# Online Supplemental Materials I

## Methods and Materials

### Subjects

GOLDN is part of the program for Genetic Interactions Network and is funded by the NIH through the University of Alabama at Birmingham and in collaboration with the University of Utah, Washington University, Tufts University, University of Texas, University of Michigan, University of Minnesota, and Fairview-University of Minnesota Medical Center. The majority of participants were re-recruited from the ongoing NHLBI Family Heart Study (FHS) (Higgins, 1996 #13) in two genetically homogeneous centers (Minneapolis and Salt Lake City) with predominantly Caucasian populations. We extended pedigrees by recruiting offspring of the probands' siblings who were not included in the original FHS sampling.

The exclusion criteria included the following: 1) fasting TGs ≥1500mg/dl; 2) recent history (6 months) of myocardial infarction; 3) history of liver, kidney, pancreas, or gall bladder diseases or malabsorption, 4) pregnant women or women of childbearing potential not using contraception, 4) abnormal serum levels of aspartate aminotransferase (AST) and alanine transaminase (ALT), 5) current insulin user; 6) individuals with known hypersensitivity to fenofibrate. Individuals who were taking lipid-lowering medications or dietary supplements were asked to consult with their physicians and, upon approval, were asked to stop taking these agents for 4 weeks prior to their participation in the study.

GOLDN comprised two study interventions. In the postprandial (PPL) intervention, we collected clinical measurements before and after a high-fat meal to assess baseline and

postprandial TGs and related phenotypes. In the fenofibrate intervention, we collected clinical measurements from participants during a three-week fenofibrate intervention. Participants were given the options of participating in one of three protocols: the PPL-only protocol, the fenofibrate-only protocol, or the full protocol, which included both interventions. Total 858 GODLN subjects participated in the fenofibrate-only protocol. Among them, total 305 subjects were defined as having the MetS. The longitudinal analysis for the current study was performed among total 290 MetS subjects with full data records for baseline and the post-treatment values.

Blood samples were collected after overnight fasting. Biochemical measurements, including those for lipid profiles and inflammatory markers, were determined before and after fenofibrate. The baseline clinical exam included anthropometric and blood pressure measurements. Weight was measured with a beam balance and waist measures at the umbilicus. Height without shoes was measured with fixed stadiometer, BMI was calculated as weight (kg)/height$^2$ ($m^2$). Blood pressure was determined as the mean of two consecutive measurements after a five-minute rest, in the right arm (except for those women who reported right-side mastectomy), with an oscillometric device (Dinamap Pro Series 100, GE Medical Systems, Waukesha, WI, USA). Questionnaires were administered to solicit demographic and lifestyle information; medical history and medication history. Physical activity was expressed as metabolic equivalent task (MET)–hours based on self-reported types and durations of activities over 24 hours. Smoking status was described in three categories including current, former and never smoking. Alcohol consumption was defined as current drinkers versus non-drinkers. Self-reported

use of hormone therapy by women included contraceptives, conjugated estrogen, estradiol and progestin.

**Biochemical measurements**

Triglycerides were measured using a glycerol blanked enzymatic method (Trig/GB, Roche Diagnostics Corporation, Indianapolis, Ind) on the Roche/Hitachi 911 Automatic Analyzer (Roche Diagnostics Corporation). Cholesterol was measured on the Hitachi 911 using a cholesterol esterase, cholesterol oxidase reaction (Chol R1, Roche Diagnostics Corporation). The same reaction was also used to measure HDL-C after precipitation of non-HDL-C with magnesium/dextran. LDL-C was measured by a homogeneous direct method (LDL Direct Liquid Select™ Cholesterol Reagent, Equal Diagnostics, Exton, PA) on the Hitachi 911. Insulin was determined by the Human Insulin Specific RIA kit, a radioimmunoassay (Linco Research, Inc. St.Charles, MO). Glucose was measured on a Hitachi 911 using the method of a hexokinase-mediated reaction (Roche Diagnostics Corporation).

**DNA isolation and genotyping**

A total of five SNPs (m772A>G, m301G>A >T, i178T>A, 3u1273C>T, 3u2131C>T) were selected based on literature reports, the potential functionality or the representation of the common haplotypes at the *CRP* locus. Among them, i178T>A (rs1417938) and 3u2131C>T (rs1205) were identified as tagging SNPs using the HapMap database (www.hapmap.org), representing common haplotypes in the *CRP* locus (6.3 kbp) including all exons, introns, 2 kbp upstream and 2 kbp downstream of the transcribed region. SNP names were designated by their positions relative to the transcription initiation site (“m” indicating the minus residing in the gene control or promoter region,

"i" indicating the intronic region and "3u" indicating the 3'UTR region). The genotype distributions did not deviate from Hardy-Weinberg equilibrium ($X^2$ test, $P$>0.05) for all SNPs in unrelated subjects.

**Online Table 1.** The description of *CRP* SNPs, primers and probes

| SNPs * | dbSNP ID | Gene position | Primers and probes and ABI assay-on demand ID |
|---|---|---|---|
| m772A>G (-757T>C) | rs3093059 | Upstream | Forward: AGTGCCAAGATGTCTAGAGAGTTCT<br>Reverse: CCCAGAGCCATGGACACA<br>Probe 1: CTCAGCCAATTGAG<br>Probe 2: TCTCAGCCGATTGAG |
| m301G>A (-286G>A) | rs3091244 | Upstream | Forward: GTTGGAGAGGCAGCTACCA<br>Reverse: TCCTGCGAAAATAATGGGAAATGGT<br>Probe1: ACATATTAAACGAGTGGCCAT<br>Probe 2: ACATATTAAACAAGTGGCCAT |
| m301G>T (-286G>T) | rs3091244 | Upstream | Forward: GTTGGAGAGGCAGCTACCA<br>Reverse: TCCTGCGAAAATAATGGGAAATGGT<br>Probe1: AACATATTAAACTAGTGGCCATC<br>Probe 2: CATATTAAACGAGTGGCCA |
| i178T>A (IVS1+29A>T) | rs1417938 | Intron 1 | C___7479322_10 |
| 3u1273C>T (1444C>T) | rs1130864 | 3'UTR | C___7479332_10 |
| 3u2131C>T (1846G>A ) | rs1205 | 3'UTR | C___7479334_10 |

* Alternative names for SNPs used in previous reports included in parenthesis [14].

**Online Table 2.** Minor allele frequency and linkage disequilibrium patterns among SNPs at the *CRP* locus *

| | m772A>G | m301G>A | m301G>T | i178T>A | 3u1273C>T | 3u2131C>T |
|---|---|---|---|---|---|---|
| Frequencies | 6% | 28% | 10% | 31% | 31% | 32% |
| LD (D'/$r^2$) | | | | | | |
| m772A>G | | 0 | 0.935($P$<0.0001) | 0.166 ($P$=0.043) | 0.166 ($P$=0.043) | 0.165 ($P$=0.044) |
| m301G>A | <0.001 | | n/a | 0.999 ($P$<0.001) | 0.999 ($P$<0.001) | 0.437 ($P$<0.001) |
| m301G>T | 0.999 | n/a | | 0 | 0 | 0.164 ($P$=0.045) |
| i178T>A | 0.994 | 1 | <0.001 | | 1 ($P$<0.001) | 0.461 ($P$<0.001) |
| 3u1273C>T | 0.994 | 1 | <0.001 | 1 | | 0.461 ($P$<0.001) |
| 3u2131C>T | 0.980 | 0.999 | 0.733 | 0.999 | 0.999 | |

* The pair -wise LD among five SNPs was estimated as correlation coefficient ($r^2$).
$r^2$ and D' were displayed above the diagonal and below the diagonal, respectively.

**Online Table 3.** Plasma CRP response to fenofibrate among subjects with MetS according to *CRP* haplotypes

| | | Before drug | | After drug | | Responses | |
|---|---|---|---|---|---|---|---|
| Haplotypes | N | Mean (95%CI) | *P* | Mean (95%CI) | *P* | % change (95%CI) | *P* |
| 1111 | 126 | 2.94(2.31-3.75) | 0.102 | 2.36(1.81-3.07) | 0.134 | -11.3(-24.5-4.3) | 0.183 |
| 1112 | 142 | 2.57(2.07-3.20) | | 2.07(1.62-2.65) | | -11.8(-24.5-3.0) | |
| 1221 | 135 | 2.95(2.35-3.70) | | 2.55(1.98-3.30) | | -3.6(-18.2-13.7) | |
| 1321 | 16 | 3.27(2.42-4.43) | | 2.99(1.99-4.49) | | 1.5(-23.6-34.8) | |
| 2311 | 33 | 3.45(2.61-4.56) | | 2.53(1.84-3.46) | | -18.3(-34.5-1.8) | |

Values were geometric means (95%CI) adjusting for baseline CRP levels, change of triglyceride, change of IL6, age, gender, BMI, smoking status, alcohol intake, physical activity, use of aspirin and NSAID, drugs for lowering cholesterol, diabetes and hypertension, and hormone treatment in women.

* Subjects with CRP>10mg/L at the baseline or after the treatment were excluded from the analysis.

**Online Table 4.** Lipids, glucose and insulin responses to fenofibrate among subjects with MetS according to *CRP* i178T>A

| | | Before drug | | After drug | | Responses | |
|---|---|---|---|---|---|---|---|
| | Genotype | Mean(95%CI) | $P^{\dagger}$ | Mean(95%CI) | $P^{\dagger}$ | % change (95%CI) | $P^{\dagger}$ |
| Total cholesterol(mg/dl) | TT | 205(194-217) | 0.717 | 189(179-199) | 0.216 | -8(-12- -3) | 0.224 |
| | TA | 202(194-211) | | 182(173-192) | | -10(-13- -6) | |
| | AA | 204(187-222) | | 188(172-204) | | -7(-12- -2) | |
| Triglycerides (mg/dl) | TT | 234(203-271) | 0.662 | 141(120-164) | 0.516 | -38(-44- -31) | 0.56 |
| | TA | 230(202-263) | | 136(117-158) | | -39(-45- -32) | |
| | AA | 245(210-286) | | 148(125-174) | | -34(-44- -23) | |
| LDL-C(mg/dl) | TT | 125(117-135) | 0.547 | 122(113-130) | 0.059 | -3(-9- 4) | 0.148 |
| | TA | 122(115-130) | | 114(106-122) | | -7(-12- -2) | |
| | AA | 127(114-141) | | 121(108-136) | | -4(-12- 5) | |
| HDL-C(mg/dl) | TT | 39(36-42) | 0.058 | 42(39-46) | 0.092 | 8(5- 11) | 0.247 |
| | TA | 41(38-44) | | 43(40-47) | | 6(2- 10) | |
| | AA | 37(34-41) | | 40(37-44) | | 8(4- 13) | |
| Glucose(mg/dl) | TT | 118(114-123) | 0.505 | 113(109-117) | 0.271 | -5(-7- -2) | 0.481 |
| | TA | 118(114-123) | | 113(109-117) | | -5(-7- -2) | |
| | AA | 124(114-135) | | 120(111-131) | | -3(-6- 1) | |
| Insulin(μU/ml) | TT | 15(13-17) | 0.598 | 14(12-16) | 0.335 | -7(-15- 2) | 0.615 |
| | TA | 15(13-16) | | 13(12-15) | | -9(-17- -1) | |
| | AA | 14(12-17) | | 13(11-16) | | -5(-17- 7) | |

Values were geometric means (95%CI) adjusting for baseline, age, gender, BMI, smoking status, alcohol intake, physical activity, drugs for lowering cholesterol, diabetes and hypertension, and hormone treatment in women. $^{\dagger}$ Additive model.
Sample size : N for TT=144, N for TA=125, N for AA=34

**Online Table 5.** Lipids, glucose and insulin responses to fenofibrate among subjects with MetS according to *CRP* m301G>A>T

| | | Before drug | | After drug | | Responses | |
|---|---|---|---|---|---|---|---|
| | Genotype | Mean(95%CI) | $P^{\dagger}$ | Mean(95%CI) | $P^{\dagger}$ | % change (95%CI) | $P^{\dagger}$ |
| Total cholesterol(mg/dl) | GG | 204(193-216) | 0.992 | 191(181-201) | 0.335 | -6(-10- -2) | 0.17 |
| | GA+GT | 203(194-213) | | 182(173-192) | | -10(-14- -7) | |
| | TA+AA | 205(191-219) | | 188(176-202) | | -7(-11- -3) | |
| Triglycerides (mg/dl) | GG | 234(200-273) | 0.843 | 147(126-171) | 0.854 | -32(-39- -25) | 0.73 |
| | GA+GT | 219(188-254) | | 134(116-155) | | -35(-41- -29) | |
| | TA+AA | 245(209-288) | | 155(133-181) | | -30(-37- -22) | |
| LDL-C(mg/dl) | GG | 124(115-134) | 0.906 | 124(115-133) | 0.145 | -1(-7- 4) | 0.069 |
| | GA+GT | 124(117-132) | | 114(106-123) | | -9(-14- -4) | |
| | TA+AA | 124(113-134) | | 119(108-131) | | -5(-11- 2) | |
| HDL-C(mg/dl) | GG | 38(36-42) | 0.591 | 42(38-45) | 0.875 | 8(5- 12) | 0.316 |
| | GA+GT | 40(38-43) | | 43(40-47) | | 7(3- 11) | |
| | TA+AA | 38(36-41) | | 41(38-45) | | 7(3- 12) | |
| Glucose(mg/dl) | GG | 116(112-120) | 0.142 | 112(108-115) | 0.084 | -3(-6- -1) | 0.472 |
| | GA+GT | 119(115-124) | | 113(109-117) | | -5(-7- -2) | |
| | TA+AA | 120(112-128) | | 117(110-124) | | -2(-6- 3) | |
| Insulin(μU/ml) | GG | 15(13-17) | 0.398 | 14(12-16) | 0.456 | -9(-17- -1) | 0.843 |
| | GA+GT | 15(13-17) | | 13(12-15) | | -12(-19- -4) | |
| | TA+AA | 14(12-16) | | 13(11-15) | | -9(-18- 1) | |

Values were geometric means (95%CI) adjusting for baseline, age, gender, BMI, smoking status, alcohol intake, physical activity, drugs for lowering cholesterol, diabetes and hypertension, and hormone treatment in women. $^{\dagger}$ Additive model.
Sample size : N for GG=114, N for GA+GT=131, N for TA+AA=53.

**Online Table 6.** Lipids, glucose and insulin responses to fenofibrate among subjects with MetS according to *CRP* 3u2131C>T

| | | Before drug | | After drug | | Responses | |
|---|---|---|---|---|---|---|---|
| | Genotype | Mean(95%CI) | $P^{\dagger}$ | Mean(95%CI) | $P^{\dagger}$ | % change (95%CI) | $P^{\dagger}$ |
| Total cholesterol(mg/dl) | CC | 202(193-212) | 0.682 | 185(176-195) | 0.659 | -8(-12- -4) | 0.733 |
| | CT | 207(197-219) | | 185(174-196) | | -10(-14- -7) | |
| | TT | 193(179-208) | | 183(171-196) | | -6(-10- -2) | |
| Triglycerides (mg/dl) | CC | 218(189-251) | 0.871 | 141(122-162) | 0.557 | -32(-38- -25) | 0.148 |
| | CT | 235(201-275) | | 136(117-158) | | -38(-44- -32) | |
| | TT | 206(164-257) | | 136(110-169) | | -32(-39- -23) | |
| LDL-C(mg/dl) | CC | 124(116-132) | 0.447 | 117(109-126) | 0.884 | -7(-12- -1) | 0.67 |
| | CT | 125(117-134) | | 116(107-126) | | -8(-13- -3) | |
| | TT | 117(106-128) | | 117(106-129) | | -3(-9- 3) | |
| HDL-C(mg/dl) | CC | 40(37-42) | 0.597 | 42(39-45) | 0.877 | 7(3- 11) | 0.723 |
| | CT | 41(38-44) | | 43(40-47) | | 7(3- 10) | |
| | TT | 37(34-41) | | 41(36-45) | | 9(4- 13) | |
| Glucose(mg/dl) | CC | 120(116-125) | 0.027 | 115(111-120) | 0.032 | -3(-6- -1) | 0.608 |
| | CT | 117(113-121) | | 112(107-116) | | -4(-6- -2) | |
| | TT | 115(110-120) | | 110(105-115) | | -4(-7- -0.2) | |
| Insulin(μU/ml) | CC | 15(13-17) | 0.981 | 14(12-16) | 0.917 | -10(-17- -4) | 0.786 |
| | CT | 14(13-16) | | 13(12-15) | | -10(-18- -2) | |
| | TT | 16(13-19) | | 14(11-17) | | -12(-22- -1) | |

Values were geometric means (95%CI) adjusting for baseline, age, gender, BMI, smoking status, alcohol intake, physical activity, drugs for lowering cholesterol, diabetes and hypertension, and hormone treatment in women. $^{\dagger}$ Additive model.
Sample size : N for CC=145, N for CT=125, N for TT=33.