

A randomized trial of personal genomics for preventive cardiology: design and challenges

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Short title: Randomized trial of personal genomics

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BACKGROUND-

Genome wide association studies (GWAS) have identified over 1500 disease-associated single nucleotide polymorphisms (SNPs), including many related to atherosclerotic cardiovascular disease (CVD).

Associations have been found for most traditional risk factors (TRFs) including lipids^{1,2}, blood pressure/hypertension^{3,4}, weight/body mass index^{5,6}, smoking behavior⁷, and diabetes⁸⁻¹³. GWAS have also identified susceptibility variants for coronary heart disease (CHD). The first, and so far the strongest, of these signals was found in the 9p21.3 locus where common variants in this region increase in the relative risk of CVD by 15-30% per risk allele in most race/ethnic groups¹³⁻²⁰. Subsequent large scale GWAS meta-analysis and replication studies in largely white/European populations have led to the reliable identification of an additional 26 loci conferring susceptibility to CHD²⁰⁻²², all with substantially lower effects sizes compared to the 9p21 locus. Many of these CVD susceptibility loci appear to be conferring risk *independent* of traditional risk factors and thus cannot currently be assessed by surrogate clinical measures (Table 2). Among the 27 independent loci identified in the most recent large meta-analyses of CVD, 21 were reported not to be associated with any of the traditional risk factors^{20,21}.

Several studies have explored whether initial CVD related genetic markers can improve risk prediction over standard models restricted to TRFs using a genetic risk score (GRS) constructed on the basis of the number of risk alleles inherited²²⁻²⁴. Results to date have been mixed. While all have shown that a GRS is strongly associated with the outcome of interest independent of TRFs, none were able to demonstrate a significant improvement in the C-statistic. Two of the three studies showed some modest improvement in newly defined discrimination indices including the integrated discrimination index, the net reclassification index (NRI), and the clinical NRI (NRI in the intermediate risk subjects). Thus, the use of these markers has not yet been shown to convincingly outperform models that include traditional risk factors and family history alone.

One important reason for the failure of these markers to demonstrate clinically meaningful

improvement of risk prediction relates to the small proportion of the genetic variance explained by these markers, a phenomenon commonly referred to as the “heritability gap”. The basis for this heritability gap is the focus of intense investigation. Despite this gap, it is still possible that knowledge of genetic risk may improve patient outcomes through means other than enhanced risk reclassification. For instance, genetic testing may improve patient adherence and CVD risk factor reduction for Mendelian disorders related to CHD such as familial hypercholesterolemia (FH) ²⁵. This effect may be due to an increase in patient motivation—e.g., people who recognize and accept their high risk are more encouraged to reduce it. However, no clinical trial to date has demonstrated that newly discovered genetic markers improve risk factor profiles by improving adherence to prescribed therapy for complex (“garden variety”) CVD.

Here we describe the design of an ongoing randomized trial to investigate whether CVD risk factor profiles can be improved by providing participants with knowledge related to their inherited risk of CVD in addition to information on their risk related to measured traditional risk factors. We also discuss some of the challenges that arise in the design and conduct of such a trial and how they were addressed.

METHODS-

Clinical Trial Registration- This trial is registered at ClinicalTrials.gov (NCT01406808).

TRIAL FLOW

Patient eligibility and recruitment:

The study population consists of new or continuing patients seen at the Stanford Preventive Cardiology Clinic (PCC) including patients that were referred to the clinic by other providers or patients that self-referred to the clinic. The PCC staff includes three attending physicians, a clinical nurse specialist, and a

dietician. Flyers describing the trial were sent to cardiologists, general internists and family physicians at Stanford University Medical Center and in the surrounding area. Inclusion and exclusion criteria are summarized in Table 1. Participants do not receive financial remuneration (or any form of payment) for their participation in the trial.

Patients are eligible if they have a $\geq 6\%$ risk over the next 10 years or a $\geq 20\%$ risk over the next 30 years of atherosclerotic CVD as determined by respective Framingham risk scores^{26,27}. These criteria will result in the inclusion of some individuals that would be classified as “low risk” based on current NCEP/ATPIII guidelines, which define risk as low, moderate and high based on cutoffs of $< 10\%$, $10\text{-}20\%$ and $> 20\%$ over 10 years²⁸. We chose these eligibility criteria for several reasons. First, we wanted to have fairly broad entry criteria to be able to ascertain whether there is a differential effect of receiving genetic information depending on CVD risk. We chose not to include individuals at very low risk ($< 6\%$ over 10 years or $< 20\%$ over 30 years) because it would be difficult to see the effect of the genetic score intervention in these patients, as these patients would be largely left untreated. Interestingly, low risk was defined as $< 6\%$ risk over 10 years until the current NCEP/ATPIII guidelines were published and this cut-off is still utilized as defining the boundary between low and moderate risk in some settings²⁹⁻³². Second, we wanted to include low risk individuals that will not qualify for statin-based therapy based on our treatment algorithm derived from NCEP/ATPIII guidelines, so that we will be able to capture whether genetic information is sufficient to motivate changes in diet and lifestyle that are sufficient to affect our primary outcome (change in LDL cholesterol). Third, there are persuasive arguments that assessing 30-year or lifetime risk of CVD should be a standard part of risk assessment³³⁻³⁵. This strategy identifies a significant number of individuals (particularly younger individuals) that have a low 10-year risk but a substantial lifetime risk. Targeting these individuals with early, preventive measures to reduce long-term risk could potentially result in substantial public health benefit^{27,33-36}.

Participants are not eligible if they have already had an atherosclerotic related cardiovascular event including myocardial infarction, stroke, coronary revascularization of any type or if they had a CVD risk equivalent condition such as documented peripheral arterial disease or carotid stenosis. For these participants, we cannot stratify future risk of recurrent events easily although we know it is high. We also concluded that the life experience related to the initial CVD event(s) suffered by these individuals is likely to serve as a far stronger motivator than any genetic risk score. Patients are excluded if they were already on lipid lowering therapy, as the inclusion of participants on different statins at various doses with different LDL targets introduces considerable heterogeneity into the analyses. In addition, inclusion of people already adherent to therapy and at goal for risk factor reduction strategies would limit our ability to detect relevant outcomes.

Finally, the SNPs included in the genetic score have been evaluated predominantly in white/European subjects. Because there is considerable overlap in the genetic architecture between white/European populations and South Asian or Hispanic/Latino populations we thought it reasonable to include these latter race/ethnic groups. In particular the recent publication from the C4D consortium demonstrated “little evidence for ancestry-specific effects”²⁰ of the known CHD/MI variants in South Asians versus white/European populations. We are limited by the paucity of published findings in Hispanic populations. However, given the high degree of white/European ancestry in these populations we would expect these markers to perform well. Furthermore, one of us (J.P.A.I) as also recently published a manuscript demonstrating that while the effect sizes for single variants may differ between white/European and Asian populations, they are on average larger in one or the other ancestry group, thus a multi-gene signature is likely to carry similar predictive ability, while this is not true for white/European (or Asian) versus African descent³⁷. Therefore, we are limiting the trial population to these three race/ethnicity groups as based on self-identification, while excluding African Americans, East Asians and Pacific islanders. We are careful to acknowledge that these SNPs may not convey the exact

same risk in South Asian or Hispanic populations in the information we give to patients. (Supplementary Appendix). We evaluated the risk score in African Americans and in white/Europeans from the ARIC cohort. In African Americans the relative risk for a standard deviation increase in the Risk Score of coronary heart disease over 10 years (controlled for age and gender) is 1.04 (0.93, 1.16), while in white/Europeans the equivalent relative risk is 1.18 (1.12, 1.25). This indicates that the SNPs that are used in the risk score are not valid in African Americans.

Initial clinic visit—eligibility and consent, guideline-based standard care:

At the initial clinic visit (time=0), eligibility screening and consent are completed (Figure 1). After consent is obtained, the participants complete a comprehensive baseline questionnaire (see Supplementary Appendix for details). Participants are then randomized to one of the two arms using a permuted block algorithm (block size 8) implemented in StudyTrax (Macon, Georgia). A saliva sample is collected for DNA preparation using kits from Oragene (Ontario, Canada), and a medical history and physical exam are also performed. All patients receive standard care including: Assessment of personal medical history, social history, family history; measurements of height, weight, blood pressure; laboratory testing such as fasting lipids and blood chemistries; and calculation of 10- and 30-year risk using Framingham models^{26, 27}. Most importantly, the patient's target LDL based on NCEP/ATPIII guidelines is determined and documented at this initial visit^{28, 38}. Based on the same guidelines, recommendations on how to optimize one's diet and physical activity levels are also made. If applicable, medications are prescribed according to guidelines (see Figure 1).

Second clinic visit—guideline based standard care and discussion of genetic testing results with intervention patients only:

The second clinic visit is scheduled 3 months after the initial visit. This schedule follows the usual practice at PCC and was chosen for several reasons. First, it allows sufficient time to gauge the effectiveness of the initial diet and lifestyle interventions and, when applicable, pharmacologic therapy. Second, the interval allows for the completion of genetic testing, which currently takes approximately 2 months. Immediately before the second clinic visit, participants fill out another study questionnaire and undergo a fasting measurement of lipids. A reassessment of risk factors such as weight, blood pressure and smoking behavior are performed to measure progress with initial recommendations. If additional evaluations were completed such as non-invasive imaging studies of atherosclerotic plaque development (e.g. coronary artery calcium scan), these studies are reviewed as this additional information may change therapeutic recommendations³⁹. Guideline-based therapeutic recommendations are discussed with the patient.

At the conclusion of the second clinic visit the clinician opens a sealed envelope that contains either the genetic risk score or a note indicating that the patient was randomized to the non-intervention arm and will receive their genetic risk score at the conclusion of the trial (6-month visit). Importantly, prior to this point the clinician is blinded to the results of the genetic testing.

The genetic test results are discussed with the patient by guiding them through an individualized genetic risk report (Figure 2). A standard script was developed to help clinicians discuss these results (Supplementary Appendix). This risk report includes: 1. A traditional estimate of both 10-year risk based on the Framingham model; 2. The absolute number of risk alleles carried by the patient (out of a possible 38 alleles); 3. An estimate of the participant's percentile of genetic risk based on the absolute number of risk alleles in relation to the frequency distribution of risk alleles observed in the ARIC cohort of whites; 4. An estimate of the participant's 10-year risk of CHD before and after the addition of the genetic data to the standard Framingham 10-year risk model; and 5. A list of SNPs tested annotated with the nearest genes. We also provide participants with a take-home Frequently Asked Questions

sheet that re-iterates much of the message conveyed by the clinician at the visit (Supplementary Appendix). We aimed for a very short session (10 minutes) to convey the genetic information. This mode of information delivery does entail the risk that participants may not fully comprehend this information and this may affect their response to it. However, it would have been difficult to advocate adoption of a genomic information intervention in the everyday busy clinical practice, if this were to take a lot of time to deliver. Moreover, a lengthy delivery of the information may have seriously unbalanced the two arms in regards to the attention they received and may have introduced a spurious effect due to greater attention alone. Furthermore, providing more time and detailed emphasis might have given the wrong impression that this information is as important as major genetic risks conferred by major mutations in monogenetic disease that require careful and in-depth genetic counseling. This mode of information delivery does entail the risk that participants may not fully comprehend this information and this may affect their response to it. We do not involve a genetic counselor in the interpretation and discussion of the results of the genetic testing with the participant. Involvement of a genetic counselor would be a different type of intervention and it would add to the complexity and cost of care. Moreover, it was felt that a genetic counselor would be unnecessary for conveying information for modest multigenetic risks, as opposed to large risks conferred by mendelian monogenetic inheritance. Furthermore, we do not perform a time-matched intervention in the non-intervention arm, since the time required to convey the genetic information is relatively minimal, and time-matching would add an extra layer of complexity to the design.

Third clinical visit—final outcome assessment:

The third clinical visit is scheduled for 3 months after the second clinic visit (6 months after enrollment at the initial clinic visit). This schedule is typical for PCC and allows adequate assessment of the success of diet, lifestyle and pharmacologic interventions. Participants once again fill out a questionnaire and

undergo testing to determine serum lipid levels immediately prior to this visit. At the clinic visit, the participant's cardiovascular risk factors are all reassessed and progress in relation to original therapeutic recommendations is documented and discussed. At this visit, participants in the non-intervention arm also receive their genetic testing results, which are reviewed in the same manner as they were reviewed for the intervention arm at the 3-month visit.

PHARMACOLOGIC ALGORITHM FOR LOWERING LDL:

A rigid LDL lowering algorithm is implemented to minimize the possibility of a channeling bias introduced by a treating physician's knowledge of the genetic testing results. Both the LDL target and the therapeutic regimen are established before the genetic results are disclosed and no change in the lipid-lowering algorithm is permitted for the duration of the trial except in the case of drug intolerance. If a statin is needed, the initial choice is either simvastatin 20mg per day for the vast majority of the cases where mild to moderate (< 35%) LDL lowering is required or atorvastatin 40mg per day if more potent LDL lowering (> 35%) is required or if there is a previously documented intolerance to simvastatin

28 .

GENOTYPING STRATEGY:

We encountered several challenges in establishing an efficient method to perform and deliver the genetic testing for this trial. Despite decreasing costs, genetic testing remains relatively expensive if a limited set of SNPs is queried in a small number of individuals (several hundred dollars per participant). In addition, FDA approved commercial genotyping assays for testing variants associated with complex diseases are not available. Furthermore, we were unable to identify an academic lab that offers CLIA certified genotyping for these markers and the cost for developing such an assay at Stanford in 2011 was estimated to be > \$25,000. Thus, this trial would have not have been possible if it were not for a waiver

granted by the Stanford IRB allowing us to deliver genetic testing results to our participants from a non-CLIA certified lab.

We elected to genotype participants in this trial using Illumina's custom iSelect Cardio-MetaboChip (CM), a 200k array designed by academic investigators in 2009. SNPs included in this array allow for wet-lab replication of the top ~2% signals emanating from several large scale GWAS meta analyses of cardiovascular related traits including BMI, lipids, blood pressure, and CHD phenotypes and an opportunity to fine map a majority of the currently published validated GWAS loci for CHD²¹. Because many investigators around the world were willing to purchase large volumes of the CM array, Illumina made the array available to the scientific community for only ~\$40 / sample. DNA is purified from saliva samples using standard protocols (PrepIt L2P, Oragene). Genotyping is performed at the HudsonAlpha Institute for Biotechnology (Huntsville, Alabama) using 250ng of genomic DNA combined with standard Illumina reagents and protocols. Genotype calls are made with Illumina's GenomeStudio software (<http://www.illumina.com>) and samples with >98% call rates across all SNPs on the array are considered for analysis. Samples with lower call rates are rerun as necessary.

ASSESSMENT OF CHD RISK USING A GENETIC RISK SCORE:

At the time this trial was implemented, the published lead SNPs at 21 out of 27 known CHD loci did not appear to be associated with traditional risk factors in the CARDIoGRAM study^{20, 21}. One may debate whether genetic variants that relate to traditional risk factors should also have been considered in the polygenic score. We opted to exclude them, since these variants in theory do not provide information that is beyond what can be gleaned already by measurements of traditional risk factors. An evaluation of the ARIC data showed that consideration of these 6 variants as well as the 3 variants that were not present in the panel that we chose for genotyping did not alter the AUC. Among these 21 loci, 19 are represented on the Cardio-MetaboChip and were considered in this study. Two loci (located at

17q21.32 and 7q22) identified by the C4D consortium did not meet the pre-specified threshold for significance in the CARDIoGRAM study and were thus not included on the Cardio-Metabochip which was exclusively populated with the top hits from the CARDIoGRAM meta-analysis.

Individual level genotype (including imputation up to ~2.5 million HapMap SNPs) and phenotype data from the Atherosclerosis Risk in Communities Study (ARIC) cohort were acquired through an application to the NIH controlled access database of Genotypes and Phenotypes (dbGaP) by one of the co-investigators of this study (TLA)^{4, 40, 41}. The ARIC data were then used to calculate 10-year risks of CHD for participants before and after incorporating their genotyping results at the 19 loci of interest. Due to pruning algorithms implemented during the design of the Cardio-Metabochip array and limitations in imputation quality in ARIC for some SNPs, only 11 out of the 19 lead SNPs were found to be present both on the Cardio-Metabochip and available in the ARIC imputation file secured from dbGaP. For three loci, the lead SNP was not present on the Cardio-Metabochip and a proxy with $r^2 > 0.9$ on the array was chosen to represent these loci. For five loci, the ARIC imputation file did not have genotype information for the lead SNP and thus a proxy imputed SNP was chosen to represent these loci in ARIC (the $r^2 = 1$ for 4 of these proxies, $r^2 = 0.97$ for one, and the $r^2 = 0.87$ for the last) (Table 2).

The 19 identified risk SNPs were abstracted from the ARIC imputation file. Using the estimated log odds ratios (ORs) from the CARDIoGRAM study as weights, a normalized risk score was derived for all Caucasians ($n = 8,734$) in ARIC. This distribution of scores served as the population based comparison for the participant's genetic risk score. To assess the 10-yr risk of CVD based on an individual's genetic risk score, a relative risk (RR) regression was estimated within the ARIC cohort, adjusting for sex and age. Within the ARIC sample, 13% were identified as having CHD within 10 years, and the RR for a 1-standard deviation increase in genetic risk was found to be 1.18 (95% confidence intervals: 1.12,1.25). For participants in this study, a genetic risk score was calculated in the same manner as it was calculated for ARIC cohort members with the caveat that genotype information for each locus was derived from the

SNPs at each locus on the CM array. Each individual's 10-year risk of CHD based on the Framingham risk calculator was computed. This 10-year risk was then multiplied by the individual's estimated genetic relative risk to generate an updated risk.

OUTCOMES:

Primary outcome: The primary outcome is the change in LDL cholesterol between the 3-month and the 6-month visits. We chose this as the primary outcome for several reasons. First, a strong relationship exists between LDL cholesterol and CVD and LDL cholesterol reduction is a major therapeutic goal in both primary and secondary prevention of CVD. Second, it is easy to measure and accurately quantify LDL cholesterol. Finally, repeated measures of LDL cholesterol are standard-of-care.

Secondary outcomes: Providing participants information on genetic risk may affect other outcomes of interest in the context of the goal of this trial. Thus, we are assessing four sets of secondary outcomes. As in the primary outcome, the main comparison for each of these sets of outcomes is the difference in these measures between the 3-month and 6-month visits.

The first set of secondary outcomes includes changes in traditional cardiovascular risk factors other than LDL such as HDL, blood pressure, weight, smoking status, glycemic control (as assessed by hemoglobin A1C) as well as physical activity and dietary patterns. We assess usual leisure-time physical activity in the past month with a single self-report item consisting of six descriptive categories ranging from inactive to very active⁴². This item, the Stanford Leisure-Time Activity **C**ategorical Item (L-Cat) has strong psychometrics in a sample of obese women in a randomized behavioral weight management trial including excellent test-retest reliability; concurrent criterion validity with pedometer steps and weight loss; and sensitivity to change. To assess usual dietary patterns in the past month, we revised a previously validated brief dietary questionnaire⁴³. Revisions included updating item content to reflect

current CVD-related dietary guidelines and simplifying response categories. The revised questionnaire consists of 11 self-report items with 10 response categories ranging from 'one time or less in the last month' to '5 plus times a day'. We will examine the internal consistency of the dietary items in this sample.

The second set of secondary outcomes involves measures of adherence to and attitudes towards taking medications. Medication adherence to cholesterol lowering drugs and anti-hypertensive medications are each assessed with a single item using a visual analog scale. These items have adequate validity with unannounced phone-based pill counts in similar clinical samples⁴⁴. Medication attitudes are assessed with 12 self-report items from the Beliefs about Medicines Questionnaire⁴⁵, which examine perceived *necessity* of medications and *concerns* taking medications as validated against the Illness Perception Questionnaire, the Reported Adherence to Medication scale, and the Sensitive Soma Scale. We will examine the internal consistency of the items in this sample.

The third set of secondary outcomes focuses on perceptions of risk. Relative and absolute perception of risk are each assessed by a single item measured on a 5-point Likert scale ranging from 'Not at all' to 'Extremely'. The items included: "Over the next 10 years, compared to others your age and sex, how would you rate your risk of having a heart attack or dying due to blocked coronary arteries?" and "Over the next 10 years how likely do you think it is that you personally will have a heart attack or die due to blocked coronary arteries?"⁴⁶. These items have validity in other clinical samples⁴⁷. We also assess whether patients discuss their risk of CVD with others and encourage others to be screened; each are assessed by a single item measured on a 5-point Likert scale with response options ranging from 'Not at all' to 'Extremely'. Informed by the transtheoretical stages of change model⁴⁸, we assess stages of change with five questions adapted from a questionnaire previously validated in a preventive cardiology setting⁴⁹. These questions focused on stages later than the first (precontemplation) stage, as we reasoned that patients coming to the PCC would have already

advanced past the precontemplation stage. We will examine the internal consistency of the items in this sample.

The final set of outcomes focuses on psychological outcomes given the potential of genetic risk information to have negative psychological impact. Informed by previous literature on the potential psychological impacts of genetic screening for pregnancy and mammogram screening⁵⁰, we assess five constructs with single or two question items adapted from previously published reports^{51,52}. To minimize participant burden, we standardize the items using 5-point Likert scales with response options ranging from 'Not at all' to 'Extremely'. The constructs include anxiety associated with testing, fatalism, depression, stress, and mood. These items have adequate validity in similar clinical samples.

SUBGROUP ANALYSES:

The size of this trial limits the ability to examine subgroups robustly. We will nevertheless examine trends of association in two important subgroups to help plan future intervention trials that build off this pilot study. The first is the subgroup with participants whose 10-year risk of CHD is increased when genetic information is incorporated into the ARIC cohort modeling compared as compared to those that have a lower updated risk. The second is the subgroup who demonstrates a clear reluctance to taking or initiating medications at the 3-month visit based on their responses to the Beliefs about Medicines Questionnaire.

STATISTICAL METHODS AND POWER CALCULATIONS:

For the primary outcome and all the secondary continuous endpoints, the mean changes between 3- and 6-month visits will be compared between the two arms, adjusting for baseline values. Scales will be analyzed assuming interval scaling. With 50 patients per arm, the trial has ~85% power to detect a difference of 8 mg/dl of LDL cholesterol at $\alpha=0.05$ (assuming post treatment LDL values of 108 and

100 mg/dl in the two arms and a standard deviation of 15mg/dl). These LDL values are representative of typical patients in the PCC with pre-treatment LDL cholesterol values ~130-160mg/dl. In general, studies have confirmed a 5-15% decrease in LDL with diet and lifestyle changes and a 30-40% decrease in LDL with statin therapy^{53,54}. Thus the trial is well powered to identify an effect of genetic testing that is in the same range as that of diet and exercise.

HUMAN SUBJECT PROTECTION:

The Stanford Institutional Review Board approved this protocol. A research exception was made to allow the return of non-CLIA certified genotypic information to patients.

DISCUSSION-

Genome-wide association studies continue to identify a growing number of susceptibility variants for various complex diseases offering hope that this information may allow for improved risk prediction and a reduction of risk through the more efficient application of proven primary and secondary prevention strategies. However, the use of these markers in clinical practice has not yet been shown to improve patient outcomes or risk factor profiles in CVD. The NIH and the US Preventive Services Task Force has indicated that genetic screening will be highly scrutinized until randomized trials demonstrate clinical benefit⁵⁵. Currently, both the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group and the ACC/AHA Taskforce on Practice Guidelines recommend against genetic testing for cardiovascular disease^{39,56} citing lack of clinical trial data supporting its use^{39,56}. Despite these recommendations, and lack of efficacy data, there is growing enthusiasm for personalized medicine approaches, fueled also by direct-to-consumer genetic testing companies. In this context, an important opportunity exists to develop well-designed clinical trials that objectively examine the population effect of providing information on complex trait susceptibility variants to individuals at risk. We seek to help

answer some of these questions by determining if genetic testing plus standard care improves cardiovascular risk profiles in the short term compared to standard care alone.

Although there are no published clinical trials using genetic testing specifically for complex cardiovascular disease, we note the recently reported results of Bloss *et al* involving over 2000 research volunteers participating in the Scripps Genomic Health Initiative⁵⁷. In this non-randomized survey, participants reported on changes in levels of anxiety, fat intake, and exercise after being provided the genotyping results of a genome wide array. The genetic risk score for a number of common conditions was provided to the participants along with genetic counseling services. The investigators found that this information had no effect on exercise and dietary habits. Furthermore, they observed no short-term adverse negative psychological impacts. Importantly, these participants were not enrolled based on a prior existing condition or set of risk factors and so may not represent the effect genetic testing would have on individuals with a specific disease or risk for disease.

Recently, Grant *et al* published a description of the methods of a randomized trial of genetic risk testing to evaluate risk of diabetes and motivate behavioral change to reduce that risk⁵⁸. The trial design of that study and ours has several similarities including the creation of a genetic risk score based on the latest set of validated susceptibility variants, enrollment of patients with a baseline elevated risk of a condition and assessment of secondary outcomes measures such as changes in weight, exercise and diet as well as psychological stages of change, and levels of anxiety. The trials also have important differences. The primary outcome for the Grant *et al* trial is attendance at 12-week diabetes prevention classes rather than change in a quantitative risk variable (e.g., LDL). In addition, the investigators used a novel allocation scheme based on the application of Mendelian randomization to exclude individuals at 'average' genetic risk of diabetes with the hypothesis that there would be a differential effect of the genetic testing on the behaviors of people at higher vs. lower genetic risk. Conversely, we have opted to enroll participants regardless of their genetic profile but we do plan to examine whether effects

between participants whose CVD risk increases because of their genetic profile vs. participants whose risk decreases.

Although the MetaboChip contains over 200K SNPs related to many cardiometabolic risk factors, participants in this study are receiving genetic risk information restricted to the 19 loci that have been unequivocally associated with CVD and appear to be mediating risk independent of traditional risk factors. We opted for this strategy for several reasons. While many true susceptibility loci likely exist among the top signals included on the MetaboChip, a GRS incorporating many of these loci for CVD has not been shown to be superior to a GRS restricted to validated loci. Similarly, the addition of information from either validated or promising susceptibility loci for risk factors of CVD such as LDL, BMI, or blood pressure to a GRS based on CVD variants alone has also not been shown to improve risk prediction substantially²⁴. Furthermore, we thought it best to focus on a discrete number of susceptibility variants for a single phenotype given the complexities of conveying the genetic information in a standard clinical visit. Thus, we chose focus on the single phenotype that would be most directly relevant from a clinical perspective and would conceivably have the largest impact on behavior.

While several trials assessing the efficacy of genetic testing have employed genetic counselors⁵⁷, we made a conscious decision to avoid that paradigm because we wanted to mimic what might happen in 'real-world' scenarios where access to genetic counselors is often limited. Involvement of a genetic counselor would change the purpose of the trial from ascertaining usefulness of genetic testing alone to ascertaining the usefulness of genetic testing combined with genetic counseling. In contrast to Mendelian disorders, where counseling is usually limited to a few family members at-risk, counseling for complex genetic disease would involve a much larger population. This approach currently appears neither efficient nor practical given the number of individuals at intermediate or high risk of CVD⁵⁹. Optimal ways of conveying complex genetic information to people without necessarily involving genetics

experts need to be explored in depth.

We do not perform a time-matched alternate intervention in control patients who do not receive their genetic data at the second visit. Time-matched alternate interventions may be necessary when testing more complex interventions where considerably more time is spent interacting with volunteers and where there is a danger that this interaction in of itself will result in a benefit that is independent of the intervention being tested. Thus, a time-matched alternate intervention was not deemed necessary given that the genetic score information can be generally conveyed in 10 minutes and the pharmacologic algorithm for lowering LDL is “locked” for the duration of the trial.

Potential strengths of our trial include the development of a comprehensive risk score based on 19 validated loci associated with CVD independent of traditional risk factors. This risk score was designed to present a fair and balanced view of the genetic risk by showing not only the effect on overall risk but also the patient-specific risk relative to the general population. Another strength is use of change in LDL cholesterol as the primary outcome, which is a quantitative measure that has a direct effect on the risk of cardiovascular disease and is a widely accepted major target for therapeutic intervention. Many of the secondary outcome measures have also been shown to markedly impact CVD risk including changes in blood pressure, weight and diet. We are also capturing information about more subtle but important ways in which genetic testing may have a positive impact such as moving patients along the spectrum of the stages of change, giving them a more realistic appraisal of their absolute and relative risk of cardiovascular disease. We tried to remove the impact of genetic testing on physician prescriptions, since there is no evidence that physicians should react to such genetic information and allowing such reaction might have added an uncontrolled source of variance, e.g. the response might have been better in the genetic information group simply because physicians prescribed higher doses of medication. However, future trials may need to try to address also the impact of such information on physician behavior. Finally, one of the key issues we are assessing is whether genetic

testing for cardiovascular disease will result in increased anxiety, depression or other psychological harm. We chose to address this issue because discussions of the potential for psychological harm are prominent in the screening literature regarding other conditions. For example, the US Preventive Services Task Force has explicitly sought and commented on evidence regarding psychological harms in its evidence reviews of the use of mammography for cancer screening⁶⁰.

The limitations of the trial include the relatively small sample size and short duration but these were considered appropriate for a “proof of concept” trial designed to demonstrate feasibility. We hope that successful completion of the trial will demonstrate that such experimental designs can be adapted to larger, multi-center trials with long term follow-up that includes hard clinical outcomes. Another potential limitation relates to the generalizability of results given the patient population being tested. Patients who present to the PCC are likely to be more motivated than the general population and more likely to undertake recommended beneficial lifestyle changes. They also may be more highly educated and more willing to embrace new technologies (as ‘early adopters’) compared to the general population. While we recognize this latter limitation, we also believe that this could be beneficial. If the trial fails to show benefit in a highly motivated population such as this one, it may be more difficult to show benefit in the general population for people who have less interest in seeking expert help and intervention for their cardiovascular health. Furthermore, we will be able to gauge the motivation level within this group in the time between the first and second clinical visits. This should provide us greater insight into the impact of the genetic testing, as we will be able to also use each individual as his or her own control. Lastly, while we have some understanding of the genetic basis for cardiovascular disease based on large GWAS studies, a large fraction of the inherited risk remains unexplained. In the future, the magnitude of changes in risk imparted by a genetic score may be much greater and this could significantly affect the patient response and outcome. We are unable to assess whether an enhanced risk score based on additional markers would substantially change the outcome. As more risk variants

are discovered, a challenge will be to keep the genetic score up-to-date. Maintaining an updated genetic risk score is less of a concern for our relatively short-term trial, but it may become an issue for larger long-term trials, where the list of validated or relevant genetic variants may grow substantially during the trial. One option in this situation may be to build an analytic pipeline that can quickly adapt the genetic risk score to include newly validated variants in the risk calculations.

There are similar challenges in the adoption of information also from other cardiovascular biomarkers, including but not limited to coronary artery calcium, intima-media thickness, and blood protein biomarkers^{61, 62}. Ideally, similar pilot trials should be performed also for other biomarkers that are contemplated for use in clinical practice. Until such trials are available, it is difficult to discern what are the relative merits of different types of genetic versus non-genetic markers, other than their discriminating ability which generally seems to be modest for all available proposed markers.

In conclusion, we have designed a trial to assess whether we can reduce risk in patients through providing them with information about CVD susceptibility loci. Trials such as this are necessary before we can advocate for widespread application of genetic testing for common, complex disease phenotypes. This type of trial may be adopted for prevention settings for other chronic diseases in which there are good treatments available but suboptimal risk discrimination that could potentially be improved by consideration of genetic information.

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Tables:

Table 1. Eligibility criteria
<p>Inclusion criteria:</p> <ul style="list-style-type: none">-Adults age > 18-White/European, South Asian, or Hispanic/Latino race/ethnicity*-Patient seeking cardiovascular risk evaluation-At intermediate (6-20%) or high risk ($\geq 20\%$) over 10 years of CVD as defined by Framingham 10 year risk score AND/OR at $\geq 20\%$ risk of CVD over 30 years using the Framingham 30 year risk calculator.
<p>Exclusion criteria:</p> <ul style="list-style-type: none">-History of myocardial infarction, angina, stroke, peripheral arterial disease, PCI, or CABG-Already on lipid lowering therapy-Anticipated survival <1 year (e.g. metastatic cancer)-Serious conditions that would limit ability to adhere to recommendations (inability to take statins, exercise)-Already had genetic testing.

*The 19 genetic loci that comprise our risk score have been evaluated predominantly in white/European subjects.

However, there is considerable overlap in genetic architecture between white/European populations and South Asians or Hispanic/Latino populations. Therefore, we have limited our study population to these three race/ethnicity groups.

Table 2. SNPs related to CVD that are independent of traditional risk factors

SNP tested	Risk		Chrom.	Nearby genes	Odds ratio*	Ref.
	Risk allele	Allele Frequency				
rs17465637	C	0.74	1	<i>MIA3</i>	1.14	14, 21, 63
rs9970807 ^a	T	0.91	1	<i>PPAP2B</i>	1.17	21
rs6725887	C	0.15	2	<i>WDR12</i>	1.17	21, 63
rs2306374	C	0.18	3	<i>MRAS</i>	1.15	21, 64
rs12190287	G	0.76	6	<i>TCF21</i>	1.08	21
rs12204265 ^b	C	0.67	6	<i>ANKS1A</i>	1.07	21
rs12526453	C	0.62	6	<i>PHACTR1</i>	1.12	21, 63
rs11556924	C	0.62	7	<i>ZC3HC1</i>	1.09	21
rs4977574	G	0.46	9	<i>CDKN2A, CDKN2B</i>	1.28	13, 15, 21, 63
rs1746048	C	0.87	10	<i>CXCL12</i>	1.17	14, 21, 63
rs2246833	C	0.42	10	<i>LIPA</i>	1.09	20
rs2505083	T	0.37	10	<i>KIAA1462</i>	1.07	20
rs974819	T	0.29	11	<i>PDGFD</i>	1.07	20
rs4773144	G	0.44	13	<i>COL4A1, COL4A2</i>	1.07	21
rs2895811	C	0.43	14	<i>HHIPL1</i>	1.07	21
rs7177699 ^c	T	0.56	15	<i>ADAMTS7</i>	1.08	21
rs12449964 ^d	C	0.56	17	<i>RASD1, SMCR3, PEMT</i>	1.07	21
rs143499 ^e	C	0.36	17	<i>SMG6, SRR</i>	1.07	21
rs9305545 ^f	A	0.16	21	<i>SLC5A3, MRPS6, KCNE2</i>	1.2	21, 63

* odds ratio based on that reported for lead SNP reported by large-scale meta-analyses^{20, 21}

^a proxy for lead CARDIOGRAM SNP rs17114036 ($r^2 = 0.90$)

^b proxy for lead CARDIOGRAM SNP rs17609940 ($r^2 = 0.91$)

^c proxy for lead CARDIOGRAM SNP rs3825807 ($r^2 = 1$)

^d proxy for lead CARDIOGRAM SNP rs12936587 ($r^2 = 1$)

^e proxy for lead CARDIOGRAM SNP rs216172 ($r^2 = 1$)

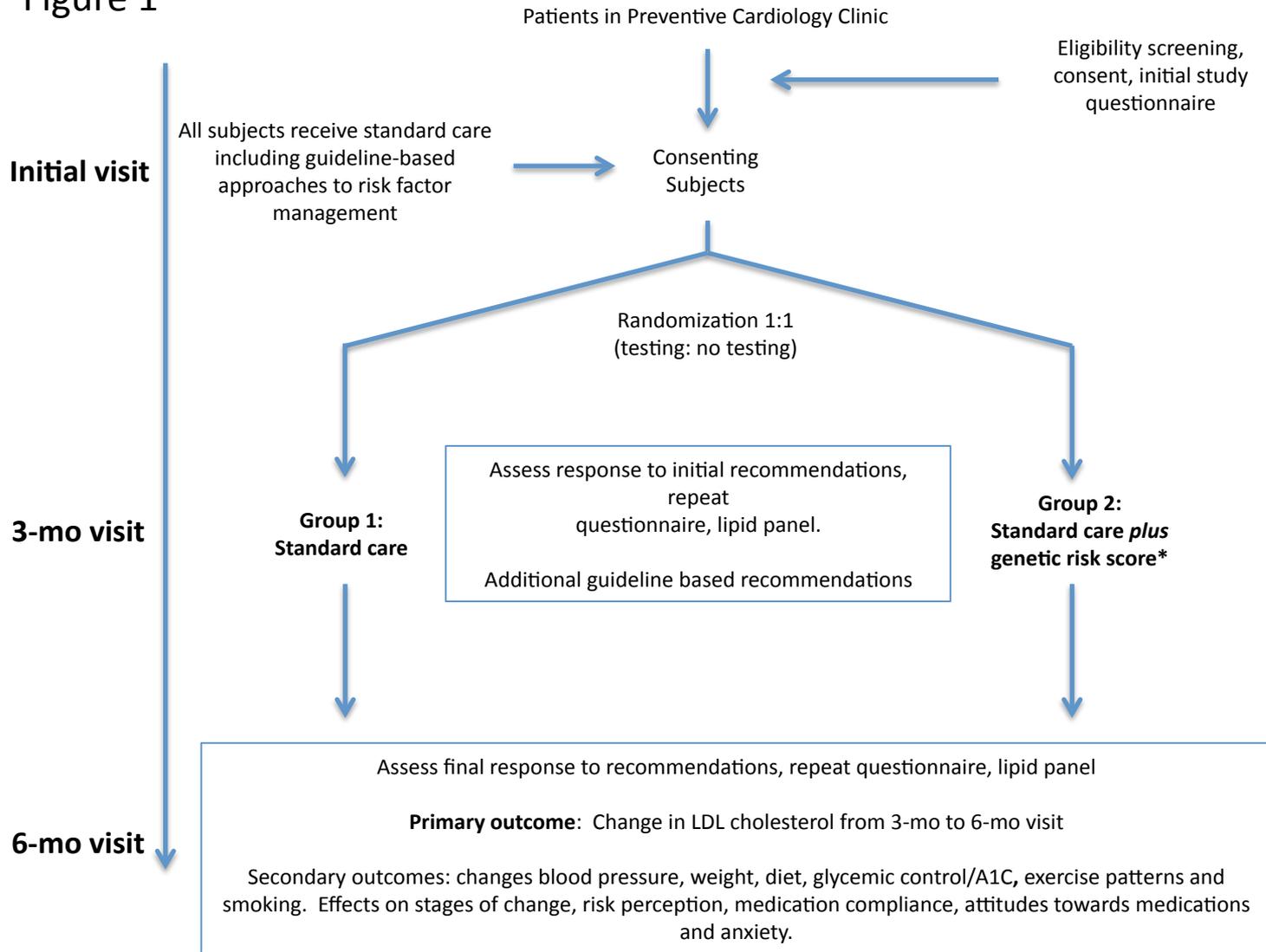
^f proxy for lead CARDIOGRAM SNP rs9982601 ($r^2 = 0.87$)

Figure Legend:

Figure 1: Study Schema

Figure 2: Genetic risk score report: This score was derived for a hypothetical patient.

Figure 1



* Genetic risk score given at the end of the 3 mo clinic visit, after all therapeutic decisions have been made and guideline based recommendations have been given. Altering therapy based on genetic test results is not permitted.

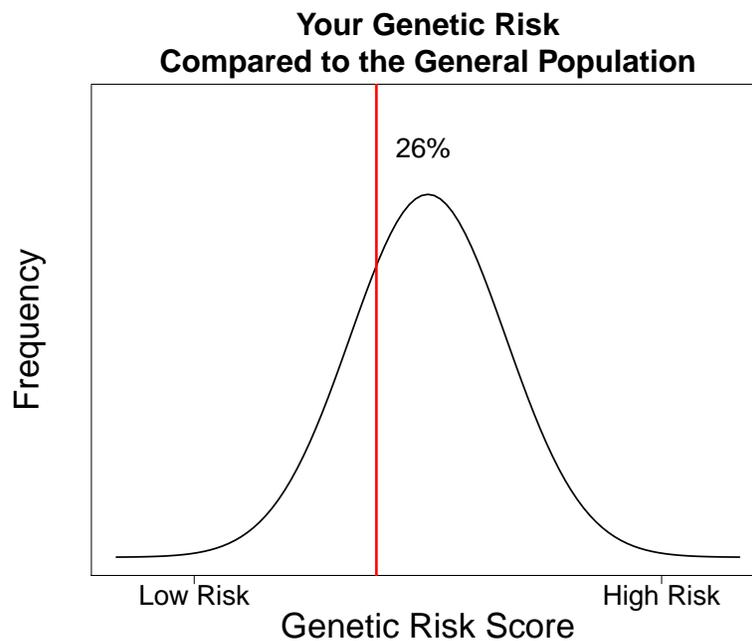
Figure 2

Your Risk Score

Based on the traditional Framingham risk score, your risk of coronary heart disease over the next 10 years is approximately 9.7%.

We tested for a total of 38 possible risk variants or alleles. Out of these 38, you carry 15 variants that are associated with higher risk.

Your genetic profile puts you in the 26 percentile for risk. This means 26% of the general population have a genetic risk score more favorable than you and 74% have a genetic risk score less favorable than you.



Based on the traditional Framingham risk score plus the genetic risk score, your risk of coronary heart disease over the next 10 years is approximately 8.7%.