

Supplementary Appendix 1

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Oguma T, Palmer LJ, Birben E, Sonna LA, Asano K, Lilly CM. Role of prostanoid DP receptor variants in susceptibility to asthma. *N Engl J Med* 2004;351:1752-63.

African-American Population Substructure Analysis

29 single nucleotide polymorphism (SNPs), including one from each of the 22 autosomes, were selected from the NCBI single nucleotide polymorphism database (<http://www.ncbi.nlm.nih.gov/SNP/>). Synonymous coding region SNPs were selected as to be nonfunctional; each was reported to have a frequency of 0.4 or greater; and when they were on the same chromosome, were separated by at least 20 MB so as to be unlinked. Genotyping was performed by PCR-RFLP analysis. Mutations were introduced into PCR primers to create SNP-dependent enzyme recognition sites when distinguishing RFLP sites were not naturally present. Where appropriate, control primers were designed to yield a product containing the specific enzyme recognition site for each SNP and were used as a positive control in the RFLP analyses. The primers and restriction endonucleases used for SNPs analysis are presented in Tables I and II, respectively. PCR reactions were carried out in 30 μ l containing 100 ng of genomic DNA in a 200 μ M $MgCl_2$ buffer (Promega) containing 200 μ M dATP, dTTP, dCTP, and dGTP, 10 pmoles of each primer, and 3 U Taq polymerase. The PCR was carried out under the following conditions: 94°C for 5 min, followed by 35 cycles comprising a for a 1-minute denaturation step at 94°C, a 1 minute annealing step at the temperatures defined below, and a 1-minute 72°C elongation step, finally followed by a 5-min elongation step at 72°C. The annealing temperature was 52°C for SNPs designated chromosomes 1(1), 2, 6 (1 and 2), 13, 17, 19 (3), and 20 (2 and 3); 55°C for SNPs designated chromosomes 4, 8, 11, 16, 19 (1), 22 (1 and 2); 62°C for SNPs designated chromosomes 1(2), 3, 5, 7, 9, 10, 12, 14, 15, 18, 20 (1), 21; and 65°C for the SNP designated 19 (2). RFLP digestion was

carried out under the conditions detailed in Table II, and the products were subjected to electrophoresis on a 3% agarose gel containing ethidium bromide and visualized by UV transillumination.

Chromatin Immunoprecipitation (ChIP) Assay

Chromatin immunoprecipitation was performed using a commercially available assay kit according to the directions of the manufacturer (Upstate, Charlottesville, VA).

Transcription factor binding was assessed in 2 distinct *PTGDR* expressing cell types: KU812 cells and human peripheral blood eosinophils. Cells were grown in RPMI 1640 media supplemented with 10% FBS and 100 µg streptomycin/100 U penicillin G at a concentration of 1 million cells/ml at 37°C, 5% CO₂. Eosinophils were isolated by Histopaque density gradient centrifugation (Histopaque 1077 and 1119, Sigma) and hypotonic lysis, followed by CD16 antibody-negative immunomagnetic selection (Dynalect, Oslo, Norway). 1 x 10⁶ cells were used in each preparation. Histone proteins were cross-linked to DNA with 1% formaldehyde and sheared to 400-800 base pairs by sonication and incubated with antibodies to specific transcription factors at 4°C overnight. Negative controls included preparations without DNA, without antibody, and without both; and incubation with an antibody to OCT-1 that did not supershift the complexes detected in the EMSA assays. After incubation with antibody, protein A or G agarose beads were added to collect the antibody-histone complexes, washed, and eluted from the beads. Cross-links between histone proteins and DNAs were reversed by heating at 65°C for 4 hours. DNA fragments thus obtained were purified using the QIAquick PCR Purification kit (Quiagen, Valencia, CA) according to the directions of the manufacturer and subjected to PCR analysis using primers specific for the *PTGDR* promoter (5'-GAAGAGGAGCTAAGGCTCAG-3' and reverse 5'-GCGCCACAGAAAAAGCTC-3'). PCR was carried out under the following conditions: 94°C for 5 min, followed by 37 cycles of 94°C for 1 minute, 53°C for 1 minute, and 72°C

for 1 minute and finally followed by a chain elongation step of 72°C for 5 min. The PCR product was subjected to electrophoresis on a 1.5% agarose gel containing ethidium

bromide and visualized by UV transillumination.

Legend for Supplementary Fig. 1

Supplementary Figure 1. **Association among variants.**

Linkage disequilibrium (D') among individual variants presented as a proportion of their maximum possible disequilibrium coefficient D' . The 4 common SNPs were all in some degree of linkage disequilibrium with each other. The synonymous coding region variant G+1044A was in almost complete linkage disequilibrium with the T-197C 5'-flanking region variant.

Table I. Primers for generating allele specific products in RFLP digestion reactions.

Chromosome 1	F	5`-GTCTGCACTCTATGAGGACTC-3`
	R	5`-ATTGGAGGTTCCATCTCTGGA-3`
Chromosome 1	F2	5`-GATTGACAGGCATCTCTTGGA-3`
	R2	5`-CCTGCCTGCCCTCCTGGCAGGAGCGCGGCGTGGGGCTCCC-3`
	TPR2	5`- CCTGCCCTCCTGGCAGGAGCGCGGCGTGGGGCTCCCGGCC-3`
Chromosome 2	F	5`-GGGCTGGTCAAGGTTTGC-3`
	R	5`-TACAGTTTCCTCTCTTGTAGATA-3`
Chromosome 3	F	5`-CTGGCAGCTCCACACACAT-3`
	R	5`-CAAGGCTTCCAGCTCCTGCTCGCTCAGCCTGGATCATCG-3`
	TPR	5`-GCTTCCAGCTCCTGCTCGCTCAGCCTGGATCATCGATAG-3`
Chromosome 4	F	5`-GAAATCCTTACAAAACCCTAG-3`
	R	5`-TAAAATATGCTGCTTCATATGAAGGATCAGATGACTTGC -3`
	TPR	5`- ATATGCTGCTTCATATGAAGGATCAGATGACTTGCATTC -3`
Chromosome 5	F	5`-TCAGTACTGCCCGTCCAGTCCAA-3`
	R	5`-GTAAACTTTGAATATGATTACTCCGAACACTGTGACCTC -3`
	TPR	5`-ACTTTGAATATGATTACTCCGAACACTGTGACCTCGAAC-3`
Chromosome 6	F	5`-TGCAGATGCAAAAGATGATCT-3`
	R	5`-GGGAATGTCTTACCTAGGTC-5`
Chromosome 6	F2	5`-AGTTTGACAGCATCCACAG-3`
	R2	5`-CCTCATTTGGACGATATTGC-3`
Chromosome 7	F	5`-CCAGAAGGAGGAGCTGCTGCTGGAGGAGATTACATGCA-3`
	TPF	5`-AAGGAGGAGCTGCTGCTGGAGGAGATTACATGCATACC-3`
	R	5`-CAGCCGCTGTAGCAGCATGGAC-3`
Chromosome 8	F	5`-CCAGCATCACCGGTCAGCCAG-3`
	R	5`-CACCACCGATGATGGCGTTC-3`
Chromosome 9	F	5`-GCTTATTGAGGGGACGAGC-3`
	R	5`-CTCAGGGAGATGTGCTCATCACCTCCTGCACGTACACCGC-3`

Chromosome 10	TPR	5`-GGGAGATGTGCTCATCACCTCCTGCACGTACACCGCGGAA-3`
	F	5`-TATGCAAGTGAAGAGCCAGCAGT-3`
Chromosome 11	R	5-TATATGGTTTGCAAAAGCAAGCAAATCCTCCACAGTTAC-3`
	TPR	5`-TGGTTTGCAAAAGCAAGCAAATCCTCCACAGTTACGTAT-3`
Chromosome 12	F	5`-CTGTGGCCTTGCAGCCAG-3`
	R	5`-CAGTTGCCTCTGGGTGGTG-3`
Chromosome 13	F	5`-TCTTCCAGGTGCTGCCGCAAAT-3`
	R	5`-GCCGTGCGGCTGTCATGACTACCAGCATGTAGGCCGACTC-3`
Chromosome 14	TPR	5`-TCGGCTGTCATGACTACCAGCATGTAGGCCGACTCGAAC-3`
	F	5`-GGAACAGGAGAAAGAGGC-3`
Chromosome 15	R	5`-AAATGGGACACTATACTCTGT-3`
	F	5`-CCTACATTGTGCCTTGCGCCCCCTTGACCATGAAAGTCA-3`
Chromosome 16	TPF	5`-CATTGTGCCTTGCGCCCCCTTGACCATGAAAGTCATGAG-3`
	R	5`-CCAAACAGTCTTACAAGGCAGC-3`
Chromosome 17	F	5`-GACTGAAGCAAGGACAAGAACC-3`
	R	5`-CCCTGGGAAGCCTGGCACCCCCGACGGACAGCAAAGCGAC-3`
Chromosome 18	TPR	5`-GGGAAGCCTGGCACCCCCGACGGACAGCAAAGCGACGGA-3`
	F	5`-GTCGAGAGTGGTTCTTCAGAGC-3`
Chromosome 19	R	5`-TCCCAGCCAGCAGCCTTCTTA-3`
	F	5`-CATAACCTGATAAAGCTCC-3`
Chromosome 19	R	5`-CTCTTCAGAAGGAGATAAAG -3`
	F	5-GGGTGCATTTACCTCCTTGGC-3`
Chromosome 20	R	5`-CCACTTACGCATTCGTCTATCTTTCGCATAGGTTGGTTC-3`
	TPR	5`-TTACGCATTCGTCTATCTTTCGCATAGGTTGGTTCGAT-3`
Chromosome 20	F	5`-TAACACTGGAGGATGTGGCTGTG-3`
	R	5`-TGTGTGGGAGCAAGAGAAGG-3`
Chromosome 20	F2	5`-GAGAGTGGCCCAGGCTC-3`
	R2	5`-GCTGAGGGCAGGAGGAC-3`
Chromosome 21	F3	5`-CTGTCAGCCACAGCTTC-3`
	R3	5`-AAAACTGGGCTGTGGCC-3`
Chromosome 21	F	5`-AGAGAAAGCTGAGGACAAGATAG-3`
	R	5`-GACTACTCACTTGTTCAACCACATAAAAATGCAACCTGCA-3`
Chromosome 22	TPR	5`-ACTCACTTGTTCAACCACATAAAAATGCAACCTGCAGGCG-3`
	F2	5`-ACTGCACGTGGTTCTCAG-3`
Chromosome 22	R2	5`-GCATAAAGACTGTGGTGTG-3`
	F3	5`-GAGATCGTGCGCAGCAAG-3`
Chromosome 22	R3	5`-AGTGCGGTAGAAGTTGAG-3`
	F	5`-GGGACTCTTAGGGCCTAAAG-3`
Chromosome 22	R	5`-GTCCACCTCCTGGGCCATGCTGAGAGCCTCTGTGGTGTG-3`
	TPR	5`-ACCTCCTGGGCCATGCTGAGAGCCTCTGTGGTGTGCGATG-3`
Chromosome 22	F	5`-CCCAGACGCAGGCCTGCGTG-3`
	R	5`-ACCTTCTTACCATGGCCCCG-3`
Chromosome 22	F2	5`-ATCCAGCGTGTCTCCAAC-3`
	R2	5`-AGAGCCATCCTAAGTGCCA-3`

F-forward primer, R-reverse primer, TPF- forward positive control primer, TPR-reverse positive control primer

Table II. RFLP digestion conditions for products derived from Table I primers.

	Enzyme	Recognition site	Cuts A not B	Digestion conditions	Product size	Digestion-product sizes
Chromosome 1	Bst-N-I	CC/A(T)GG	T not G	60°C,NE2+BSA	232	142 + 90
Chromosome 1	Msp-I	C/CGG	C not T	37°C,NE2	268	227+41
Chromosome 2	Alu-I	AG/CT	T not C	37°C,NE2	279	172 + 107
Chromosome 3	BspD-I	AT/CGAT	T not C	37°C,NE4	210	171 + 39
Chromosome 4	Bsm-I	GAATGCN/	T not C	65°C,NE2	239	203 + 36
Chromosome 5	Taq-I	T/CGA	C not T	65°C, Taq 1 +BSA	272	232 + 40
Chromosome 6	Mse-I	T/TAA	T not C	37°C,NE2 +BSA	270	180 + 90
Chromosome 6	Mbo-II	GAAGA(N)8/	C not T	37°C,NE2	339	221+118
Chromosome 7	Nsi-I	ATGCA/T	T not C	37°C, Nsi B.	202	163 + 39
Chromosome 8	Aci-I	C/CGC	C not T	37°C,NE3	275	129 + 90 + 56
Chromosome 9	Sac-II	CCGC/GG	C not T	37°C,NE4	185	147 + 38
Chromosome 10	SnaB-I	TAC/ GTA	C not T	37°C,NE4+BSA	173	134 + 39
Chromosome 11	Xcm-I	CA(N)5/(N)4TGG	C not T	37°C,NE2	283	188 + 95
Chromosome 12	Taq-I	T/CGA	C not T	65°C, Taq 1+BSA	143	103 + 40
Chromosome 13	Rsa-I	GT/AC	T not C	37°C,NE1	351	235+116
Chromosome 14	BspH-I	T/CATGA	T not C	37°C,NE4	222	185 + 37
Chromosome 15	Hpy99-I	CGA(T)CG/	C not T	37°C,NE4+BSA	254	218 + 36
Chromosome 16	Mbo-II	GAAGA(N)8/	T not C	37°C,NE2	258	147 + 111
Chromosome 17	Mbo-II	GAAGA(N)8/	T not C	37°C,NE2	226	132 + 94
Chromosome 18	Taq-I	T/CGA	C not T	65°C, Taq 1 +BSA	198	158 + 40
Chromosome 19	BstF5-I	GGATGNN/	T not C	65°C,Y	253	167 + 86
Chromosome 19	BstF5-I	GGATGNN/	T not C	65°C,Y	253	175+149
Chromosome 19	Eci-I	GGCGGA(N)1	C not T	37°C,NE2+BSA	322	232 +90
Chromosome 20	Sbf-I	CCTGCA/GG	C not T	37°C,NE4	260	225 + 35
Chromosome 20	Mnl-I	CCTC(N)7/	T not C	37°C,NE2+BSA	337	201 +136
Chromosome	Nla-III	CATG/	T not C	37°C,NE4+BSA	240	136 + 104

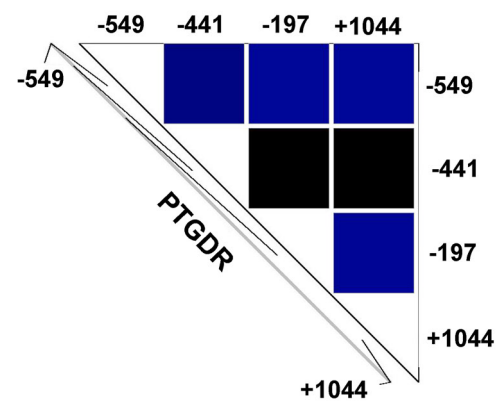
20 Chromosome	Taq-I	T/CGA	C not T	65°C, Taq 1+BSA	217	177 + 40
21 Chromosome	Bsm-A-I	GTCTC(N)1/1	T not C	55°C,NE3	242	168 + 74
22 Chromosome	Sac-I	GAGCT/C	C not T	37°C,NE1+BSA	322	207 + 115

Legend for Supplementary Fig. 1

Supplementary Figure 1. **Association among variants.**

Linkage disequilibrium (D') among individual variants presented as a proportion of their maximum possible disequilibrium coefficient D' . The 4 common SNPs were all in some degree of linkage disequilibrium with each other. The synonymous coding region variant G+1044A was in almost complete linkage disequilibrium with the T-197C 5'-flanking region variant.

Pairwise LD (D') European-Americans



Pairwise LD (D') African-Americans

