Genetic variation in the serotonin receptor gene affects immune responses in rheumatoid arthritis

O Snir, E Hesselberg, P Amourdruz, Klareskog, L Zarea-Ganjii, A Catrina, N Padyukov, V Malmström and M Seddighzadeh

INTRODUCTION

Detection of association in human complex diseases has been facilitated by the development of new tools for genome-wide genotyping. During the last 5 years, over 1300 genome-wide association studies have been performed, however, most risk alleles have not been translated to specific causal effects. Indeed, due to high abundance of genetic polymorphisms and common linkage disequilibrium in genetic loci, it is an enormous task to identify causality in genetics. The difficulty may also relate to the involvement of less common genetic variants, which calls for an efficient approach to the sample selection for functional study. Two inclusion criteria are most preferable as grounds for experimental mechanistic studies: (i) an association with a disease-related subphenotype. (ii) availability of data for association of this locus with a disease-related subphenotype.

Serotonin (5-hydroxytryptamine; 5-HT) is an important neurotransmitter that besides its involvement in a large number of central nervous system processes also regulates many physiological functions and displays immunomodulatory effects. Several hematopoietic cell lineages such as platelets, monocytes and lymphocytes can store and release 5-HT when stimulated. Seven main groups of 5-HT receptors (5-HT1–7) have been identified; all are G protein-coupled receptors apart from 5-HT3 which is a ligand-gated ion channel. The 5-HT2A receptor is not only expressed by neurons but is also expressed on fibroblasts, platelets and human peripheral blood mononuclear cells (PBMCs). It has an important role in mediating physiological processes such as smooth muscle contraction, platelet aggregation and the modulation of mood and perception. The expression of 5-HT2A receptor mRNA has been observed in dendritic cells, monocytes and lymphocytes. This receptor is encoded by HTR2A (MIM 182135) that is localized on the human chromosome 13q14-q21 and consists of three exons with known five non-synonymous and two synonymous variations and two introns with >200 known variations.

The function of the 5-HT2A receptor in the periphery is still largely unknown, but an increasing body of evidence points to effects of this receptor on immune responses. The production of tumor necrosis factor alpha (TNF-α) from PBMCs following lipopolysaccharide (LPS) stimulation is inhibited by 5-HT via its 5-HT2A receptor and activation of this receptor suppresses TNF-α-induced inflammation in primary aortic smooth muscle cells. The 5-HT2A receptor is also involved in cis-urocanic acid-induced immune suppression, and it was recently shown that treatment of cytometric bead array (CBA) mice with a selective serotonin agonist caused immune suppression, an effect that was reversed with a 5-HT2A antagonist.

Rheumatoid arthritis (RA (MIM 180300)) is a common chronic inflammatory disease resulting from the complex interaction between genes and environment. To date, >30 RA-associated genetic loci have been defined with the HLA-DRB1 being the major one. Recently, we demonstrated that a haplotype in HTR2A composed of the protective alleles of two SNPs in HTR2A is associated with protection against RA. Furthermore, this
haplotype interacts with HLA-DRB1 shared epitope alleles and the 5-HT2A receptor is colocalized with HLA-DR molecules in synovial tissue cells from patients with RA. These findings prompted us to investigate whether this haplotype also associates with the changes in function of the immune system. To this end, we studied the impact of the TC haplotype and the effect of selective modulation of the 5-HT2A receptor on effector functions of RA-derived immune cells.

RESULTS

The TC haplotype of HTR2A affects cytokine secretion from T cells and monocytes

Since the 5-HT2A receptor is expressed on PBMC, we first investigated whether the specific TC haplotype in HTR2A that associates with RA influences T-cell and/or monocyte functions. The production of TNF-α, interleukin-17 (IL-17) and interferon gamma (IFN-γ) was monitored both in culture supernatants and intracellularly following the short-term stimulation of PBMCs with the superantigen staphylococcal enterotoxin B (SEB) and further compared between TC and non-TC patients. Interestingly, a clear tendency for higher production of TNF-α, IL-17 and IFN-γ was shown in patients carrying the TC HTR2A haplotype as was monitored both in culture supernatants and intracellularly (Figures 1a–c and e–g, respectively). When considered altogether, these cytokines indicated significantly higher levels of proinflammatory cytokines in culture supernatants from TC patients as compared with non-TC patients (Figure 1d). These data were further corroborated by intracellular cytokine measurements in CD4+ T cells. However, this was not statistically significant (Figure 1h). Also, the overall activity levels of CD4 T cells illustrated by the levels of CD40L were similar in the two haplotype groups (Figure 1l). Gating strategy for detection of intracellular cytokine in CD4 T cells is shown in Supplementary Figure 1.

A short-term stimulation of PBMCs with LPS, which specifically stimulates monocytes, resulted in an increase in TNF-α and IL-6 production both in culture supernatants and intracellularly (Figure 2). Similarly to SEB stimulation, the secreted levels of TNF-α following LPS stimulation were significantly higher in the TC group compared with the non-TC group (Figures 2a and c), whereas for IL-6 an opposite effect was observed (Figures 2b and d). Gating strategy for detection of intracellular cytokine in monocyte following stimulation with LPS is shown in Supplementary Figure 2.

T-cell and monocyte cytokine secretion is inhibited via 5-HT2A receptor

A major aim was to address the question of whether direct activation of 5-HT2A using the selective receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) differently affects the function of T cells and monocytes from RA patients with or without the TC haplotype. We first examined whether DOI can induce apoptosis and/or necrosis of CD4+ T cells and monocytes using Annexin V/7AAD staining. The results demonstrated that the T-cell and monocyte viability was not affected in the presence of up to 75 μM of DOI (Supplementary Figure 3). Next, PBMCs were cultured with 50 μM of DOI and further stimulated with either SEB or LPS to achieve T-cell and monocyte stimulation, respectively.

Figure 1. Increased cytokine production by T cells from RA patients carrying the TC haplotype. PBMCs from RA patients were stimulated with SEB. (a) TNF-α, (b) IL-17 and (c) IFN-γ were measured in culture supernatant following SEB stimulation. (d) The mean of the pooled levels of T-cell cytokines in supernatant (TNF-α, IL-17 and IFN-γ). Production of (e) TNF-α, (f) IL-17 and (g) IFN-γ was also measured intracellularly using flow cytometry and presented as a percentage of total CD4 T-cell population. (h) Mean of pooled cytokine levels that were monitored by ICS and CD40L levels in CD4 T cells (i). Significance of differences was determined by Mann–Whitney test. *P < 0.05.
DOI inhibited CD4+ T-cell activation following SEB stimulation as monitored by reduced levels of CD40L in both TC and non-TC RA patients. Of note, the inhibitory effect induced by DOI was significantly stronger in the TC group compared with the non-TC group (Figure 3a). We further examined and compared the effects of DOI on the production of TNF-α, IL-17 and IFN-γ by T cells in the two haplotype groups in culture supernatants and intracellularly. Indeed, DOI inhibited the production of these cytokines, however, significant differences were found between the two TC-haplotype groups. The secretion of TNF-α in the TC-patient group was reduced significantly more in comparison with the non-TC group (Figure 3b) and a similar trend was also found for IL-17 and IFN-γ (Figures 3c and d). Also, the overall inhibition of T-cell cytokines was significantly stronger in the TC group in comparison with the non-TC group, both in culture supernatants and intracellularly (Figure 3e).

DOI treatment before LPS stimulation compared with LPS stimulation alone resulted in significant inhibition of TNF-α (P < 0.0001) and a slight increase in IL-6, both primarily produced by monocytes (Supplementary Figure 4). Also here, the measured concentrations of TNF-α in supernatants were significantly lower in the TC group compared with non-TC group (Figure 3f). This effect, however, could not be detected using intracellular staining (ICS) (Figure 3f). In contrast, IL-6 was less inhibited in the TC group, however, this difference was not significant (Supplementary Figure 5).

To find out whether the inhibition of the cytokine production by DOI is specifically mediated via the 5-HT2A receptor or whether 5-HT2C receptor also is involved in the cytokine secretion, we pretreated PBMCs from healthy controls with receptor selective antagonists before stimulation with DOI and LPS or SEB. We could see that the inhibition of cytokine production in monocytes and T cells is partly mediated through both 5-HT2A and 5-HT2C receptors (Figures 4a-d).

**DISCUSSION**

Serotonin (5-HT) is primarily known as a neurotransmitter involved in the regulation of mood, sleep, appetite and cognition. However, it also influences the immune system and can regulate inflammation and cytokine secretion. Indeed, it has been demonstrated that 5-HT inhibits TNF-α synthesis from human monocytes and PBMCs through several 5-HT receptors. A specific haplotype in the HTR2A was previously demonstrated to be associated with reduced risk for the development of RA, that is, the TC haplotype. We therefore hypothesized that the TC haplotype may control the 5-HT regulatory effects on the immune system and examined its potential effects on T cells and monocytes in RA using a selective 5-HT2A receptor agonist (DOI).

Our data demonstrate that upon stimulation, T cells and monocytes from patients carrying the TC haplotype in HTR2A produce more proinflammatory cytokines compared with patients who do not. The stimulation, however, was inhibited by the 5-HT2A receptor agonist DOI and this effect was more pronounced in the TC group. These findings speak for a regulatory role of 5-HT on cytokines production by T cells and monocytes. Of interest, it further shows the greater inhibitory effect of serotonin in individuals carrying the TC haplotype, which is in agreement with the protective feature of this haplotype in RA, indicating a possible functional explanation for this genetic polymorphism. To strengthen our studies, T-cell and monocyte function was measured by intracellular CD40L in both TC and non-TC RA patients. Ketanserin, a non-selective 5-HT2A receptor antagonist was used. Thereby the involvement of the 5-HT2C receptor could not be excluded. To find whether in our experimental settings DOI

---

**Figure 2.** Increased cytokine production by monocytes from RA patients carrying the TC haplotype. PBMCs from RA patients were stimulated with LPS. (a) TNF-α and (b) IL-6 production by monocytes was monitored in culture supernatant and intracellularly (c, d), respectively. TC group (n = 11–13) and non-TC group (n = 10–11). Significance of differences was determined by Mann–Whitney test. *P < 0.05, **P < 0.01.
inhibits cytokine production via the 5-HT2A and/or 5-HT2C receptors, we treated PBMCs with two different antagonists, specific to the 5-HT2A or 5-HT2C receptor before adding DOI and further stimulated with either (a–e) SEB or (f) LPS and cytokine production was measured in culture supernatant and intracellularly. (a) T-cell activation was monitored using CD40L and (b) TNF-α, (c) IL-17 and (d) IFN-γ production was measured. The overall effect of DOI on T-cell cytokine secretion is shown in (e) (f) TNF-α produced by monocytes. TC group (n = 11–13) and non-TC group (n = 10–11). Inhibition index: the ratio of the levels of cytokine secretion following stimulation divided by stimulation response with agonist. Data are presented as box plots, where the boxes represent the 25th to 75th percentiles and the lines within the boxes represent the median. Significance of differences was determined by Mann–Whitney test. *P < 0.05, **P < 0.01, ***P < 0.001.
106 cells per well, in the presence or absence of 50 \mu M PBMCs were thawed and cultured in flat-bottom 96-well plates, 1.0. Antibodies and intracellular flow cytometry

Isolation of PBMCs

PBMCs were isolated from blood of RA patients using Ficoll separation media (Ficoll-Paque, GE Healthcare, St Louis, MO, USA) and thereafter frozen in fetal calf serum supplemented with 10% DMSO (Merck KGaA, Darmstadt, Germany) and kept at -150°C until use.

Isolation of PBMCs

PBMCs were isolated from blood of RA patients using Ficoll separation media (Ficoll-Paque Plus, GE Healthcare, St Louis, MO, USA) and thereafter frozen in fetal calf serum supplemented with 10% DMSO (Merck KGaA, Darmstadt, Germany) and kept at -150°C until use.

Antibodies and intracellular flow cytometry

PBMCs were thawed and cultured in flat-bottom 96-well plates, 1.0 \times 10^5 cells per well, in the presence or absence of 50 \mu M of (±)DOI (Sigma-Aldrich, St Louis, MO, USA) for 16 h. Cells were then stimulated with either 50 ng ml^{-1} of SEB (Sigma-Aldrich) or 50 pg ml^{-1} of LPS (Sigma-Aldrich) for 5 or 4 h, respectively, together with 10 \mu g ml^{-1} of brefeldin A (Sigma-Aldrich). Following stimulation, cells were treated with LIVE/DEAD Fixable Green Dead Cell Stain (Invitrogen, Carlsbad, CA, USA), washed and permeabilized using a Cytofix/CytoPerm fixation/permeabilization solution kit (BD Biosciences). Samples were acquired on a CyAn ADP Analyzer (Dako, Glostrup, Denmark) and the data were analyzed by FlowJo software version 7.6.1 (Tree star, Ashland, OR, USA).

Figure 4. Selective 5-HT2A receptor and 5-HT2C receptor antagonists partially inhibit the effect of DOI. The intracellular TNF-\(\alpha\) produced by monocytes was measured in (a) PBMCs from healthy controls treated with 1 \mu M of MDL100907 (selective 5-HT2A receptor antagonist) or 1 \mu M RS 102221 (selective 5-HT2C receptor antagonist) for 30 min before treatment with 50 \mu M of DOI for 1 h and 50 pg ml^{-1} of LPS for 4 h. (b-d) PBMCs from healthy controls were treated with 1 \mu M of MDL100907 or 1 \mu M of RS 102221 for 30 min and thereafter treated with 10 \mu M of DOI for 1 h and with 10 ng ml^{-1} of SEB overnight and the intracellular production of (b) TNF-\(\alpha\), (c) IFN-\(\gamma\) and (d) CD40L by CD4+ T cells was measured. These results are representative of two independent experiments. Values are the mean and s.e.m. D, DOI; L, lipopolysaccharide; M, MDL100907; S, SEB; R, RS 102221; U, unstimulated.

### Table 1. Characteristics and treatment(s) of eligible rheumatoid arthritis patients included in the study

| TC patienta | Age (years) | Treatment(s)b | Non-TC patienta | Age (years) | Treatment(s)b |
|-------------|-------------|----------------|-----------------|-------------|----------------|
| 1           | 63          | Adalimumab + MTX + Prednisolone | 1c          | 57          | Etanercept + MTX + Prednisolone |
| 2           | 70          | Etanercept + MTX + Prednisolone | 2            | 57          | Infliximab + Azathioprine |
| 3           | 49          | Etanercept + Azathioprine | 3            | 41          | Etanercept + MTX |
| 4           | 43          | Etanercept + Metoject | 4            | 57          | Etanercept + MTX |
| 5           | 53          | Etanercept + MTX | 5            | 51          | Infliximab + Salazopyrin |
| 6           | 58          | Etanercept + Salazopyrin | 5            | 50          | Adalimumab |
| 7           | 49          | Etanercept | 6            | 56          | Adalimumab |
| 8           | 78          | Untreated | 7c           | 77          | Untreated |
| 9           | 83          | NSAID | 10            | 63          | Infliximab + MTX |
| 11          | 70          | Infliximab | 9            | 64          | Infliximab |
| 12          | 70          | Abatacept + MTX + NSAID | 10           | 65          | Abatacept + MTX |
| 13          | 82          | Prednisolone | 11           | 84          | Prednisolone |

Abbreviations: MTX, methotrexate; NSAID, non-steroidal anti-inflammatory drug. aAll are women. bThe non-TC patients were matched for the following treatments; MTX/Metoject, Salazopyrin, Abatacept and Azathioprine (DMARDs), Etanercept, Infliximab and Adalimumab (Biologics), Prednisolone (Corticosteroids). cEach of these patients was used as a match for two TC patients.

Hospital approved this study, and all subjects gave informed consent before participation in the study.

Genotyping

Genomic DNA was extracted from EDTA-treated peripheral white blood cells using salting-out method. The genotyping for rs6314 and rs1328674 was done using TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA, USA). The results were analyzed using the Sequence Detection System v 2.2 software (Applied Biosystems). The call rate was above 95% for both SNPs.

Detection of cytokines in culture supernatant

In parallel to each cell culture described above, an identical culture was set up in the absence of brefeldin A, allowing cytokine secretion. Culture supernatants were collected and the levels of TNF-\(\alpha\), IL-17, IFN-\(\gamma\) and IL-6 were determined using CBA according to manufacturer’s instructions (BD Biosciences). Samples were acquired using CyAn flow cytometer and

© 2013 Macmillan Publishers Limited Genes and Immunity (2013) 83 – 89
the concentrations were determined using FCAP Array v1.0.1 software (BD Biosciences). Cytokine levels in culture supernatants were monitored in pg ml⁻¹ and the background levels were subtracted. However, to reduce variability between samples as well as between different cytokines the measured cytokine levels were ranked from 1 to 25, with the highest level being 25 and the lowest being 1. To confirm that this ranking did not introduce an error in our analysis, a pair-wise comparison was performed for cytokine levels measured in supernatant from samples treated with either LPS or SEB and subsequent treatments with DOI. TC group (n = 11–13) and non-TC group (n = 10–11). Significance of differences between the groups was determined using Wilcoxon signed rank test. NS, not significant.

**Figure 5.** DOI causes a greater cytokine inhibition from monocytes and T cells of patients carrying the TC haplotype. The effect of DOI on TNF-α production from LPS-stimulated monocytes isolated from TC patients (a) and non-TC patients (b) Pooled T-cell cytokine levels (that is, TNF-α, IL-17 and IFN-γ) in culture supernatants of patients carrying the TC haplotype (c) in comparison with patients who do not (d) following stimulation with SEB and subsequent treatments with DOI. TC group (n = 11–13) and non-TC group (n = 10–11).

**Statistics**

The distribution of genotypes was in agreement with Hardy–Weinberg equilibrium (P > 0.05). The haplotypes, comprising rs6314 and rs1328674, for each individual were assigned using PHASE software. Whereas inhibition is usually expressed as a percentage of stimulation, in the present study we have inverted this index to circumvent values that lay outside the maximum threshold (100%). Thus, the reduction in cytokine secretion following treatment with DOI was assessed using an inhibition index, calculated as the ratio between an SEB/LPS-stimulated sample and its corresponding DOI-pre-treated one. Due to the limited number of samples that met the criteria to be included in the study, cumulative analysis of proinflammatory T-cell cytokines (TNF-α, IL-17 and IFN-γ) was used to increase the statistical power. Differences in cytokine secretion or inhibition between two groups were analyzed by unpaired, non-Gaussian two-tailed t-tests (Mann–Whitney) and values of P < 0.05 were considered as significant. All the graphics were performed using Graphpad Prism (GraphPad Software Inc., La Jolla, CA, USA).

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ACKNOWLEDGEMENTS**

We would like to thank the patients for their contribution to the study, and Eva Jemseby, Gull-Britt Almgren and Julia Bostroem for organizing administration of biomaterial and Hawa Camara for excellent data collection. This study was supported by grants from the King Gustaf V's 80-year Foundation, the EU-supported AutoCure project, the Swedish Combine project and the Swedish Agency Vinnova.

**REFERENCES**

1. Wright AF. Genomics of common diseases: approaching the tipping point. Genome Med 2011; 3: 70.

2. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ et al. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol Rev 1994; 46: 157–203.

3. Abdouh M, Storring JM, Riad M, Paquette Y, Albert PR, Drobetsky E et al. Transcriptional mechanisms for induction of S-HT1A receptor mRNA and protein in activated B and T lymphocytes. J Biol Chem 2001; 276: 4382–4388.

4. Izdki M, Panther E, Stratz C, Muller T, Bayer H, Zissel G et al. The serotoninergic receptors of human dendritic cells: identification and coupling to cytokine release. J Immunol 2004; 172: 6011–6019.

5. Leon-Ponte M, Ahern GP, O’Connell PJ. Serotonin provides an accessory signal to enhance T-cell activation by signaling through the S-HT7 receptor. Blood 2007; 109: 3139–3146.

6. Matsuha H, Ushio H, Geba GP, Askenase PW. Human platelets can initiate T cell-dependent contact sensitivity through local serotonin release mediated by IgE antibodies. J Immunol 1997; 158: 2891–2897.

7. O’Connell PJ, Wang X, Leon-Ponte M, Griffiths C, Pingle SC, Ahern GP. A novel form of immune signaling revealed by transmission of the inflammatory mediator serotonin between dendritic cells and T cells. Blood 2006; 107: 1010–1017.

8. Stemberg EM, Trial J, Parker CW. Effect of serotonin on murine macrophages: suppression of IL-1α expression by serotonin and its reversal by S-HT2 serotoninergic receptor antagonists. J Immunol 1986; 137: 276–282.

9. Messner R, Lesch KP. Role of serotonin in the immune system and in neuroimmune interactions. Brain Behav Immun 1998; 12: 249–271.

10. Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of S-HT receptors. Pharmacol Biochem Behav 2002; 71: 533–554.

11. Cloez-Tayarani I, Petit-Bertron AF, Venters HD, Cavaillon JM. Differential effect of serotonin on cytokine production in lipopolysaccharide-stimulated human peripheral blood mononuclear cells: involvement of S-hydroxytryptamine2A receptors. Int Immunol 2003; 15: 359–366.

12. Roth BL, Williams DL, Kristiansen K, Kroeze WK. S-Hydroxytryptamine2-family receptors (5-hydroxytryptamine2A, 5-hydroxytryptamine2B, 5-hydroxytryptamine2C): where structure meets function. Pharmacol Ther 1998; 79: 231–257.

13. de Clerck F, David JL, Janssen PA. Inhibition of 5-hydroxytryptamine-induced and -amplified human platelet aggregation by ketanserin (R 41 468), a selective S-HT2-receptor antagonist. Agents Actions 1982; 12: 388–397.

14. Roth BL, Berry SA, Kroeze WK, Williams DL, Kristiansen K. Serotonin S-HT2 receptors: molecular biology and mechanisms of regulation. Crit Rev Neurobiol 1998; 12: 319–338.

15. Durk T, Panther E, Muller T, Sorichter S, Ferrari D, Pizzirani C et al. 5-Hydroxytryptamine modulates cytokine and chemokine production in LPS-primed human monocytes via stimulation of different S-HT receptor subtypes. Int Immunol 2005; 17: 599–606.

16. Stefuji J, Jemej B, Cicin-Sain L, Rinner I, Schauenstein K. mRNA expression of serotonin receptors in cells of the immune tissues of the rat. Brain Behav Immun 2000; 14: 219–224.

17. Inoue M, Okazaki T, Kitaono T, Mizushima M, Omata M, Ozaki S. Regulation of antigen-specific CTL and Th1 cell activation through S-Hydroxytryptamine 2A receptor. Int Immunopharmacol 2011; 11: 67–73.
18 Guillet-Denau I, Burnol AF, Girard J. Identification and localization of a skeletal muscle serotonin 5-HT2A receptor coupled to the Jak/STAT pathway. J Biol Chem 1997; 272: 14825–14829.
19 Yu B, Becnel J, Zerfaoui M, Rohatgi R, Boulares AH, Nichols CD. Serotonin 5-hydroxytryptamine (5-HT2A) receptor activation suppresses tumor necrosis factor-alpha-induced inflammation with extraordinary potency. J Pharmacol Exp Ther 2008; 327: 316–323.
20 Walterscheid JP, Nghiem DX, Kazimi N, Nutt LK, McConkey DJ, Norval M et al. Cis-urocanic acid, a sunlight-induced immunosuppressive factor, activates immune suppression via the 5-HT2A receptor. Proc Natl Acad Sci USA 2006; 103: 17420–17425.
21 Davydova SM, Cheido MA, Gevorgyan MM, Idova GV. Effects of 5-HT2A receptor stimulation and blocking on immune response. Bull Exp Biol Med 2010; 150: 219–221.
22 Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP et al. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat Genet 2010; 42: 508–514.
23 Stastny P. Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. N Engl J Med 1978; 298: 869–871.

24 Seddighzadeh M, Korotkova M, Kallberg H, Ding B, Daha N, Kurreeman FA et al. Evidence for interaction between 5-hydroxytryptamine (serotonin) receptor 2A and MHC type II molecules in the development of rheumatoid arthritis. Eur J Hum Genet 2010; 18: 821–826.
25 Nelson DL, Lucaites VL, Wainscott DB, Glennon RA. Comparisons of hallucinogenic phenylisopropylamine binding affinities at cloned human 5-HT2A, -HT2B and 5-HT2C receptors. Naunyn Schmiedebergs Arch Pharmacol 1999; 359: 1–6.
26 Nichols DE, Nichols CD. Serotonin receptors. Chem Rev 2008; 108: 1614–1641.
27 Arzt E, Costas M, Finkielman S, Nahmod VE. Serotonin inhibition of tumor necrosis factor-alpha synthesis by human monocytes. Life Sci 1991; 48: 2557–2562.
28 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31: 315–324.
29 Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003; 73: 1162–1169.

Supplementary Information accompanies the paper on Genes and Immunity website (http://www.nature.com/gene)