



- (51) **International Patent Classification:**
C12Q 1/68 (2006.01) *C07K 16/28* (2006.01)
- (21) **International Application Number:**
PCT/GB2013/051518
- (22) **International Filing Date:**
10 June 2013 (10.06.2013)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
1210328.9 12 June 2012 (12.06.2012) GB
- (71) **Applicant:** **ISIS INNOVATION LIMITED** [GB/GB];
Ewert House, Ewert Place, Summertown, Oxford, Oxfordshire OX2 7SG (GB).
- (72) **Inventors:** **FUGGER, Lars**; The Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, Oxfordshire OX3 9DS (GB). **MCVEAN, Gilean**; Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, Oxfordshire OX3 7BN (GB). **DENDRON, Calliopi, Athena**; The Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe

Hospital, Oxford, Oxfordshire OX3 9DS (GB). **GREGORY, Adam, Patrick**; The Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, Oxfordshire OX3 9DS (GB). **ATTFIELD, Kathrine, Elizabeth**; The Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, Oxfordshire OX3 9DS (GB).

- (74) **Agent:** **CHAPMAN, Lee, Phillip**; 14 South Square, Gray's Inn, London, Greater London WC1R 5JJ (GB).
- (81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH,

[Continued on next page]

- (54) **Title:** BIOMARKER FOR DETERMINING THE SUITABILITY OF ANTI -TUMOUR NECROSIS FACTOR (ANTI-TNF) THERAPY IN THE TREATMENT OF AUTOIMMUNE DISEASES

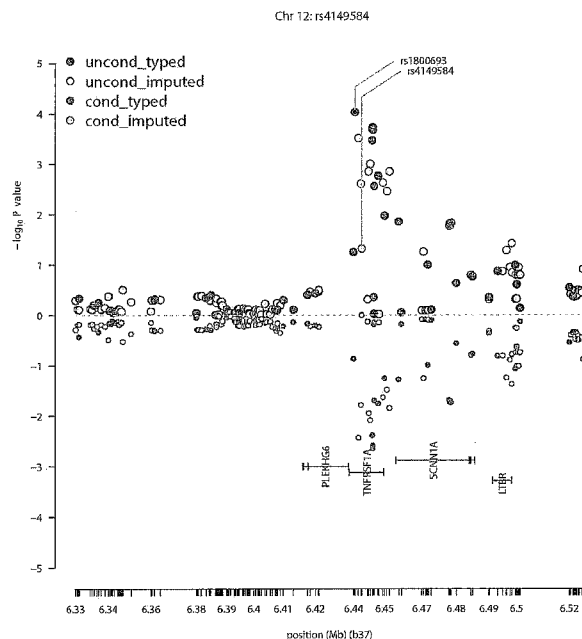


Figure 1

(57) **Abstract:** The invention relates to methods of determining whether or not a patient is suitable for anti-tumour necrosis factor (anti-TNF) therapy. The invention also relates to methods of treating autoimmune diseases and methods of identifying whether or not a patient is at risk of developing multiple sclerosis (MS) or MS-like symptoms or whether or not a patient has MS. The invention further provides novel polypeptide TNF antagonists and related polynucleotides, expression vectors, host cells and antibodies.



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

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

BIOMARKER FOR DETERMINING THE SUITABILITY OF ANTI-TUMOUR NECROSIS FACTOR
(ANTI-TNF) THERAPY IN THE TREATMENT OF AUTOIMMUNE DISEASES**Field of the invention**

The invention relates to methods of determining whether or not a patient is
5 suitable for anti-tumour necrosis factor (anti-TNF) therapy. The invention also relates to methods
of treating autoimmune diseases and methods of identifying whether or not a patient is at risk of
developing multiple sclerosis (MS) or MS-like symptoms or whether or not a patient has MS.
The invention further provides novel polypeptide TNF antagonists and related polynucleotides,
expression vectors, host cells and antibodies.

Background of the invention

Tumour necrosis factors (or TNFs) are a group of cytokines which can cause cell death
(apoptosis). Anti- TNF therapies have been used to treat many autoimmune diseases, including
rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and
15 Crohn's disease. However, the use of anti-TNF therapy is not without risk, and may lead to
certain adverse side effects in patients (as reviewed in Bosch et al. 2011, Nature Reviews
Neurology 7, 165-172). For example, there may be an increased risk of infections such as
tuberculosis (TB) or of developing certain lymphomas after anti-TNF therapy. Dysimmune
disorders may also be associated with anti-TNF therapies. For example, central nervous system
20 (CNS) disorders such as the induction or aggravation of multiple sclerosis (MS) or optic neuritis,
progressive multifocal leukoencephalopathy (PML), myasthenia gravis and various forms of
peripheral demyelinating neuropathy are associated with the administration of monoclonal
antibodies (mAbs) targeting TNF and its receptors.

Summary of the invention

The inventors have surprisingly shown that the single nucleotide polymorphism (SNP)
rs1800693 directs the expression of a soluble isoform of the TNF receptor 1 (TNFR1) which is
capable of TNF antagonism and mimicking the MS-like side effects of anti-TNF therapies.
Detection of the SNP rs1800693 or the soluble isoform of the TNFR1 in a patient can therefore
30 be used to determine whether or not a patient is suitable for treatment with anti-TNF therapy or
whether or not a patient is at risk of developing MS or MS-like symptoms.

Accordingly, the invention provides a method of determining whether or not a patient is
suitable for anti-tumour necrosis factor (anti-TNF) therapy, comprising detecting in a sample
from the patient the presence or absence of:

- (a) the single nucleotide polymorphism (SNP) rs1800693 in the patient's genome;
- (b) one or more polymorphisms in linkage disequilibrium with the SNP of (a) in the patient's genome;
- (c) the tumour necrosis factor receptor 1 (TNFR1) isoform as set forth in SEQ ID NO: 2; and/or

(d) a variant of the TNFR1 isoform in (c) which comprises a sequence having at least 95% homology to SEQ ID NO: 2 based on amino acid identity over the entire length of SEQ ID NO: 2; and thereby determining whether or not the patient is suitable for anti-TNF therapy, wherein the presence of (a), (b), (c) and/or (d) indicates that the patient is not suitable for anti-TNF therapy and wherein the absence of (a), (b), (c) and/or (d) indicates that the patient is suitable for anti-TNF therapy.

The invention also provides:

- a method of treating or preventing an autoimmune disease in a patient in need thereof comprising (i) determining whether or not the patient is suitable for anti-TNF therapy using a method of the invention, and (ii), if the patient is suitable for anti-TNF therapy, administering an anti-TNF therapy to the patient and thereby treating or preventing the autoimmune disease;
- a method of treating or preventing an autoimmune disease in a patient identified as suitable for anti-TNF therapy using a method of the invention, comprising administering an anti-TNF therapy to the patient and thereby treating or preventing the autoimmune disease;
- a method of determining whether or not a patient is at risk of developing multiple sclerosis (MS) or MS-like symptoms or whether or not a patient has MS, comprising detecting in a sample from the patient the presence or absence of:

(a) the TNFR1 isoform as set forth in SEQ ID NO:2; and/or

(b) a variant of the TNFR1 isoform in (a) which comprises a sequence having at least 95% homology to SEQ ID NO: 2 based on amino acid identity over the entire length of SEQ ID NO: 2;

and thereby determining whether or not the patient is at risk of developing MS or MS-like symptoms or whether or not the patient has MS,

wherein the presence of (a) or (b) indicates that the patient is at risk of developing MS or MS-like symptoms or indicates that the patient has MS and

wherein the absence of (a) or (b) indicates that the patient is not at risk of developing MS or MS-like symptoms or that the patient does not have MS;

- a polypeptide TNF antagonist which comprises:

(a) the sequence of the TNFR1 isoform as set forth in SEQ ID NO:2; or
(b) a sequence which has at least 85% homology to the sequence of SEQ ID NO: 2 based on amino acid identity over the entire sequence of SEQ ID NO: 2 and which retains its ability to bind TNF;

- 5 - a polynucleotide encoding a polypeptide TNF antagonist of the invention;
- an expression vector comprising polynucleotide of the invention operably linked to a promoter sequence;
- a host cell comprising a polynucleotide of the invention or a vector of the invention;
- a polypeptide TNF antagonist, polynucleotide or expression vector of the invention for
- 10 use in the treatment or prevention of an autoimmune condition in a patient in need thereof;
- an antibody which specifically recognises an epitope within a polypeptide TNF antagonist of the invention;
- an immunoassay plate comprising an antibody of the invention;
- a method for producing an antibody of the invention comprising (a) immunising a non-
- 15 human mammal with an immunogen comprising a polypeptide of the invention; and (b) obtaining an antibody preparation from said mammal and deriving therefrom monoclonal antibodies that specifically recognise said epitope;
- a hybridoma which produces an antibody of the invention;
- an antibody of the invention for use in a method of determining whether or not a patient
- 20 is suitable for anti-TNF therapy;
- a method of preventing MS or MS-like symptoms in a patient in need thereof comprising administering to the patient a prophylactically effective amount of an antibody of the invention and thereby preventing MS or MS-like symptoms in the patient;
- a method of treating or preventing an autoimmune disease in a patient in need thereof
- 25 comprising (i) determining whether or not the patient is suitable for anti-TNF therapy using a method of the invention and (ii), if the patient is not suitable for anti-TNF therapy, administering to the patient a prophylactically effective amount of an antibody of the invention in conjunction with anti-TNF therapy and thereby treating or preventing the autoimmune disease;
- a method of treating or preventing an autoimmune disease in a patient identified as not
- 30 suitable for anti-TNF therapy using a method of the invention, comprising administering to the patient a prophylactically effective amount of an antibody of the invention in conjunction with anti-TNF therapy and thereby treating or preventing the autoimmune disease;
- an antibody of the invention for use in preventing a patient undergoing anti-TNF therapy from developing MS or MS-like symptoms; and

- a method of identifying whether or not a patient is at risk of developing multiple sclerosis (MS) or MS-like symptoms or whether or not a patient has MS, comprising detecting in a sample from the patient the presence or absence of a soluble isoform of TNFR1 and thereby determining whether or not the patient is at risk of developing MS or MS-like symptoms or whether or not the patient has MS,

wherein the presence of the soluble isoform of TNFR1 indicates that the patient is at risk of developing MS or MS-like symptoms or that the patient has MS and

wherein the absence of the soluble isoform of TNFR indicates that the patient is not at risk of developing MS or MS-like symptoms or that the patient does not have MS.

Brief description of the Figures

Figure 1 shows analysis of association signal within the *TNFRSF1A* region. Top shows the primary signal (measured by $-\log_{10} P$ -value under an additive logistic model, including gender as a covariate). Bottom shows signal conditional on variation at rs4149584 (imputed).

Figure 2 shows MS-associated *TNFRSF1A* region and rs1800693-dependent splicing. (a) MS association signal plot for the UK cohort. Top: primary association with strongest signal at rs1800693 (blue: typed variants; light blue: variants imputed from 1000 Genomes Project). Bottom: signal conditional on rs1800693 (red: typed; orange: imputed). Inset: linkage disequilibrium to rs1800693 in 1000 Genomes Project (black: typed; untyped - red: $r^2 \geq 0.05$; blue: $r^2 < 0.05$). (b) Rs1800693-dependent TNFR1 splicing using a minigene system. SD/SA: vector splice donor/acceptor site. (c) Relative expression of transcripts lacking exon 6 in primary human cells. Data = mean $\pm$ SEM; for A/A, A/G, G/G, n = 15, 28 and 13 donors.

Figure 3 shows inter-cell subset correlations in the relative expression of transcripts lacking exon 6 (TNFR1_Δ6). The relative expression level of TNFR1_Δ6 transcripts was correlated for (a) CD14+ monocytes and polymorphonuclear cells, (b) CD14+ monocytes and CD3+ T cells, and (c) polymorphonuclear and CD3+ T cells by linear regression analysis. The residuals for the linear regression analysis of the variation in relative TNFR1_Δ6 expression by rs1800693 genotype (see Fig. 2c) were also correlated for (d) CD14+ monocytes and polymorphonuclear cells, (e) CD14+ monocytes and CD3+ T cells, and (f) polymorphonuclear and CD3+ T cells. r^2 values were calculated for each genotype group (black: A/A; blue: A/G; red: G/G), and for all donors (black dotted line and r^2 in bold). For genotype A/A, n = 15; for A/G, n = 28; for G/G, n = 13 donors.

Figure 4 shows a polysome profiling assay for TNFR1 transcript translation. The chromatogram shows a representative A260 sedimentation profile obtained from post-nuclear,

stimulated PBMC lysate supernatants centrifuged through a linear 20-47% sucrose gradient containing 5 mM MgCl₂; the arrow denotes the direction of sedimentation. Monosome and polysome fractions are indicated and roughly correspond to fractions 1-3 and 4-9, respectively. The detection of transcripts in the polysome fractions is indicative of active translation. Graphs show levels of TNFR1 transcripts lacking (TNFR1_Δ6) or containing exon 6 (TNFR1_6-7), in the different fractions, as detected by quantitative PCR. The PBMCs were obtained from rs1800693 A/A protective or G/G susceptible homozygous individuals. No transcripts lacking exon 6 were detectable in the A/A homozygote, however, such transcripts were present in G/G homozygotes in the monosome and also the polysome fractions, the latter indicating translation. As a negative control, a sample from a G/G homozygote was subjected to a gradient containing 10 mM EDTA, which promotes release of RNA from polysome and thus an enrichment of transcripts in the monosome fractions. CT = cycle threshold.

Figure 5 shows expression of transcripts containing exon 6 (TNFR1_6-7) does not vary by genotype at rs1800693. TNFR1_6-7 expression relative to house-keeping gene expression is shown for three human, primary immune cell subsets (a-c). Data shown are mean ± SEM; for genotype A/A, n = 15; for A/G, n = 28; for G/G, n = 13 donors.

Figure 6 shows TNFR1 protein levels by donor genotype at rs1800693 at the surface of human peripheral blood immune cell subsets. TNFR1 cell surface levels were measured by flow cytometry on (a) HLA-DR⁺ CD14⁺, (b) CD14⁺ CD16⁺, and (c) CD16⁺ monocytes, (d) CD11b⁺ CD16⁺ neutrophils, and (e) CD11c⁺ CD123⁻ myeloid and (f) CD11c⁻ CD123⁺ plasmacytoid dendritic cells (DCs). Histograms show representative anti-TNFR1 (light blue) and isotype control (gray) staining. “Iso” = isotype control median fluorescence intensity (MedFI); “TNFR1” = anti-TNFR1 MedFI; “Norm” = anti-TNFR1 MedFI/isotype control MedFI and normalized to control for day-to-day variation in flow cytometer function using fluorospheres. This normalized anti-TNFR1 MedFI/isotype control MedFI ratio is shown in the plots below the histograms. % of Max = cell number in each bin divided by cell number in the bin containing the largest cell number; this statistic represents the normalization performed to account for the different number of events collected for each sample that is overlaid. For the plots, 1-d.f. P-values are shown. A/G = protective/risk allele at rs1800693; in the U.K. non-MS control population, the risk allele frequency = 0.40 (Sawcer et al. 2011 Nature). For genotype A/A, n = 15; for A/G, n = 28; for G/G, n = 13 donors.

Figure 7 shows peptides unique to Δ6-TNFR1 are detected in the cell culture supernatant of transfected cells by MS/MS. Top: Schematic of the novel 45 amino acid sequence unique to Δ6-TNFR1. The peptide used for MS/MS analysis is underlined. Annotated fragmentation

spectra obtained for SLPFIPPLHWF from (middle) purified recombinant $\Delta 6$ -TNFR1 protein and (bottom) cell culture supernatant of $\Delta 6$ -TNFR1 transfected HEK 293T cells. Inlay shows the mass spectrum. y-ion = charge retained on C-terminal fragment, b-ion = charge retained on N-terminal fragment; ion numbering represents the number of amino acids present.

5 Figure 8 shows $\Delta 6$ -TNFR1 localization and analysis of isoform association. (a) TNFR1 subcellular localization. (b) FL/ $\Delta 6$ -TNFR1 localization analysis. Data = mean $\pm$ SEM; FL-TNFR1, n = 20, $\Delta 6$ -TNFR1, n = 27 cells; scale bar: 1 μ m. (c) FL/ $\Delta 6$ -TNFR1 association analysis. HEK 293T double-positive cells expressing ECFP/EYFP-tagged TNFR1 isoforms were analyzed for FRET to assess isoform association. FL-TNFR1-ECFP + FAS180-EYFP co-
10 transfection was used for background signal definition with non-interacting proteins. Percentage of FRET-positive events (indicating protein association) is shown. (d) HEK 293T cells expressing HA-tagged FL- or $\Delta 6$ -TNFR1-EYFP were analyzed for cell surface and total protein expression. ECFP/EYFP: enhanced cyan/yellow fluorescent protein.

Figure 9 shows localization of $\Delta 6$ -TNFR1-EYFP and FL-TNFR1-ECFP. HeLa cells were
15 co-transfected with the indicated fluorescent fusion protein constructs and analyzed 48 h later by confocal microscopy.

Figure 10 shows analysis of TNFR1 association by BRET. (a) HEK 293T cells were transfected with FL-TNFR1-GFP2 and $\Delta 6$ -TNFR1-RLuc at a ratio of 12:1. GFP2 expression, luciferase activity and BRETeff were measured in cells taken at hourly time-points from 10 h
20 post-transfection. au = arbitrary units. (b) Varying ratios of FL-TNFR1-GFP2:FL-TNFR1-RLuc and $\Delta 6$ -TNFR1-GFP2: $\Delta 6$ -TNFR1-RLuc were transfected to analyze self-association of FL-TNFR1 and $\Delta 6$ -TNFR1 respectively. GFP2 fluorescence, luciferase activity and BRETeff were measured at 24 h. Data for FL-TNFR1 approximate to the BRET equation for a trimer (solid line), and of $\Delta 6$ -TNFR1 data (for [GFP2]/[Luc] > 2) to a constant value, as predicted for a
25 monomer (dotted line).

Figure 11 shows analysis of spontaneous signaling in TNFR1-transfected cells. (a, left panel) Firefly luciferase NF- κ B reporter activity in TNFR1-EYFP-transfected HEK 293T cells. Firefly luciferase activity was normalized to Renilla luciferase (RLTK; transfection efficiency and viability control). (a, right panel) IL-8 levels in culture supernatant of TNFR1-transduced
30 cells. IL-8 levels were detected by sandwich ELISA. Transfection and transduction efficiencies were similar (typically >90%). (b) Analysis of spontaneous apoptosis by tetramethylrhodamine methyl ester (TMRM; Rasola & Guena 2001 Cytometry; Gross et al. 1999 J Biol Chem). HeLa cells were transfected with the indicated TNFR1-ECFP constructs. H₂O₂ and ZVAD.fmk were used as controls to promote and inhibit apoptosis, respectively. TMRM fluorescence was

analyzed in ECFP-positive cells (except for non-transfected (NT) cells) by flow cytometry. Data shown are mean $\pm$ SEM; n = 3.

Figure 12 shows spontaneous apoptosis in TNFR1-transduced cells as measured using propidium iodide (PI). HEK 293T cells were transduced with the indicated TNFR1 lentivirus at a multiplicity of infection of 50. 72 h later cells were harvested, permeabilized, stained with PI and analyzed by flow cytometry. Cells with a sub-G1 DNA content were deemed apoptotic. NT = non-transfected. Data shown are mean $\pm$ SEM; n = 3.

Figure 13 shows unfolded protein response in TNFR1-transfected cells. (a) HT1080 cells were co-transfected with an XBP1 firefly luciferase reporter construct, RLTK and the indicated TNFR1-EYFP constructs. 24 h later cells were analyzed for firefly luciferase activity, which was normalized to Renilla luciferase. (b) HT1080 cells were transfected with the indicated constructs and 24 h later RNA was isolated, cDNA was made and XBP1 splicing was analyzed. NT = non-transfected. Data shown are mean $\pm$ SEM; n = 3.

Figure 14 shows Δ 6-TNFR1 solubility and TNF binding and neutralization. (a) Soluble TNFR1 detected by anti-HA ELISA using TNFR1-transfected HEK 293T supernatants. AFU: absolute fluorescence units; NT: non-transfected. (b) TNF-FLAG pulldown using TNFR1-Fc proteins. Inset: negative control for TNF binding (DR5.CRD1-Fc). (c) Surface plasmon resonance analysis of TNFR1-Fc protein TNF binding. Sensorgrams show resonance units (RU) over time and model fit for dissociation constant (Kd) derivation. (d) TNF neutralization by TNFR1-Fc proteins using HEK-Blue™ TNF α SEAP reporter cells. Dotted lines = IC50 for TNFR1-Fc and Δ 6-TNFR1-Fc (158 and 3,035 ng/ml, respectively). Data = mean $\pm$ SEM; n = 3.

Figure 15 shows triplicate anti-HA Western blots of lysates (upper panel) and supernatants (lower panel) of HA-TNFR1-EYFP-transfected HEK 293 T cells. Whole cell lysates and supernatants of HEK 293T cells transiently transfected with the indicated HA-TNFR1-EYFP construct were resolved by SDS-PAGE and immunoblotted using anti-HA.11. * = non-specific bands.

Figure 16 shows commercially available anti-TNFR1 antibodies cannot specifically detect Δ 6-TNFR1 by ELISA. Plates were coated with supernatants from transfected HEK cells (open bars) or with unconjugated anti-TNFR1 antibodies (16805 = horizontal; H398 = vertical; H-5 = slanted striped bars). The supernatants tested were from HA-tagged FL- (a) or Δ 6- (b) TNFR1-expressing cells. Biotinylated ("bio") anti-HA.11 (grey) or anti-TNFR1 antibodies (blue = H398-bio; red = BAF225-bio) were used for detection. Anti-TNFR1 clone 16805 and a polyclonal anti-TNFR1 antibody ("BAF225") were from R&D Systems, while anti-TNFR1 clones H398 and H-5 were from Santa Cruz Biotechnology, Inc. Data shown are normalized to

the signal obtained using anti-HA.11-bio as the detection antibody in the absence of a capture reagent (with this signal being taken as 1) and are mean $\pm$ SEM; n = 2.

Figure 17 shows specific $\Delta 6$ -TNFR1 detection by ELISA. (a) ELISA performed at AbD Serotec to verify the specificity of anti- $\Delta 6$ -TNFR1 monoclonal antibodies (x-axis). Test substrates (z-axis) were coated on the plate. Clone AbD19050.1 showed the best binding and specificity to $\Delta 6$ -TNFR1 ("hTNFR1-45aa-Fc") (b) Using the anti- $\Delta 6$ -TNFR1-specific clone AbD19050.1 as the capture antibody and an anti-TNFR1 monoclonal antibody (clone 16805, R&D Systems) as the detection antibody (as it binds the splice isoform well; see Supplementary Fig. 13), $\Delta 6$ -TNFR1 was detected in the sera of both healthy individuals (n = 12) and relapsing-remitting MS patients (n = 13). Using a recombinant $\Delta 6$ -TNFR1 as a standard, the sensitivity limit of the assay was determined to be ~ 1.25 ng/ml. Data shown are mean $\pm$ SEM.

Figure 18 shows patterns of association between SNPs within the *TNFRSF1A* region to the focal SNP (rs1800693; blue) from the MS GWAS (Sawcer et al. 2011 Nature), and the focal SNP (rs11616188; red) from the AS GWAS (Evans et al. 2011 Nature Genet), estimated from 381 individuals of European ancestry sequenced within the 1000 Genomes Project. The two SNPs are in distinct regions, separated by two recombination hotspots, providing strong evidence that the two GWAS association signals are not driven by the same variant. The x-axis denotes position on human chromosome 12 based on the GRCh36 genome assembly.

Brief description of the sequences

SEQ ID NO: 1 shows the polynucleotide sequence of the human *TNFRSF1A* region encompassing the SNP rs1800693.

SEQ ID NO: 2 shows the amino acid sequence of the soluble TNFR1 isoform (called $\Delta 6$ -TNFR1 in the Example) whose expression is directed by the SNP rs1800693.

SEQ ID NO: 3 shows the amino acid sequence of the wild-type human TNFR1 (called full-length TNFR1 or FL-TNFR1 in the Example).

Detailed description of the invention

Method of determining suitability for anti-TNF therapy

The present invention relates to a method of determining whether or not a patient is suitable for anti-TNF therapy. The patient is typically an individual for whom treatment using an anti-TNF therapy is being considered. The patient is preferably human. The patient to be treated typically suffers from or is suspected of having an autoimmune disease associated with

the presence of TNF. Typically, the autoimmune disease is associated with the presence of TNF α and treatment using an anti-TNF α therapy is being considered.

Any disease that results in failure of an organism to recognize its own constituent parts, resulting in an immune response against the organism's own cells and tissues from such an aberrant immune response, is termed an autoimmune disease. Autoimmune diseases treated using anti-TNF therapy include rheumatoid arthritis, psoriatic arthritis, psoriasis, ankylosing spondylitis, ulcerative colitis and Crohn's disease. The method of the present invention may be used to determine whether a patient having or suspected of having any one of these diseases, or any other disease, condition or symptom that is treatable using an anti-TNF therapy, is suitable for anti-TNF therapy.

The phrase "suitable for anti-TNF therapy" typically means whether a patient is likely to develop an adverse reaction to TNF therapy. As outlined above, anti-TNF therapy may lead to adverse side effects in a patient such as the onset of certain infections, diseases or disorders. For instance, anti-TNF therapy may lead to the onset of tuberculosis (TB), development of lymphomas, the induction or aggravation of multiple sclerosis (MS) or optic neuritis, progressive multifocal leukoencephalopathy (PML), myasthenia gravis and various forms of peripheral demyelinating disease. Anti-TNF therapy is discussed in more detail below.

The method of the invention thus allows the evaluation of the potential risk of anti-TNF therapy based on the genotype and/or phenotype of an individual patient, before determining whether or not to proceed and treat the patient with anti-TNF therapy. The method of the invention is particularly suitable for determining whether or not a patient is likely to develop MS or MS-like symptoms if they are treated with anti-TNF therapy. This is discussed in more detail below.

The presence in a sample from the patient of any combination of one or more of: (a) the SNP rs1800693 in the patient's genome; (b) one or more polymorphisms in linkage disequilibrium with the SNP rs1800693; (c) the TNFR1 isoform as set forth in SEQ ID NO: 2; and (d) a variant of the TNFR1 isoform which comprises a sequence having at least 95%, preferably at least 96%, at least 97%, at least 98% or at least 99%, homology to SEQ ID NO: 2, based on amino acid identity over the entire length of SEQ ID NO: 2; indicates that the patient is not suitable for anti-TNF therapy. For example, the presence of one or more of (a) to (d) may indicate an increased risk of the patient developing an adverse reaction to TNF therapy such as the potential side effects and symptoms as outlined above. Patients identified as not suitable for anti-TNF therapy (i.e. determined as not being suitable for anti-TNF therapy) in accordance with the invention are typically not treated with such therapy and an alternative therapy is used.

Accordingly, the absence in a sample from the patient of any combination of one or more of (a) to (d) above indicates that the patient is suitable for anti-TNF therapy. For example, the patient may be at a decreased risk of developing an adverse reaction to TNF therapy such as the potential side effects and symptoms as outlined above. Patients identified as suitable for anti-TNF therapy (i.e. determined as being suitable for anti-TNF therapy) in accordance with the invention are typically then treated with such therapy alone or in combination with other therapies.

The method of the invention may comprise the detection of the presence or absence of any combination of (a) to (d) as defined above. The method may comprise the detection of the presence or absence of (a); (b); (c); (d); (a) and (b); (a) and (c); (a) and (d); (b) and (c); (b) and (d); (c) and (d); or (a), (b), (c) and (d).

The method is carried out on a sample from the patient of interest. The method is carried out *in vitro* on a sample that has been obtained from the patient. Preferably the sample is obtained from the patient before any treatment for the disease or condition is started. For example, the sample may be obtained from the patient before any treatment with an anti-TNF therapy has begun.

The sample may be from any tissue or bodily fluid. The sample typically comprises a body fluid and/or cells of the patient and may, for example, be obtained using a swab, such as a mouth swab. The sample may be, or be derived from, blood, urine, saliva, skin, cheek cell or hair root samples. The sample is typically processed before the method is carried out, for example DNA extraction may be carried out. The polynucleotide or protein in the sample may be cleaved either physically or chemically, for example using a suitable enzyme. In one embodiment the part of polynucleotide in the sample is copied or amplified, for example by cloning or using a PCR based method prior to detecting the polymorphism. Methods for obtaining samples from patients are well known in the art.

As outlined above, the method of the invention may comprise detecting in a sample from the patient the presence or absence of the single nucleotide polymorphism (SNP) rs1800693 in the patient's genome. This SNP comprises an A to G point mutation at position 11,275 of the *TNFRSF1A* region. The wild-type *TNFRSF1A* region is shown in SEQ ID NO: 3. The *TNFRSF1A* region including the SNP is set forth in SEQ ID NO:1. The method of the invention preferably comprises detecting the presence or absence of the sequence set forth in SEQ ID NO: 1. However, the method may also comprise detecting a variant of this sequence as long as the variant comprises the SNP rs1800693 (i.e. an A to G point mutation at the corresponding position in the variant). Typically, the variant comprises a sequence having at least 95%

homology, preferably at least 96% homology, at least 97% homology, at least 98% homology or at least 99% homology, to SEQ ID NO:1 based on nucleotide identity over the entire sequence of SEQ ID NO: 1 and which comprises the SNP rs1800693. Homology is discussed in more detail below. The variant encodes a soluble isoform of TNFR1 which is capable of TNF antagonism.

5 This can be measured as described in the Examples. The position of the SNP in the variant could for example vary from position 11, 275 because of deletions or additions of nucleotides elsewhere in the sequence of the variant. Those skilled in the art will be able to determine a position in a variant that corresponds to or is equivalent to position 11, 275 in SEQ ID NO: 1, using for example the alignment programs that are described in greater detail below.

10 The method of the invention may comprise detecting the presence or absence of one or more polymorphisms, such as two, three, four or five, in linkage disequilibrium with the single nucleotide polymorphism (SNP) rs1800693 in the patient's genome. Therefore, determining whether or not a patient is suitable for anti-TNF therapy is not limited to the detection of the causal variant in the *TNFRSF1A* region, i.e the SNP rs1800693. For instance, determining
15 whether a patient is susceptible to any adverse effects of anti-TNF therapy (or at risk of developing MS), is not limited to the detection of a polymorphism that is causal for susceptibility to any adverse effects of anti-TNF therapy (or risk of developing MS). Polymorphisms include, but are not limited to, SNPs, microsatellite or repeat polymorphisms, insertion polymorphisms and deletion polymorphisms.

20 As a result of linkage disequilibrium, a polymorphism that is not the causal variant in the *TNFRSF1A* region (i.e the SNP rs1800693), but is in linkage disequilibrium with the SNP, may act as a marker indicating whether a patient is susceptible to any adverse effects of anti-TNF therapy. A polymorphism that is in linkage disequilibrium with SNP rs1800693 is therefore indicative of whether or not a patient is suitable for anti-TNF therapy.

25 Accordingly, the SNP rs1800693 may be typed directly, in other words by determining the nucleotide present at that position, or indirectly, for example by determining the nucleotide present at another polymorphic position that is in linkage disequilibrium with said polymorphic position.

Linkage disequilibrium is the non-random gametic association of alleles at different loci
30 in a population. Polymorphisms that have a tendency to be inherited together instead of being inherited independently by random assortment are in linkage disequilibrium. Polymorphisms are randomly assorted or inherited independently of each other if the frequency of the two polymorphisms together is the product of the frequencies of the two polymorphisms individually. For example, if two polymorphisms at different polymorphic sites are present in 50% of the

chromosomes in a population, then they would be said to assort randomly if the two alleles are present together on 25% of the chromosomes in the population. A higher percentage would mean that the two alleles are linked. It follows that a first polymorphism is in linkage disequilibrium with a second polymorphism if the frequency of the two polymorphisms together is greater than the product of the frequencies of the two polymorphisms individually in a population. Preferably, a first polymorphism is in linkage disequilibrium with a second polymorphism if the frequency of the two polymorphisms together is more than 10% greater, for example more than 30%, more than 50% or more than 70% greater, than the product of the frequencies of the two polymorphisms individually.

Polymorphisms which are in linkage disequilibrium are often in close physical proximity, which is why they are co-inherited. Polymorphisms which are in linkage disequilibrium with the polymorphisms mentioned herein are located on the same chromosome. Polymorphisms which are in linkage disequilibrium may be typically within 5mb, preferably within 2mb, within 1mb, within 700kb, within 600kb, within 500kb, within 400kb, within 200kb, within 100kb, within 50kb, within 10kb, within 5kb, within 1kb, within 500bp, within 100bp, within 50bp or within 10bp of the polymorphism.

It would be within the capability of the skilled person to use routine techniques to identify polymorphisms that are in linkage disequilibrium with SNP rs1800693 as defined herein. Once a potential polymorphism has been selected, the skilled person can readily determine whether this polymorphism, and what version or allele of the polymorphism, is significantly correlated with SNP rs1800693.

In more detail, to determine whether a polymorphism is in linkage disequilibrium with the SNP defined herein, the skilled person should genotype the candidate polymorphism and the SNP defined herein in a panel of patients. The size of the panel should be adequate enough to achieve a statistically significant result. Typically, samples from at least 100, preferably at least 150 or at least 200, different patients should be genotyped. Once the polymorphisms have been genotyped in the panel of patients, linkage disequilibrium between one or more pairs of polymorphisms can be measured using any one of a number of readily available statistical packages. An example of a free software package is Haploview (Haploview: analysis and visualisation of LD and haplotype maps, Barrett et al, 2005, Bioinformatics, 21(2): 263-265), downloadable at <http://www.broadinstitute.org/haploview/haploview>. Another example of software that can be used is PLINK (<http://pngu.mgh.harvard.edu/purcell/plink/>).

A measure of linkage disequilibrium is D' . A range of 0.5 to 1 for D' is indicative of a pair of polymorphisms being in linkage disequilibrium, with 1 indicating the most significant

linkage disequilibrium. Therefore if D' is found to be from 0.5 to 1, preferably from 0.6 to 1, 0.7 to 1, from 0.8 to 1, from 0.85 to 1, from 0.9 to 1, from 0.95 to 1 or most preferably 1, for a candidate polymorphism and the specific SNP defined herein, the candidate polymorphism may be said to be predictive of SNP rs1800693 and will thus indicate whether a patient is suitable for anti-TNF therapy. In a preferred method of the invention, a polymorphism that is in linkage disequilibrium with SNP rs1800693 defined herein is within 680 kb and on the same chromosome as SNP rs1800693, chromosome 12, and the calculated measure of linkage disequilibrium between the pair of polymorphisms, D' , is greater than or equal to 0.9.

Another measure of linkage disequilibrium is R-squared, where R is the correlation coefficient. R-squared, which is also known as the 'Coefficient of determination', is the fraction of the variance in the genotypes of the first polymorphism which is accounted for in the genotypes of the second polymorphism. Therefore an R-squared of 0.5 for a candidate polymorphism and SNP rs1800693 would mean that the candidate polymorphism accounts for 50% of the variance in the specific polymorphism. R-squared is producible from standard statistical packages such as Haploview. Typically, an R-squared of 0.25 or greater (R of >0.5 or ≤ -0.5) is considered a large correlation. Therefore if R-squared is found to be 0.5 or more, preferably 0.75 or more, 0.8 or more, 0.85 or more, 0.9 or more, or 0.95 or more for a candidate polymorphism and SNP rs1800693, the candidate polymorphism may be said to be predictive of SNP rs1800693 and will thus indicate whether or not a patient is suitable for anti-TNF therapy. In a preferred method of the invention, a polymorphism that is in linkage disequilibrium with SNP rs1800693 defined herein is within 680 kb and on the same chromosome as SNP rs1800693 and the calculated measure of linkage disequilibrium between the pair of polymorphisms, R-squared, is greater than or equal to 0.5.

It is also possible to build a haplotype of polymorphisms in LD with SNP rs1800693. Even if one or more polymorphisms are individually only weakly in LD with the polymorphisms of the invention, they may be in strong LD if they are used in combination. For example, any one polymorphism may have an R-squared value below 0.25. However, two or more mutations individually having an R-squared of below 0.25 may in combination have an R-squared of greater than 0.5. Therefore, these polymorphisms may be used in combination to determine the suitability of the patient for anti-TNF treatment.

The method may comprise detecting the presence or absence of two or more polymorphisms, such as three, four, five or more, in linkage disequilibrium with the specific polymorphism defined herein, wherein R-squared for each of said two or more polymorphisms

individually may be less than or equal to 0.25, but R-squared for the combination of said two or more polymorphisms is greater than or equal to 0.5.

The inventors have surprisingly characterised the SNP rs1800693 in the human genome as directing the expression of a soluble TNFR1 isoform as set forth in SEQ ID NO: 2. Without wishing to be bound by theory, it is believed that the soluble isoform sequesters TNF, but does not activate intracellular second messenger systems responsible for TNF signalling. It therefore inhibits or antagonises TNF signalling. As outlined above, the method of the invention may therefore comprise detecting in a sample from the patient the presence or absence of the TNFR1 isoform as set forth in SEQ ID NO: 2.

The TNFR1 isoform as set forth in SEQ ID NO: 2 comprises the N-terminal 183 amino acids of the human wild-type TNFR1 (i.e amino acids 1 to 183 of SEQ ID NO: 3), followed by a novel 45 amino acid sequence (i.e corresponding to amino acid numbers 184 to 228 of SEQ ID NO: 2).

The method of the invention may comprise detecting in a sample from the patient the presence or absence of a variant of the TNFR1 isoform, which comprises a sequence having at least 95%, preferably at least 96%, at least 97%, at least 98% or at least 99% homology to SEQ ID NO: 2 based on amino acid identity over the entire length of SEQ ID NO: 2. Methods of determining homology are described below. The variant preferably comprises amino acids 184 to 228 of SEQ ID NO: 2.

The phrase “detecting the presence or absence of” typically means determining whether or not the SNP rs1800693 or a polymorphism in linkage disequilibrium with the SNP is present in the patient’s genome; or determining whether or not the TNFR1 isoform or a variant thereof is present in the patient’s proteome.

The presence of the SNP, one or more polymorphisms, the isoform and/or a variant thereof (in any combination) is indicative that the patient is not suitable (i.e. unsuitable) for anti-TNF therapy. A patient producing the soluble TNFR1 isoform will inherently have some inhibition or antagonism of TNF signalling. Further inhibition or antagonism using anti-TNF therapy will typically lead to the side effects mentioned above, such as the induction or aggravation of MS or MS-like symptoms. These are discussed in more detail below.

The absence of the SNP, one or more polymorphisms, the isoform and/or a variant thereof (in any combination) is indicative that the patient is suitable for anti-TNF therapy. A patient that is not producing the soluble TNFR1 isoform will not inherently have some inhibition or antagonism of TNF signalling. Inhibition or antagonism of TNF signalling using anti-TNF

therapy will typically not lead to the side effects mentioned above, such as the induction or aggravation of MS or MS-like symptoms. These are discussed in more detail below.

As outlined above, polymorphisms include SNPs, microsatellite or repeat polymorphisms, insertion polymorphisms and deletion polymorphisms. Detecting the presence or absence of a SNP means genotyping the SNP or typing the nucleotide(s) present in the genome for the SNP. Typically, the nucleotide present at the same position on both homologous chromosomes will be determined. A patient may therefore be determined to be homozygous for a first allele, heterozygous or homozygous for a second allele of the SNP. When the polymorphism is a microsatellite or repeat sequence, typically the method will involve determining the number of repeats.

Methods for detecting the presence or absence of the SNP, polymorphism in linkage disequilibrium, isoform and/or variant in accordance with the invention are well known in the art. Polymorphisms, such as the SNP or polymorphism in linkage disequilibrium, are typically detected by directly determining the presence of the polymorphic sequence in a polynucleotide of the patient. Such a polynucleotide is typically genomic DNA, mRNA or cDNA. The polymorphism may be detected by any suitable method such as those mentioned below.

Methods for detecting proteins, such as the isoform or a variant thereof, include, but are not limited to, protein immunostaining microscopy, protein immunoprecipitation, immunoelectrophoresis, immunoblotting, bicinchoninic acid (BCA) Protein Assay, Western blotting, spectrophotometry and enzyme assays. Any of these methods may be used in the invention.

Detection of the polymorphism, isoform and/or variant may comprise contacting a polynucleotide or protein in a sample from the patient with a specific binding agent for the polymorphism, isoform or variant and determining whether the agent binds to the polynucleotide or protein, wherein binding of the agent indicates the presence of the polymorphism, isoform or variant, and lack of binding of the agent indicates the absence of the polymorphism, isoform or variant.

A specific binding agent is an agent that binds with preferential or high affinity to the SNP, polymorphism, isoform or variant, but does not bind or binds with only low affinity to other polynucleotides or proteins. The specific binding agent may be a probe or primer. The probe may be a protein (such as an antibody as described below) or an oligonucleotide. The probe may be labelled or may be capable of being labelled indirectly. The binding of the probe to the polynucleotide or protein may be used to immobilise either the probe or the polynucleotide or protein.

Generally in the method, the SNP, polymorphism, isoform or variant can be detected by determining the binding of the agent to the polymorphic polynucleotide or protein of the patient. However in one embodiment the agent is also able to bind the corresponding wild-type sequence, for example by binding the nucleotides or amino acids which flank the variant position, although
5 the manner of binding to the wild-type sequence will be detectably different to the binding of a polynucleotide or protein containing the polymorphism.

The method may be based on an oligonucleotide ligation assay in which two oligonucleotide probes are used. These probes bind to adjacent areas on the polynucleotide that contains the polymorphism, allowing after binding the two probes to be ligated together by an
10 appropriate ligase enzyme. However the presence of a single mismatch within one of the probes may disrupt binding and ligation. Thus ligated probes will only occur with a polynucleotide that contains the polymorphism, and therefore the detection of the ligated product may be used to determine the presence of the polymorphism.

In one embodiment the probe is used in a heteroduplex analysis based system. In such a
15 system when the probe is bound to a polynucleotide sequence containing the polymorphism it forms a heteroduplex at the site where the polymorphism occurs and hence does not form a double strand structure. Such a heteroduplex structure can be detected by the use of a single or double strand specific enzyme. Typically the probe is an RNA probe, the heteroduplex region is cleaved using RNAase H and the polymorphism is detected by detecting the cleavage products.

20 The method may be based on fluorescent chemical cleavage mismatch analysis which is described for example in PCR Methods and Applications 3, 268-71 (1994) and Proc. Natl. Acad. Sci. 85, 4397-4401 (1998).

In one embodiment a PCR primer is used that primes a PCR reaction only if it binds a polynucleotide containing the polymorphism, for example a sequence-specific PCR system, and
25 the presence of the polymorphism may be determined by detecting the PCR product. Preferably the region of the primer that is complementary to the polymorphism is at or near the 3' end of the primer. The presence of the polymorphism may be determined using a fluorescent dye and quenching agent-based PCR assay such as the Taqman PCR detection system.

The specific binding agent may be capable of specifically binding the amino acid
30 sequence encoded by a polymorphic sequence, i.e the TNFR1 isoform as set forth in SEQ ID NO: 2, and not the wild-type TNFR1 as set out in SEQ ID NO: 3. For example, the agent may be an antibody or antibody fragment. The antibody is preferably an antibody of the invention as described in more detail below. A detector antibody is an antibody that is specific for one polymorphism but does not bind to any other polymorphism. Detector antibodies are for

example useful in purification, isolation or screening methods involving immunoprecipitation techniques.

The detection method may be based on an ELISA system. An ELISA system is disclosed in the Examples. The method may be an RFLP based system. This can be used if the presence of the polymorphism in the polynucleotide creates or destroys a restriction site that is recognised by a restriction enzyme.

The presence of the polymorphism may be determined based on the change that the presence of the polymorphism makes to the mobility of the polynucleotide or protein during gel electrophoresis. In the case of a polynucleotide, single-stranded conformation polymorphism (SSCP) or denaturing gradient gel electrophoresis (DDGE) analysis may be used. In another method of detecting the polymorphism, a polynucleotide comprising the polymorphic region is sequenced across the region that contains the polymorphism to determine the presence of the polymorphism.

The presence of the polymorphism may be detected by means of fluorescence resonance energy transfer (FRET). In particular, the polymorphism may be detected by means of a dual hybridisation probe system. This method involves the use of two oligonucleotide probes that are located close to each other and that are complementary to an internal segment of a target polynucleotide of interest, where each of the two probes is labelled with a fluorophore. Any suitable fluorescent label or dye may be used as the fluorophore, such that the emission wavelength of the fluorophore on one probe (the donor) overlaps the excitation wavelength of the fluorophore on the second probe (the acceptor). A typical donor fluorophore is fluorescein (FAM), and typical acceptor fluorophores include Texas red, rhodamine, LC-640, LC-705 and cyanine 5 (Cy5).

In order for fluorescence resonance energy transfer to take place, the two fluorophores need to come into close proximity on hybridisation of both probes to the target. When the donor fluorophore is excited with an appropriate wavelength of light, the emission spectrum energy is transferred to the fluorophore on the acceptor probe resulting in its fluorescence. Therefore, detection of this wavelength of light, during excitation at the wavelength appropriate for the donor fluorophore, indicates hybridisation and close association of the fluorophores on the two probes. Each probe may be labelled with a fluorophore at one end such that the probe located upstream (5') is labelled at its 3' end, and the probe located downstream (3') is labelled at its 5' end. The gap between the two probes when bound to the target sequence may be from 1 to 20 nucleotides, preferably from 1 to 17 nucleotides, more preferably from 1 to 10 nucleotides, such as a gap of 1, 2, 4, 6, 8 or 10 nucleotides.

The first of the two probes may be designed to bind to a conserved sequence of the gene adjacent to a polymorphism and the second probe may be designed to bind to a region including one or more polymorphisms. Polymorphisms within the sequence of the gene targeted by the second probe can be detected by measuring the change in melting temperature caused by the resulting base mismatches. The extent of the change in the melting temperature will be dependent on the number and base types involved in the nucleotide polymorphisms.

Polymorphism typing may also be performed using a primer extension technique. In this technique, the target region surrounding the polymorphic site is copied or amplified for example using PCR. A single base sequencing reaction is then performed using a primer that anneals one base away from the polymorphic site (allele-specific nucleotide incorporation). The primer extension product is then detected to determine the nucleotide present at the polymorphic site. There are several ways in which the extension product can be detected. In one detection method for example, fluorescently labelled dideoxynucleotide terminators are used to stop the extension reaction at the polymorphic site. Alternatively, mass-modified dideoxynucleotide terminators are used and the primer extension products are detected using mass spectrometry. By specifically labelling one or more of the terminators, the sequence of the extended primer, and hence the nucleotide present at the polymorphic site can be deduced. More than one reaction product can be analysed per reaction and consequently the nucleotide present on both homologous chromosomes can be determined if more than one terminator is specifically labelled.

The invention further provides primers or probes that may be used in the detection of the SNP, polymorphism in linkage disequilibrium, isoform or variant. These probes and primers may be used for determining whether or not a patient is suitable for anti-TNF therapy in accordance with the invention. Polynucleotide sequences disclosed herein may also be used in the design of primers for primer extension reactions to detect the SNP rs1800693.

Such primers, probes and other polynucleotide fragments will preferably be at least 10, preferably at least 15 or at least 20, for example at least 25, at least 30 or at least 40 nucleotides in length. They will typically be up to 40, 50, 60, 70, 100 or 150 nucleotides in length. Probes and fragments can be longer than 150 nucleotides in length, for example up to 200, 300, 400, 500, 600, 700 nucleotides in length, or even up to a few nucleotides, such as five or ten nucleotides, short of a full length polynucleotide sequence of the invention.

Primers and probes for genotyping SNP rs1800693 may be designed using any suitable design software known in the art using regions of the sequence set forth in SEQ ID NO: 1. Homologues of these polynucleotide sequences would also be suitable for designing primers and

probes. Such homologues typically have at least 70% homology, preferably at least 80, at least 90%, at least 95%, at least 97% or at least 99% homology, for example over a region of at least 15, 20, 30, 100 more contiguous nucleotides. The homology may be calculated on the basis of nucleotide identity (sometimes referred to as "hard homology"). Methods for determining
5 homology are described below.

The polynucleotides, such as primers or probes, may be present in an isolated or substantially purified form. They may be mixed with carriers or diluents that will not interfere with their intended use and still be regarded as substantially isolated. They may also be in a substantially purified form, in which case they will generally comprise at least 90%, e.g. at least
10 95%, at least 98% or at least 99%, of polynucleotides of the preparation.

Also provided is a kit that comprises means for typing one or more of the polymorphisms defined herein, such as SNP rs1800693. In particular, such means may include a specific binding agent, probe, primer, pair or combination of primers, or antibody, including an antibody fragment, as defined herein which is capable of detecting or aiding detection of the
15 polymorphisms defined herein. The primer or pair or combination of primers may be sequence specific primers that only cause PCR amplification of a polynucleotide sequence comprising the polymorphism to be detected, as discussed herein. The primer or pair of primers may alternatively not be specific for the polymorphic nucleotide, but may be specific for the region upstream (5') and/or downstream (3'). These primers allow the region encompassing the
20 polymorphic nucleotide to be copied. A kit suitable for use in the primer-extension technique may specifically include labelled dideoxynucleotide triphosphates (ddNTPs). These may for example be fluorescently labelled or mass modified to enable detection of the extension product and consequently determination of the nucleotide present at the polymorphic position.

The kit may also comprise a specific binding agent, probe, primer, pair or combination of
25 primers, or antibody that is capable of detecting the presence or absence of the polymorphism. The kit may further comprise buffers or aqueous solutions.

The kit may additionally comprise one or more other reagents or instruments that enable any of the embodiments of the method mentioned above to be carried out. Such reagents or instruments may include one or more of the following: a means to detect the binding of the agent
30 to the polymorphism, a detectable label such as a fluorescent label, an enzyme able to act on a polynucleotide, typically a polymerase, restriction enzyme, ligase, RNase H or an enzyme which can attach a label to a polynucleotide, suitable buffer(s) or aqueous solutions for enzyme reagents, PCR primers which bind to regions flanking the polymorphism as discussed herein, a positive and/or negative control, a gel electrophoresis apparatus, a means to isolate DNA from

sample, a means to obtain a sample from the individual, such as swab or an instrument comprising a needle, or a support comprising wells on which detection reactions can be carried out. The kit may be, or include, an array such as a polynucleotide array comprising the specific binding agent, preferably a probe, of the invention. The kit typically includes a set of

5 instructions for using the kit.

Also provided is a patient identified as suitable for, or determined as being suitable for, anti-TNF therapy using the method of the invention. Also provided is a patient identified as not suitable (i.e. unsuitable) for, or determined as being not suitable for, anti-TNF therapy using the method of the invention.

10

Method of treating autoimmune diseases

The present invention relates to a method of treating or preventing an autoimmune disease in a patient in need thereof. The method comprises determining whether or not the patient is suitable for anti-TNF therapy using the method of the invention. If the patient is

15 suitable for anti-TNF therapy, the method comprises administering an anti-TNF therapy to the patient and thereby treating or preventing the autoimmune disease.

The invention also provides a method of treating or preventing an autoimmune disease in a patient identified as suitable for, or determined as being suitable for, anti-TNF therapy using the method of the invention. The method comprises administering an anti-TNF therapy to the

20 patient and thereby treating or preventing the autoimmune disease.

The autoimmune disease is preferably rheumatoid arthritis (RA), psoriatic arthritis, psoriasis, ankylosing spondylitis, ulcerative colitis or Crohn's disease.

Suitable anti-TNF therapies and conditions to be treated are described below. The dose of anti-TNF therapy to be used in accordance with the invention will depend upon the nature of the specific therapy. A suitable dose can be determined by a skilled practitioner based on his

25 common general knowledge, taking into account, for example, the regime and dose that would be used for *in vivo* treatment using that therapy. For example, a suitable dose may be selected to reflect the level of a therapeutic agent that would be present in the blood circulatory system of a patient after *in vivo* administration.

30 Tumor necrosis factor α (TNF α) is a cytokine produced by monocytes and macrophages. For example, TNF α mediates the immune response by increasing the transport of white blood cells to sites of inflammation, and through additional molecular mechanisms which initiate and amplify inflammation. Biological activities that are attributed to TNF α include induction of proinflammatory cytokines such as IL-1 and IL-6; enhancement of leukocyte movement or

migration from the blood vessels into the tissues by increasing the permeability of endothelial layer of blood vessels; and increasing the release of adhesion molecules. Because of its key role in the regulation of inflammation, TNF α is a key target for a number of therapies, particularly those directed at preventing or reducing inflammation or autoimmune diseases.

5 By an anti-TNF therapy is meant a therapy or treatment that is directed against TNF, preferably TNF α . For example, an anti-TNF therapy may prevent or reduce the production or release of TNF α . An anti-TNF therapy may prevent or reduce the activity of TNF α . An anti-TNF therapy may involve the use of a molecule that specifically binds to TNF α . Such a molecule may prevent the activity of TNF α . An anti-TNF therapy may be capable of
10 neutralising or removing extracellular TNF α and/or transmembrane TNF α and/or receptor bound TNF α . An anti-TNF therapy may utilise a molecule that binds to soluble TNF α (e.g. free floating in the blood) and/or transmembrane TNF α (that may be present at the surface of T cells and other immune cells). An anti-TNF therapy may utilise a molecule that inhibits or prevents effective binding of TNF α with its receptors. For example, in one embodiment the anti-TNF
15 therapy neutralises, binds to or removes membrane bound TNF α . An anti-TNF therapy is preferably specific to TNF α , that is it preferably acts exclusively on TNF α , or acts on TNF α in preference to other molecules. For example, an anti-TNF therapy preferably acts on TNF α , but not TNF β , even though the two types of TNF can utilise the same receptors.

In accordance with the present invention, the anti-TNF therapy preferably utilizes a
20 molecule that is capable of binding to, and neutralizing, TNF α . Preferred molecules are capable of neutralizing all forms of TNF α , for example extracellular TNF α , transmembrane TNF α and receptor-bound TNF α . In particular, preferred anti-TNF therapies for use in accordance with the present invention are capable of neutralizing receptor-bound TNF α . Anti-TNF α therapies for use in accordance with the present invention preferably have the capacity of lysing cells involved in
25 the inflammatory process.

Accordingly, a preferred group of anti-TNF therapies for use in accordance with this invention are neutralising antibodies to TNF (or anti-TNF antibodies), preferably neutralising antibodies to TNF α (or anti-TNF α antibodies). Examples of neutralising antibodies to TNF include infliximab, adalimumab, certolizumab and golimumab.

30 Infliximab and adalimumab are examples of antibodies capable of neutralising all forms (extracellular, transmembrane, and receptor-bound) of TNF α . Accordingly, in a preferred aspect of the invention, the anti-TNF therapy comprises, consists essentially or consists of the administration of infliximab and/or adalimumab.

Infliximab (sold under the brand name Remicade®) is a drug used to treat inflammatory and autoimmune diseases. Infliximab is a chimeric monoclonal antibody comprising murine binding VK and VH domains and human constant Fc domains.

5 Infliximab neutralizes the biological activity of TNF α by binding with high affinity to the soluble (free floating in the blood) and transmembrane (located on the outer membranes of T cells and similar immune cells) forms of TNF α and inhibits or prevents the effective binding of TNF α with its receptors. Infliximab has high specificity for TNF α , and does not neutralize TNF β , although TNF β utilizes the same receptors as TNF α . Infliximab has been approved by the U.S. Food and Drug Administration for the treatment of, for example, psoriasis, pediatric
10 Crohn's disease, ankylosing spondylitis, Crohn's disease, psoriatic arthritis, rheumatoid arthritis, and ulcerative colitis.

Adalimumab (sold under the brand name Humira®) also binds to TNF α , preventing it from activating TNF receptors. Adalimumab was constructed from a fully human monoclonal antibody, while infliximab is a mouse-human chimeric antibody. Adalimumab has been
15 approved by the United States Food and Drug Administration (FDA) for the treatment of, for example, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and Crohn's disease, and an application has been made to the FDA for approval in the treatment of plaque psoriasis.

In another preferred aspect of the invention, the anti-TNF therapy comprises, consists essentially or consists of the administration of a neutralising antibody to a TNF receptor.
20 Typically, the neutralising antibody to the TNF receptor is a neutralising antibody to the TNFR1 receptor, for example, the human wild-type TNFR1 receptor as set forth in SEQ ID NO:3. Examples of neutralising antibodies to the TNFR1 receptor include, but are not limited to, atosab. Atosab binds to amino acids 1 to 70 of human TNFR1 and selectively inhibits TNFR1-mediated signal transduction (see Zettlitz *et al.* (2010) MABs 2:6, 639-647).

25 In another preferred aspect of the invention, the anti-TNF therapy comprises, consists essentially or consists of the administration of soluble TNF receptor chimeras. Examples of soluble TNF receptor chimeras include, but are not limited to, lenercept and etanercept.

Etanercept binds to TNF α and decreases its role in diseases involving excess inflammation in humans and other animals, including autoimmune diseases such as ankylosing
30 spondylitis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, rheumatoid arthritis, and, potentially, in a variety of other diseases mediated by excess TNF α .

In another preferred aspect of the invention, the anti-TNF therapy comprises, consists essentially or consists of the administration of modulators of TNF production. Examples of modulators of TNF production include pentoxifylline or bupropion.

The method may be for treating the disease. In the case of treating, the patient typically
5 has the disease, i.e. has been diagnosed as having the disease, or is suspected as having the disease, i.e. shows the symptoms of the disease. As used herein, the term “treating” includes any of following: the prevention of the disease or of one or more symptoms associated with the disease; a reduction or prevention of the development or progression of the disease or symptoms; and the reduction or elimination of an existing disease or symptoms.

10 The method may be for preventing the disease. In this embodiment, the patient can be asymptomatic. The patient can have a genetic predisposition to the autoimmune disease. The patient may have one or more family member(s) with an autoimmune disease. As used herein, the term “preventing” includes the prevention of the onset of the disease or of one or more symptoms associated with the disease.

15 Therapy and prevention includes, but is not limited to, preventing or eliciting an effective anti-TNF α response and/or preventing, alleviating, reducing, curing or at least partially arresting symptoms and/or complications resulting from or associated with TNF α . When provided therapeutically, the therapy is typically provided at or shortly after the onset of a symptom of disease. Such therapeutic administration is typically to prevent or ameliorate the progression of,
20 or a symptom of the disease or to reduce the severity of such a symptom or disease. When provided prophylactically, the treatment is typically provided before the onset of a symptom of disease. Such prophylactic administration is typically to prevent the onset of symptoms of the disease.

Specific routes, dosages and methods of administration of anti-TNF therapies may be
25 routinely determined by the medical practitioner. Typically, a therapeutically effective or a prophylactically effective amount of the anti-TNF therapy is administered to the patient. A prophylactically effective amount is an amount which prevents the onset of one or more symptoms of the disease. A therapeutically effective amount of the compound is an amount effective to ameliorate one or more symptoms of the disease.

30 For example, infliximab is currently sold under the brand name Remicade®. Remicade® is sold for administration by intravenous infusion, typically at 2-month intervals and at a clinic or hospital. The recommended dose of Remicade® for treatment of rheumatoid arthritis is 3 mg/kg, followed by additional similar doses at 2 and 6 weeks after the first infusion and then every 8 weeks thereafter. For Crohn’s disease, ankylosing spondylitis, psoriatic arthritis, psoriasis or

ulcerative colitis, the recommended dose of Remicade® is 5 mg/kg over a similar administration schedule.

Adalimumab is currently sold under the brand name Humira®. Humira® is marketed in both preloaded 0.8ml syringes and in preloaded pen devices, both for injection subcutaneously, typically by the patient at home. In accordance with the present invention, an anti-TNF α therapy may be administered via any suitable route in any suitable dose and in any suitable administration regime. The recommended dose of Humira® for adult patients with rheumatoid arthritis, psoriatic arthritis or ankylosing spondylitis is 40 mg administered every other week. The recommended Humira® dose regimen for adult patients with Crohn's disease is 160 mg initially at Week 0 (dose can be administered as four injections in one day or as two injections per day for two consecutive days), 80 mg at Week 2, followed by a maintenance dose of 40 mg every other week beginning at Week 4.

An anti-TNF therapeutic agent may be employed alone as part of a composition, such as but not limited to a pharmaceutical composition or a vaccine composition or an immunotherapeutic composition to prevent and/or treat a condition associated with TNF α activity.

An anti-TNF therapy may be used in combination with one or more other therapies intended to treat the same patient. By a combination is meant that the therapies may be administered simultaneously, in a combined or separate form, to a patient. The therapies may be administered separately or sequentially to a patient as part of the same therapeutic regimen. For example, an anti-TNF therapy may be used in combination with another therapy intended to treat an inflammatory or autoimmune disease. The other therapy may be a general therapy aimed at treating or improving the condition of a patient with an inflammatory or autoimmune disease. For example, treatment with methotrexate, glucocorticoids, salicylates, nonsteroidal anti-inflammatory drugs (NSAIDs), analgesics, other DMARDs, aminosalicylates, corticosteroids, and/or immunomodulatory agents (e.g., 6-mercaptopurine and azathioprine) may be combined with an anti-TNF therapy. For example, infliximab is commonly used in combination with methotrexate in the treatment of rheumatoid arthritis.

The other therapy may be a specific treatment directed at the particular disease or condition suffered by the patient, or directed at a particular symptom of such a disease or condition. For example, where the patient has rheumatoid arthritis, the treatment may comprise treatment with an anti-TNF therapy, and also treatment with a further therapy specifically intended to treat, prevent or reduce the symptoms of the rheumatoid arthritis.

Method of identifying whether or not a patient is at risk of developing MS or has MS

The invention further provides a method of identifying whether or not a patient is at risk of developing multiple sclerosis (MS) or MS-like symptoms or whether or not a patient has MS. The method comprises detecting in a sample from the patient the presence or absence of the TNFR1 isoform, and/or a variant of the TNFR1 isoform. The method of the invention may therefore concern the diagnosis or prognosis of MS.

The phrase “at risk of developing MS or MS-like symptoms” typically means that the patient is significantly likely to develop MS or develop MS-like symptoms. For instance, MS may lead to the demyelination of axons of the brain and/or spinal cord, leading to symptoms in a patient such as loss of sensitivity or tingling, pricking or numbness (hypoesthesia and paresthesia), muscle weakness, clonus, muscle spasms, or difficulty in moving; difficulties with coordination and balance (ataxia); problems in speech (dysarthria) or swallowing (dysphagia), visual problems (nystagmus, optic neuritis including phosphenes, or diplopia), fatigue, acute or chronic pain, and bladder and bowel difficulties. Cognitive impairment of varying degrees and emotional symptoms of depression or unstable mood are also common. The phrase “has MS” typically means that the patient already has developed MS or MS-like symptoms, such as those outlined above.

The presence of the TNFR1 isoform or variant thereof indicates that the patient is at risk of developing MS or MS-like symptoms or has MS. The absence of the TNFR1 isoform or variant thereof indicates that the patient is not at risk of developing MS or MS-like symptoms or does not have MS. Methods of detecting the presence or absence of the TNFR1 isoform or variant thereof are described above. Any of the embodiments discussed above with reference to the method of determining the suitability of a patient for anti-TNF therapy equally apply here.

The patient may be any of those discussed above. The patient is preferably undergoing anti-TNF therapy, such as anti-TNF α therapy. As discussed above, anti-TNF therapy can induce or exacerbate MS and MS-like symptoms. Anti-TNF therapy is also described above.

The invention also relates to a method of identifying whether or not a patient is at risk of developing multiple sclerosis (MS) or MS-like symptoms or whether or not a patient has MS, comprising detecting in a sample from the patient the presence or absence of a soluble isoform of TNFR1 and thereby determining whether or not the patient is at risk of developing MS or MS-like symptoms or whether or not the patient has MS. This method therefore relates to determining the presence or absence of different soluble isoforms of TNFR1 from the one identified by the inventors. The soluble isoform typically inhibits or antagonises TNF signaling.

Methods for measuring this are known in the art. One method is disclosed in the Example. All of the embodiments discussed above equally apply here.

The presence of the soluble isoform of TNFR1 indicates that the patient is at risk of developing MS or MS-like symptoms or that the patient has MS. The absence of the soluble
5 isoform of TNFR1 indicates that the patient is not at risk of developing MS or MS-like symptoms or that the patient does not have MS.

Polypeptide TNF antagonists, polynucleotides and therapy

The invention provides a polypeptide TNF antagonist which comprises the sequence of
10 the TNFR1 isoform as set forth in SEQ ID NO:2 or a sequence which has at least 85%, preferably at least 90%, at least 95%, at least 98% or at least 99%, homology to SEQ ID NO:2 based on amino acid identity over its entire sequence and which retains its ability to bind TNF, preferably TNF α .

The ability of polypeptide to bind TNF can be determined using any method. Binding
15 assays for TNF are known in the art. For instance, dissociation constants may be measured using radioactively labelled TNF and/or surface plasmon resonance techniques (see Grell *et al.*, 1998, *Eur. J. Immunol.* and the Examples)

Preferably, the polypeptide TNF antagonist comprises amino acids 184 to 228 as set forth
20 in SEQ ID NO:2 (i.e the novel amino acid tail of the soluble TNFR1 receptor identified by the inventors).

The above mentioned homology is calculated on the basis of amino acid identity (sometimes referred to as "hard homology"). The UWGCG Package provides programs including GAP, BESTFIT, COMPARE, ALIGN and PILEUP that can be used to calculate homology or line up sequences (for example used on their default settings). The BLAST
25 algorithm can also be used to compare or line up two sequences, typically on its default settings. Software for performing a BLAST comparison of two sequences is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm is further described below. Similar publicly available tools for the alignment and comparison of sequences may be found on the European Bioinformatics Institute website
30 (<http://www.ebi.ac.uk>), for example the ALIGN and CLUSTALW programs.

A BLAST analysis is preferably used for calculating identity. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that

either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al.*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each
 5 sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of
 10 the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci. USA* 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90: 5873-5787. One
 15 measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two polynucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than
 20 about 0.01, and most preferably less than about 0.001.

The homologous sequences typically differ by at least 1, 2, 5, 10, 20 or more mutations (which may be substitutions, deletions or insertions of amino acids). These mutations may be measured across any of the regions mentioned above in relation to calculating identity. In the case of proteins as above, the substitutions are preferably conservative substitutions. These are
 25 defined according to the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar – uncharged	C S T M
		N Q
	Polar – charged	D E
		K R
AROMATIC		H F W Y

Any of the polypeptide TNF antagonists useful in the invention may be in the form of a dimer. Any of the polypeptides useful in the invention may further be chemically-modified to form a derivative. Derivatives include polypeptides that have lipid extensions or have been glycosylated. Derivatives also include polypeptides that have been detectably labelled. Detectably labelled polypeptides have been labelled with a labelling moiety that can be readily detected. Examples of labelling moieties include, but are not limited to, radioisotopes or radionucleotides, fluorophores such as green fluorescent protein (GFP), electron-dense reagents, quenchers of fluorescence, enzymes, affinity tags and epitope tags. Preferred radioisotopes include tritium and iodine. Affinity tags are labels that confer the ability to specifically bind a reagent onto the labelled molecule. Examples include, but are not limited to, biotin, histidine tags and glutathione-S-transferase (GST). Labels may be detected by, for example, spectroscopic, photochemical, radiochemical, biochemical, immunochemical or chemical methods that are known in the art.

Any of the polypeptides useful in the invention may also comprise additional amino acids or polypeptide sequences. Any of the polypeptides useful in the invention may comprise additional polypeptide sequences such that they form fusion proteins. The additional polypeptide sequences may be fused at the amino terminus, carboxy terminus or both the amino terminus and the carboxy terminus. Examples of fusion partners include, but are not limited to, GST, maltose binding protein, alkaline phosphatases, thiorexin, GFP, histidine tags and epitope tags (for example, Myc or FLAG). CCRL2 polypeptides may be fused to a GTP-binding protein (G protein)

The present invention also provides polynucleotide sequences which encode any of the polypeptide TNF antagonists of the invention. It is straightforward to generate polynucleotide sequences of the invention using standard techniques. The polynucleotide sequence encodes a construct comprising SEQ ID NO: 2 or a variant thereof. The variants of SEQ ID NO: 2 may be any of those discussed above. Preferably, the polynucleotide comprises a sequence encoding amino acids 184 to 228 as set forth in SEQ ID NO:2. The polynucleotide of the invention may comprise the nucleotide sequence set forth in SEQ ID NO:1 or a sequence which has at least 85%, preferably at least 90%, at least 95%, at least 98% or at least 99%, homology to SEQ ID NO:1 based on nucleotide identity over the entire sequence of SEQ ID NO: 1.

Polynucleotide sequences may be isolated and replicated using standard methods in the art. Chromosomal DNA may be extracted from a receptor producing organism, such as a human comprising SNP rs1800693. The gene encoding a TNF polypeptide antagonist of the invention

may be amplified using PCR involving specific primers. The amplified sequences may then be incorporated into a recombinant replicable vector such as a cloning vector. The vector may be used to replicate the polynucleotide in a compatible host cell. Thus polynucleotide sequences encoding the TNF polypeptide antagonist of the invention may be made by introducing a
5 polynucleotide encoding the TNF polypeptide antagonist of the invention into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under conditions which bring about replication of the vector. The vector may be recovered from the host cell. Suitable host cells for cloning of polynucleotides are known in the art and described in more detail below.

10 The polynucleotide sequence may be cloned into any suitable expression vector. In an expression vector, the polynucleotide sequence encoding a construct is typically operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell. Such expression vectors can be used to express a construct.

The term “operably linked” refers to a juxtaposition wherein the components described
15 are in a relationship permitting them to function in their intended manner. A control sequence “operably linked” to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. Multiple copies of the same or different polynucleotide may be introduced into the vector.

The expression vector may then be introduced into a suitable host cell. Thus, a construct
20 can be produced by inserting a polynucleotide sequence encoding a construct into an expression vector, introducing the vector into a compatible bacterial host cell, and growing the host cell under conditions which bring about expression of the polynucleotide sequence. The recombinantly-expressed construct may self-assemble into a pore in the host cell membrane. Alternatively, the recombinant construct produced in this manner may be isolated from the host
25 cell and inserted into another membrane. When producing an oligomeric pore comprising a construct of the invention and at least one different subunit, the construct and different subunits may be expressed separately in different host cells as described above, removed from the host cells and assembled into a pore in a separate membrane, such as a rabbit cell membrane.

The vectors may be for example, plasmid, virus or phage vectors provided with an origin
30 of replication, optionally a promoter for the expression of the said polynucleotide sequence and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene. Promoters and other expression regulation signals may be selected to be compatible with the host cell for which the expression vector is designed. A T7, *trc*, *lac*, *ara* or λ_L promoter is typically used.

The host cell typically expresses the construct at a high level. Host cells transformed with a polynucleotide sequence encoding a construct will be chosen to be compatible with the expression vector used to transform the cell. The host cell is typically bacterial and preferably *E. coli*. Any cell with a λ DE3 lysogen, for example C41 (DE3), BL21 (DE3), JM109 (DE3), B834
5 (DE3), TUNER, Origami and Origami B, can express a vector comprising the T7 promoter.

A polypeptide TNF antagonist of the invention, polynucleotide of the invention encoding said polypeptide TNF antagonist, or expression vector of the invention comprising said polynucleotide may be used in to treat or prevent an autoimmune condition in a patient in need thereof. Autoimmune conditions are described above, and include rheumatoid arthritis (RA),
10 psoriatic arthritis, psoriasis, ankylosing spondylitis, ulcerative colitis or Crohn's disease. Patients, treating and preventing are described above. The patient has preferably been identified as being suitable for, or has been determined as being suitable for, anti-TNF therapy in accordance with the invention.

The polypeptide, polynucleotide or expression vector may be administered to the patient
15 in order to treat the disease or prevent the disease. A therapeutically or prophylactically effective amount of the polypeptide, polynucleotide or vector is preferably administered to the patient. These concepts are defined above.

The polypeptide, polynucleotide or expression vector can be administered to the patient by any suitable means. The polypeptide, polynucleotide or expression vector can be
20 administered by enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intra-arterial, intramuscular, intraperitoneal, intraarticular, topical or other appropriate administration routes.

The formulation will depend upon factors such as the nature of the polypeptide, polynucleotide or expression vector and the disease to be treated. The polypeptide,
25 polynucleotide or expression vector may be administered in a variety of dosage forms. It may be administered orally (e.g. as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules), parenterally, subcutaneously, intravenously, intramuscularly, intrasternally, transdermally or by infusion techniques. The polypeptide, polynucleotide or expression vector may also be administered as a suppository. A physician will be able to
30 determine the required route of administration for each particular patient.

Typically, the polypeptide, polynucleotide or expression vector is formulated for use with a pharmaceutically acceptable carrier or diluent and this may be carried out using routine methods in the pharmaceutical art. The pharmaceutical carrier or diluent may be, for example, an isotonic solution. For example, solid oral forms may contain, together with the active

compound, diluents, e.g. lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents; e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone; disaggregating agents, e.g. starch, alginic acid, 5 alginates or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulations. Such pharmaceutical preparations may be manufactured in known manner, for example, by means of mixing, granulating, tableting, sugar-coating, or film coating processes.

10 Liquid dispersions for oral administration may be syrups, emulsions and suspensions. The syrups may contain as carriers, for example, saccharose or saccharose with glycerine and/or mannitol and/or sorbitol.

 Suspensions and emulsions may contain as carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. The 15 suspensions or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

 Solutions for intravenous or infusions may contain as carrier, for example, sterile water or preferably they may be in the form of sterile, aqueous, isotonic saline solutions.

20 For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1% to 2%.

 Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, 25 cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10% to 95% of active ingredient, preferably 25% to 70%. Where the pharmaceutical composition is lyophilised, the lyophilised material may be reconstituted prior to administration, e.g. a suspension. Reconstitution is preferably effected in buffer.

30 Capsules, tablets and pills for oral administration to a patient may be provided with an enteric coating comprising, for example, Eudragit "S", Eudragit "L", cellulose acetate, cellulose acetate phthalate or hydroxypropylmethyl cellulose.

 Pharmaceutical compositions suitable for delivery by needleless injection, for example, transdermally, may also be used.

A therapeutically or prophylactically effective amount of the compound is administered. The dose may be determined according to various parameters, especially according to the compound used; the age, weight and condition of the patient to be treated; the route of administration; and the required regimen. Again, a physician will be able to determine the required route of administration and dosage for any particular patient. A typical daily dose is from about 0.1 to 50mg per kg, preferably from about 0.1mg/kg to 10mg/kg of body weight, according to the activity of the specific inhibitor, the age, weight and conditions of the patient to be treated, the type and severity of the disease and the frequency and route of administration. Preferably, daily dosage levels are from 5mg to 2g.

A polynucleotide of the invention may be administered. Preferably, the polynucleotide, such as RNA or DNA, in particular DNA, is provided in the form of an expression vector, which may be expressed in the cells of the patient to be treated. The polynucleotides may be naked nucleotide sequences or be in combination with cationic lipids, polymers or targeting systems. The polynucleotides may be delivered by any available technique. For example, the polynucleotide may be introduced by needle injection, preferably intradermally, subcutaneously or intramuscularly. Alternatively, the polynucleotide may be delivered directly across the skin using a polynucleotide delivery device such as particle-mediated gene delivery. The polynucleotide may be administered topically to the skin, or to mucosal surfaces for example by intranasal, oral, intravaginal or intrarectal administration.

Uptake of polynucleotide constructs may be enhanced by several known transfection techniques, for example those including the use of transfection agents. Examples of these agents includes cationic agents, for example, calcium phosphate and DEAE-Dextran and lipofectants, for example, lipofectam and transfectam. The dosage of the nucleic acid to be administered can be altered. Typically the polynucleotide is administered in the range of 1pg to 1mg, preferably to 1pg to 10µg nucleic acid for particle mediated gene delivery and 10µg to 1mg for other routes.

Antibodies of the invention

The invention provides antibodies which specifically recognise an epitope within a polypeptide TNF antagonist of the invention. Preferably, the antibody recognises an epitope within amino acids 184 to 228 of SEQ ID NO:2 or the corresponding region in the variant thereof. Preferably, the antibody recognises an epitope within the TNFR1 isoform as set forth in SEQ ID NO:2 but not an epitope within the wild-type TNFR1 polypeptide as set forth in SEQ ID NO:3. Preferably, the antibody stimulates TNF signalling *in vivo*. The antibody stimulates TNF signalling by neutralising the TNF antagonistic effect of the soluble TNFR1 isoform. The

ability of an antibody of the invention to stimulate TNF signalling *in vivo* can be determined by administering the antibody to a patient and investigating the effect on TNF signalling. Methods for doing this are known in the art. The ability of an antibody of the invention to stimulate TNF signalling *in vivo* can also be predicted from *in vitro* experiments.

5 An antibody, or other compound, “specifically binds” to a polypeptide when it binds with preferential or high affinity to the protein for which it is specific (for example, the TNFR1 isoform set forth in SEQ ID NO:2) but does not substantially bind, not bind or binds with only low affinity to other polypeptides (for example, the wild-type TNFR1 polypeptide set forth in SEQ ID NO:3). A variety of protocols for competitive binding or immunoradiometric assays to
10 determine the specific binding capability of an antibody are well known in the art (see for example Maddox *et al*, J. Exp. Med. 158, 1211-1226, 1993). Such immunoassays typically involve the formation of complexes between the specific protein and its antibody and the measurement of complex formation.

 The antibody may be a monoclonal antibody or a polyclonal antibody. For the purposes
15 of this invention, the term “antibody”, unless specified to the contrary, includes fragments that bind a polypeptide of the invention. Such fragments include Fv, F(ab’) and F(ab’)₂ fragments, as well as single chain antibodies. Furthermore, the antibodies and fragment thereof may be chimeric antibodies, CDR-grafted antibodies or humanised antibodies.

 Antibodies may be used in a method for detecting the TNFR1 isoform of the invention in
20 a biological sample (such as any such sample mentioned herein), which method comprises:

- I providing an antibody of the invention;
- II incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and
- III determining whether antibody-antigen complex comprising said antibody is formed.

25 Antibodies of the invention can be produced by any suitable method. Means for preparing and characterising antibodies are well known in the art, see for example Harlow and Lane (1988) “Antibodies: A Laboratory Manual”, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. For example, an antibody may be produced by raising an antibody in a host animal against the whole polypeptide or a fragment thereof, for example an antigenic epitope
30 thereof, hereinafter the “immunogen”. The fragment may be any of the fragments mentioned herein (typically at least 10 or at least 15 amino acids long).

 A method for producing a polyclonal antibody comprises immunising a suitable host animal, for example an experimental animal, with the immunogen and isolating immunoglobulins from the animal’s serum. The animal may therefore be inoculated with the

immunogen, blood subsequently removed from the animal and the IgG fraction purified. A method for producing a monoclonal antibody comprises immortalising cells which produce the desired antibody. Hybridoma cells may be produced by fusing spleen cells from an inoculated experimental animal with tumour cells (Kohler and Milstein (1975) *Nature* **256**, 495-497).

5 An immortalized cell producing the desired antibody may be selected by a conventional procedure. The hybridomas may be grown in culture or injected intraperitoneally for formation of ascites fluid or into the blood stream of an allogenic host or immunocompromised host. Human antibody may be prepared by *in vitro* immunisation of human lymphocytes, followed by transformation of the lymphocytes with Epstein-Barr virus.

10 For the production of both monoclonal and polyclonal antibodies, the experimental animal is suitably a goat, rabbit, rat, mouse, guinea pig, chicken, sheep or horse. If desired, the immunogen may be administered as a conjugate in which the immunogen is coupled, for example via a side chain of one of the amino acid residues, to a suitable carrier. The carrier molecule is typically a physiologically acceptable carrier. The antibody obtained may be
15 isolated and, if desired, purified.

The antibodies of the invention may be used in a method of determining whether or not a patient is suitable for anti-TNF therapy as described above. The antibodies of the invention may alternatively be used in a method of identifying whether or not a patient is at risk of developing MS or MS-like symptoms or whether or not a patient has MS as described above. In each case, the
20 antibody of the invention is used to specifically detect in a sample from the patient the presence or absence of the TNFR1 isoform as set forth in SEQ ID NO:2 or a variant of said TNFR1 isoform which comprises a sequence having at least 95%, preferably at least 96%, at least 97%, at least 98% or at least 99%, homology to SEQ ID NO:2 based on amino acid identity over the entire length of SEQ ID NO: 2.

25 The antibodies of the invention may be used to prevent a patient undergoing anti-TNF therapy from developing MS or MS-like symptoms. The antibodies of the invention may be administered to the patient as discussed above. The antibodies neutralise the antagonistic effects of the soluble TNFR1 isoform and thereby prevent the development of MS or MS-like symptoms. Patients in need of such therapy can be identified as discussed above. The patient is
30 preferably undergoing anti-TNF therapy. A prophylactically effective amount of the antibody is administered to the patient. A prophylactically effective amount is an amount which prevents the onset of MS or of one or more symptoms of MS.

The invention also relates to a method of treating or preventing an autoimmune disease in a patient in need thereof. The method comprises determining whether or not the patient is

suitable for anti-TNF therapy using a method of the invention. If the patient is not suitable for anti-TNF therapy, the method comprises administering to the patient a prophylactically effective amount of an antibody of the invention in conjunction with anti-TNF therapy and thereby treating or preventing the autoimmune disease. The antibody and anti-TNF therapy may be administered simultaneously, separately or sequentially. They may be combined or mixed in the same composition administered to the patient. The antibodies neutralise the antagonistic effects of the soluble TNFR1 isoform present in the patient and thereby prevent the anti-TNF therapy from inducing or exacerbating MS or MS-like symptoms. In other words, the antibody of the invention mitigates the effects of the soluble isoform of TNFR1.

The invention also provides a method of treating or preventing an autoimmune disease in a patient identified as not suitable for, or determined as being not suitable for, anti-TNF therapy using the method of the invention. The method comprises administering to the patient a prophylactically effective amount of an antibody of the invention in conjunction with anti-TNF therapy to the patient and thereby treating or preventing the autoimmune disease. Any of the embodiments discussed above equally apply here.

The invention is illustrated by the following Example:

Example

1. Methods

Statistical analysis of disease association. SNP genotype data from the UK cohort of the IMSGC and WTCCC2 MS GWAS study⁴ were used to localize primary association signals. SNP and sample exclusion lists were as in the original study. SNPs that were not in Hardy-Weinberg equilibrium (HWE) within either control cohort ($P < 0.001$) were also excluded. Inferred haplotypes for 379 samples of European ancestry (EUR) from the March 2012 release of the 1000 Genomes Project were used for analysis of haplotype structure and imputation of untyped variants. Only SNPs typed successfully in case and control cohorts were used for imputation with IMPUTE Version 2^{Ref12}, removing SNPs whose minor allele frequencies (MAF) differed by more than 0.1 between control cohorts. Association analysis with a logistic model accounting for uncertainty in genotype imputation, including gender as a covariate, was carried out using SNPTTEST²⁸, on SNPs with MAF > 0.01 that had an imputation information score greater than 0.9. A Bayesian, additive test was used with the score method to measure evidence

for association and we report the Bayes factor comparing the models of association and no association. Default priors of SNPTEST were assumed for the analysis, namely the standard normal for the intercept and a normal distribution with mean = 0 and standard deviation = 0.2 for the genotypes (which were coded as 0, 1 or 2 for the minor homozygous, heterozygous and major homozygous sites respectively). Gender had a normal prior as well, with mean = 0 and variance = 1000. Under the assumption of a single associated variant in the region, the Bayes factor for a variant is a proportional (under weak assumptions) to the posterior probability that it is driving the association. The conditional analysis was performed in the same way, including the genotype at rs1800693 as an additional covariate (with an additive effect).

Minigene assays. *TNFRSF1A* minigene constructs were made using a ~1.5 kb genomic region comprising rs1800693 and spanning the region from introns 5 to 8. Site-directed mutagenesis was performed using the QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene). RNA was isolated from HEK 293T cells (ATCC) using the RNeasy Micro Kit (Qiagen) and cDNA was synthesized using the High Capacity RNA-to-cDNA Kit (Applied Biosystems), according to the manufacturer's instructions. Minigene splicing was analyzed by PCR amplification of cDNA using primers specific to the SD and SA sites. All resulting amplification products were sequenced.

Peripheral blood donors. With informed consent and ethics committee (NRES Committee South Central – Oxford B; REC Ref. No.: 10/H0605/5;v.1) approval, blood samples were obtained from healthy white European volunteers (without self-reported autoimmune diseases); their ages ranged from 20 - 61 years (mean = 33.38 years) and 70% were females. Relapsing-remitting MS patient serum samples were obtained in accordance with the Ruhr-University Bochum (Germany) ethics committee.

Genotyping. Rs1800693 was genotyped using a TaqMan® SNP Genotyping Assay (Applied Biosystems) according to the manufacturer's instructions. Genotyping data were scored independently by two operators to minimize error.

Isolation of human blood cell subsets. Peripheral blood mononuclear cells (PBMCs) were separated on Lymphoprep™ (Axis-Shield). Polymorphonuclear cells were obtained following erythrocyte pellet lysis. CD14-positive monocytes were isolated from PBMCs using anti-CD14 MicroBeads (Miltenyi Biotec); CD3-positive T-cells were isolated using anti-CD3 MicroBeads (Miltenyi Biotec), according to the manufacturer's instructions.

Human TNFR1 transcript quantification. TNFR1 transcript levels were measured by real-time quantitative PCR (qRT-PCR) with an ABI Prism 7900HT Sequence Detection System and analyzed using SDS v.2.4 software (Applied Biosystems). GAPDH house-keeping gene

transcripts and transcripts containing exons 6 and 7 were detected using Taqman® Gene Expression Assays Hs02758991_g1 and Hs00533565_g1, respectively (Applied Biosystems). A custom primer/probe set spanning the exon 5-7 boundary was designed for specific detection of transcripts lacking exon 6. Relative expression is expressed as $2^{-\Delta\Delta CT}$, where ΔCT = (cycle threshold for the transcript of interest) – (cycle threshold for the house-keeping gene transcript).

Polysome profiling assay. PBMCs obtained from peripheral blood samples of healthy volunteers were cultured in RPMI containing 10% FCS, 4 ng/ml IL-2, 50 ng/ml IL-4 and 50 ng/ml GM-CSF for 72 h, followed by stimulation with 10 ng/ml PMA and 100 ng/ml LPS for 16h. Cells were then treated with 10 μ g/ml of cycloheximide, lysed and post-nuclear supernatants were centrifuged at 40,000 rpm for 2 h 15 min through 12 ml linear 20-47% (w/v) sucrose gradients containing 20 mM Tris-HCl pH 8.0, 5 mM MgCl₂, 140 mM KCl. EDTA control gradients contained 20 mM Tris-HCl pH 8.0, 10 mM EDTA, 140 mM KCl. 1 ml fractions were collected and absorbance at 260 nm was used to generate chromatograms of sedimentation profiles. RNA was extracted from the fractions and cDNA was analysed by qRT-PCR.

Human immune cell TNFR1 surface expression. The anti-human monoclonal antibodies used for immunostaining were Alexa-Fluor®647 anti-CD120a/TNFR1 (clone H398; AbD Serotec), Alexa-Fluor®700 anti-CD11c, e-Fluor®450 anti-CD123 (clones 3.9 and 6h6 respectively; eBioscience), FITC and PE anti-HLA-DR (clone L243, BioLegend), PerCP-Cy5.5 anti-CD14 and Pacific Blue™ anti-CD16 (clones HCD14 and 3G8 respectively; BioLegend) and FITC Lin-1 cocktail (BD Biosciences). Alexa-Fluor®647 mouse IgG2a (AbD Serotec) was used as an isotype control for CD120a/TNFR1 staining. Data obtained were analyzed using FlowJo (Tree Star, Inc.). TNFR1 surface expression was calculated by dividing the median fluorescence intensity (MedFI) of the anti-TNFR1 staining by the MedFI of the isotype control for each sample. Each day that donor samples were evaluated, FluoroSpheres were also analyzed (Blank Beads and Calibration Beads, Dakocytomation). Data normalization was performed using the Calibration Beads to control for day-to-day variation in flow cytometer function.

Confocal microscopy. HeLa cells (ATCC) were transfected with constructs expressing, TNFR1 fluorescent fusion proteins, a DsRed2-tagged beta subunit of the signal particle receptor (SR β) as a marker of the ER, and an ECFP-tagged *trans*-Golgi-resident protein *N*-acetylgalactosaminyltransferase-2 (GalNAc-T2) stalk region as a marker of the Golgi. TO-PRO®-3 iodide (Molecular Probes, Invitrogen) was used for nuclear staining, with staining being performed 48 h after transfection. Images were taken with an LSM510 confocal

microscope (Zeiss) and processed using Zeiss LSM510 v.3.2 and Image J software.

Colocalization was quantified using MetaMorph software (Molecular Devices).

FRET. FRET analysis by flow cytometry was performed as described previously²⁹.

Briefly, HEK 293T cells were transfected with the indicated ECFP and EYFP fusion protein

5 constructs and harvested 24 h later for FRET analysis. FRET signals were examined on viable, ECFP/EYFP double-positive cells.

BRET. BRET assays were performed and analyzed as described by previously³⁰. GFP² and RLuc fusion protein constructs for BRET studies were generated by cloning TNFR1 sequences into pGFP²-N3 and pRLuc-N3 vectors (BioSignal Packard).

10 **Flow cytometric analysis of TNFR1-transfected cells.** HEK 293T cells were transfected with the indicated N-terminally HA-tagged TNFR1-EYFP fusion protein constructs. TNFR1 expression was measured in EYFP-positive cells using anti-HA.11 (clone 16B12; Covance) and APC-polyclonal goat anti-mouse IgG secondary antibody (BD Pharmingen™). For total anti-HA.11 staining, cells were permeabilized with ice-cold 70% ethanol for 30 min
15 before incubation with the primary antibody.

Spontaneous NFκB signaling analysis. HEK 293T cells were transfected with the indicated TNFR1 construct along with a *Renilla* luciferase construct (pGL4.74, Promega) and the *luc2*/NFκB/RE reporter (pGL4.32, Promega). Cells were analyzed for firefly normalized to *Renilla* luciferase activity using the Dual Luciferase Reporter Assay System (Promega). IL-8
20 levels in culture supernatant were measured by immunoassay using the Human CXCL8/IL-8 Quantikine® ELISA kit (R & D Systems), according the manufacturer's instructions.

Lentivirus preparation. The lentiviral expression plasmid pHRsinUbEm was a gift from Paul J. Lehner (University of Cambridge). TNFR1 expression plasmids were co-transfected with the vesicular stomatitis virus-G envelope plasmid pMD2.G and packaging plasmid psPAX2
25 (containing HIV-1 Gag and Rev) into HEK 293T cells to package lentiviral particles. Viral titers were determined by serial dilution and transduction of HEK 293T cells.

Spontaneous apoptosis analysis. For spontaneous apoptosis measurement by mitochondrial integrity analysis, HeLa cells were transfected with the indicated TNFR1-ECFP construct. Mitochondrial membrane potentials ($\Delta\psi_M$) were analyzed by staining cells with the
30 mitochondrion-selective probe tetramethylrhodamine methyl ester (TMRM). The TMRM signal was measured by flow cytometry in ECFP-positive cells. For spontaneous apoptosis measurement by cell cycle analysis, HEK 293T cells were transduced with the indicated TNFR1 lentivirus at a multiplicity of infection of 50. Cell DNA content was measured by flow cytometry

using propidium iodide (PI) staining. Following exclusion of debris and cell doublets, cells with a sub-diploid (G_0/G_1) DNA content were considered apoptotic.

UPR analysis. The XBP1 luciferase reporter assay was performed as for the NF κ B/RE luciferase reporter assay but with the use of an XBP1 luciferase reporter construct and HT1080
5 cells (ATCC). ER stress-induced splicing of the 26-bp intron of human XBP1 was analyzed.

Cell culture supernatant TNFR1 measurement. HEK 293T cells were transfected with the indicated N-terminally HA-tagged TNFR1-EYFP fusion protein constructs. Culture supernatants or anti-TNFR1 antibodies (R&D Systems; Santa Cruz Biotechnology, Inc.) were used to coat Nunc™ microtitre plates. HA-tagged TNFR1 proteins were detected using
10 biotinylated anti-HA.11 (clone 16B12; Covance) or biotinylated anti-TNFR1 antibodies (R&D Systems; Santa Cruz Biotechnology, Inc.) and Europium-conjugated Streptavidin (Perkin Elmer). For Western blots, HA-tagged TNFR1 proteins detected using anti-HA.11 (clone 16B12; Covance) and IRDye® 800CW goat-anti-mouse IgG secondary antibody, and visualized using an Odyssey Infrared Imaging System (LI-COR Biosciences).

Δ 6-TNFR1 measurement in human serum. Endogenous Δ 6-TNFR1 was measured in control and patient sera by ELISA. An anti- Δ 6-TNFR1-specific monoclonal Fab antibody was generated using HuCAL® antibody technology (AbD Serotec) that employs a human combinatorial antibody library and the CysDisplay® method of phage display. The anti- Δ 6-TNFR1-specific antibody was coated onto Nunc™ microtitre plates at a concentration of 2 μ g/ml
15 overnight. The anti-human TNFR1 mouse monoclonal antibody (clone 16805; R&D Systems) was used at 2 μ g/ml for detection, in conjunction with Europium-conjugated anti-mouse IgG. The sensitivity limit of the assay was determined to be ~1.25 ng/ml.

Recombinant protein expression, purification and TNF immunoprecipitation. HEK 293T cells were transiently transfected with TNF-FLAG or F_c fusion constructs. F_c fusion
25 proteins were purified from culture supernatant using protein A Sepharose (Sigma). Following F_c-fusion protein pre-incubation with TNF-FLAG, immunoprecipitation was performed using protein A Sepharose. An F_c fusion of the first cysteine-rich domain of DR5 (DR5.CRD1-F_c) was used as a negative control for TNF binding.

Tandem mass spectrometry. Purified Δ 6-TNFR1-F_c protein and transfected cell
30 supernatants were solubilized and digested with chymotrypsin (Roche). The digested protein mixture was separated with a 140 min gradient from 5 to 60 % acetonitrile (uHPLC, Proxeon) and loaded onto a LTQ-Orbitrap or a Q-Exactive mass spectrometer (Thermo Fisher). The instrument was operated in a data-dependent top10 acquisition modus. Raw data were searched using the MaxQuant software suite (version 1.2.2.0) against the complete IPI human database

(v3.68, 87061 entries) with an additional entry for the $\Delta 6$ -TNFR1 isoform. The fragmentation spectra were plotted and annotated by the Viewer interface of MaxQuant.

Surface plasmon resonance. Surface plasmon resonance spectroscopy was performed using the BIAcore T100 system. TNF-FLAG was directly immobilized onto anti-FLAG-coated chips from culture supernatants. Experiments were carried out using serial dilutions of the indicated purified F_c-fusion proteins. Binding curves were analyzed using BIAcore T100 Evaluation software to fit the data and determine dissociation constants (K_d), assuming 1:1 binding.

TNF neutralization assay. Varying concentrations of purified F_c fusion proteins ranging from 1-10,000 ng/ml were incubated with 5×10^4 HEK-Blue™ TNF α /IL-1 β cells (Invivogen) in the presence of 25 ng/ml of TNF. The assay was performed according to the manufacturer's instructions.

Statistical analysis of functional data. All statistical tests were performed using GraphPad Prism, GraphPad StatMate 2.0, and the R statistical software package. Regression analysis was used to test for correlations between rs1800693 genotype and TNFR1 transcript levels and TNFR1 cell surface expression in human immune cell subsets, assuming a linear genotype-to-phenotype relationship (1 d.f. F -test); age and sex were not included as covariates as for all data sets no age or sex effects were observed ($P > 0.05$). For the TNFR1_ $\Delta 6$ transcripts the slope calculated by the linear regression analysis for the CD14⁺ monocytes, polymorphonuclear cells and CD3⁺ T cells was 0.0014, 0.0026 and 0.0003, respectively. Using a Bonferroni correction for multiple testing (taking $P = 0.05$ and considering three independent hypotheses for the three different human immune cell subsets for RNA-level analyses), the significance threshold estimated was $P = 0.017$. At this significance threshold >90% power was obtained to detect the differences observed with the sample size used in the study. The percentage of TNFR1_ $\Delta 6$ transcript level variation accounted for by genotype at rs1800693 was estimated by least-squares regression analysis at 67, 52 and 55% for the CD14⁺ monocytes, polymorphonuclear cells and CD3⁺ T cells, respectively. Linear regression analysis was used to quantify the correlation in TNFR1_ $\Delta 6$ transcript levels between the different immune cell subsets. For all regression analyses there was no significant departure from linearity ($P > 0.05$) as determined using a runs test. For the immune cell TNFR1 surface expression, using a Bonferroni correction (considering six independent hypotheses for six independent immune cell subsets), the significance threshold was $P = 0.008$; at this threshold >80% power was obtained to detect a 50% difference between the homozygous groups, given the sample size of the study. For the statistical analysis of the confocal colocalization quantification, two-tailed, paired Student's T

tests were performed. For all other analyses, two-tailed, unpaired Student's T tests were performed. For all Student's T tests a 5% significance threshold was used.

2. Results

Summary

Although there has been much success in identifying genetic variants associated with common diseases using genome-wide association studies (GWAS)¹, it has been difficult to demonstrate which variants are causal and what role they play in disease. Moreover, the modest contribution these variants make to disease risk has raised questions regarding their medical relevance². A single nucleotide polymorphism (SNP) has been investigated in the *TNFRSF1A* gene, that encodes TNF receptor 1 (TNFR1), which was discovered through GWAS to be associated with multiple sclerosis (MS)^{3,4}, but not with other autoimmune conditions such as rheumatoid arthritis (RA)⁵, psoriasis⁶ and Crohn's disease⁷. By analyzing MS GWAS^{3,4} data in conjunction with the 1000 Genomes Project data⁸ genetic evidence is provided that strongly implicates this SNP, rs1800693, as the causal variant in the *TNFRSF1A* region. This is further substantiated through functional studies showing that the MS risk allele directs expression of a novel, soluble form of TNFR1 that can block TNF. Importantly, TNF blocking drugs can promote onset or exacerbation of MS⁹⁻¹¹, but they have proven highly efficacious in the treatment of autoimmune diseases for which there is no association with rs1800693. This indicates that the clinical experience with these drugs parallels the disease association of rs1800693, and that the MS-associated TNFR1 variant mimics the effect of TNF blocking drugs. Hence, the experiments described herein demonstrate that clinical practice can be informed by comparing GWAS across common autoimmune diseases and by investigating the functional consequences of the disease-associated genetic variation.

SNP rs1800693 is primarily responsible for the MS association in the *TNFRSF1A* region

The largest MS GWAS⁴ reports rs1800693 as the most associated SNP in the *TNFRSF1A* region by over two orders of magnitude (odds ratio for risk allele = 1.12 (1.11-1.14); $P = 4.1 \times 10^{-14}$). To assess whether this SNP is mainly driving the association, the haplotype structure across the region in 379 individuals of European ancestry was examined using whole genome sequence from the 1000 Genomes Project⁸ and statistical imputation into a UK cohort of 1,853 MS patients and 5,174 controls⁴ performed. Among genotyped SNPs the strongest signal is seen at

rs1800693 and the variants in strongest association with this SNP were also genotyped in the study. Statistical imputation¹² revealed no other variant with stronger association to MS within the region, including the previously reported³ nonsynonymous SNP rs4149584 (Fig. 1), and analysis of association after controlling for the effect of rs1800693 removed almost all of the signal (Fig. 2a). These observations all support variation at rs1800693 as being primarily responsible for the MS association in the *TNFRSF1A* region.

Functional and genetic characterization of rs1800693

To further substantiate the causality of rs1800693, the functional consequences of the variation at this SNP was investigated. As rs1800693 is proximal to the *TNFRSF1A* exon 6/intron 6 boundary, it was hypothesized that rs1800693 may influence splicing of TNFR1 exon 6¹³. In an *in vitro* minigene splicing assay, only the risk 'G' allele resulted in skipping of exon 6 (Fig. 2b). In primary human immune cells it was found that the presence of the risk allele correlated with increased expression of transcripts lacking exon 6, with the gene dosage effect observed being consistent with the genetic effect on disease risk (Fig. 2c and Fig. 3). Moreover, these transcripts are translated in the primary immune cells, as binding of polysomes to these transcripts demonstrated active translation (Fig. 4). As full-length (FL-) TNFR1 expression did not vary in a genotype-dependent fashion (Figs. 5 and 6), this indicates that the effect of rs1800693 may be specifically mediated by its influence on the generation of a functional protein isoform lacking exon 6 ($\Delta 6$ -TNFR1), and the properties of this molecule were therefore investigated.

TNFR1 exon 6 skipping results in a frameshift and a premature stop codon, which translates into a protein comprising only the N-terminal 183 amino acids of FL-TNFR1, followed by a novel 45 amino acid sequence, as confirmed by tandem mass spectrometry (Fig. 7). Consequently, $\Delta 6$ -TNFR1 lacks the extracellular C-terminal portion of the fourth cysteine-rich domain (CRD) of FL-TNFR1, the transmembrane domain, and the intracellular region that is essential for appropriate subcellular localization¹⁴. While FL-TNFR1 localizes to the Golgi apparatus, $\Delta 6$ -TNFR1 demonstrated a more diffuse intracellular distribution (Fig. 8), consistent with the absence of the Golgi-retention motif. Nevertheless, partial subcellular colocalization of FL- and $\Delta 6$ -TNFR1 was observed (Fig. 9), suggesting the potential for an interaction between the two isoforms. As $\Delta 6$ -TNFR1 retains the pre-ligand assembly domain (PLAD/CRD1), required for TNFR1 trimerization at the cell surface^{14,15}, it could exert a functional effect on FL-TNFR1 by associating with it to form heteromers that would therefore have modified properties. To investigate the existence of such an interaction, we used FL- and $\Delta 6$ -TNFR1 proteins fused at their C-termini with fluorescent or luciferase proteins in fluorescence and bioluminescence

resonance energy transfer (FRET and BRET, respectively). This demonstrated that $\Delta 6$ -TNFR1 associates neither with the full-length protein nor with itself (Fig. 8c and Fig. 10). Consistent with this and with the lack of transmembrane and cytoplasmic domains, $\Delta 6$ -TNFR1 was not observed at the surface of transfected cells (Fig. 8d). Hence, as $\Delta 6$ -TNFR1 cannot interact with FL-TNFR1, it is unlikely to have a direct impact on the latter, intracellularly or at the cell surface.

To assess whether $\Delta 6$ -TNFR1 has some intracellular function, regardless of its inability to associate with FL-TNFR1, TNFR1-mediated signaling in $\Delta 6$ -TNFR1-transfected cells was investigated. As predicted by the absence of a death domain, which is necessary for both NF- κ B-mediated signal transduction and apoptosis^{14,15}, no significant spontaneous NF- κ B signaling or TNFR1-mediated apoptosis were observed upon $\Delta 6$ -TNFR1 expression (Fig. 11 and Fig. 12). However, $\Delta 6$ -TNFR1 could potentially retain some intracellular activity by accumulating in the endoplasmic reticulum (ER) and evoking a stress response¹⁵. Nevertheless, there was no evidence for increased ER-localization of $\Delta 6$ -TNFR1 (Fig. 8a and Fig. 8b), or induction of the unfolded protein response in $\Delta 6$ - compared with FL-TNFR1-transfected cells (Fig. 13).

Given that no intracellular $\Delta 6$ -TNFR1 activity was observed, and that this isoform has no transmembrane region and does not associate with FL-TNFR1, it was hypothesized that $\Delta 6$ -TNFR1 could exist as a soluble, functional molecule. Soluble TNFR1 generation has been previously described through exosomal release of full-length receptor and through metalloprotease-dependent cleavage of the FL-TNFR1 extracellular domain. Here, it is demonstrated that skipping of exon 6 constitutes a novel mechanism of stable, soluble TNFR1 production: a higher level of soluble protein was found in supernatants of $\Delta 6$ - compared to FL-TNFR1-transfected cells (Fig. 14 and Fig. 15). Given the importance of determining the presence of $\Delta 6$ -TNFR1 protein in primary human samples, an ELISA for this molecule was established. This required the generation of an anti- $\Delta 6$ -TNFR1-specific monoclonal antibody, using a human combinatorial antibody library in conjunction with phage display, as commercially available anti-TNFR1 antibodies are not specific for the splice isoform (Fig. 16). This ELISA allowed the detection of $\Delta 6$ -TNFR1 in the sera of both healthy individuals (range = <1.25-14.8 ng/ml; n = 12) and MS patients (range = <1.25-37.3 ng/ml; n = 13) (Fig. 17). Furthermore, soluble $\Delta 6$ -TNFR1 can bind to TNF, as shown in TNF pull-down assays using TNFR1-F_c and $\Delta 6$ -TNFR1-F_c fusion proteins (Fig. 14b). Using surface plasmon resonance the TNF binding ability of $\Delta 6$ -TNFR1-F_c was determined to be in the nanomolar range (K_d = 15.6 nM), indicating that the isoform has a high affinity for TNF (Fig. 14c). Critically, $\Delta 6$ -TNFR1-F_c proved to have a TNF antagonistic function as it can bind and neutralize the ability of TNF to signal through

membrane-bound TNFR1 (Fig. 14d), with the neutralization capacity of $\Delta 6$ -TNFR1-F_c being consistent with the surface plasmon resonance data. The combined genetic and functional analyses described herein strongly implicate rs1800693 as the causal SNP in the MS-associated *TNFRSF1A* region, with the risk allele directing an increased expression of $\Delta 6$ -TNFR1. Since

5 $\Delta 6$ -TNFR1 protein is soluble and is capable of TNF antagonism, the evidence described herein is consistent with the reported worsening of MS upon anti-TNF therapy^{10,11}. TNF antagonists were originally used in MS patients partly because increased levels of TNF were detected in both active lesions and cerebrospinal fluid from MS patients^{16,17}, and partly because of reports on animal models of MS where blockade of TNF prevented or delayed disease onset^{18,19}. However,

10 opposing findings have also been obtained from animal studies²⁰, and the paradoxical outcomes from these pre-clinical models, as well as the precise mechanism of TNF antagonist action, are unexplained but remain an active area of research^{21,22}.

TNF antagonists are largely beneficial in the treatment of other autoimmune conditions, including RA, psoriasis, Crohn's disease, and ankylosing spondylitis (AS)²³. In accordance with

15 this, common genetic variants in the *TNFRSF1A* region have not been identified for these conditions⁵⁻⁷ with the exception of AS²⁴, that has a distinct association signal compared to MS (Fig. 18), indicating that $\Delta 6$ -TNFR1 is unlikely to influence the pathology of these conditions. Notably, the MS-associated rs1800693 SNP is also associated with primary biliary cirrhosis²⁵, but there is no controlled clinical study of TNF antagonists in this disease. Interestingly, side

20 effects associated with the use of TNF antagonists in treating non-MS autoimmune diseases include clinical onset of MS and isolated demyelinating diseases, such as optic neuritis, which is often an early manifestation of MS^{9,26}. These side effects are relatively rare, indicating that they may only arise in individuals with a propensity for demyelinating disease that is unmasked upon treatment.

3. Discussion

A disease-associated genetic variant has been identified that directs increased expression of a molecule that is analogous to drugs whose adverse effects can promote or exacerbate disease. This finding has broader implications. A criticism against the potential clinical value of

30 GWAS findings has been the modest effect on disease risk conferred by most associated genetic variants². However, this notion does not consider that drugs targeting the same pathways as the genetic variants are likely to have a larger functional impact and thus may be clinically relevant²⁷. Experiments described herein show that naturally-occurring variation and therapeutic

agents can have the same target, but their effect differs in magnitude: $\Delta 6$ -TNFR1 and a TNF antagonist both neutralized TNF, but the antagonist had a greater ability to do so. Therefore, the inventors results suggests that a signal of disease association for rs1800693 could be predictive of an adverse effect of TNF antagonist treatment. In conclusion, the study described herein

5 shows that, through genetic and functional follow-up investigations, GWAS can serve to directly inform therapeutic choice in the treatment of a common disease.

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CLAIMS

1. A method of determining whether or not a patient is suitable for anti-tumour necrosis factor (anti-TNF) therapy, comprising detecting in a sample from the patient the presence or absence of:

- (a) the single nucleotide polymorphism (SNP) rs1800693 in the patient's genome;
- (b) one or more polymorphisms in linkage disequilibrium with the SNP of (a) in the patient's genome;
- (c) the tumour necrosis factor receptor 1 (TNFR1) isoform as set forth in SEQ ID NO: 2; and/or
- (d) a variant of the TNFR1 isoform in (c) which comprises a sequence having at least 95% homology to SEQ ID NO: 2 based on amino acid identity over the entire length of SEQ ID NO: 2; and thereby determining whether or not the patient is suitable for anti-TNF therapy, wherein the presence of (a), (b), (c) and/or (d) indicates that the patient is not suitable for anti-TNF therapy and wherein the absence of (a), (b), (c) and/or (d) indicates that the patient is suitable for anti-TNF therapy.

2. A method according to claim 1, wherein the method comprises detecting the presence or absence of (i) the sequence set forth in SEQ ID NO: 1 or (ii) a variant thereof which comprises a sequence having at least 95% homology to SEQ ID NO: 1 based on nucleotide identity over the entire length of SEQ ID NO: 1 and which comprises the SNP rs1800693.

3. A method of treating or preventing an autoimmune disease in a patient in need thereof comprising (i) determining whether or not the patient is suitable for anti-TNF therapy using a method according to claim 1 or 2 and (ii), if the patient is suitable for anti-TNF therapy, administering an anti-TNF therapy to the patient and thereby treating or preventing the autoimmune disease.

4. A method of treating or preventing an autoimmune disease in a patient identified as suitable for anti-TNF therapy using a method of according to claim 1 or 2, comprising administering an anti-TNF therapy to the patient and thereby treating or preventing the autoimmune disease.

5. A method of determining whether or not a patient is at risk of developing multiple sclerosis (MS) or MS-like symptoms or whether or not a patient has MS, comprising detecting in a sample from the patient the presence or absence of:

- (a) the TNFR1 isoform as set forth in SEQ ID NO:2; and/or
- (b) a variant of the TNFR1 isoform in (a) which comprises a sequence having at least 95% homology to SEQ ID NO: 2 based on amino acid identity over the entire length of SEQ ID NO: 2;

and thereby determining whether or not the patient is at risk of developing MS or MS-like symptoms or whether or not the patient has MS,

wherein the presence of (a) or (b) indicates that the patient is at risk of developing MS or MS-like symptoms or indicates that the patient has MS and

wherein the absence of (a) or (b) indicates that the patient is not at risk of developing MS or MS-like symptoms or that the patient does not have MS.

6. A method according to claim 5, wherein the patient is undergoing anti-TNF therapy.

7. A method according to any one of claims 1, 2, 3, 4 or 6, wherein anti-TNF therapy comprises anti-TNF α therapy.

8. A method according to any one of the preceding claims, wherein the anti-TNF therapy comprises the administration of a neutralising antibody to TNF, a neutralising antibody to the TNF receptor, a soluble TNF receptor chimera and/or a modulator of TNF production.

9. A method according to claim 8, wherein:

- (a) the neutralising antibody to TNF is selected from infliximab, adalimumab, certolizumab and golimumab;
- (b) the neutralising antibody to the TNF receptor is a neutralising antibody to the TNFR1 receptor, preferably wherein the antibody is atrosab;
- (c) the soluble TNF receptor chimera is selected from lenercept and etanercept; and/or
- (d) the modulator of TNF production is selected from pentoxifylline and bupropion.

10. A method according to any of the preceding claims, wherein the patient has or is suspected of having rheumatoid arthritis (RA), psoriatic arthritis, psoriasis, ankylosing spondylitis, ulcerative colitis or Crohn's disease.

11. A polypeptide TNF antagonist which comprises:
 - (a) the sequence of the TNFR1 isoform as set forth in SEQ ID NO:2; or
 - (b) a sequence which has at least 85% homology to the sequence of SEQ ID NO: 2 based on amino acid identity over the entire sequence of SEQ ID NO: 2 and which retains its ability to bind TNF.
12. A polypeptide TNF antagonist according to claim 11, wherein the polypeptide comprises amino acids 184 to 228 of SEQ ID NO: 2.
13. A polynucleotide encoding a polypeptide according to claim 11 or 12.
14. A polynucleotide according to claim 13, wherein the polynucleotide comprises the sequence set forth in SEQ ID NO: 1 or a sequence having at least 85% homology to SEQ ID NO: 1 based on nucleotide identity over the entire length of SEQ ID NO: 1.
15. An expression vector comprising a polynucleotide according to claim 13 or 14 operably linked to a promoter sequence.
16. A host cell comprising a polynucleotide according to claim 13 or 14 or a vector according to claim 15.
17. A polypeptide TNF antagonist according to claim 11 or 12, a polynucleotide according to claim 13 or 14 or an expression vector according to claim 15 for use in the treatment or prevention of an autoimmune condition in a patient in need thereof.
18. Use of a polypeptide TNF antagonist according to claim 11 or 12, a polynucleotide according to claim 13 or 14 or an expression vector according to claim 15 in the manufacture of a medicament for the treatment or prevention of an autoimmune condition in a patient in need thereof.
19. A method of treating or preventing an autoimmune condition in a patient in need thereof comprising administering to the patient a therapeutically or prophylactically effective amount of a polypeptide TNF antagonist according to claim 11 or 12, a polynucleotide according

to claim 13 or 14 or an expression vector according to claim 15 and thereby treating or preventing the autoimmune disease.

20. A polypeptide TNF antagonist for use according to claim 17, use according to claim 18 or a method according to claim 19, wherein the autoimmune condition is selected from rheumatoid arthritis (RA), psoriatic arthritis, psoriasis, ankylosing spondylitis, ulcerative colitis or Crohn's disease.

21. An antibody which specifically recognises an epitope within a polypeptide TNF antagonist according to claim 11.

22. An antibody according to claim 21, wherein the epitope is within amino acids 184 to 228 of SEQ ID NO: 2 or the corresponding region in the homologous sequence.

23. An antibody according to claim 21 or 22, wherein the antibody does not specifically recognize an epitope within the wild-type TNFR1 polypeptide as set forth in SEQ ID NO: 3.

24. An antibody according to any one of claims 21 to 23, wherein the antibody stimulates TNF signaling *in vivo*.

25. An immunoassay plate comprising an antibody according to any one of claims 21 to 24.

26. A method for producing an antibody according to any one of claims 21 to 24, comprising:

- (a) immunising a non-human mammal with an immunogen comprising a polypeptide according to claim 11 or 12; and
- (b) obtaining an antibody preparation from said mammal and deriving therefrom monoclonal antibodies that specifically recognise said epitope.

27. A hybridoma which produces an antibody according to any one of claims 21 to 24.

28. An antibody according to any one of claims 21 to 24 for use in a method of determining whether or not a patient is suitable for anti-TNF therapy.

29. A method of preventing MS or MS-like symptoms in a patient in need thereof comprising administering to the patient a prophylactically effective amount of an antibody according to any one of claims 21 to 24 and thereby preventing MS or MS-like symptoms in the patient.

30. A method of treating or preventing an autoimmune disease in a patient in need thereof comprising (i) determining whether or not the patient is suitable for anti-TNF therapy using a method according to claim 1 or 2 and (ii), if the patient is not suitable for anti-TNF therapy, administering to the patient a prophylactically effective amount of an antibody according to any one of claims 21 to 24 in conjunction with anti-TNF therapy and thereby treating or preventing the autoimmune disease.

31. A method of treating or preventing an autoimmune disease in a patient identified as not suitable for anti-TNF therapy using a method according to claim 1 or 2, comprising administering to the patient a prophylactically effective amount of an antibody according to any one of claims 21 to 24 in conjunction with anti-TNF therapy and thereby treating or preventing the autoimmune disease.

32. An antibody according to any one of claims 21 to 24 for use in preventing a patient undergoing anti-TNF therapy from developing MS or MS-like symptoms.

33. A method of identifying whether or not a patient is at risk of developing multiple sclerosis (MS) or MS-like symptoms or whether or not a patient has MS, comprising detecting in a sample from the patient the presence or absence of a soluble isoform of TNFR1 and thereby determining whether or not the patient is at risk of developing MS or MS-like symptoms or whether or not the patient has MS,

wherein the presence of the soluble isoform of TNFR1 indicates that the patient is at risk of developing MS or MS-like symptoms or that the patient has MS and

wherein the absence of the soluble isoform of TNFR indicates that the patient is not at risk of developing MS or MS-like symptoms or that the patient does not have MS.

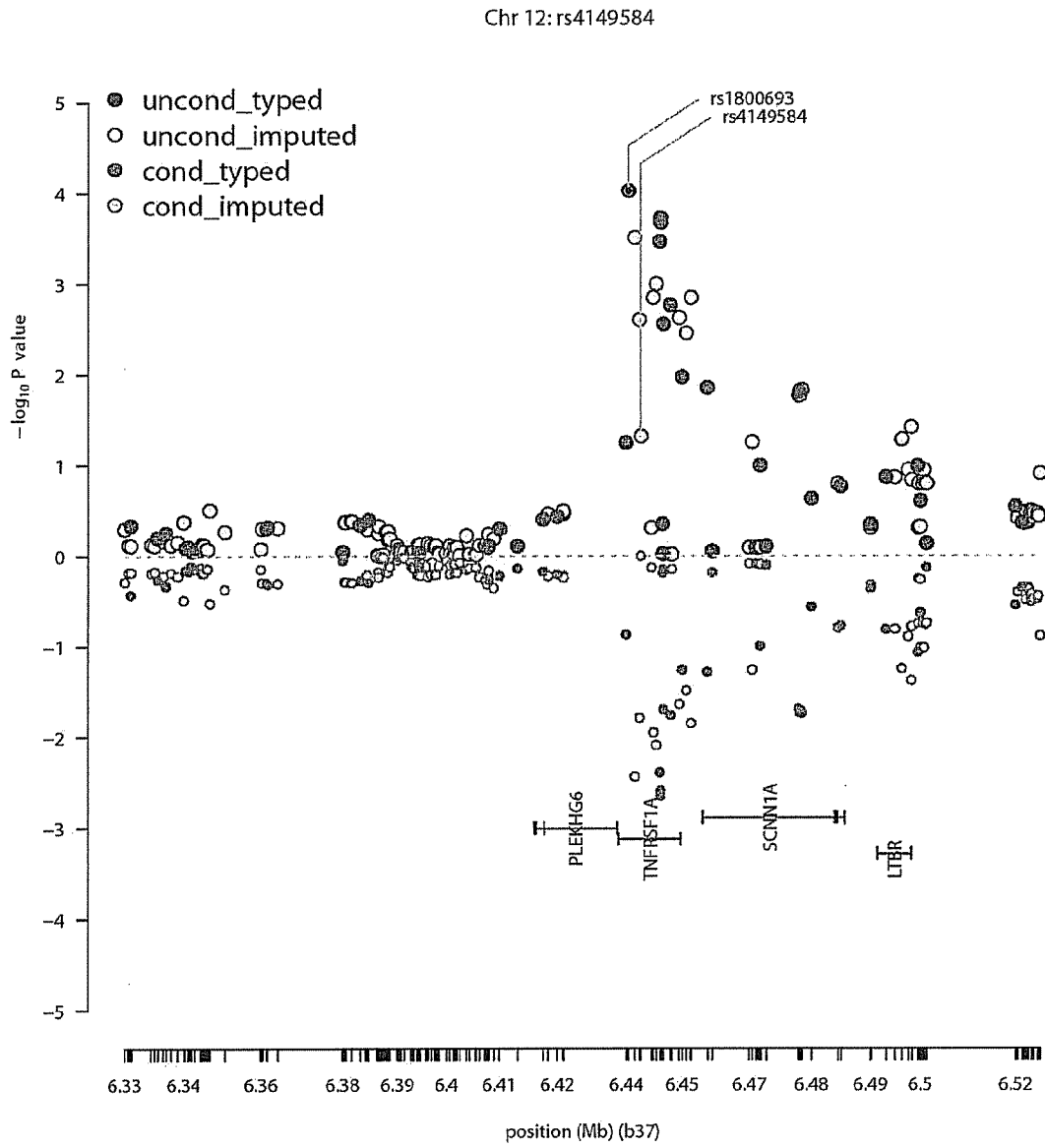


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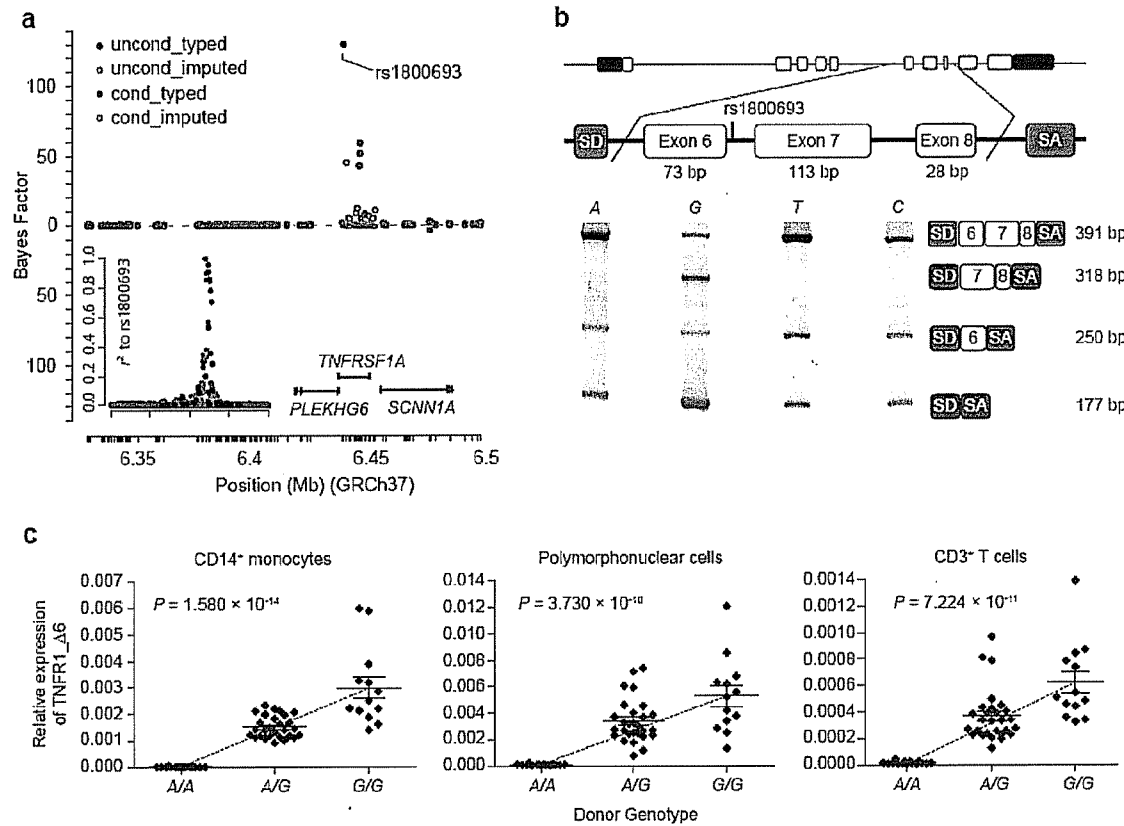


Figure 2

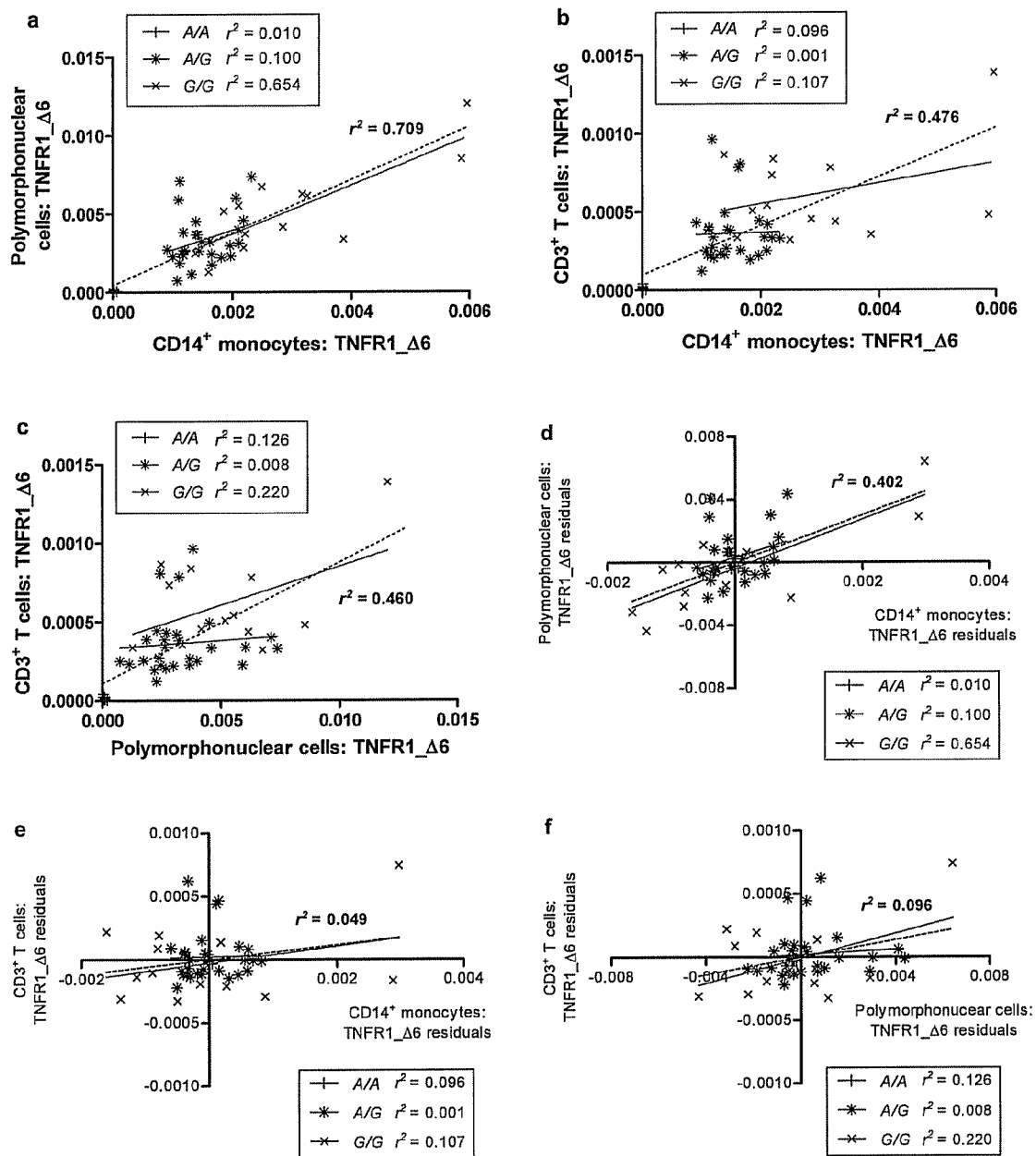


Figure 3

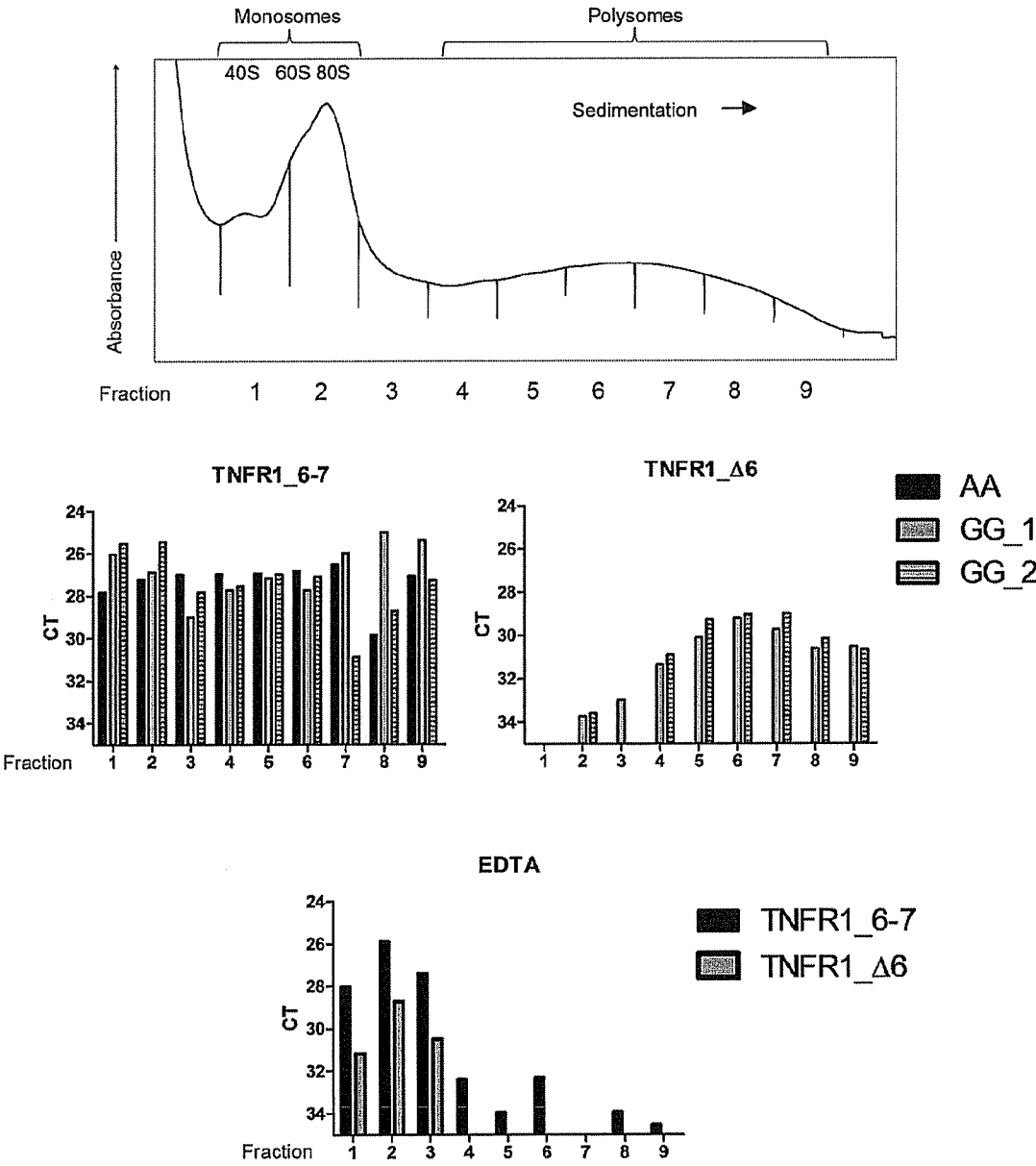


Figure 4

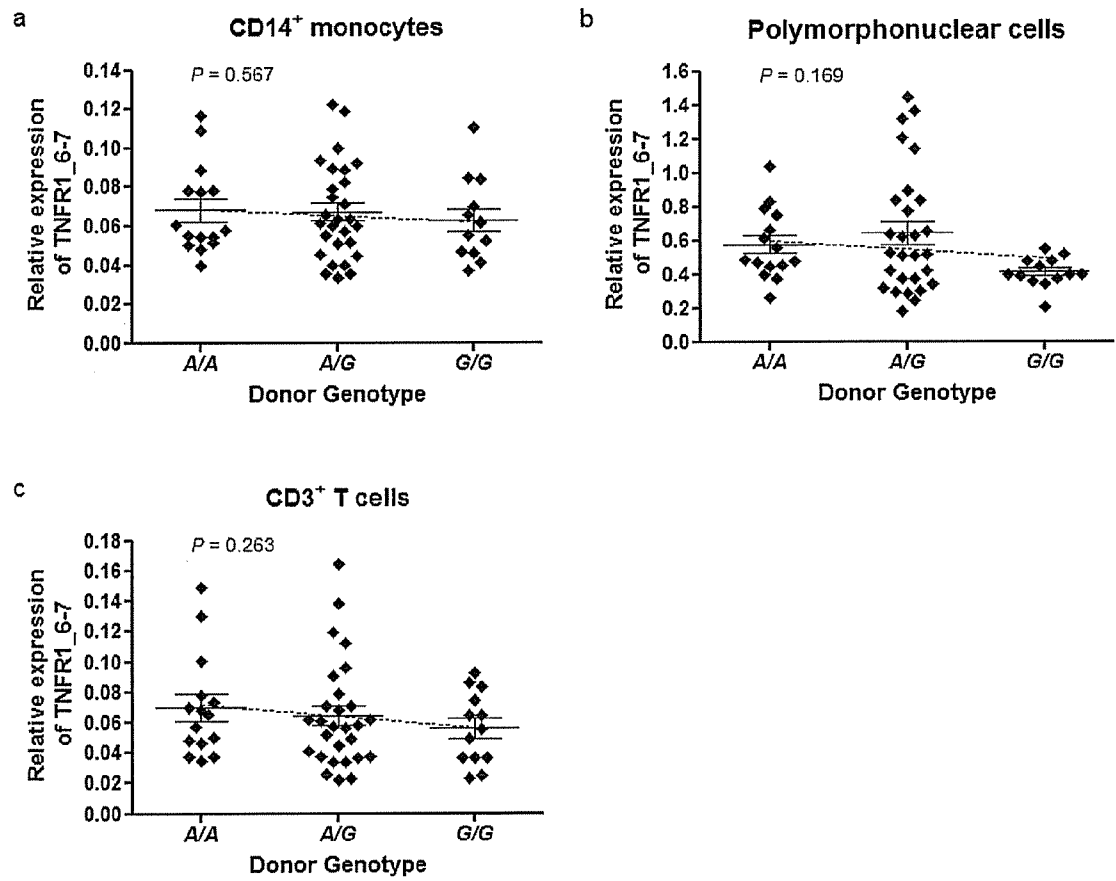


Figure 5

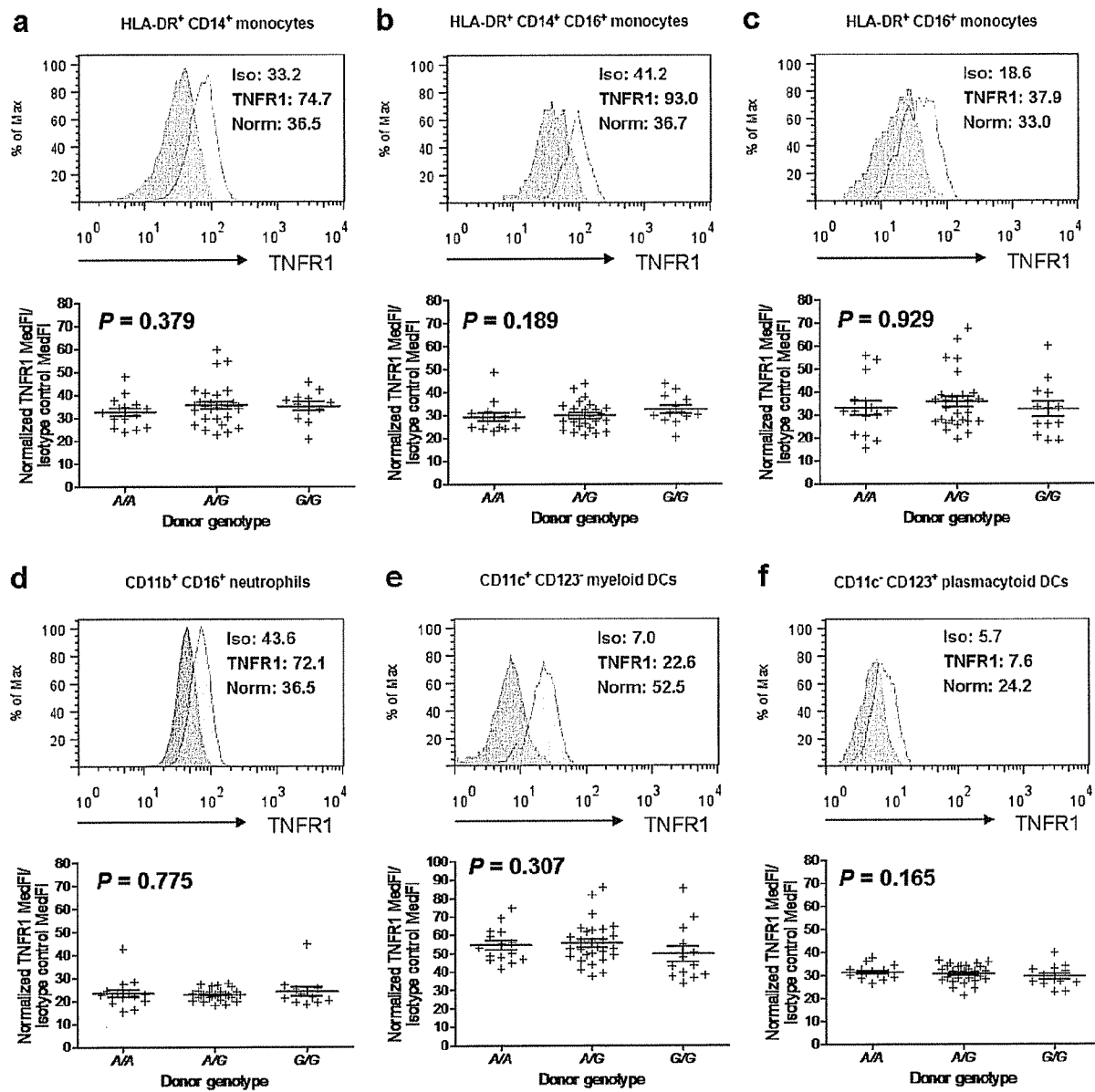


Figure 6

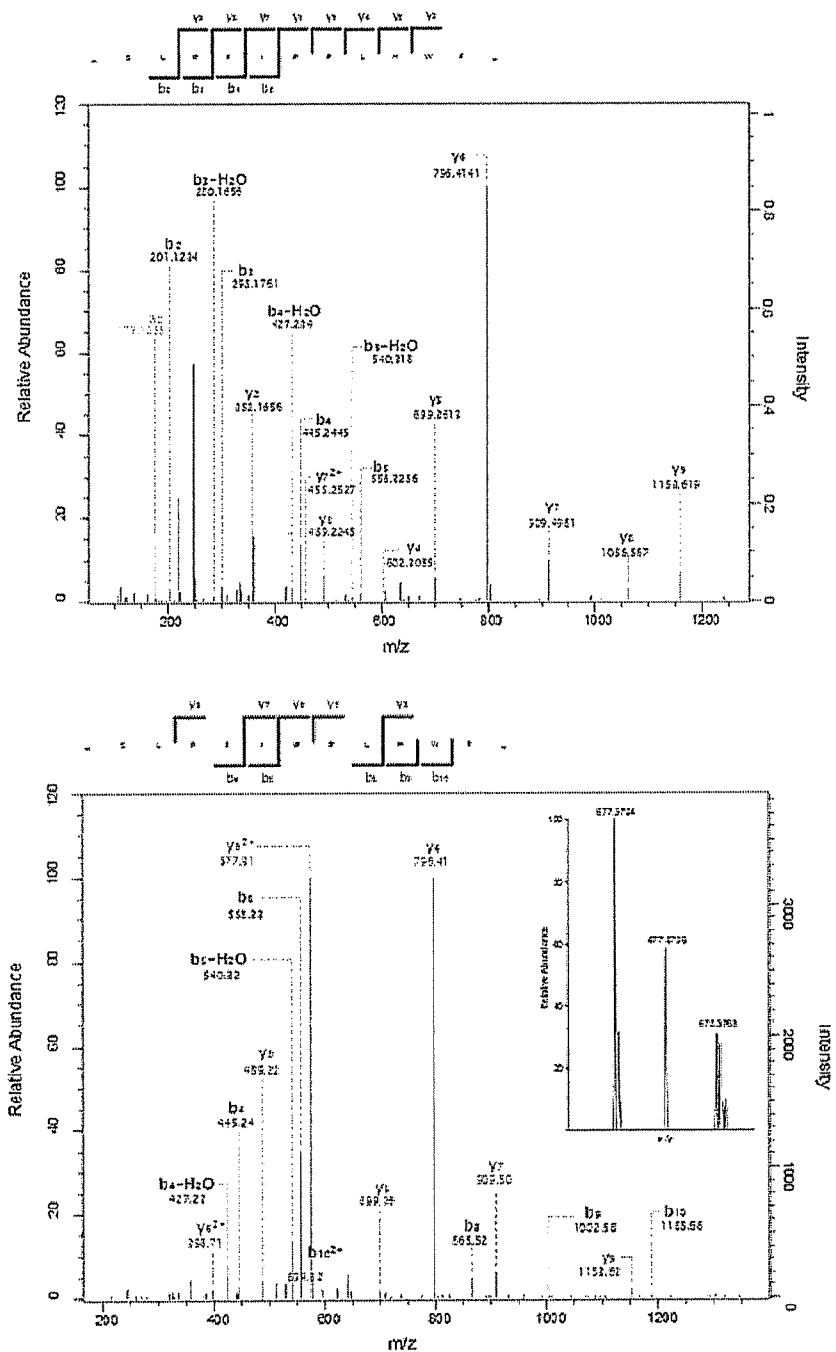
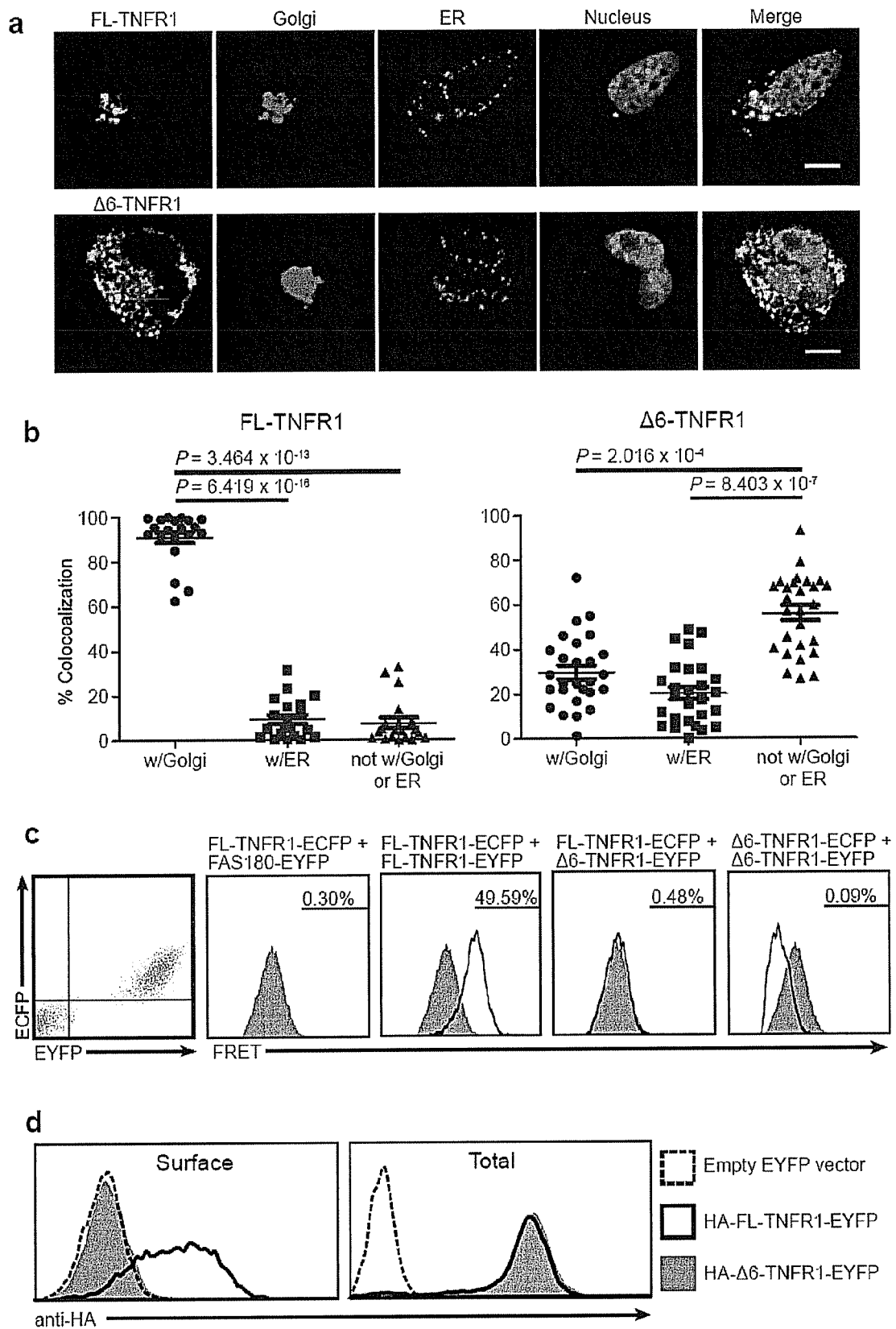


Figure 7

8/17



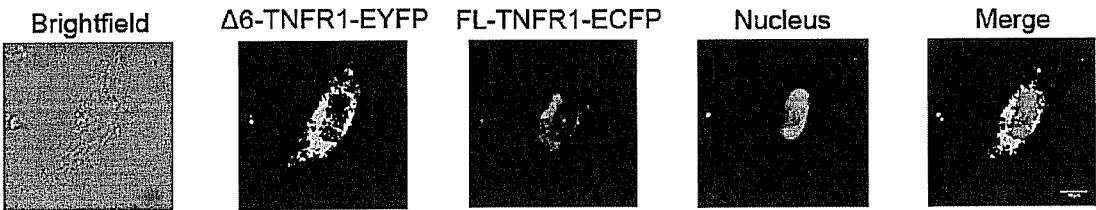


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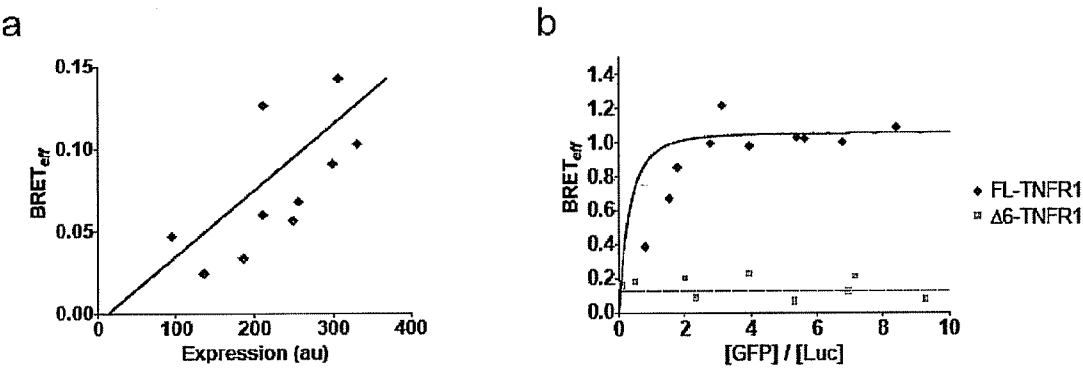


Figure 10

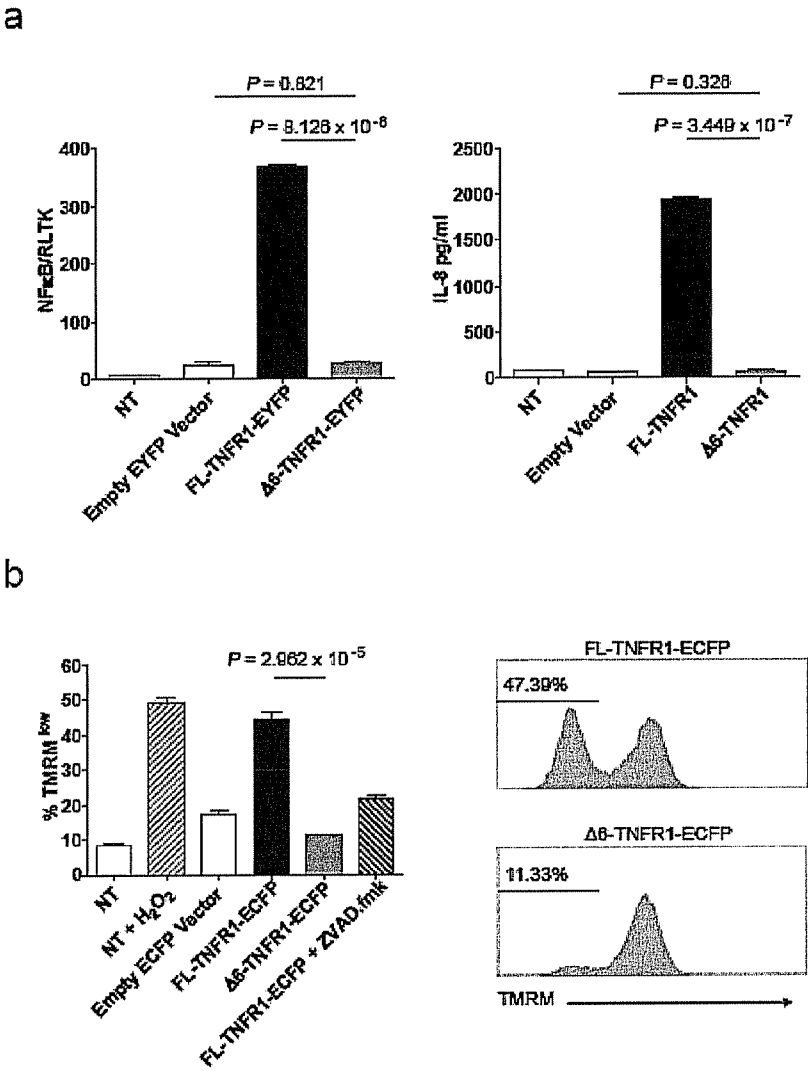


Figure 11

11/17

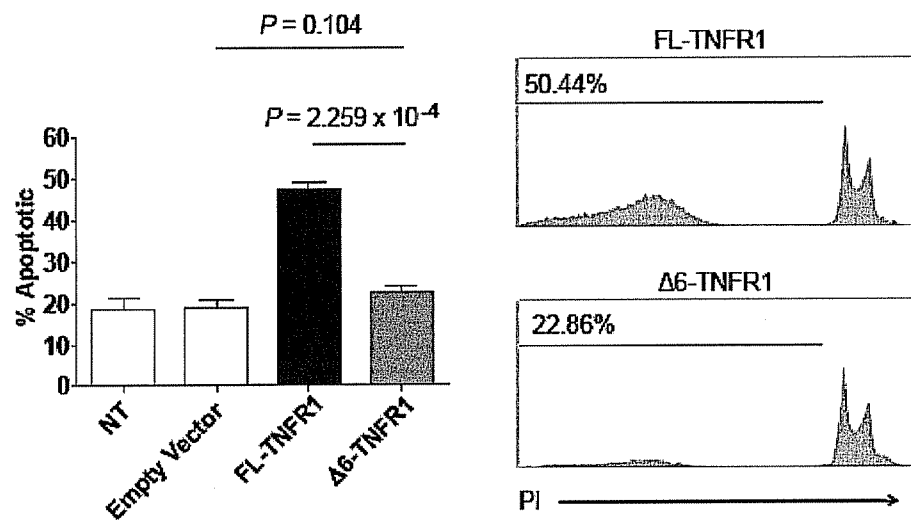
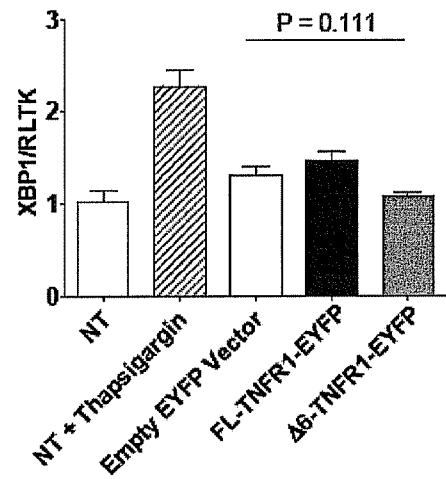


Figure 12

a



b

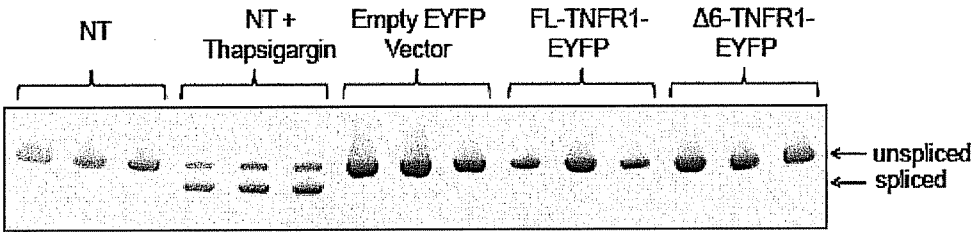


Figure 13

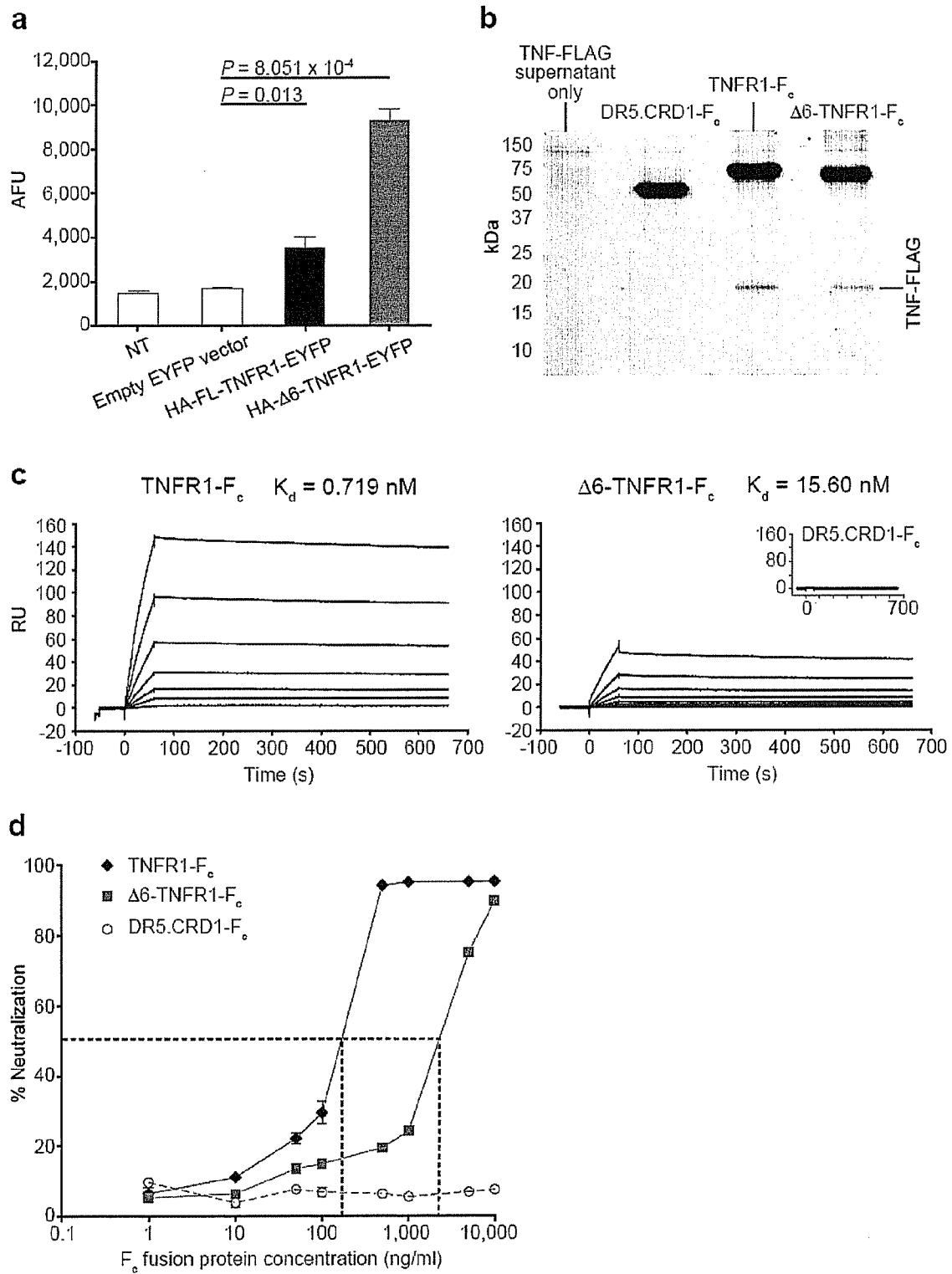


Figure 14

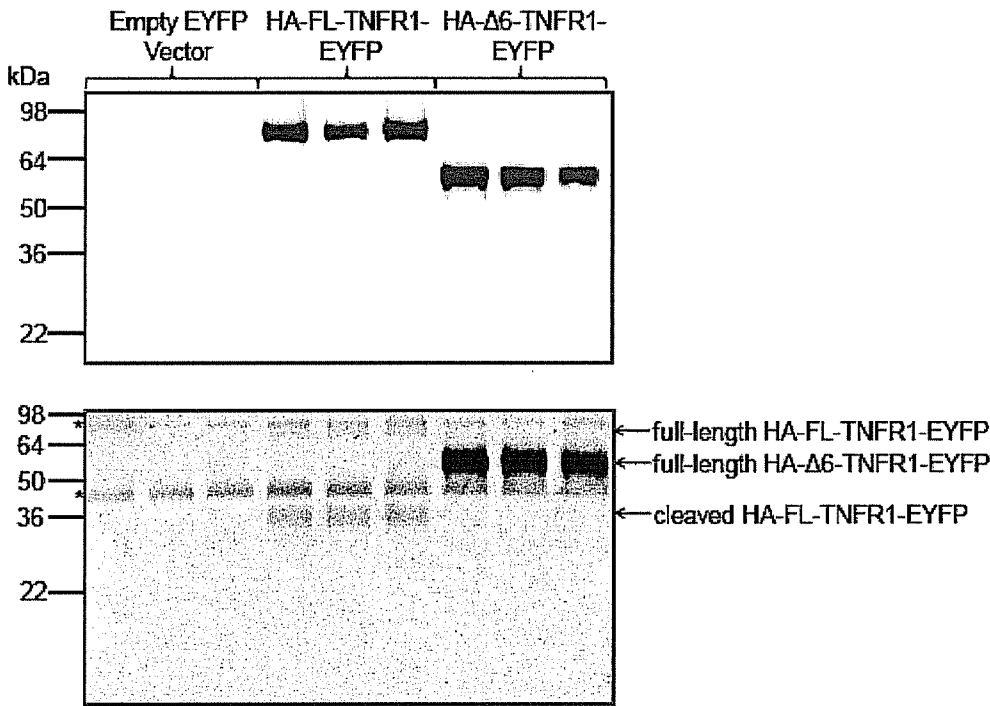
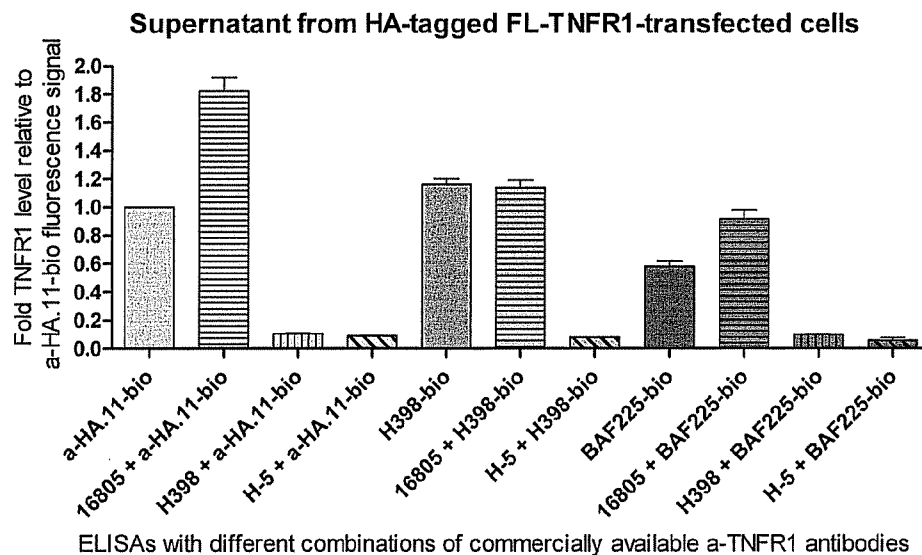
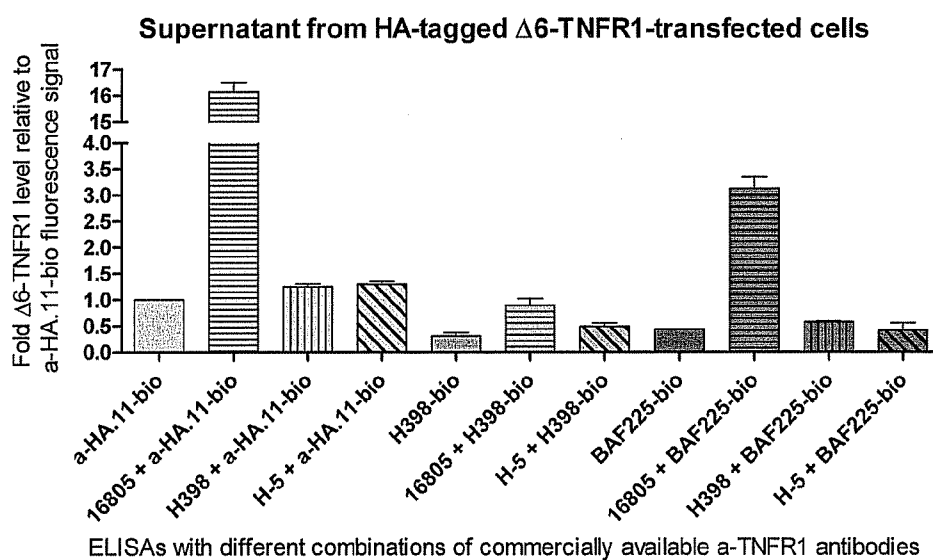
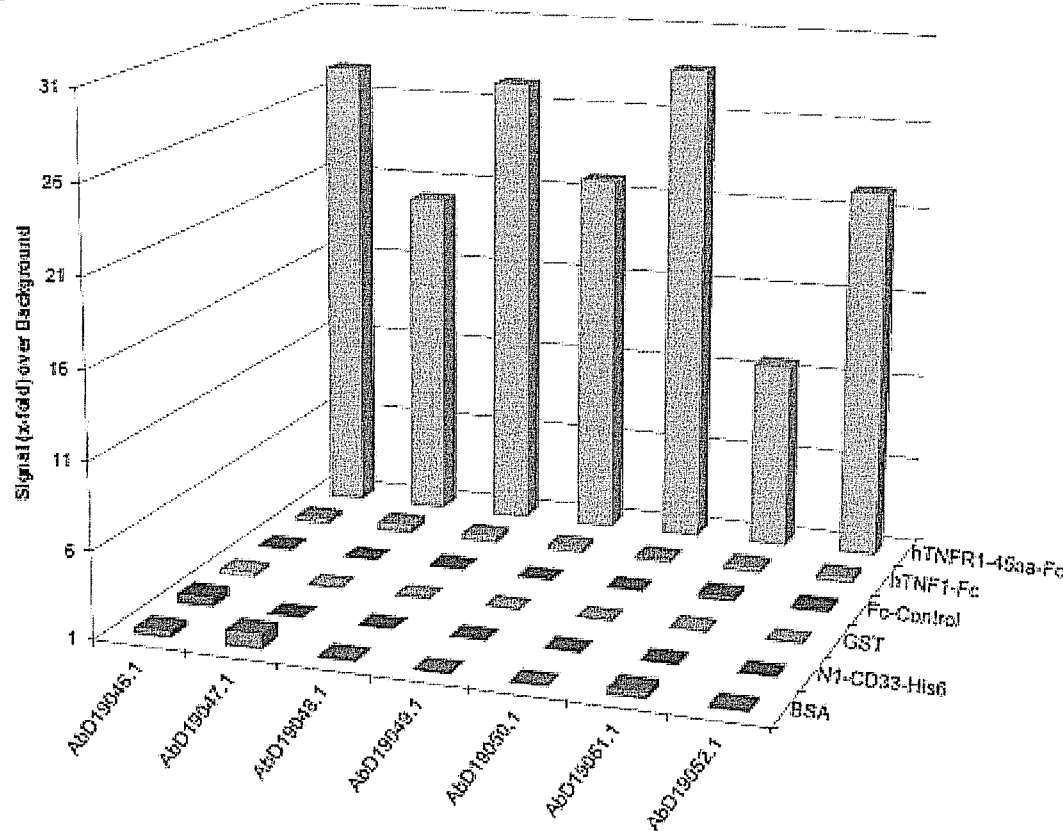


Figure 15

a**b****Figure 16**

a



b

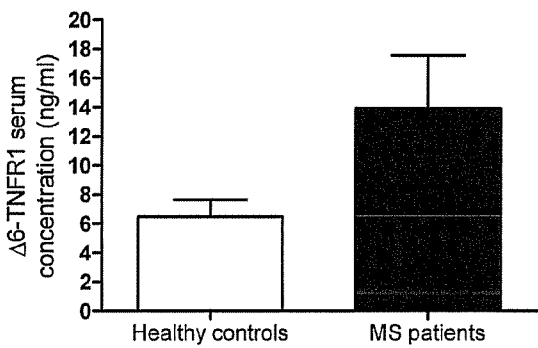
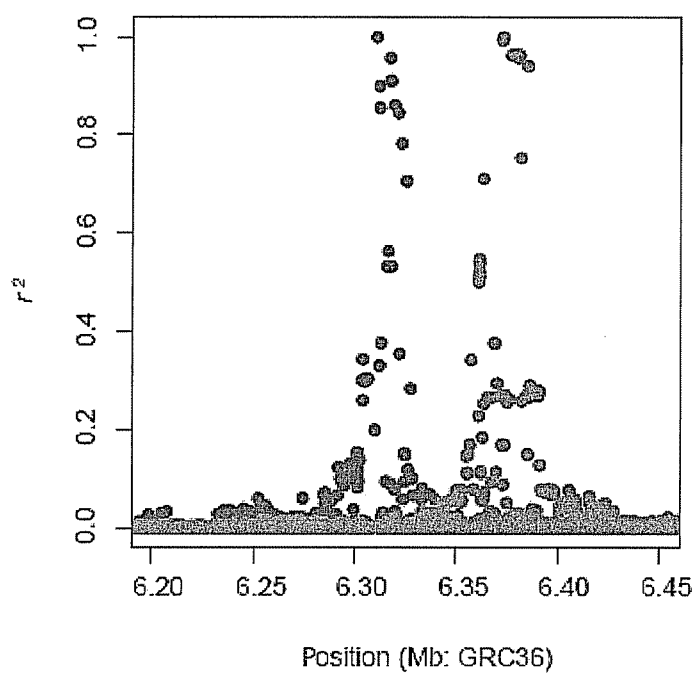


Figure 17

17/17

**Figure 18**

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2013/051518

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12Q1/68 C07K16/28
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12Q C07K

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

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

EPO-Internal, WPI Data, BIOSIS, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/072340 A2 (COMPUGEN LTD [IL]; COMPUGEN INC [US]; AYALON-SOFFER MICHAL [IL]; LEVIN) 11 August 2005 (2005-08-11) SEQ ID NO:571 (SNP position 317 A/G) SEQ ID NO:633 variant polypeptide SEQ ID NO:1565 Epitope; page 508, line 7; tables 156,165 page 503 - page 504 -----	11-17, 20-28,32
X	WO 2008/093323 A2 (COMPUGEN LTD [IL]; WALLACH SHIRA [IL]; SAMEAH-GREENWALD SHIRLEY [IL];) 7 August 2008 (2008-08-07) SEQ ID NO:99 (variant polypeptide); page 145; claims 1-6,19,20,29 ----- -/--	11-17, 20-28,32

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

4 July 2013

Date of mailing of the international search report

18/07/2013

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Gabriels, Jan

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2013/051518

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	HIMMLER A ET AL: "MOLECULAR CLONING AND EXPRESSION OF HUMAN AND RAT TUMOR NECROSIS FACTOR RECEPTOR CHAIN (P60) AND ITS SOLUBLE DERIVATIVE, TUMOR NECROSIS FACTOR-BINDING PROTEIN", DNA AND CELL BIOLOGY, MARY ANN LIEBERT, NEW YORK, NY, US, vol. 9, no. 10, 1 January 1990 (1990-01-01), pages 705-715, XP001118652, ISSN: 1044-5498 page 713, right-hand column, last paragraph -----	1-10,18, 19, 29-31,33
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INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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	& NEPHRON 1994, vol. 66, no. 4, 1994, pages 386-390, ISSN: 0028-2766 -----	
Y	ENGELMANN H ET AL: "TWO TUMOR NECROSIS FACTOR-BINDING PROTEINS PURIFIED FROM HUMAN URINE. EVIDENCE FOR IMMUNOLOGICAL CROSS-REACTIVITY WITH CELL SURFACE TUMOR NECROSIS FACTOR RECEPTORS", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 265, no. 3, 25 January 1990 (1990-01-25), pages 1531-1536, XP000673304, ISSN: 0021-9258 the whole document	1-10,18, 19, 29-31,33
Y	US 2007/249538 A1 (SAZANI PETER L [US] ET AL) 25 October 2007 (2007-10-25) claims 1-5 -----	1-10,18, 19, 29-31,33
A	STEPHEN SAWCER ET AL: "Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis", NATURE, vol. 476, no. 7359, 10 August 2011 (2011-08-10), pages 214-219, XP055068833, ISSN: 0028-0836, DOI: 10.1038/nature10251 the whole document -----	1
A	AN GORIS ET AL: "TNFRSF1A coding variants in multiple sclerosis", JOURNAL OF NEUROIMMUNOLOGY, ELSEVIER SCIENCE PUBLISHERS BV, NL, vol. 235, no. 1, 15 April 2011 (2011-04-15), pages 110-112, XP028228530, ISSN: 0165-5728, DOI: 10.1016/J.JNEUROIM.2011.04.005 [retrieved on 2011-04-22] the whole document -----	1
T	ADAM P. GREGORY ET AL: "TNF receptor 1 genetic risk mirrors outcome of anti-TNF therapy in multiple sclerosis", NATURE, vol. 488, no. 7412, 8 July 2012 (2012-07-08), pages 508-511, XP055068853, ISSN: 0028-0836, DOI: 10.1038/nature11307 the whole document -----	

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Information on patent family members

International application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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