a personalized therapy for asthma patients, i.e., they allow determination of whether to selectively treat the patient with an IL-13 antagonist. In this way, a clinician can maximize the benefit and minimize the risk of IL-13 antagnoism in the entire population of patients afflicted with asthma. It will be understood that IL-13 antagonists are useful for the treatment, prevention, or amelioration of asthma, including moderate or severe asthma. The IL-13 antagonists, may be used *in vitro*, *ex vivo*, or incorporated into pharmaceutical compositions and administered to individuals (e.g., human patients) *in vivo* to treat, ameliorate, or prevent asthma, e.g., in patients who have an AIR marker. A pharmaceutical composition will be formulated to be compatible with its intended route of administration (e.g., oral compositions generally include an inert diluent or an edible carrier). Other nonlimiting examples of routes of administration include parenteral (e.g., intravenous), intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. The pharmaceutical compositions compatible with each intended route are well known in the art.

The IL-13 antagonists, may be used as a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may contain, in addition to an IL-13 antagonist, carriers, various diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The characteristics of the carrier will depend on the route of administration.

Antibodies, e.g., antibodies to IL-13, are typically formulated either in aqueous form ready for parenteral administration or as lyophilisates for reconstitution with a suitable diluent prior to administration. In some embodiments of the disclosed methods and uses, the IL-13 antagonist, e.g., IL-13 antibody, is formulated as a lyophilisate. Suitable lyophilisate formulations can be reconstituted in a small liquid volume (e.g., 2ml or less) to allow subcutaneous administration and can provide solutions with low levels of antibody aggregation. The use of antibodies as the active ingredient of pharmaceuticals is now widespread, including the products HERCEPTIN® (trastuzumab), RITUXAN® (rituximab), SYNAGIS®

(palivizumab), etc. Techniques for purification of antibodies to a pharmaceutical grade are well known in the art. When a therapeutically effective amount of an IL-13 antagonistis administered by intravenous, cutaneous or subcutaneous injection, the IL-13 antagonist will be in the form of a pyrogen-free, parenterally acceptable solution. A pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection may contain, in addition to the IL-13 antagonist, an isotonic vehicle such as sodium chloride, Ringer's, dextrose, dextrose and sodium chloride, lactated Ringer's, or other vehicle as known in the art.

The appropriate dosage will, of course, vary depending upon, for example, the particular IL-13 antagonists to be employed, the host, the mode of administration and the nature and severity of the condition being treated, and on the nature of prior treatments that the patient has undergone. Ultimately, the attending health care provider will decide the amount of the IL-13 antagonist with which to treat each individual patient.

In practicing some of the methods of treatment or uses of the present disclosure, a therapeutically effective amount of an IL-13 antagonistis administered to a patient, e.g., a mammal (e.g., a human). While it is understood that the disclosed methods provide for selective treatment of patients depending on the presence of an AIR marker, this does not preclude that, if the patient is ultimately treated with an IL-13 antagonist, such IL-13 antagonist therapy is necessarily a monotherapy. Indeed, if a patient is selected for treatment with an IL-13 antagonist, then the IL-13antagonist may be administered in accordance with the method of the disclosure either alone or in combination with other therapeutics for treating asthma. When coadministered with one or more additional therapeutics, an IL-13 antagonist may be administered either simultaneously with the other therapeutic, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering the IL-13 antagonist in combination with other therapeutics, as well as the appropriate dosages for co-delivery.

An IL-13 antagonist, is conveniently administered parenterally, intravenously, e.g., into the antecubital or other peripheral vein, intramuscularly, or subcutaneously. The duration of intravenous (i.v.) therapy using a pharmaceutical composition of the present disclosure will vary, depending on the severity of the disease being treated and the condition and personal response of each individual patient. Also contemplated is subcutaneous (s.c.) therapy using a pharmaceutical composition of the present disclosure. The health care provider will decide on the appropriate duration of i.v. or s.c. therapy and the timing of administration of the therapy, using the pharmaceutical composition of the present disclosure.

Doses may be delivered based on weight, e.g., 3 mg/kg, 10 mg/kg, 15 mg/kg, or as a fixed amount, e.g., 75 mg, 150 mg, 300 mg, 1000 mg depending on the severity of the disease.

Kits

The invention also encompasses kits for detecting an AIR marker or polymorphism, expression level, protein level or activity, in a biological sample (a test sample) from an asthma patient. Such kits can be used to predict if a patient having asthma is likely to respond (or have a higher response) to treatment with an IL-13 antagonist. For example, the kit can comprise a probe (e.g., an oligonucleotode, antibody, labeled compound or other agent) capable of detecting an AIR marker or polymorphism, products of those alleles and/or an equivalent genetic marker of those alleles in a biological sample. The kit may also comprise instructions for providing a prediction of the likelihood that the patient will respond to treatment with the IL-13 antagonist.

Probes may specifically hybridize to genomic sequences, nucleic acid products, or polypeptide products. Exemplary probes are oligonucleotides or conjugated oligonucleotides that specifically hybridizes to the responsive alleles of Table 1; an antibody that is capable of differentiating between polypeptide products encoded by the disclosed alleles; primer-extension oligonucleotides, allele-specific primers, a combination of allele-specific primers, allele-specfic probes, and primer extension primers, etc. Optionally, the kit can contain a probe that targets an internal control allele, which can be any allele presented in the general population. Detection of an internal control allele is designed to assure the performance of the kit. The disclosed kits can also comprise, e.g., a buffering agent, a preservative, or a protein stabilizing agent. The kit can also comprise components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples that can be assayed and compared to the test sample contained. Each component of the kit is usually enclosed within an individual container, and all of the various containers are within a single package along with instructions for use.

Such kits may also comprise an IL-13 antagonist or a pharmaceutical composition comprising the IL-13 antagonist. Such kits are useful in the selective treatment of asthma patients using an IL-13 antagonist. Additionally, such kits may comprise means for administering the IL-13 antagonist (e.g., a syringe and vial, a prefilled syringe, a prefilled pen) and instructions for use. These kits may contain additional therapeutic agents for treating asthma, e.g., for delivery in combination with the enclosed IL-13 antagonist..

The phrase "means for administering" is used to indicate any available implement for systemically administering a drug top a patient, including, but not limited to, a pre-filled syringe, a vial and syringe, an injection pen, an autoinjector, an i.v. drip and bag, a pump, etc. With such items, a patient may self-administer the drug (i.e., administer the drug on their own behalf) or a physician may administer the drug.

The details of one or more embodiments of the disclosure are set forth in the accompanying description above. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. Other features, objects, and advantages of the disclosure will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms include plural referents unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. All numerical ranges in this disclosure are inclusive of the endpoints and of all integers, decimals and fractions therebetween whether specifically stated or not.All patents and publications cited in this specification are incorporated by reference. The following Examples are presented in order to more fully illustrate the preferred embodiments of the disclosure. These examples should in no way be construed as limiting the scope of the disclosed patient matter, as defined by the appended claims.

EXAMPLES

List of abbreviations

Abbreviation	Explanation
ACQ	Asthma Control Questionnaire
DNA	Deoxyribonucleic Acid
FEV	Forced Expiratory Volume
IgE	Immunoglobulin E
IL-4	Interleukin 4
IL4-Rα	Interleukin 4 Receptor, Alpha
IL-13	Interleukin 13
LD	Linkage Disequilibrium
SNP	Single Nucleotide Polymorphism
UTR	Untranslated Region

EXAMPLE 1:

Identification of Il-13 antagonists

For the purposes of the invention, IL-13 antagonists that compete with a specified anti-IL-13 antibody set forth herein, including antibody 01951/G12 (SEQ ID NO:14 and 16) for binding to IL-13 can be identified by methods known in the art, and further as described in the following methodology.

All experiments are performed using a Biacore T200 instrument. Biacore T200 Control software and Biacore T200 Evaluation software are used for the control and analysis of experiments, respectively.

PART 1: The anti-human IL-13 antibody ANTIBODY 01951/G12 is immobilized to a CM5 sensorchip surface using the amine coupling method. Briefly, the surface of the measuring flow cell is activated with EDC/NHS, followed by a 700 second addition of 50 μg/mL ANTIBODY

01951/G12 in 10 mM sodium acetate pH 4.5. Based on previous experiments, these buffers and injection times are sufficient to allow saturation of the chip surface with ANTIBODY 01951/G12 , though exposure time could be reduced if saturation was achieved prior to 700 seconds. Immobilization buffers that are potentially more suitable could be determined by buffer scouting. Any remaining active surface groups are subsequently blocked with ethanolamine. The surface of the reference flow cell is activated with EDC/NHS and subsequently blocked with ethanolamine without immobilization of protein (blank immobilization) or by the addition of an isotype control antibody. Based on previous studies using ANTIBODY 01951/G12 immobilization, an appropriate running buffer is HBS-EP+ containing 10 mM HEPES, 150 mM NaCl, 0.005 % (w/v) polysorbate 20 and 3 mM EDTA at pH 7.4.

PART 2: A concentration-response curve for human IL-13 is performed to determine the appropriate concentration of IL-13 to use in the subsequent epitope competition studies. IL-13 is diluted in running buffer and, beginning at 25 nM, is flowed over the reference and measuring flow cell at a flow rate of 30 μL/min. The optimal rate can be adjusted experimentally. The association time is 120 seconds and dissociation time is 120 seconds, although dissociation time could be extended to 180 seconds, or 600 seconds or more if an affinity (K_D) measurement is required. Regeneration of the surface with a 30 second injection of 10 mM Glycine at pH 2.0, at a flow rate of 10 μL/min, is necessary to remove IL-13 from ANTIBODY 01951/G12 after the dissociation step. A second regeneration step immediately after the first is sometimes required. These steps are repeated over the following dilution series of IL-13: 25, 12.5, 6.25, 3.13, 1.56 and 0 nM. Based on previous studies, this dilution series would be suitable for preliminary experiments, but may need to be changed accordingly. The dilution series is run twice to provide n = 2. Analysis temperature is 25 °C. Running buffer is HBS-P+ containing 10 mM HEPES, 150 mM NaCl, 0.005 % (w/v) and polysorbate 20 at pH 7.4. Data is evaluated using a 1:1 binding model with Biacore T200 Evaluation software.

PART 3: Epitope competition studies are done using a dual binding method. Based on the data from Part 2, a concentration of IL-13 that gives approximately 50 RU binding to ANTIBODY 01951/G12 is injected for 120 seconds at 30 μL/min. There is NO dissociation phase; instead a second injection of a competitor anti-IL-13 antibody immediately follows, at a 10-fold* greater concentration than IL-13. Previous studies with respect to IL-25 have used an association phase of 120 seconds, with a flow rate of 30 µL/min, mirroring the IL-13 addition. Any competitor that binds to IL-13 using a different epitope to that of ANTIBODY 01951/G12 will give a binding response. Those that bind to IL-13 using the same epitope as ANTIBODY 01951/G12 will give no binding response. A variable dissociation period can apply, followed by an acidic wash step to remove both the IL-13 and the competitor addition. This step can be reversed, with competitor antibody immobilized to the chip surface and ANTIBODY 01951/G12 as the second addition, to confirm results. Running buffer is HBS-P+ containing 10 mM HEPES, 150 mM NaCl, 0.005 % (w/v) and polysorbate 20 at pH 7.4.

EXAMPLE 2

Identification of the antibody 01951/G12 epitope residues:

Peptide mapping:

The sequence of IL-13 was probed at the peptide level to identify the binding site with ANTIBODY 01951/G12 . Thirty four 15-mer peptides were synthesized in order to scan the entire sequence with 12 residue overlap starting from the N-terminus. The synthesis of the peptides, preparation of the peptide-array slides, incubation with antibodies and data analysis where done as described by Maksimov et al. (PLoS ONE 7(3): e34212).

The results are shown in Table 4. The binding signal intensity (light unit) observed is reported for each peptide, in column 1 against a control unrelated human antibody and in column 2 against ANTIBODY 01951/G12 . Signals above 10000 Light Units are considered as being significant. 2 overlapping peptides in the set of the 34 peptides produced a signal above that threshold, peptide 22 (TQRMLSGFCPHKVSA) (SEQ ID NO: 31) and peptide 23 (MLSGFCPHKVSAGQF) (SEQ ID NO: 32). The overlapping sequence between both peptides is MLSGFCPHKVSA (SEQ ID NO: 33).

In order to verify that this sequence is important for the binding of IL-13 to ANTIBODY 01951/G12 and eventually narrow it down further, a substitution analysis was performed.

For this substitution analysis, a peptide array was created where each residue of the sequence MLSGFCPHKVSA was exchanged against every possible 20 standard amino acid, creating a set of 240 peptides in which 228 peptides differ from the original sequence by 1 amino acid at a time. The result of the binding study with ANTIBODY 01951/G12 is presented in Figure 1. The boxed region indicates residues for which binding is strongly reduced or abolished when they are replaced by almost any amino acid. Therefore this sequence stretch, FCPHKV (SEQ ID NO: 67), represent the minimal binding region of IL-13 to ANTIBODY 01951/G12.

Sequence of IL-13 used for peptide mapping:
SPGPVPPSTALRELIEELVNITQNQKAPLCNGSMVWSINLTAGMYCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLFREGRFN (SEQ ID NO: 34).

Table 4: Signal intensities (Light Unit) of peptides of IL-13 of control antibody and target antibody (ANTIBODY 01951/G12) incubations.

Index	peptide sequence	SEQ ID NO:	Corrected Mean control-antibody	Corrected Mean ANTIBODY 01951/G12
1	SPGPVPPSTALRELI	35	44.3	-1072.3
2	PVPPSTALRELIEEL	36	-9	-1133.3
3	PSTALRELIEELVNI	37	-7.3	-210.5
4	ALRELIEELVNITQN	38	-16.7	-207
5	ELIEELVNITQNQKA	39	-2.7	-131
6	EELVNITQNQKAPLC	40	1.5	1
7	VNITQNQKAPLCNGS	41	28	-405
8	TQNQKAPLCNGSMVW	42	41.7	-350.5
9	QKAPLCNGSMVWSIN	43	27.7	-518
10	PLCNGSMVWSINLTA	44	-6.5	-982
11	NGSMVWSINLTAGMY	45	-5	-131.5
12	MVWSINLTAGMYCAA	46	19	56.5
13	SINLTAGMYCAALES	47	-1.5	-757
14	LTAGMYCAALESLIN	48	1	-99.7
15	GMYCAALESLINVSG	49	1	-987
16	CAALESLINVSGCSA	50	1	-586.7
17	LESLINVSGCSAIEK	51	18.7	-340.3
18	LINVSGCSAIEKTQR	52	12.5	-539.3
19	VSGCSAIEKTQRMLS	53	15	-830.3
20	CSAIEKTQRMLSGFC	54	26	-721.3
21	IEKTQRMLSGFCPHK	55	101	2207
22	TQRMLSGFCPHKVSA	31	123	38568
23	MLSGFCPHKVSAGQF	32	23	48125.7

24	GFCPHKVSAGQFSSL	56	3.3	492.7
25	PHKVSAGQFSSLHVR	57	-24.7	-386.5
26	VSAGQFSSLHVRDTK	58	3.7	-778.5
27	GQFSSLHVRDTKIEV	59	-10.5	-291
28	SSLHVRDTKIEVAQF	60	-6	-170
29	HVRDTKIEVAQFVKD	61	-17	-734.3
30	DTKIEVAQFVKDLLL	62	-13	-438.5
31	IEVAQFVKDLLLHLK	63	-11	-810
32	AQFVKDLLLHLKKLF	64	25	-746.5
33	VKDLLLHLKKLFREG	65	19	-203
34	LLLHLKKLFREGRFN	66	322	7152.3

EXAMPLE 3

Pharmacogenetic analysis of IL4-Rα SNPs and discovery of AIR markers 1-9:

ANTIBODY 01951/G12 is a fully human IgG1/κ monoclonal antibody. CQAX576A2207 was a Phase II clinical study to evaluate the effectiveness and safety of ANTIBODY 01951/G12 in patients with moderate to severe persistent asthma following multiple doses, when added to existing asthma therapy.

To assess associations of several single nucleotide polymorphisms (SNPs) in the IL4-Rα gene and risk of asthma exacerbation in patients treated with ANTIBODY 01951/G12, DNA was collected from patients enrolled in the CQAX576A2207 trial and nine SNPs in the IL4-Rα gene were genotyped in these samples. Statistical tests of association were performed to assess the evidence for association of SNPs in the IL4-Rα gene with risk of asthma exacerbation, as well as with other asthma clinical endpoints, in patients treated with ANTIBODY 01951/G12.

In this manner, a pharmacogenetic analysis was undertaken, with the objective:

- To evaluate whether certain SNPs in the IL4-Rα gene are associated with risk of asthma exacerbation in patients treated with ANTIBODY 01951/G12.
- To evaluate whether certain SNPs are associated with change from baseline in ACQ7 score, FEV1, or IgE level in patients treated with ANTIBODY 01951/G12.
- To evaluate whether the aforementioned SNPs are associated with risk of asthma exacerbation in patients treated with placebo.

Study population

The study population included all patients who met the following criteria:

- Were included in the final analysis population of the CQAX576A2207 clinical study, and
- Did not have a discrepancy between gender as recorded in the clinical database vs. as determined genetically

Clinical endpoints evaluated

Statistical tests were performed to evaluate evidence for association between IL4-Rα SNPs and the following asthma clinical endpoints:

- Risk of occurrence of at least one asthma exacerbation from baseline to Week 24
- Mean absolute change from baseline to Week 24 in ACQ7 score
- Mean percent change from baseline to Week 24 in FEV1 level
- Mean percent change from baseline to Week 24 in IgE level

Genetic markers evaluated:

The following strategies were employed to identify genetic variants associated with response to ANTIBODY 01951/G12.

Candidate SNPs

The 9 SNPs utilized in this pharmacogenetic analysis are listed in Table 5 together with their location relative to the gene structure and, where applicable, their effect on the gene product. They are listed in their physical order along the gene. The SNPs were genotyped using the Taqman platform.

Table 5

SNP	Reported association with pitrakinra exacerbation risk	Location	Effect
rs1110470	yes	intron	unknown
rs3024530	yes	intron	unknown
rs1805010	yes	exon	amino acid change (I→V)

SNP	Reported association with pitrakinra exacerbation risk	Location	Effect
rs2239347	yes	intron	unknown
rs1805011	no	exon	amino acid change (E→A)
rs1801275	no	exon	amino acid change (Q→R)
rs8832	yes	3' UTR	some UTR variants affect gene transcription level
rs1029849	yes	3' proximal	unknown
rs4787956	yes	3' proximal	unknown

UTR, untranslated region

Genome-wide association study (GWAS)

A genome-wide association study (GWAS) was performed using the Illumina Omni5Exome chip. All SNPs that map to the IL4-Rα gene were evaluated.

Resequencing of the IL4-Rα gene

Sanger sequencing was performed on most exons in the IL4-Rα gene, as well as the 3' untranslated region (UTR), to determine whether any additional variants could be identified that were not included on the Omni5Exome chip.

Genotyping methods:

Candidate SNPs

Sample Reactions were prepared following the Protocol recommended by the vendor, TaqMan SNP Genotyping Assays Protocol (PN 4332856D), and using Taqman Universal Master mix (PN 4326614), with the following exceptions:

- 2.00 uL of DNA sample per reaction
- 0.25ul of Water was added per reaction

PCR reactions were conducted on the GeneAmp 9700 (Applied Biosystems) using the program specified in the vendor protocol with the following exception:

- Standard thermal profile is used with 50 cycles, rather than 40

Endpoint reads were conducted on the ABI PRISM 7900HT Sequence Detection System, with data analysis by SNP Manager and SDS 2.2.2 (Applied Biosystems).

GWAS

Genotype data for the GWAS were generated using the Illumina HumanOMNI5Exome microarray platform.

Resequencing of the IL4-Rα gene

SNPs were identified by Sanger sequencing in IL4Rα (NT_010393.15). PCR was performed using unique primers carrying M13 universal sequence tags. Amplicons for each coding region were bi-directionally sequenced with unique or universal primers and BigDye Terminator v3.1 (Applied Biosystems, 4337456). Sequencing products were cleaned up with CleanSEQ (Beckman, A29154) and run on a 3730xl Genetic Analyzer (Applied Biosystems) with POP-7 for sequence detection. Traces were aligned to reference sequences for each amplicon from NCBI using PhredPhrap (University of Washington) and were then visually inspected for discrepancies from canonical sequence using Consed (University of Washington, version 19.0). Sequence variations were noted and any corresponding coding changes characterized where possible.

Statistical methods:

Analysis of asthma exacerbation risk

All tests of association between SNPs and risk of asthma exacerbations were conducted by way of a conditional logistic regression model using PROC LOGISTIC in SAS version 9.3, with country as the conditioning factor. All tests were designed to evaluate the hypothesis of an additive relationship between genotype and exacerbation risk (on the logit scale), meaning that the risk among heterozygotes is intermediate to that within the two homozygous classes. The additive hypothesis was tested by specifying genotype as a continuous variable equal to the number of copies of the minor (i.e., less common in the CQAX576A2207 sample) allele carried by the patient.

Race and ethnicity are known to be common confounding factors in genetic association studies; as such, it is advisable to adjust for race and ethnicity in association tests where possible. In the CQAX576A2207 study the distributions of race and ethnicity were too imbalanced to enable adjustment for these factors, so the logistic regression was instead conditioned on country as a proxy for those factors.

The score statistic was used to assess statistical significance. No adjustment was made for multiple testing.

Analysis of change from baseline in ACQ7, FEV1, and IgE

All tests for association between SNPs and absolute change from baseline in ACQ7, percent change from baseline in FEV1, and percent change from baseline in IgE were conducted by way of a linear model using PROC GENMOD in SAS version 9.3. All tests evaluated the hypothesis of an additive relationship between genotype and the endpoint, as described in Section 3.4.1. Country, history of atopy, maintenance oral corticosteroid use, and baseline level of the corresponding endpoint were included in the model as covariates. A Wald chi-square test was used to assess statistical significance. No adjustment was made for multiple testing.

Permutation test to assess overall significance

A permutation test was performed to assess the likelihood of finding that each of the top 7 SNPs in Table 5 (i.e. excluding the bottom 2 SNP's: rs1029849, and rs4787956) would be found to have a homozygous genotype class in which there were no exacerbations among ANTIBODY 01951/G12-treated patients, if in reality there were no association between any of the SNPs and ANTIBODY 01951/G12 exacerbation risk.

The permutation test proceeded according to the following steps:

- For the first permutation, exacerbation status (yes/no) was randomly permuted for the 94 ANTIBODY 01951/G12-treated patients, such that it remained the case that only 12 of the 94 patients experience an exacerbation but these 12 patients were selected at random. All genotype data remained unchanged.
- Using this permuted dataset, each SNP was evaluated to determine whether it had a homozygous genotype class in which there were no exacerbations. If all 7 SNPs were found to have such a genotype class, the permuted dataset was flagged to record that finding.
- The first 2 steps were iterated 1000 times. The percentage of permuted datasets that were flagged to indicate that each of the 7 SNPs had a homozygous genotype class with no exacerbations represented the empirical significance level of this finding. Thus, a small percentage of flagged datasets would indicate a low likelihood of observing such a result by random chance, and would be taken as evidence that risk of asthma exacerbation among ANTIBODY 01951/G12-treated patients is influenced by SNPs in the IL4-Rα gene.

Calculation of r-squared values in the linkage disequilibrium graph

The r^2 values in the LD graph were computed using Haploview software (Barrett et al, 2004).

Cross-validation analysis to assess optimal number of SNPs for prediction

Cross-validation is a statistical method that can be used to compare alternative approaches to building predictive models to assess which approach is most likely to yield the model with highest predictive accuracy. The method entails dividing the available set of patients into a training set and a test set, applying alternative model-building approaches to the training set only, using the resulting models to predict the outcome of the patients in the test set, and determining which model predicted most accurately. This procedure is repeated many times, the predictions are averaged across the test sets. If a particular approach tends to result in more accurate predictions than the other approaches, it suggests that this approach may also result in a model with the highest predictive accuracy when applied to future studies.

In this application of cross-validation, once the selected SNPs were determined for each of the model-building approaches listed below, linear discriminant analysis (LDA) was applied to build the predictive model. The set of markers available for selection consisted of 6 of the top 7 IL4-Rα SNPs listed in Table 5. rs3024530 was removed because it was almost perfectly correlated with rs1805010. The procedure was then applied to patients treated with ANTIBODY 01951/G12 to compare the following model-building approaches:

- Include the most significant SNP from each of the 2 linkage disequilibrium (LD) blocks
- Include the 2 SNPs selected above, as well as the next most significant SNP
- Include the 2 most significant SNPs from each of the 2 LD blocks
- Include the 4 SNPs selected above, as well as the next most significant SNP
- Include all 6 SNPs.

The results were compared to evaluate whether a predictive model that includes additional SNPs would be likely to have substantially higher predictive accuracy than one which contains only the single most significant SNP from each of the 2 LD blocks.

Results:

Genotype frequencies of IL4-Rα SNPs

A total of 94 ANTIBODY 01951/G12-treated patients and 102 placebo-treated patients were genotyped for the 9 SNPs in the IL4-Rα gene listed in Table 5. Two patients, both placebo-treated, had a discrepancy between gender as recorded in the clinical database vs. as determined genetically, and were removed from the analysis. Both were from the same country, suggesting a possible sample switch.

Twelve of the 94 ANTIBODY 01951/G12-treated patients (12.8%) experienced at least one asthma exacerbation during the study, compared to 23 of 100 placebo-treated patients (23.0%). The distribution was consistent with Hardy-Weinberg equilibrium for each SNP.

Table 0-1 Genotype frequency distributions in ANTIBODY 01951/G12 and pitrakinra studies

SNP	Genotype frequency distribution	ANTIBODY 01951/G12 study n (%)
rs1110470	GG	61 (31.4%)
	AG	97 (50.0%)
	AA	36 (18.6%)
rs3024530	AA	48 (24.7%)
	AG	106 (54.6%)
	GG	40 (20.6%)
rs1805010	AA	47 (24.5%)
	AG	105 (54.7%)
	GG	40 (20.8%)
rs2239347	AA	48 (24.7%)
	AC	98 (50.5%)
	CC	48 (24.7%)
rs1805011	AA	159 (82.4%)
	AC	31 (16.1%)
	CC	3 (1.6%)
rs1801275	AA	127 (65.5%)
	AG	56 (28.9%)
	GG	11 (5.7%)
rs8832	GG	58 (29.9%)
	AG	93 (47.9%)
	AA	43 (22.2%)
rs1029849	GG	68 (35.2%)
	AG	86 (44.6%)
	AA	39 (20.2%)
rs4787956	AA	71 (36.6%)
	AG	99 (51.0%)
	GG	24 (12.4%)

Genotype frequencies were calculated by combining the ANTIBODY 01951/G12 and placebo arms.

Association between IL4-Rα SNPs and risk of exacerbation

The proportion of ANTIBODY 01951/G12-treated patients who experienced at least one asthma exacerbation during the study is shown in Figure 2 by genotype class for each of the 9 genotyped SNPs, along with significance levels for the corresponding tests of association between SNP and exacerbation risk. Six of these 7 SNPs were also significantly associated with response to ANTIBODY 01951/G12, with p-values ranging from 0.005 to 0.015, while the remaining SNP approached significance with a p-value of 0.066. Moreover, the SNPs generally showed an additive effect on exacerbation risk in both studies, meaning that the risk for heterozygotes was generally intermediate to that for the two homozygous classes, suggesting that exacerbation risk for both therapies increases with the number of risk alleles carried by an individual at an associated SNP.

Since race is well known to be a common confounding factor in genetic association studies, the association tests for ANTIBODY 01951/G12-treated patients were repeated using only self-reported Caucasian patients, but the p-values were unchanged. This is likely because 89 of the 94 patients (94.7%) were Caucasian, and the 5 non-Caucasians were all from countries in which there were no exacerbations.

Of particular note in the ANTIBODY 01951/G12 study, for each of the 7 SNPs there was one homozygous class for which no exacerbations were observed among ANTIBODY 01951/G12-treated patients, a result that would be unlikely to occur by random chance.

The results for the two bottom SNPs listed in Table 5, which code for amino acid changes in the IL-4RA receptor, showed weaker evidence for association with p-values 0.091 and 0.10.

The proportion of placebo-treated patients in the ANTIBODY 01951/G12 study who experienced at least one asthma exacerbation is shown in Figure 3 by genotype class for the 9 genotyped SNPs. Unlike the results for the ANTIBODY 01951/G12 arm, placebo-treated patients show no evidence of association between genotype and exacerbation risk for any of the SNPs.

The SNPs shown in all tables and figures for this pharmacogenetic analysis are ordered according to their physical location within the gene.

Association between IL4-Rα SNPs and other asthma endpoints

The 9 genotyped SNPs were also evaluated in ANTIBODY 01951/G12-treated patients for evidence of association with 3 other asthma endpoints: change from baseline in ACQ7 score, percent change from baseline in FEV1, and percent change from baseline in IgE. All endpoints were evaluated at Week 24. Since all association tests evaluated the hypothesis of an additive allelic effect (i.e., that the mean response among heterozygotes is intermediate to those of the two homozygous classes), Table 6 shows the effect of one additional copy of the minor allele (i.e., less common allele in this sample) on each of these 3 endpoints. For example, under the additive model, carrying one additional copy of the minor allele at rs8832 -- whether carrying 1 copy instead of 0, of 2 copies instead of 1 -- is associated with a mean increase from baseline in FEV1 of 7.6%. Thus, carrying 2 copies instead of 0 is associated with a mean increase of 15.2%.

The corresponding results for the asthma exacerbation endpoint are also provided for comparison. In this case the effect size represents the odds ratio associated with one additional copy of the minor allele. Hence, carrying one additional copy of the minor allele at rs8832 is associated with 3.8-fold increase in the odds of experiencing an exacerbation. Since odds ratios are multiplicative, carrying 2 copies instead of 0 is associated with a 3.8^2, or 14.6-fold increase in odds of experiencing an exacerbation.

No SNPs attained or approached statistical significance for the ACQ7 or IgE endpoints, even without any adjustment for multiple testing. For the FEV1 endpoint, 4 SNPs attained significance, including a cluster of 3 SNPs with $p<0.002$ (rs8832, rs1029489, and rs4787956). However, the effect of these 3 SNPs on response to ANTIBODY 01951/G12 was in the opposite direction of that observed for asthma exacerbations: specifically, for each of the 3 SNPs the minor (less common in the sample) allele was associated with higher risk of exacerbation but also with a mean increase from baseline in FEV1. Another SNP, rs1110470, also attained borderline significance, but also in the opposite direction of that observed for exacerbations, and no other SNPs in its neighborhood attained or approached significance.

Table 6 **Results of tests of association between SNPs and asthma endpoints at Week 24**

	ACQ7 (Change from BL)		FEV1 (% Change from BL)		Serum IgE (% Change from BL)		Asthma Exacerbations	
SNP (minor allele)	Effect Size[1] (mean Δ with 95% CI)	P-value	Effect Size[2] (mean Δ with 95% CI)	P-value	Effect Size[2] (mean Δ with 95% CI)	P-value	Effect Size[3] (odds ratio with 95% CI)	P-value
rs1110470 (A)	-0.039 ± 0.23	0.73	-5.4 ± 5.3	0.047	+4.7 ± 13.9	0.51	0.15 (0.036,0.64)	0.0056
rs3024530 (G)	+0.142 ± 0.24	0.24	+2.7 ± 5.8	0.37	+0.6 ± 14.7	0.94	4.2 (1.2,14.6)	0.015
rs1805010 (G)	+0.172 ± 0.24	0.16	+2.7 ± 5.9	0.38	-0.6 ± 14.9	0.94	5.1 (1.3,19.9)	0.010
rs2239347 (C)	+0.023 ± 0.22	0.84	+3.2 ± 5.4	0.25	-0.2 ± 13.7	0.98	2.6 (0.9,8.7)	0.066
rs1805011 (C)	-0.106 ± 0.35	0.55	+0.1 ± 8.5	0.98	-4.6 ± 21.4	0.87	NC[4]	0.091
rs1801275 (G)	-0.042 ± 0.25	0.74	+2.9 ± 6.3	0.36	-3.7 ± 15.6	0.64	0.30 (0.066,1.4)	0.10
rs8832 (A)	-0.049 ± 0.20	0.63	+7.6 ± 4.7	0.0014	-7.2 ± 12.5	0.26	3.8 (1.2,11.9)	0.013
rs1029489 (A)	-0.037 ± 0.20	0.72	+7.0 ± 4.6	0.0033	-6.3 ± 12.4	0.32	4.2 (1.4,12.9)	0.0054
rs4787956 (G)	-0.081 ± 0.22	0.47	+7.2 ± 5.1	0.0064	-5.4 ± 13.7	0.44	3.9 (1.3,11.7)	0.0080

[1] Mean absolute change in ACQ7 from baseline to Week 24 associated with one additional copy of the minor allele

[2] Mean percent change in FEV1 or IgE from baseline to Week 24 associated with one additional copy of the minor allele

[3] Odds ratio for risk of experiencing an asthma exacerbation between baseline and Week 24 associated with one additional copy of the minor allele

[4] Not calculable because all patients with an exacerbation had the same genotype (AA)

Permutation test to evaluate overall significance of exacerbation associations A permutation test was performed to evaluate how likely it would be to obtain association results similar to the ANTIBODY 01951/G12 exacerbation results if there were actually no association between the SNPs and exacerbation risk – i.e., as an artifact of random chance. Of particular interest was the finding that for each of the top 7 SNPs listed in Table 5, the ANTIBODY 01951/G12 analysis yielded one homozygous class in which there were no exacerbations; therefore, the permutation test was focused on how often this would be expected to occur by random chance. Among 1000 permuted datasets generated, only 2 yielded a result in which each of the 7 SNPs had one homozygous class with no exacerbations. Hence the probability of observing this result if there were no true associations was estimated to be 0.002.

Linkage disequilibrium analysis of the SNPs

Alleles that are located near each other on a chromosome tend to be inherited together. When these alleles also have similar population frequencies, they are likely to be correlated with each other, such that knowing a subject's genotype at a given SNP can enable one to predict the same subject's genotype at a nearby SNP with relatively high accuracy. This concept is referred to as "linkage disequilibrium" (LD). The relevance to genetic association studies is that multiple SNPs in a small region may show evidence of association with a phenotype; however, because these SNPs may be in LD with each other, any given SNP may not provide a substantial amount of independent evidence of association with the phenotype beyond that already obtained from other nearby SNPs.

LD is known to occur in a "block" structure throughout the genome. Each block, or "cluster", contains a group of SNPs such that the correlations between pairs of SNPs within the same block are relatively high whereas the correlations between pairs of SNPs in different blocks are relatively low. An analysis of LD between the top 7 SNPs listed in Table 5 and ANTIBODY 01951/G12 exacerbation risk revealed the presence of 2 LD blocks, as illustrated in Figure 4. The numerical values in the cell represent the r^2 value (multiplied by 100), a standard measure of correlation – or strength of LD – between the corresponding pair of SNPs. An r^2 value of 100 indicates that 2 SNPs are perfectly correlated, while 0 indicates complete independence.

As can be seen from the figure, the first 4 SNPs form one block, within which correlations between pairs of SNPs range from 56 to 95. SNPs rs3024530 and rs1805010 are almost perfectly correlated. The last 3 SNPs form the second block, with pairwise correlations ranging from 59 to 80. In contrast, no SNP from the first block shows high correlation with any SNP from the second block, the highest pairwise correlation being 32.

Cross-validation analysis to assess optimal number of SNPs for prediction

A cross-validation analysis was conducted to compare strategies for utilizing the 6 of the top 7 IL4-Rα SNPs listed in Table 5 (rs3024530 was excluded because it was nearly perfectly correlated with rs1805010) to predict a patient's risk of asthma exacerbation after treatment with ANTIBODY 01951/G12. Because the LD analysis identified 2 largely independent blocks of

SNPs that each show evidence of association with exacerbation risk, it is reasonable to expect that a predictive model with representation from both blocks would likely have greater ability to identify patients at low risk of exacerbation than one with representation from only one block. However, this raised the question of whether it would be sufficient to select only one SNP from each block or whether it would be beneficial to include additional SNPs. The cross-validation analysis showed that the expected ability of a predictive model to identify patients at low risk of asthma exacerbation would not improve substantially if additional SNPs were included beyond one from each block.

Additional information

Genotype frequency distributions for the 7 SNPs are listed by country and ethnic population in Tables 7 and 8.

Table 7 Genotype frequency distribution of IL4-Rα SNPs by country

SNP	Genotype	QAX576A2207	ARG	BEL	CZE	DEU	POL	RUS	USA
	N=	194	25	12	30	57	25	35	12
rs1805010	AA	0.24	0.20	0.00	0.25	0.37	0.24	0.12	0.36
	AG	0.55	0.64	0.67	0.46	0.53	0.60	0.53	0.45
	GG	0.21	0.16	0.33	0.29	0.11	0.16	0.35	0.18
rs8832	GG	0.30	0.36	0.17	0.29	0.30	0.28	0.31	0.33
	AG	0.48	0.48	0.50	0.50	0.47	0.60	0.46	0.25
	AA	0.22	0.16	0.33	0.21	0.23	0.12	0.23	0.42
rs4787956	AA	0.37	0.40	0.08	0.32	0.39	0.44	0.37	0.42
	AG	0.51	0.56	0.67	0.50	0.47	0.52	0.51	0.42
	GG	0.12	0.04	0.25	0.18	0.14	0.04	0.11	0.17
rs3024530	AA	0.25	0.20	0.00	0.25	0.37	0.24	0.14	0.33
	AG	0.55	0.64	0.67	0.46	0.51	0.60	0.54	0.50
	GG	0.21	0.16	0.33	0.29	0.12	0.16	0.31	0.17
rs1029489	GG	0.35	0.36	0.18	0.36	0.37	0.40	0.34	0.33
	AG	0.45	0.48	0.46	0.43	0.46	0.48	0.43	0.33
	AA	0.20	0.16	0.36	0.21	0.18	0.12	0.23	0.33
rs1110470	GG	0.31	0.36	0.42	0.39	0.25	0.24	0.37	0.25
	AG	0.50	0.48	0.50	0.46	0.51	0.56	0.49	0.50
	AA	0.19	0.16	0.08	0.14	0.25	0.20	0.14	0.25
rs2239347	AA	0.25	0.24	0.08	0.25	0.32	0.24	0.14	0.42
	AC	0.51	0.48	0.42	0.46	0.56	0.48	0.54	0.42
	CC	0.25	0.28	0.50	0.29	0.12	0.28	0.31	0.17

Table 8 Genotype frequency distribution of IL4-Rα SNPs by ethnic population

SNP	Genotype	QAX576A2207	ASW	CHB	CHD	GIH	LWK	MEX	MKK	TSI	CEU	HCB	JPT	YRI
	N=	194	49	41	84	88	90	49	142	88	112	42	86	112
rs1805010	AA	0.24	0.24	0.27	0.23	0.39	0.24	0.34	0.20	0.32	0.24	0.50	0.19	0.30
	AG	0.55	0.51	0.46	0.54	0.45	0.46	0.43	0.43	0.55	0.54	0.33	0.34	0.49
	GG	0.21	0.24	0.27	0.24	0.16	0.30	0.33	0.37	0.14	0.22	0.17	0.48	0.21
rs8832	GG	0.30	0.00	0.20	0.25	0.35	0.00	0.27	0.03	0.32	0.26	0.24	0.19	0.02
	AG	0.48	0.33	0.44	0.52	0.49	0.24	0.53	0.27	0.52	0.47	0.43	0.39	0.25
	AA	0.22	0.67	0.37	0.24	0.16	0.76	0.20	0.69	0.16	0.28	0.33	0.42	0.73
rs4787956	AA	0.37	0.43	0.17	0.24	0.40	0.37	0.30	0.38	0.38	0.39	0.26	0.16	0.41
	AG	0.51	0.49	0.44	0.50	0.45	0.56	0.52	0.52	0.50	0.43	0.44	0.41	0.43
	GG	0.12	0.08	0.39	0.26	0.15	0.08	0.18	0.10	0.13	0.18	0.30	0.43	0.16
rs3024530	AA	0.25	0.22	0.27	0.26	0.39	0.26	0.34	0.17	0.30	0.23	0.49	0.19	0.27
	AG	0.55	0.53	0.49	0.55	0.42	0.42	0.44	0.54	0.57	0.54	0.30	0.31	0.50
	GG	0.21	0.24	0.24	0.19	0.19	0.32	0.32	0.29	0.14	0.23	0.21	0.50	0.24
	N=										60	45	45	60
rs1029489	GG	0.35									0.48	0.22	0.18	0.03
	AG	0.45									0.38	0.44	0.31	0.22
	AA	0.20									0.13	0.33	0.51	0.75
rs1110470	GG	0.31									0.27	0.41	0.60	0.27
	AG	0.50									0.41	0.27	0.36	0.49
	AA	0.19									0.32	0.32	0.04	0.24
rs2239347	AA	0.25									0.28	0.40	0.20	0.32
	AC	0.51									0.50	0.42	0.49	0.45
	CC	0.25									0.22	0.18	0.31	0.23

Population	Description	Population	Description
ASW	African ancestry in Southwest USA	MKK	Maasai in Kinyawa, Kenya
CHB	Han Chinese in Beijing, China (current)	TSI	Toscan in Italy
CHD	Chinese in Metropolitan Denver, Colorado	CEU	Utah residents with Northern and Western European ancestry
GIH	Gujarati Indians in Houston, Texas	HCB	Han Chinese in Beijing, China (obsolete)
LWK	Luhya in Webuye, Kenya	JPT	Japanese in Tokyo, Japan
MEX	Mexican ancestry in Los Angeles, California	YRI	Yoruba in Ibadan, Nigeria

Source: dbSNP database, National Center for Biotechnology Information

The 7 top SNPs listed in Table 5 were found in this study to associate with exacerbation risk among patients treated with ANTIBODY 01951/G12. Six of the 7 SNPs yielded significant p-values ranging from 0.005 to 0.015, while the remaining SNP approached significance. The same p-values were obtained when the analysis was restricted to Caucasian patients to mitigate the risk of confounding. Moreover, the particular pattern of association with response to ANTIBODY 01951/G12 with most SNPs exhibited an additive relationship with exacerbation risk; i.e., the risk within heterozygotes was observed to be intermediate to that within the 2 homozygous classes. Finally, it was observed that among ANTIBODY 01951/G12-treated patients each of the 7 SNPs had a particular homozygous class in which there were no exacerbations. A permutation test showed that the probability of observing this result in the absence of any true associations is 0.002. Taken together, along with the importance of the IL4-Rα gene to the mechanism of ANTIBODY 01951/G12, these results suggest a strong likelihood that the risk of asthma exacerbations in patients treated with ANTIBODY 01951/G12 is influenced by SNPs in the IL4-Rα gene.

In addition, the two other SNPs in the IL4-Rα gene (listed as bottom two SNPs in Table 5) were evaluated for possible association with exacerbation risk in ANTIBODY 01951/G12-treated patients because of their reported association with asthma disease phenotypes and because they code for amino acid changes. Although, both were less significant than the other 7 SNPs (p=0.091 and 0.10).

No evidence was found for association between SNPs in the IL4-Rα gene and exacerbation risk among patients treated with placebo in the ANTIBODY 01951/G12 study. Taken together with knowledge of the ANTIBODY 01951/G12 mechanism, this suggests that the influence of these SNPs on exacerbation risk is specific to the drug mechanism and is not reflective of a more general disease severity association. Moreover, no evidence was found for association between these SNPs and change from baseline in ACQ7 score or IgE level, and only a few SNPs were significantly associated with change in FEV1, with these associations pointing in the opposite direction of that seen for exacerbation risk. This suggests that the genetic mechanism influencing patient's susceptibility to exacerbations is different from that for other asthma endpoints.

The strong evidence for this pharmacogenetic association among ANTIBODY 01951/G12-treated patients suggests the use of particular genotypes or combination of genotypes at these SNPs to predict a patient's risk of asthma exacerbation after treatment with ANTIBODY 01951/G12.

As shown in Table 5, rs1805010 causes an amino acid substitution, while rs8832 is in the 3' untranslated region (UTR), a region known to influence gene transcription levels. Since rs1805010 lies in the first LD block while rs8832 lies in the second, these 2 SNPs are useful in developing a predictive model for exacerbation risk. The cross-tabulated genotype frequencies for these 2 SNPs in the CQAX576A2207 study are shown in Table 9.

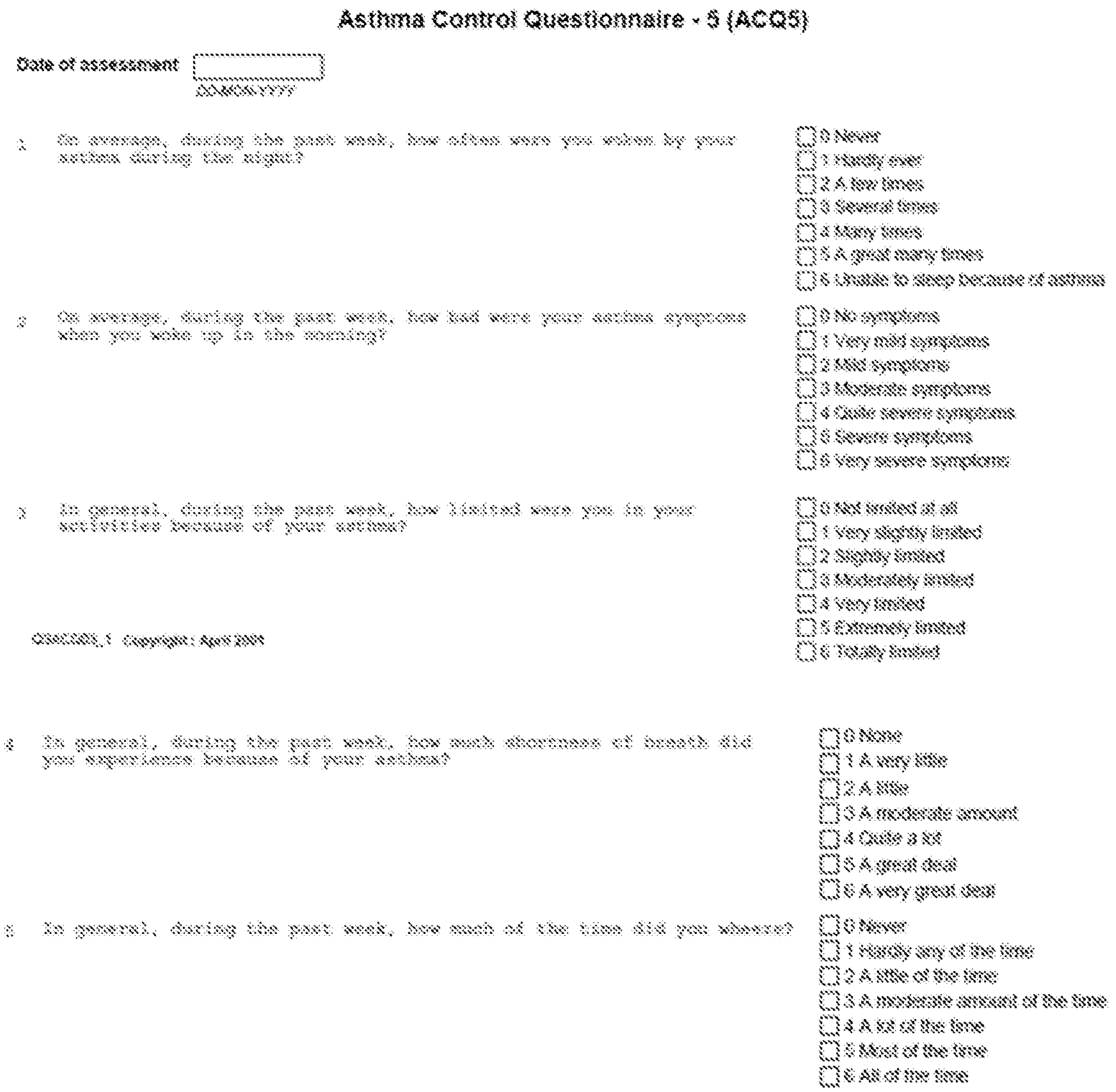
Table 9 Cross-tabulated genotype frequencies for rs1805010 and rs8832 in the

		rs8832		
		GG (lower exacerbation risk)	AG	AA (higher exacerbation risk)
rs1805010	AA (lower exacerbation risk)	33 (17.2%)	9 (4.7%)	5 (2.6%)
	AG	20 (10.4%)	71 (37.0%)	14 (7.3%)
	GG (higher exacerbation risk)	5 (2.6%)	11 (5.7%)	24 (12.5%)

Genotype frequencies were calculated by combining the ANTIBODY 01951/G12 and placebo arms.

EXAMPLE 4

Methods to evaluate reduction in asthma exacerbations will be known to the skilled practitioner and include use of ACQ-5 and/or AQLQ-S:



Asthma Control Questionnaire - 5 (ACQ5)

Date of assessment ☐
DD/MON/YYYY

1. On average, during the past week, how often were you woken by your asthma during the night?
   - ☐ 0 Never
   - ☐ 1 Hardly ever
   - ☐ 2 A few times
   - ☐ 3 Several times
   - ☐ 4 Many times
   - ☐ 5 A great many times
   - ☐ 6 Unable to sleep because of asthma

2. On average, during the past week, how bad were your asthma symptoms when you woke up in the morning?
   - ☐ 0 No symptoms
   - ☐ 1 Very mild symptoms
   - ☐ 2 Mild symptoms
   - ☐ 3 Moderate symptoms
   - ☐ 4 Quite severe symptoms
   - ☐ 5 Severe symptoms
   - ☐ 6 Very severe symptoms

3. In general, during the past week, how limited were you in your activities because of your asthma?
   - ☐ 0 Not limited at all
   - ☐ 1 Very slightly limited
   - ☐ 2 Slightly limited
   - ☐ 3 Moderately limited
   - ☐ 4 Very limited
   - ☐ 5 Extremely limited
   - ☐ 6 Totally limited

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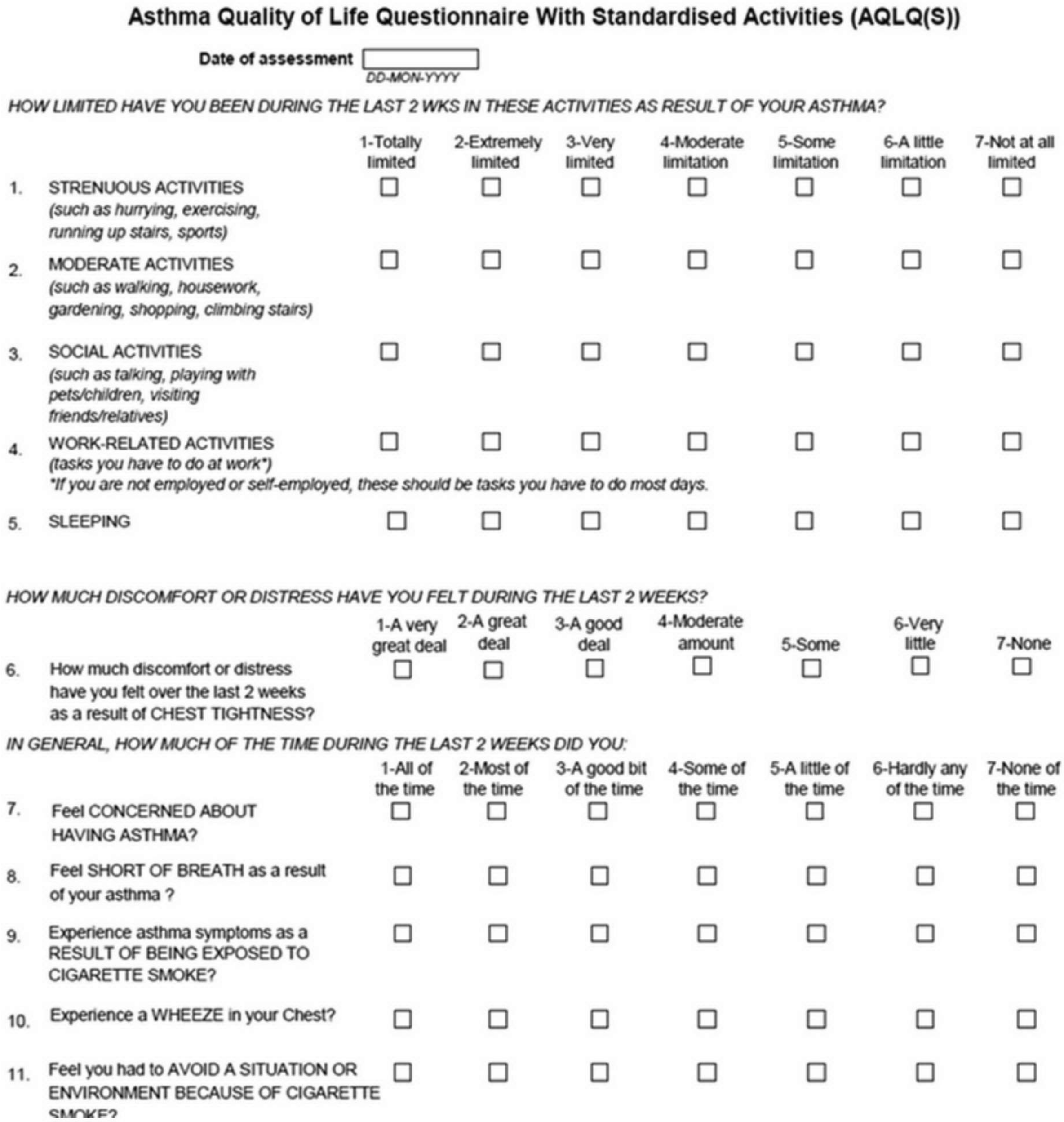
4. In general, during the past week, how much shortness of breath did you experience because of your asthma?
   - ☐ 0 None
   - ☐ 1 A very little
   - ☐ 2 A little
   - ☐ 3 A moderate amount
   - ☐ 4 Quite a lot
   - ☐ 5 A great deal
   - ☐ 6 A very great deal

5. In general, during the past week, how much of the time did you wheeze?
   - ☐ 0 Never
   - ☐ 1 Hardly any of the time
   - ☐ 2 A little of the time
   - ☐ 3 A moderate amount of the time
   - ☐ 4 A lot of the time
   - ☐ 5 Most of the time
   - ☐ 6 All of the time



Asthma Quality of Life Questionnaire With Standardised Activities (AQLQ(S))

Date of assessment ☐
DD-MON-YYYY

HOW LIMITED HAVE YOU BEEN DURING THE LAST 2 WKS IN THESE ACTIVITIES AS RESULT OF YOUR ASTHMA?

		1-Totally limited	2-Extremely limited	3-Very limited	4-Moderate limitation	5-Some limitation	6-A little limitation	7-Not at all limited
1.	STRENUOUS ACTIVITIES *(such as hurrying, exercising, running up stairs, sports)*	☐	☐	☐	☐	☐	☐	☐
2.	MODERATE ACTIVITIES *(such as walking, housework, gardening, shopping, climbing stairs)*	☐	☐	☐	☐	☐	☐	☐
3.	SOCIAL ACTIVITIES *(such as talking, playing with pets/children, visiting friends/relatives)*	☐	☐	☐	☐	☐	☐	☐
4.	WORK-RELATED ACTIVITIES *(tasks you have to do at work*)*	☐	☐	☐	☐	☐	☐	☐
5.	SLEEPING	☐	☐	☐	☐	☐	☐	☐

**If you are not employed or self-employed, these should be tasks you have to do most days.*

HOW MUCH DISCOMFORT OR DISTRESS HAVE YOU FELT DURING THE LAST 2 WEEKS?

		1-A very great deal	2-A great deal	3-A good deal	4-Moderate amount	5-Some	6-Very little	7-None
6.	How much discomfort or distress have you felt over the last 2 weeks as a result of CHEST TIGHTNESS?	☐	☐	☐	☐	☐	☐	☐

IN GENERAL, HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS DID YOU:

		1-All of the time	2-Most of the time	3-A good bit of the time	4-Some of the time	5-A little of the time	6-Hardly any of the time	7-None of the time
7.	Feel CONCERNED ABOUT HAVING ASTHMA?	☐	☐	☐	☐	☐	☐	☐
8.	Feel SHORT OF BREATH as a result of your asthma ?	☐	☐	☐	☐	☐	☐	☐
9.	Experience asthma symptoms as a RESULT OF BEING EXPOSED TO CIGARETTE SMOKE?	☐	☐	☐	☐	☐	☐	☐
10.	Experience a WHEEZE in your Chest?	☐	☐	☐	☐	☐	☐	☐
11.	Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF CIGARETTE SMOKE?	☐	☐	☐	☐	☐	☐	☐

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The claims defining the invention are as follows:

1. A method of selectively treating a patient having asthma, comprising

   i) identifying a patient having at least one AIR marker selected from the group consisting of AIR marker- 1, 2, 3, 4, 5, 6, 7, 8, and 9,

   ii) determining whether the patient is homozygous or heterozygous for said AIR marker, and

   iii) selectively administering a therapeutically effective amount of an IL-13 antagonist to the patient that is

      a. homozygous for one of AIR marker 3 and AIR marker 7 and heterozygous at the other;

      b. homozygous for both AIR marker 3 and AIR marker 7; or

      c. homozygous for AIR marker 3.

2. The method according to claim 1, wherein

   said identification comprises assaying a biological sample from the patient for the presence of at least one AIR marker selected from said group.

3. A method of selectively treating a patient having asthma, comprising

   i) assaying a biological sample from the patient for the presence or absence of at least one AIR marker selected from said group consisting of AIR marker- 1, 2, 3, 4, 5, 6, 7, 8, and 9.

   ii) detecting the presence of at least one AIR marker selected from said group in said sample and thereby determining that the patient is positive for said AIR marker, and

iii) determining whether the patient is homozygous or heterozygous for said AIR marker, and selectively administering a therapeutically effective amount of an IL-13 antagonist to the patient that is

a. homozygous for one of AIR marker 3 and AIR marker 7 and heterozygous at the other;

b. homozygous for both AIR marker 3 and AIR marker 7; or

c. homozygous for AIR marker 3.

4. A method of predicting the likelihood that a patient having asthma will respond to treatment with an IL-13 antagonist, comprising the steps of:

a) assaying a biological sample from the patient for the presence or absence of at least one AIR marker, and

b) determining whether said AIR marker is present in homozygous or heterozygous form, wherein

the presence of the at least one AIR marker in homozygous form is indicative of an increased likelihood that the patient will respond to treatment with the IL-13 antagonist, wherein said at least one AIR marker is selected from the group consisting of:

i) AIR marker 3 and AIR marker 7 each present in homozygous form,

ii) AIR marker 3 present in homozygous form and AIR marker 7 in heterozygous form,

a. AIR marker 7 present in homozygous form and AIR marker 3 in heterozygous form, and

b. AIR marker 3 in homozygous form.

5. The method according to any one of the preceding claims wherein said IL-13 antagonist competes with antibody antibody 01951/G12 (SEQ ID NO:14 and 16) for binding to IL-13 under conditions that promotes said competition.

6. The method according to any one of the preceding claims wherein said IL-13 antagonist is an antibody or a fragment thereof that binds to an epitope of IL-13 comprising residues FCPHKV (SEQ ID NO: 67) set forth as residues 103 to 107 of SEQID NO: 1.

7. The method according to any one of the preceding claims wherein said IL-13 antagonist is an antibody comprising a heavy chain as set forth in SEQ ID NO: 20 and a light chain as recited in SEQ ID NO: 18.

8. The method according to any one of the preceding claims

wherein the IL-13 antagonist is an antibody selected from the group consisting of:

i. an antibody comprising a heavy chain variable region CDR1 of SEQ ID NO: 2; a heavy chain variable region CDR2 of SEQ ID NO: 3; a heavy chain variable region CDR3 of SEQ ID NO: 4; a light chain variable region CDR1 of SEQ ID NO: 8; a light chain variable region CDR2 of SEQ ID NO: 9; and a light chain variable region CDR3 of SEQ ID NO: 10;

ii. an antibody comprising a heavy chain variable region CDR1 of SEQ ID NO: 5; a heavy chain variable region CDR2 of SEQ ID NO: 6; a heavy chain variable region CDR3 of SEQ ID NO: 7; a light chain variable region CDR1 of SEQ ID NO: 11; a light chain variable region CDR2 of SEQ ID NO: 12; and a light chain variable region CDR3 of SEQ ID NO: 13,

iii. an antibody comprising a heavy chain variable region as recited in SEQ ID NO: 14 and a light chain variable region as recited in SEQ ID NO: 16,

iv. an antibody comprising a heavy chain as recited in SEQ ID NO: 20 and a light chain as recited in SEQ ID NO: 18.

Figure 1

Substitution Analysis:

Replacement of each residue from sequence MLSGFCPHKVSA against all 20 standard amino acids.

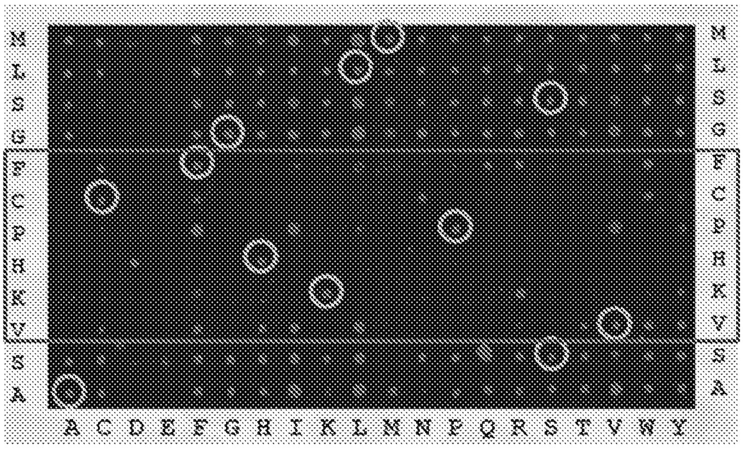


Figure 2 Exacerbation risk by genotype class for ANTIBODY 01951/G12-treated patients

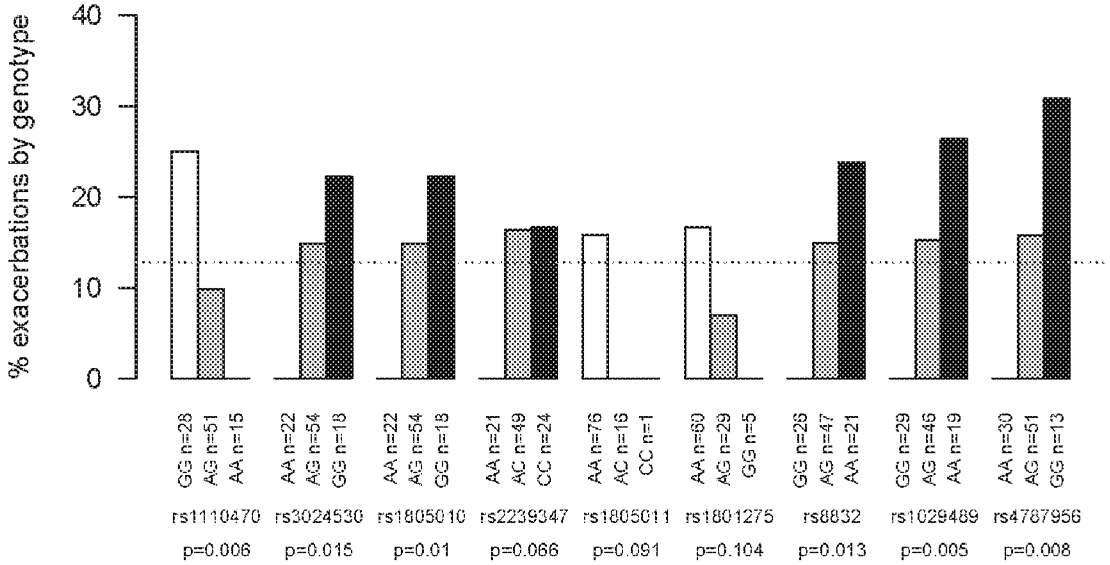


Figure 3 **Exacerbation risk by genotype class for placebo-treated patients in the ANTIBODY 01951/G12 study**

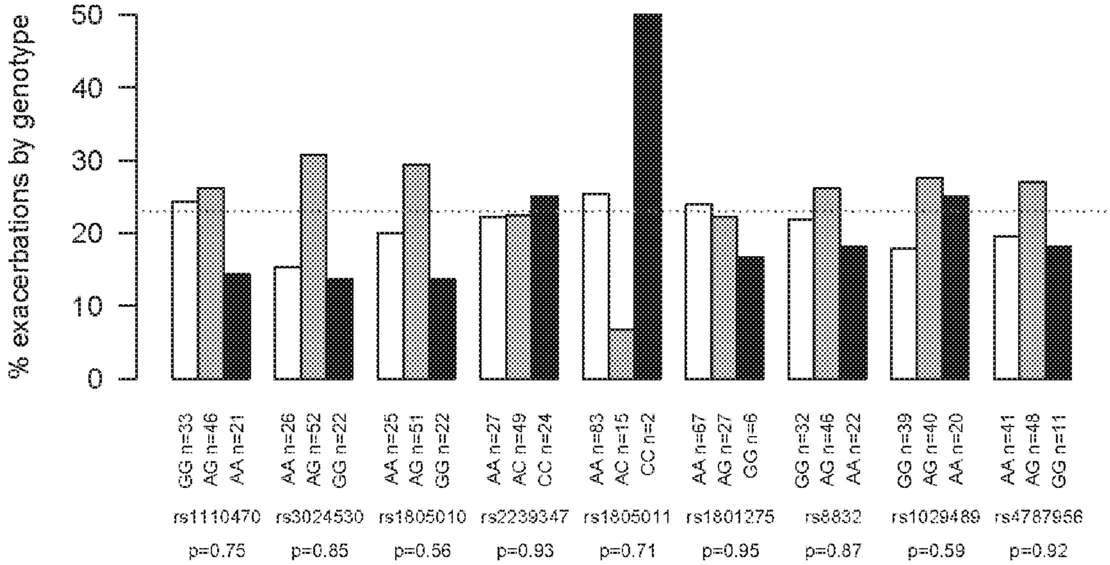
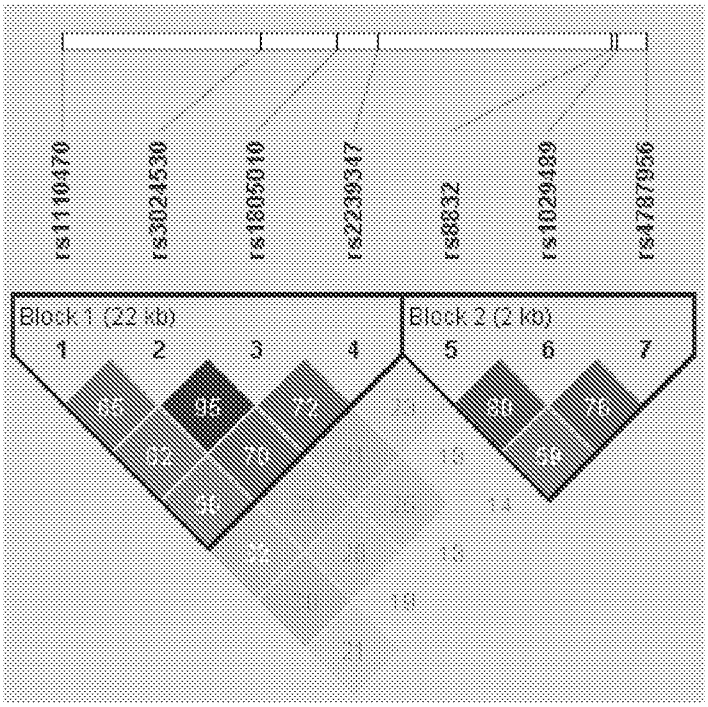


Figure 4

Identification of two linkage disequilibrium blocks among IL4-Rα SNPs



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<110> NOVARTIS AG

<120> METHODS OF SELECTIVELY TREATING ASTHMA USING IL-13 ANTAGONISTS

<130> PAT056217-WO-PCT

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<150> 61/978,604
<151> 2014-04-11

<160> 67

<170> PatentIn version 3.5

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            20                  25                  30


Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Glu Leu Ile Glu
        35                  40                  45


Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys Asn Gly
    50                  55                  60


Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys Ala Ala
65                  70                  75                  80


Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu Lys Thr
                85                  90                  95


Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala Gly Gln
            100                 105                 110


Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala Gln Phe
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Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu Gly Arg
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<211> 11
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30


Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ala Ile Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
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```
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Leu Trp Phe Gly Asp Leu Asp Ala Phe Asp Ile Trp Gly Gln
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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

tcc ctg aga ctc tcc tgt gca gcg tct gga ttc acc ttc agt agc tat         96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

ggc atg cac tgg gtc cgc cag gct cca ggc aag ggg ctg gag tgg gtg        144
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

gca att ata tgg tat gat gga agt aat aaa tac tat gcg gac tcc gtg        192
Ala Ile Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

aag ggc cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat        240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

ctg caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt        288
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

gcg agg cta tgg ttc ggg gac tta gat gct ttt gat atc tgg ggc caa        336
Ala Arg Leu Trp Phe Gly Asp Leu Asp Ala Phe Asp Ile Trp Gly Gln
            100                 105                 110

ggg aca atg gtc acc                                                    351
Gly Thr Met Val Thr
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            20                  25                  30


Leu Val Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45


Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80


Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Ser Trp Pro Pro
                85                  90                  95


Val Tyr Thr Phe Gly Gln Gly Thr
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1               5                   10                  15

gaa aga gcc atc ctc tcc tgc agg gcc ggt cag agt gtt agc agt tac         96
Glu Arg Ala Ile Leu Ser Cys Arg Ala Gly Gln Ser Val Ser Ser Tyr
            20                  25                  30

tta gtc tgg tac caa cag aaa cct ggc cag gct ccc agg ctc ctc atc        144
Leu Val Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

tat gat gca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc        192
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Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60

agt ggg tct ggg aca gac ttc act ctc acc atc agc agc cta gag cct        240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

gaa gat ttt gca gtt tat tac tgt cag cag cgc agc agc tgg cct ccg        288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Ser Trp Pro Pro
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gtg tac act ttt ggc cag ggg acc                                        312
Val Tyr Thr Phe Gly Gln Gly Thr
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Gly Thr Arg Cys Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
            20                  25                  30


Leu Ser Pro Gly Glu Arg Ala Ile Leu Ser Cys Arg Ala Gly Gln Ser
        35                  40                  45


Val Ser Ser Tyr Leu Val Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
    50                  55                  60


Arg Leu Leu Ile Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala
65                  70                  75                  80


Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
                85                  90                  95


Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser
            100                 105                 110


Ser Trp Pro Pro Val Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
        115                 120                 125


Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
    130                 135                 140


Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145                 150                 155                 160


Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
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                165                 170                 175


Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
            180                 185                 190


Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
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Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
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Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
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1               5                   10                  15

ggt acg cgt tgt gaa att gtg ttg acg cag tct cca gcc acc ctg tct          96
Gly Thr Arg Cys Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
            20                  25                  30

ttg tct cca ggg gaa aga gcc atc ctc tcc tgc agg gcc ggt cag agt         144
Leu Ser Pro Gly Glu Arg Ala Ile Leu Ser Cys Arg Ala Gly Gln Ser
        35                  40                  45

gtt agc agt tac tta gtc tgg tac caa cag aaa cct ggc cag gct ccc         192
Val Ser Ser Tyr Leu Val Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
    50                  55                  60

agg ctc ctc atc tat gat gca tcc aac agg gcc act ggc atc cca gcc         240
Arg Leu Leu Ile Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala
65                  70                  75                  80

agg ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc agc         288
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
                85                  90                  95

agc cta gag cct gaa gat ttt gca gtt tat tac tgt cag cag cgc agc         336
Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser
            100                 105                 110

agc tgg cct ccg gtg tac act ttt ggc cag ggg acc aag ctt gaa atc         384
Ser Trp Pro Pro Val Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
        115                 120                 125

aaa cga act gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat         432
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Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
    130                 135                 140

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Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145                 150                 155                 160

ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat aac gcc ctc        528
Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
                165                 170                 175

caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac agc aag gac        576
Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
            180                 185                 190

agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac        624
Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
        195                 200                 205

gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc        672
Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
    210                 215                 220

tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tag                    711
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225                 230                 235


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            20                  25                  30


Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
        35                  40                  45


Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
    50                  55                  60


Glu Trp Val Ala Ile Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala
65                  70                  75                  80


Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
                85                  90                  95


Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
            100                 105                 110


Tyr Tyr Cys Ala Arg Leu Trp Phe Gly Asp Leu Asp Ala Phe Asp Ile
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        115                 120                 125


Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly
    130                 135                 140


Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
145                 150                 155                 160


Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
                165                 170                 175


Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
            180                 185                 190


Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
        195                 200                 205


Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
    210                 215                 220


Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
225                 230                 235                 240


Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
                245                 250                 255


Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
            260                 265                 270


Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
        275                 280                 285


Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
    290                 295                 300


Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
305                 310                 315                 320


Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
                325                 330                 335


Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
            340                 345                 350


Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
        355                 360                 365


Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
    370                 375                 380


Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
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                405                 410                 415


Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
            420                 425                 430


Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
        435                 440                 445


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gtc cag gca gaa gtg cag ctg gtg gag tct ggg gga ggc gtg gtc cag         96
Val Gln Ala Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
            20                  25                  30

cct ggg agg tcc ctg aga ctc tcc tgt gca gcg tct gga ttc acc ttc        144
Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
        35                  40                  45

agt agc tat ggc atg cac tgg gtc cgc cag gct cca ggc aag ggg ctg        192
Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
    50                  55                  60

gag tgg gtg gca att ata tgg tat gat gga agt aat aaa tac tat gcg        240
Glu Trp Val Ala Ile Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala
65                  70                  75                  80

gac tcc gtg aag ggc cga ttc acc atc tcc aga gac aat tcc aag aac        288
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
                85                  90                  95

acg ctg tat ctg caa atg aac agc ctg aga gcc gag gac acg gct gtg        336
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
            100                 105                 110

tat tac tgt gcg agg cta tgg ttc ggg gac tta gat gct ttt gat atc        384
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Tyr Tyr Cys Ala Arg Leu Trp Phe Gly Asp Leu Asp Ala Phe Asp Ile
        115                 120                 125

tgg ggc caa ggg aca atg gtc acc gtc tcc tca gcc tcc acc aag ggc        432
Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly
    130                 135                 140

cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggg ggc        480
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
145                 150                 155                 160

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Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
                165                 170                 175

acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc        576
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
            180                 185                 190

ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc gtc gtg        624
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
        195                 200                 205

acc gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac gtg        672
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
    210                 215                 220

aat cac aag ccc agc aac acc aag gtg gac aag aga gtt gag ccc aaa        720
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
225                 230                 235                 240

tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc        768
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
                245                 250                 255

ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc        816
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
            260                 265                 270

ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg        864
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
        275                 280                 285

agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg        912
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
    290                 295                 300

gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc        960
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
305                 310                 315                 320

acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg       1008
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
                325                 330                 335

aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc       1056
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
            340                 345                 350

ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca       1104
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
        355                 360                 365

cag gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag       1152
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
    370                 375                 380

gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc       1200
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Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
385                 390                 395                 400

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg       1248
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
                405                 410                 415

cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tat agc aag ctc       1296
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
            420                 425                 430

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc       1344
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
        435                 440                 445

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc       1392
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
    450                 455                 460

ctg tcc ccg ggt aaa tga                                               1410
Leu Ser Pro Gly Lys
465


<210> 22
<211> 51
<212> DNA
<213> Homo sapiens

<400> 22
gaaggttggc aggccaggga caacaycgtc tgccaagcca tggcagtaga c                51


<210> 23
<211> 51
<212> DNA
<213> Homo sapiens

<400> 23
taaggtattt ttgttatagc agcctrtatg gactaagctg acttgtaacg t                51


<210> 24
<211> 51
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<213> Homo sapiens

<400> 24
ctgtgtctgc agagcccaca cgtgtrtccc tgagaacaac ggaggcgcgg g                51


<210> 25
<211> 51
<212> DNA
<213> Homo sapiens

<400> 25
accccaggtc ccatatgtcc agagaktgtc cctccaatgg gaatgtgagg a                51


<210> 26
<211> 51
<212> DNA
<213> Homo sapiens

<400> 26
agggatgact tccaggaggg aagggmgggc attgtggccc ggctaacaga g                51
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<210> 27
<211> 51
<212> DNA
<213> Homo sapiens

<400> 27
gtctcggccc ccaccagtgg ctatcrggag tttgtacatg cggtggagca g                51


<210> 28
<211> 51
<212> DNA
<213> Homo sapiens

<400> 28
gcaacagagg acatgaaaaa ttgctrtgac taaagcaggg acaatttgct g                51


<210> 29
<211> 51
<212> DNA
<213> Homo sapiens

<400> 29
cttgtatggg gaacccaaac ccagayggca agtttcttaa cctcttgcat c                51


<210> 30
<211> 51
<212> DNA
<213> Homo sapiens

<400> 30
gcttatgtca tcctgacacc tacgcrgatg tcggctcgaa tccactttgc c                51


<210> 31
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic
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<400> 31
Thr Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala
1               5                   10                  15


<210> 32
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic
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<400> 32
Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala Gly Gln Phe
1               5                   10                  15


<210> 33
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<211> 12
<212> PRT
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<220>
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<400> 33
Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala
1               5                   10


<210> 34
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
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<400> 34
Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Glu Leu Ile Glu
1               5                   10                  15


Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys Asn Gly
            20                  25                  30


Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys Ala Ala
        35                  40                  45


Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu Lys Thr
    50                  55                  60


Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala Gly Gln
65                  70                  75                  80


Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala Gln Phe
                85                  90                  95


Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu Gly Arg
            100                 105                 110


Phe Asn



<210> 35
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic
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<400> 35
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Ser Pro Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Glu Leu Ile
1               5                   10                  15


<210> 36
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<223> /note="Description of Artificial Sequence: Synthetic
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<400> 36
Pro Val Pro Pro Ser Thr Ala Leu Arg Glu Leu Ile Glu Glu Leu
1               5                   10                  15


<210> 37
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<400> 37
Pro Ser Thr Ala Leu Arg Glu Leu Ile Glu Glu Leu Val Asn Ile
1               5                   10                  15


<210> 38
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<400> 38
Ala Leu Arg Glu Leu Ile Glu Glu Leu Val Asn Ile Thr Gln Asn
1               5                   10                  15


<210> 39
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<400> 39
Glu Leu Ile Glu Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala
1               5                   10                  15


<210> 40
<211> 15
<212> PRT
<213> Artificial Sequence
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<220>
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<400> 40
Glu Glu Leu Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys
1               5                   10                  15


<210> 41
<211> 15
<212> PRT
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<220>
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<400> 41
Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys Asn Gly Ser
1               5                   10                  15


<210> 42
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<400> 42
Thr Gln Asn Gln Lys Ala Pro Leu Cys Asn Gly Ser Met Val Trp
1               5                   10                  15


<210> 43
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<400> 43
Gln Lys Ala Pro Leu Cys Asn Gly Ser Met Val Trp Ser Ile Asn
1               5                   10                  15


<210> 44
<211> 15
<212> PRT
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<220>
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<400> 44
Pro Leu Cys Asn Gly Ser Met Val Trp Ser Ile Asn Leu Thr Ala
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1   5   10   15

<210> 45
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<212> PRT
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<220>
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<400> 45
Asn Gly Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr
1   5   10   15

<210> 46
<211> 15
<212> PRT
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<400> 46
Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys Ala Ala
1   5   10   15

<210> 47
<211> 15
<212> PRT
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<220>
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<400> 47
Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys Ala Ala Leu Glu Ser
1   5   10   15

<210> 48
<211> 15
<212> PRT
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<220>
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<400> 48
Leu Thr Ala Gly Met Tyr Cys Ala Ala Leu Glu Ser Leu Ile Asn
1   5   10   15

<210> 49
<211> 15
<212> PRT
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<400> 49
Gly Met Tyr Cys Ala Ala Leu Glu Ser Leu Ile Asn Val Ser Gly
1               5                   10                  15


<210> 50
<211> 15
<212> PRT
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<220>
<221> source
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<400> 50
Cys Ala Ala Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala
1               5                   10                  15


<210> 51
<211> 15
<212> PRT
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<220>
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<400> 51
Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu Lys
1               5                   10                  15


<210> 52
<211> 15
<212> PRT
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<220>
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<400> 52
Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu Lys Thr Gln Arg
1               5                   10                  15


<210> 53
<211> 15
<212> PRT
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<220>
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<400> 53
Val Ser Gly Cys Ser Ala Ile Glu Lys Thr Gln Arg Met Leu Ser
1               5                   10                  15
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<211> 15
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<400> 54
Cys Ser Ala Ile Glu Lys Thr Gln Arg Met Leu Ser Gly Phe Cys
1               5                   10                  15


<210> 55
<211> 15
<212> PRT
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<220>
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<400> 55
Ile Glu Lys Thr Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys
1               5                   10                  15


<210> 56
<211> 15
<212> PRT
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<220>
<221> source
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<400> 56
Gly Phe Cys Pro His Lys Val Ser Ala Gly Gln Phe Ser Ser Leu
1               5                   10                  15


<210> 57
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<400> 57
Pro His Lys Val Ser Ala Gly Gln Phe Ser Ser Leu His Val Arg
1               5                   10                  15


<210> 58
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<221> source
<223> /note="Description of Artificial Sequence: Synthetic
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<400> 58
Val Ser Ala Gly Gln Phe Ser Ser Leu His Val Arg Asp Thr Lys
1               5                   10                  15


<210> 59
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<221> source
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<400> 59
Gly Gln Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val
1               5                   10                  15


<210> 60
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<400> 60
Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala Gln Phe
1               5                   10                  15


<210> 61
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<400> 61
His Val Arg Asp Thr Lys Ile Glu Val Ala Gln Phe Val Lys Asp
1               5                   10                  15


<210> 62
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
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<400> 62
Asp Thr Lys Ile Glu Val Ala Gln Phe Val Lys Asp Leu Leu Leu
1               5                   10                  15
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<210> 63
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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      peptide"

<400> 63
Ile Glu Val Ala Gln Phe Val Lys Asp Leu Leu Leu His Leu Lys
1               5                   10                  15


<210> 64
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 64
Ala Gln Phe Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe
1               5                   10                  15


<210> 65
<211> 15
<212> PRT
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<220>
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<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 65
Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu Gly
1               5                   10                  15


<210> 66
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
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<400> 66
Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu Gly Arg Phe Asn
1               5                   10                  15


<210> 67
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> source
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<223> /note="Description of Artificial Sequence: Synthetic
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<400> 67
Phe Cys Pro His Lys Val
1               5
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