SUPPLEMENTARY DATA

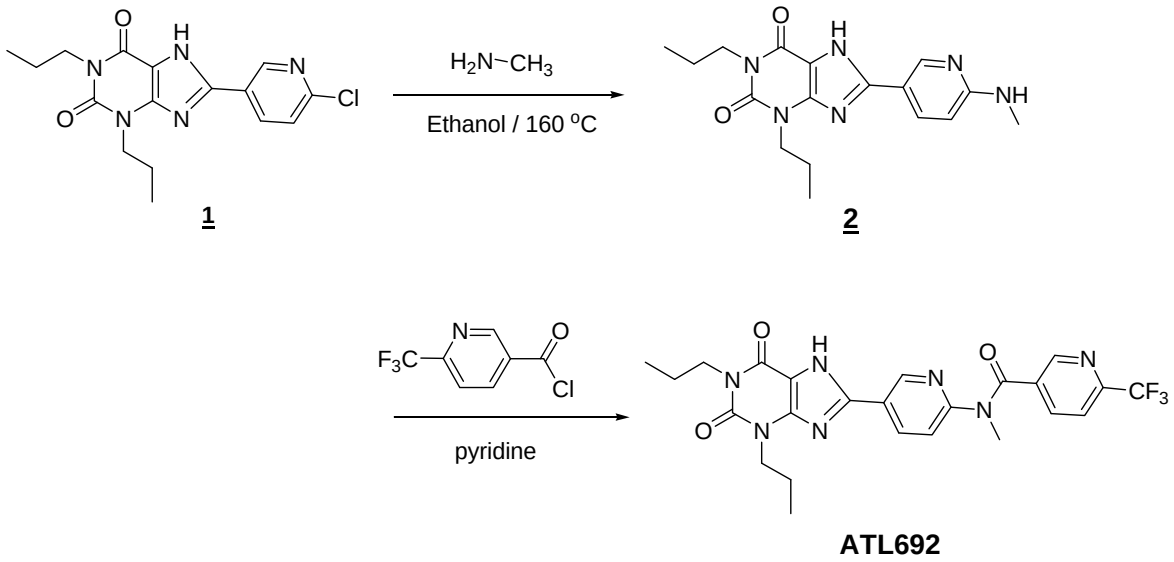
ATL692 Synthesis

Proton nuclear magnetic resonance spectroscopy was performed on a Varian-300 MHz spectrometer and spectra were taken in DMSO-d_6 . Chemical shifts are expressed as ppm downfield from relative ppm from DMSO (2.5 ppm). Electro-spray-ionization (ESI) mass spectrometry was performed with a ThermoFinnigan LCQ mass spectrometer.

All xanthine derivatives were homogeneous as judged using TLC (MK6F silica, 0.25 mm, glass backed, Whatman Inc., Clifton, NJ) and HPLC (Shimadzu) using Varian C18 5 micron analytical column (4.6 mm x 150 mm) in linear gradient solvent system, at a flow rate of 1 ml / min. The solvent system used was MeOH (0.1% formic acid) : H_2O (0.1% formic acid). Peaks were detected by UV absorption at 300 nm and 254 nm. All xanthine derivatives tested in binding assays were shown to be homogeneous by TLC (MK6F silica, 0.25 mm, glass backed, Whatman Inc., Clifton, NJ) and HPLC. NMR and mass spectra were shown to be consistent with the assigned structure.

The synthesis of **ATL692** is shown in the Reaction Scheme. 8-(6-chloropyridin-3-yl)-1,3-dipropyl-1H-purine-2,6(3H,7H)-dione (**1**) was synthesized as previously described (1). **1** reacted with excess amount of 33% methylamine in ethanol at 160 ^oC in a pressure tube for 60 h to afford 8-(6-(methylamino)pyridin-3-yl)-1,3-dipropyl-1H-purine-2,6(3H,7H)-dione (**2**). The reaction of **2** with 6-trifluoromethylnicotinoyl chloride in pyridine gave 6-(trifluoromethyl)-N-(5-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)pyridin-2-yl)-N-methylpyridine-3-carboxamide (**ATL692**).

Reaction Scheme



8-(6-chloropyridin-3-yl)-1,3-dipropyl-1H-purine-2,6(3H,7H)-dione (1): 1 was prepared based on the method reported by P. J. Scammells, *et al*. [ref: *J. Med. Chem*. 37, 2704-2712 (**1994**)]. The product was collected and washed with water, methanol and ether to provide an off-white solid. The product was used in the next step without further purification. ^1H NMR: 0.89(m, 6H), 1.59(m, 2H), 1.73(m, 2H), 3.88(t, 2H, J=7.2Hz), 4.00(t, 2H, J=7.2Hz), 7.68(d, 1H, J=8.4Hz), 8.50(dd, 1H, J_1=8.4 Hz, J_2=2.4Hz), 9.07(d, 1H, J=2.4Hz). MS: m/z 348 $(M+H)^+$. HPLC condition: MeOH/H_2O (containing 0.1% formic acid) 40% to 95% 10 minute linear gradient then 95% MeOH. Retention time = 10.75 min.

SUPPLEMENTARY DATA

8-(6-(methylamino)pyridin-3-yl)-1,3-dipropyl-1H-purine-2,6(3H,7H)-dione (2): **1** (9.7g, 28 mmol) and 200 mL of 33% methylamine in ethanol was heated with stirring at 160 ^{o}C in a sealed glass pressure bottle for 60 hours. The reaction was monitored by HPLC. After cooling, the solid was collected and washed with methanol to give the product (8.4 g, 88%). ^{1}H NMR (DMSO, d_6): δ 0.88(m, 6H), 1.56(m, 2H), 1.72(m, 2H), 2.81(d, 3H, J=4.5Hz), 3.85(t, 2H, J=7.5Hz), 3.99(t, 2H, J=7.5Hz), 6.52(d, 1H, J=8.7Hz), 7.08(q, 1H, J=4.5 Hz), 8.01(dd, 1H, J_1=2.4 Hz, J_2=8.7Hz), 8.73(d, 1H, J=2.4Hz). MS: m/z 343 $(M+H)^+$. HPLC condition: MeOH/H_2O (containing 0.1% formic acid) 40% to 95% 10 minute linear gradient then 95% MeOH. Retention time = 6.20 min.

6-(trifluoromethyl)-N-(5-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)pyridin-2-yl)-N-methylpyridine-3-carboxamide (ATL692): 2 (0.55 g, 1.6 mmol) was dissolved in dry pyridine (150 ml) at 80°C. This solution was cooled to room temperature and 6-trifluoromethylnictonyl chloride (0.67 g, 3.2 mmol) was added. The reaction was stirred at room temperature for 24 hours. The reaction progress was monitored by HPLC. A small amount of ice was added to quench the reaction. The solvents were removed in vacuo and the resulting solid was washed with methanol and purified by silica gel column using CH_2Cl_2 96%/MeOH 4% as the eluent to give **ATL692** (4.8 g, 86%). ^{1}H NMR (DMSO, d_6): 0.89(m, 6H), 1.59(m, 2H), 1.73(m, 2H), 3.54(s, 3H), 3.87(t, 2H, J=7.5Hz), 4.01(t, 2H, J=7.5 Hz), 7.55(d, 1H, J=8.4 Hz), 7.90(d, 1H, J=8.4Hz), 8.01(d, 1H, J=8.4Hz), 8.40(dd, 1H, J_1=8.4Hz, J_2=2.4 Hz), 8.71(s, 1H), 8.96(d, 1H, J=2.4Hz). MS: m/z 516 $(M+H)^+$. HPLC condition: MeOH/H_2O (containing 0.1% formic acid) 40% to 95% 10 minute linear gradient then 95% MeOH. Retention time = 10.65 min.

Reference

1. Scammells,P.J., Baker,S.P., Belardinelli,L., and Olsson,R.A. 1994. Substituted 1,3-dipropylxanthines as irreversible antagonists of A1 adenosine receptors. J. Med. Chem. 37:2704-2712.

SUPPLEMENTARY DATA

Supplemental Materials - MESA

Phenotyping in MESA- Participants had detailed medical histories (including medication history and smoking history) and underwent examinations for anthropometry, blood pressure and vascular imaging. Fasting blood samples were taken for DNA and biomarkers (including cytokines and lipids). Participants were asked to fast for 12 h, avoid smoking in the morning of the examination, and avoid heavy exercise 12 h before the examination. Diabetes mellitus (DM) was defined as a fasting glucose >6.88 mmol/L or use of anti-diabetic medication. HOMA-IR (a measure of insulin resistance) was calculated as the product of fasting insulin x fasting glucose, divided by the constant 22.5. BMI was calculated as weight divided by the square of the height (kg/m^2). The Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT, USA) measured interleukin-6 (IL-6), interleukin 2 soluble receptor α chain (IL2sR), C-reactive protein (CRP), and plasminogen activator inhibitor-1 (PAI-1) concentrations in blood samples collected at baseline, processed with the use of a standardized protocol based on that used in the Cardiovascular Health Study (CHS), and stored at –80 °C until analyzed. Concentrations of plasma IL-6 were measured by ultrasensitive enzyme-linked immunosorbent assay (Qantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN). CRP was measured in plasma with a particle enhanced immunonephelometric assay with a BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc, Deerfield, IL). PAI-1 levels were measured using a two-site ELISA. IL2sR was measured using an ultra-sensitive ELISA (Quantikine Human IL-2 sRα Immunoassay; R&D Systems, Minneapolis, MN) at the Laboratory for Clinical Biochemistry Research. Insulin and glucose concentrations were measured with reagents from Roche Diagnostics (Indianapolis, IN) and analyzed at the Collaborative Studies Clinical Laboratory (Fairview-University Medical Center; Minneapolis, MN).

Genotyping in MESA-Genomic DNA was extracted from peripheral leukocytes isolated from packed cells of anticoagulated blood by use of a commercially available DNA isolation kit (Puregene; Gentra Systems, Minneapolis, MN). The DNA was quantified by determination of absorbance at 260 nm followed by PicoGreen analysis (Molecular Probes, Inc., Eugene, OR).

Because of linkage disequilibrium (LD) in the human genome, genotyping only a few, chosen SNPs (tagSNPs) in a region will provide sufficient information to assess the remainder of the common SNPs and to construct each of the common haplotypes in that region. Using International HapMap data on NCBI Build 35 assembly for the Caucasian, Asian, and Yoruban populations, 11 SNPs in *ADORA2B* were selected to tag major haplotypes using Haploview v4. The threshold for minor allele frequency (MAF) was 0.05, and a threshold of 0.8 was used for r^2 to tag another SNP with MAF 0.05 (multilocus option of Haploview v4). The 11 tagSNPs (major/minor allele) covered ~42 kb, encompassing the *ADORA2B* locus on human chromosome 17. All 11 *ADORA2B* SNPs that were selected were successfully typed (genotype calling rate 100%).

A series of post-genotyping metrics, including use of blinded duplicates, was employed for initial quality control to identify samples and SNPs that failed genotyping according to proprietary protocols, and sporadic failed genotypes with gencall quality score <0.25. Of 156 duplicate pairs included in 33 plates of samples typed, Illumina was blinded to 92 pairs. Both unblinded and blinded sample replicate concordance rates were > 99.99%. After removal of failed SNPs and samples, the genotype call-rate was 99.93%, with a maximum missing data rate per sample of 2.1%, and maximum missing rate per SNP of 4.98%. Genetic data were checked for cryptic sample duplicates and discrepancies in genetically predicted sex (using X markers) versus reported sex. Samples with unresolved duplicate and sex discrepancies were excluded. For *ADORA2B* genotyping, the 11 tagging SNPs selected passed all criteria.

Eight *ADORA2B* SNPs had low (less than 5%) minor allele frequency, despite selection for high MAF based upon HapMap. For SNPs with low MAF, none occurred in AFA, 5 were in CHN (rs7225585, rs2779193, rs758858, rs2041458, rs8069362), 6 were in EUA (rs7225575, rs2779193,

rs758858, rs2041458, rs8069362, rs17715109), and 1 was in HIS (rs17715109). Two SNPs in HIS (rs758857, rs2041458) were excluded due to significant deviation from Hardy-Weinberg expectations. There were 4 individuals (3 CHN, 1 EUA) removed for low genotyping rates (<90% of SNPs genotyped for that DNA sample). A total of 712 AFA samples were genotyped, with 710 assayed for diabetes status, 680 for IL-6, 202 for IL2sR, 704 for CRP, and 195 for PAI-1 levels. A total of 718 CHN samples were genotyped, with 718 for diabetes status, 709 for IL-6, 97 for IL2sR, 717 for CRP, and 95 for PAI-1 levels. There were 712 EUA samples genotyped, with 711 assayed for diabetes status, 701 for IL-6, 440 for IL2sR, 709 for CRP, and 428 for PAI-1 levels. In the HIS group, 705 were genotyped and 705 were assayed for diabetes status, and 689 for IL-6, 219 for IL2sR, 701 for CRP, and 214 for PAI-1 levels. The reduced sample size with IL2sR and PAI-1 measurements results in reduced statistical power in the resulting analyses.

Statistical Analysis- Comparisons of the demographic characteristics, categorical data and phenotypic levels of inflammatory markers among ethnic groups within MESA were performed by ANOVA or the χ^2-test where appropriate. *Post hoc* comparisons were made using Fisher's Least Significant Difference procedure at $P < 0.05$.

Single SNP analyses consisted of testing for association between each phenotype with each *ADORA2B* tagged SNP in order to account for differences in SNP minor allele frequencies. An additive (1 df) model was used for each MESA phenotype that consisted of the SNP, with age, sex, ethnic group, site of ascertainment, pack-years smoking, diabetes status and the first five principal components obtained from ancestry-informative markers (AIMs) as covariates. All analyses were performed using PLINK software (1).

Reference

1. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC: PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559-575, 2007