American Journal of Human Genetics, Volume *92*

Supplemental Data

ADAMTS7 Cleavage and Vascular Smooth Muscle Cell Migration Is Affected by a Coronary-Artery-Disease-Associated Variant

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Supplemental Inventory

1. Supplemental Figures

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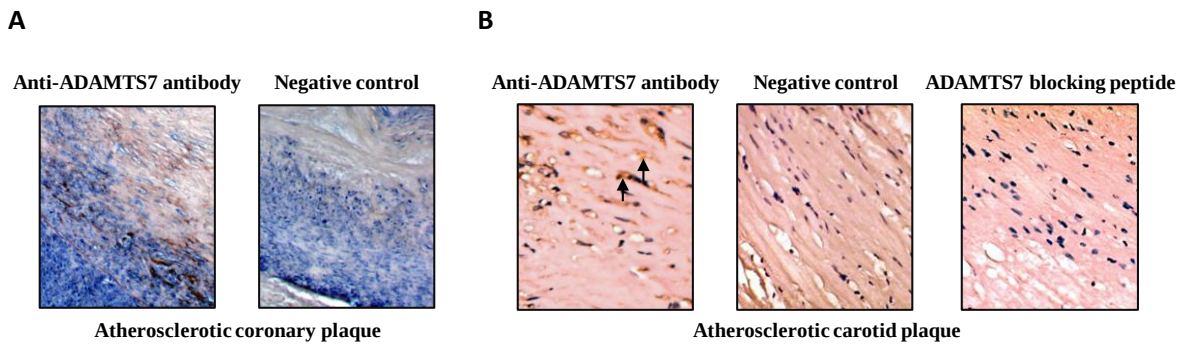

Figure S1. ADAMTS7 Immunostaining and Controls

(A) ADAMTS7 immunostaining (left) and negative control (with secondary antibody but no ADAMTS7 primary antibody, right) in human atherosclerotic coronary artery sections. Purple color (NBT/BCIP) indicates smooth muscle α-actin staining, and dark brown color with DAB indicates ADAMTS7 staining (positive in left hand panel but negative in right hand panel).

(B) ADAMTS7 immunostaining (left), negative control (with secondary antibody but no ADAMTS7 primary antibody, middle), and blocking peptide control (with ADAMTS7 primary antibody and secondary antibody, in the presence of an ADAMTS7 blocking peptide [Abcam, ab41240]), in human atherosclerotic carotid artery sections. Arrows indicate ADAMTS7 staining (positive in left hand panel but negative in middle and right hand panels).

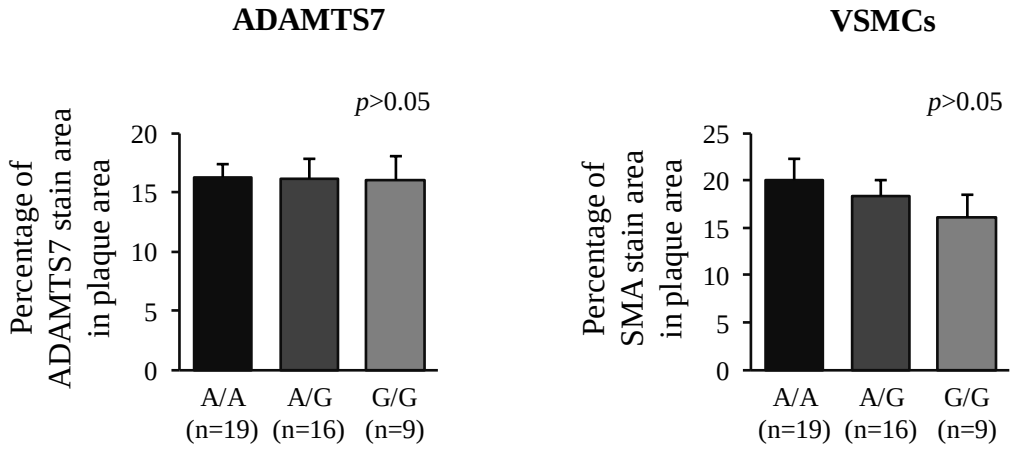

Figure S2. Percentage of ADAMTS7 Stain Area in Plaque Area and Percentage of SMA Stain Area in Plaque Area by rs3825807 Genotypes

Data shown are values of mean ± standard deviation of mean.

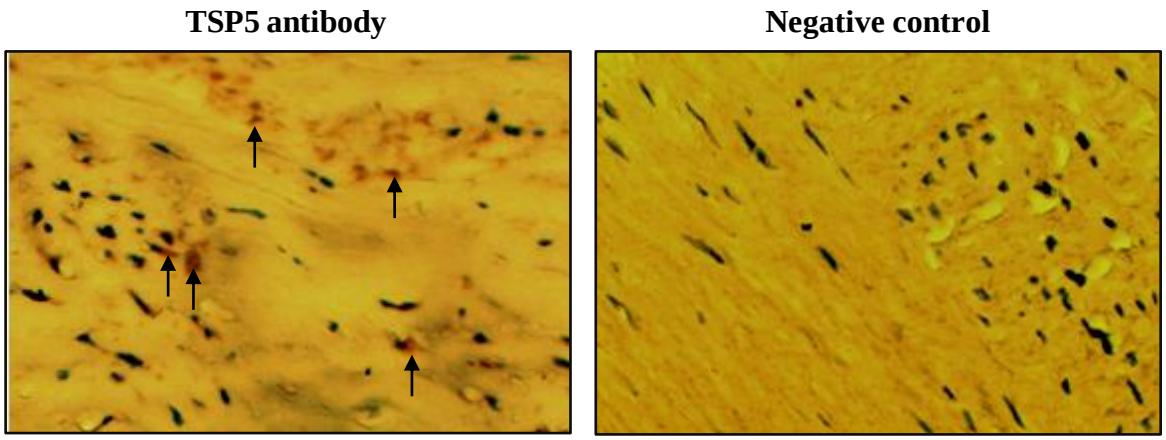

Figure S3. TSP5 in Coronary Atherosclerotic Plaques

Left panel: human atherosclerotic coronary artery sections were subjected to immunostaining of TSP5. Arrows indicate TSP5 staining. Right panel: negative control with secondary antibody but no TSP5 primary antibody.

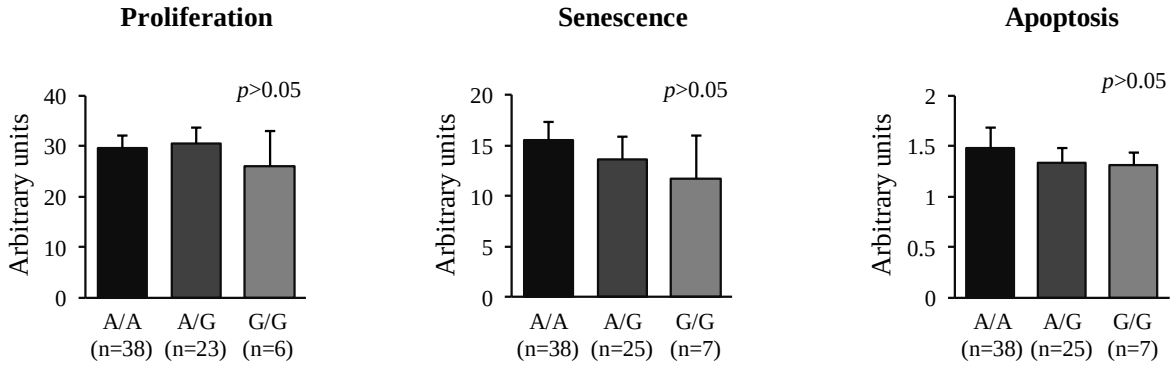

Figure S4. Proliferation, Senescence, and Apoptosis in VSMCs by rs3825807 Genotypes

VSMCs were seeded in duplicate in 96 well-plates (5,000 cells per well) and cultured overnight. For proliferation assay, cells were cultured for a further 12 hours in fresh medium in the presence of 10μM 5-bromo-2'-deoxy-uridine (BrdU), followed by BrdU detection assay with the use of 5-Bromo-2'-deoxy-uridine Labeling and Detection Kit III (Roche, 11444611001). For senescence assay, after the overnight culture, cell senescence was assessed using a 96-Well Cellular Senescence Assay Kit (Cell Biolabs Inc. CBA-231) to quantify senescence-associated beta-galactosidase activity. For apoptosis assay, after the overnight culture, cell apoptosis was measured using a Cell Death Detection ELISAPLUS kit (Roche, 11774425001) to quantify histone-complexed DNA fragments. Data shown are values of mean ± standard deviation of mean.

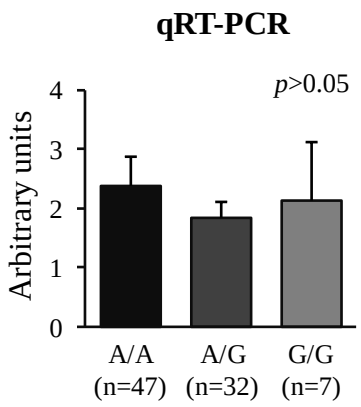

Figure S5. Relative *ADAMTS* Expression Levels in VSMCs by rs3825807 Genotypes

Total RNA samples were prepared from primary cultures of VSMCs, with the use of the SV Total RNA Isolation System (Promega). RNA was reverse transcribed into cDNA using random primers and M-MLV reverse transcriptase, followed by real-time PCR of *ADAMTS7* and the housekeeping gene *β-actin*, with TaqMan gene expression assays (Applied Biosystems, Hs00276223_ml and Hs03023943_g1, respectively). The ΔΔCT method was used to analyze the data, with *β-actin* as the reference gene. Data shown are values of mean ± standard error of mean.

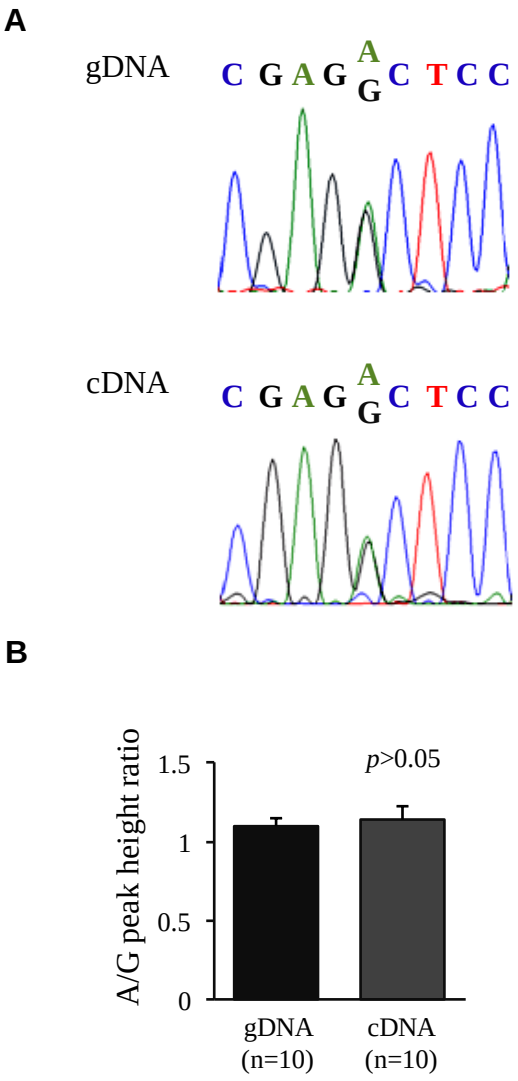

Figure S6. Allelic Expression Imbalance Analysis of *ADAMTS7* rs3825807

Genomic DNA (gDNA) and RNA were isolated from VSMCs that were heterozygous for rs3825807. RNA was reverse transcribed to complement DNA (cDNA). PCR to amply the region encompassing the site of rs3825807 was carried out using gDNA and cDNA respectively as templates, and the PCR products were subjected to fluorescent dideoxy sequencing (primer sequence: 5'-CCATGTTCATGATGGTCAGC-3'). Sequencing chromatograms were analyzed using the PeakPicker program[1] which calculated relative peak heights of the A and G nucleotides at the SNP site in the chromatograms deriving from cDNA and gDNA respectively, standardized against reference peaks in flanking sequences. Mann-Whitney test was performed to test a difference between the ratio of standardized A nucleotide peak height over standardized G nucleotide peak height from cDNA and the corresponding ratio from gDNA.

(A) Chromagrams from gDNA and cDNA, respectively.

(**B)** Schematic representation of mean (and standard error of mean) of the A/G ratios for gDNA and cDNA.

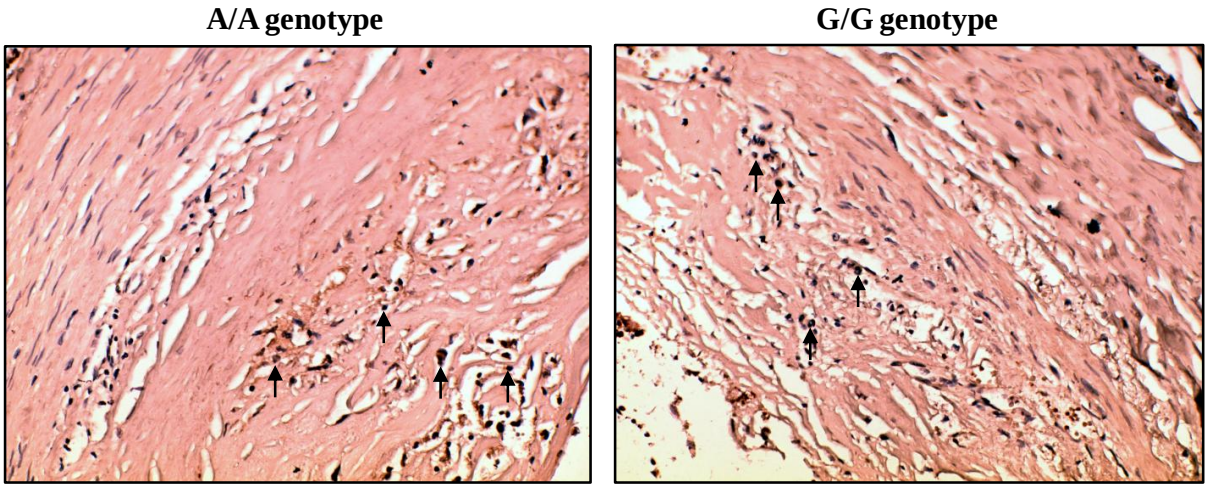

Figure S7. CD68-Positive Cells in Atherosclerotic Coronary Plaques

Human atherosclerotic coronary artery sections were subjected to immunostaining of CD68. Arrows indicate CD68-positive cells. Images shown are representatives of images from 3 individuals of the A/A genotype and 3 individuals of the G/G genotype examined. No apparent difference in the abundance of CD68 positive cells was observed between the A/A and G/G genotypes.

Supplemental References

1. Ge B, Gurd S, Gaudin T, Dore C, Lepage P, Harmsen E, Hudson TJ, Pastinen T. Survey of allelic expression using EST mining. *Genome Res* 2005;15:1584-91.