

# Challenges of Precision Medicine for Atherosclerotic Cardiovascular Disease Based on Human Genome Information

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Precision or personalized medicine is currently gaining a lot of attention. Clinical evidence for its effectiveness has been established based on randomized clinical trials accounting for classical risk factors, such as hypertension, diabetes, and serum lipids. However, besides such classical risk factors, the genetic background should be considered, at least for heritable traits, including atherosclerotic cardiovascular disease (ASCVD). Such classical risk factors are almost always incidents that have already occurred in which it may be too late to start treatment, instead of indicators of presymptomatic state. Human genome information is associated with most traits, including ASCVD. Two methods of implementing precision medicine for ASCVD using human genome information are currently being investigated: the use of rare genetic variations that have large effect sizes and polygenic risk scores that are composed of multiple common genetic variations. This review article emphasizes the importance of clinical as well as genetic diagnoses when implementing precision medicine. Precision medicine should be considered based on comprehensive genetic analyses, encompassing rare to common genetic variations.

**Key words:** Precision medicine, Genetics, Cholesterol

## Introduction

The human genome comprises approximately 3 billion DNA base pairs, including protein-coding regions and noncoding regions. The number of different patterns of DNA sequences is unlimited, making us quite diverse. Countless traits are heritable in which DNA sequences are associated with outcomes. Atherosclerotic cardiovascular disease (ASCVD) is one of the major causes of death worldwide<sup>1</sup>. It is well known that ASCVD is a heritable trait in which family history information is robustly associated with ASCVD<sup>2</sup>. Accordingly, it seems quite reasonable to investigate which particular genetic variations are associated with ASCVD development and what the mechanisms are. Familial hypercholesterolemia (FH) is a good model that can be easily understood. Typically, FH is caused by a rare loss-of-function genetic mutation, leading to a reduced number of low-density lipoprotein (LDL) receptors and a reduction in their activity, resulting in elevated serum LDL cholesterol levels<sup>3</sup>. Untreated FH

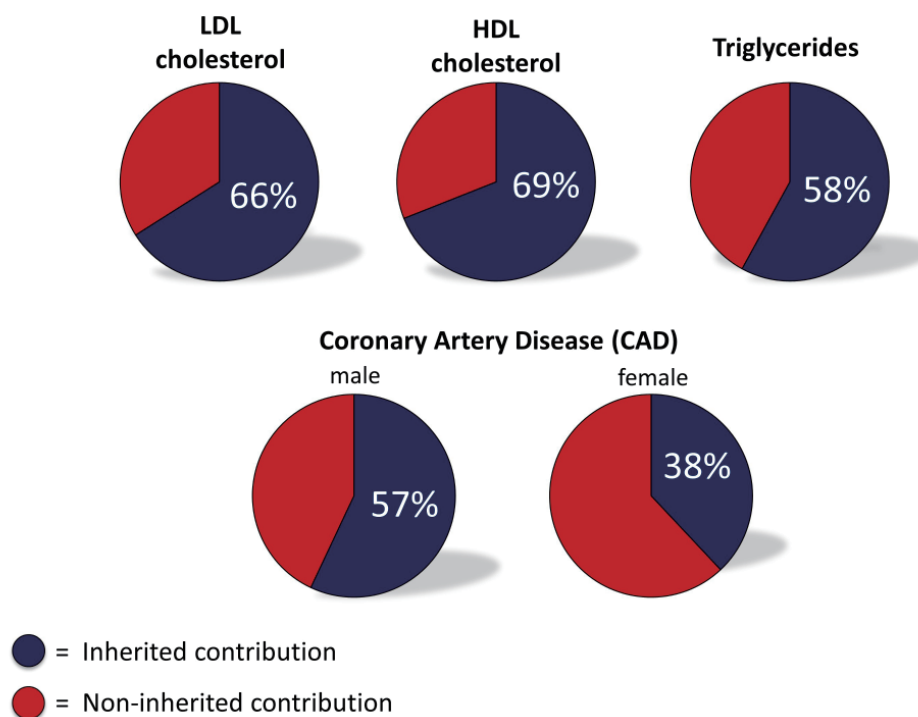
is strongly associated with premature ASCVD<sup>4</sup>. A single loss-of-function genetic mutation in the LDL receptor gene is associated with ASCVD via elevation of LDL cholesterol because LDL cholesterol is one of the causal factors of ASCVD<sup>5</sup>. However, there are a substantial number of people who are at extremely high risk for inherited ASCVD that is not associated with serum lipids. Causal links between biology and ASCVD development in such inherited risk factors exist.

## Aim

The following review describes conditions beginning with FH in which precision medicine for ASCVD seems to be quite effective, to other conditions in which precision medicine for ASCVD is currently being rigorously examined.

## What is the Human Genome? How Useful is the Human Genome in Precision Medicine for ASCVD?

In 2001, the International Human Genome



**Fig. 1.** Heritable and nonheritable factors for lipid traits and coronary artery disease

Dark blue indicates the proportion explained by heritable factors. Red indicates the proportion explained by non-heritable factors. Reproduced with permission from Zdravkovic S, *et al.* *J Intern Med*, 2002; 252: 247-254, and Kathiresan S, *et al.* *BMC Med Genet*, 2007; 8: S17.

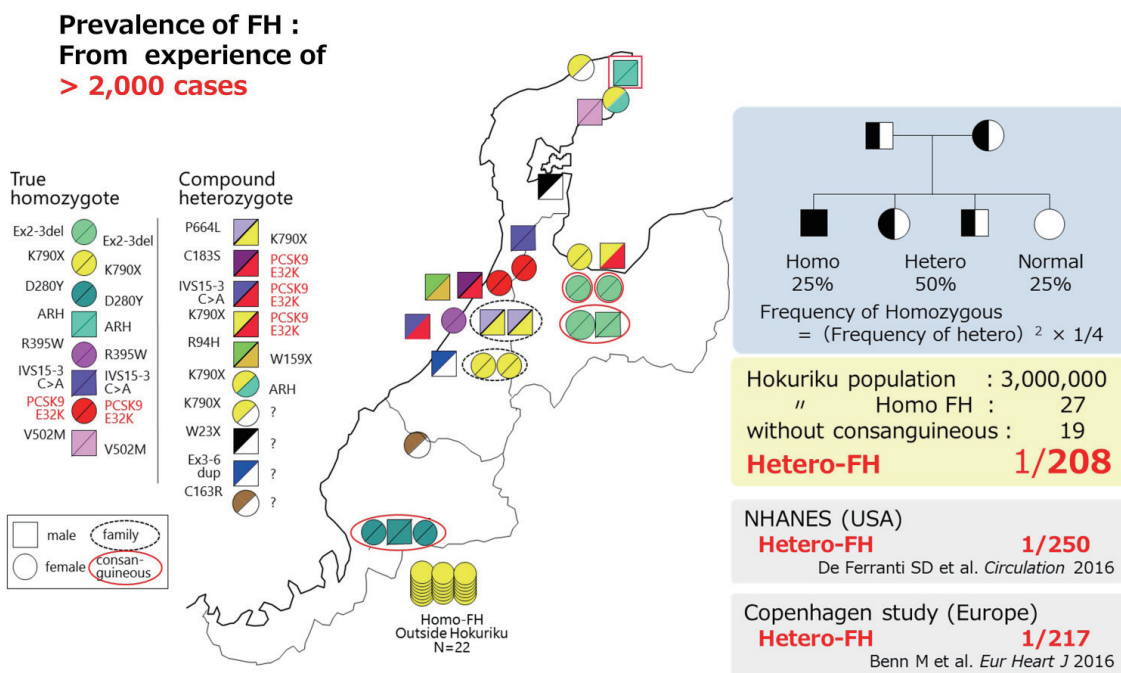
Sequencing Consortium reported a draft sequence of the euchromatic portion of the human genome<sup>6</sup>. Subsequently, an international collaborative effort worked to convert this draft into a genome sequence with high accuracy and nearly complete coverage, which was completed in 2004<sup>7</sup>. After the initial report of the entire human genome, millions of individuals have had their whole genomes sequenced. It is estimated that single nucleotide variations (SNVs) occur 1 in 1000 base pairs, on average, although they do not occur at a uniform density. Therefore, we are quite similar to one another: we are all, regardless of race, 99.9% identical genetically. Because of this similarity, it is sometimes quite difficult to identify the causative mutation or mutations associated with a disease. Conversely, “heritability” is a concept that describes the degree in which a certain trait can be attributed to a genetic variation.

Heritability has been estimated from so-called twin studies. We can estimate heritability by comparing a trait in monozygotic twins and dizygotic twins. Serum lipids as well as ASCVD have been shown to be highly heritable traits, with a heritability of approximately 60%<sup>8, 9</sup> (**Fig. 1**). Because of this high heritability, precision medicine for serum lipids and ASCVD based on the human genome should be

quite useful.

### FH as the Primary Model of Precision Medicine for ASCVD

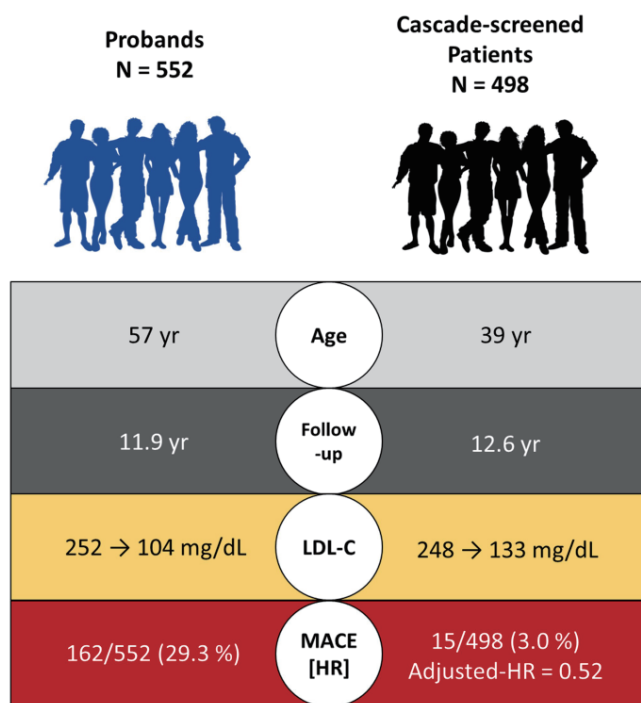
If we have a chance to see only a single patient with homozygous FH who has double loss-of-function mutations in the LDL receptor or its associated gene, it is quite easy to understand the causal relationship between LDL cholesterol and ASCVD<sup>10</sup>. Probands who have double deleterious mutations have percutaneous and tendon xanthomas associated with extremely high LDL cholesterol, almost always leading to premature cardiac death, whereas their unaffected relatives do not exhibit such complications<sup>11</sup>. Those of their relatives who have a single mutation, i.e., patients with heterozygous FH, exhibit the exact middle phenotype<sup>3</sup>. These observations in general show that a deleterious mutation in the LDL receptor is the cause of this condition. The prevalence of this condition in the general population had been considered as approximately 1 in 500 cases<sup>12</sup>. However, we found a prevalence of homozygous FH of 1 in 208 cases in the Hokuriku district of Japan in 2011<sup>13</sup>. Following this report, other groups have found similar prevalence in the United States and Europe<sup>14, 15</sup> (**Fig. 2**). Accordingly, identifying and



**Fig. 2.** Prevalence of heterozygous familial hypercholesterolemia (FH) in Hokuriku, Japan

The prevalence of heterozygous FH in the Hokuriku area in Japan is estimated to be around 1 in 208 cases, according to the number of patients with homozygous FH in this region. Similarly, the prevalence of heterozygous FH in estimated to be around 1 in 217 cases and that in the US to be around 1 in 250 cases. Reproduced with permission from Mabuchi, *et al.* *Atherosclerosis*, 2011; 214: 404-407.

treating FH are now considered public health problems. Additionally, there are a substantial number of studies that suggested that early intervention in patients with FH can be beneficial for ASCVD prevention<sup>16-19</sup>. For example, a recent study showed that pediatric FH patients who were treated starting at 13 years of age had quite good prognoses, despite their high achieved LDL cholesterol level (160 mg/dL)<sup>18</sup>. The present study strongly suggests that earlier intervention can eliminate the need for aggressive therapies, such as proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. There are two major methods of detecting FH early. The first is cascade screening, which is based on finding relatives of the probands who seek medical care. In our experience, we have found several cascade-screened patients with FH through the clinical diagnosis of FH, because we need information on the family history of FH to diagnose FH accurately. There are many published reports of the clinical impact of cascade screening of FH<sup>20-23</sup>. We have found that FH patients detected by cascade screening have much better prognoses than the probands (their relatives), even under milder therapies<sup>24</sup> (**Fig. 3**). These results strongly support the notion that finding FH by cascade screening is a good method of treating ASCVD through precision



**Fig. 3.** Impact of cascade screening on the prognosis of familial hypercholesterolemia (FH)

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medicine. The second method of detecting FH early is universal screening. There are several current programs of universal screening for FH globally<sup>25-27</sup>. Theoretically, such universal screening for FH can lead to ASCVD treatment via precision medicine.

### Genetic Diagnosis of FH: Is it Necessary, and Why?

An essential question for clinicians caring for FH patients is whether genetic testing for FH is necessary or useful. Genetic testing is fundamental for the diagnosis because FH is a genetic disorder; some experts said that clinical diagnostic criteria are sufficient. However, there are several important reasons for conducting genetic testing for FH:

- Genetic testing is useful in cases in which the clinical diagnosis is unclear<sup>28</sup>. This is true for many patients because family structure is changing, leading to uncertainty regarding family history information.

- Genetic testing can differentiate between heterozygous and homozygous FH. Although the LDL cholesterol phenotypes of normal individuals, individuals heterozygous for FH, and individuals homozygous for FH show a trimodal distribution, there is a substantial overlap between heterozygous and homozygous FH<sup>9</sup>. We typically classify these cases as “severe FH”<sup>29</sup>. However, genetic testing can diagnose homozygous FH accurately so that patients homozygous for FH can be treated by special medications, including microsomal triglyceride transfer protein inhibitors<sup>30</sup>, angiopoietin-like protein 3 (ANGPTL3) inhibitor<sup>31</sup>, and LDL apheresis<sup>9</sup>. Accurate diagnosis through genetic testing should be quite important among homozygous FH because such treatments are usually expensive. Note that as of September 2020, ANGPTL3 inhibitor is not still officially approved to use for the patients of homozygous FH in Japan.

- Genetic testing can differentiate other phenocopies, such as sitosterolemia<sup>32</sup>, autosomal recessive hypercholesterolemia (ARH)<sup>33</sup>, and cerebrotendinous xanthomatosis<sup>34</sup>. Differential diagnosis of these conditions is critically important because the optimal treatment strategy is different for different diagnoses.

- Genetic testing is also useful for the detection of double heterozygotes with LDL receptor and its related genes.

- The genetic status of FH is associated with ASCVD beyond LDL cholesterol level; thus, the determination of genetic status can be used as a biomarker for ASCVD<sup>35</sup>. Based on these, genetic testing for FH is useful. Currently, several types of panel sequencing covering known FH genes have been developed, and the cost of sequencing has been

gradually reduced over the years<sup>36</sup>. We are now conducting a randomized, waiting list-controlled, open-label study to see if genetic testing and disclosure of the results will lead to a better prognosis<sup>37</sup>.

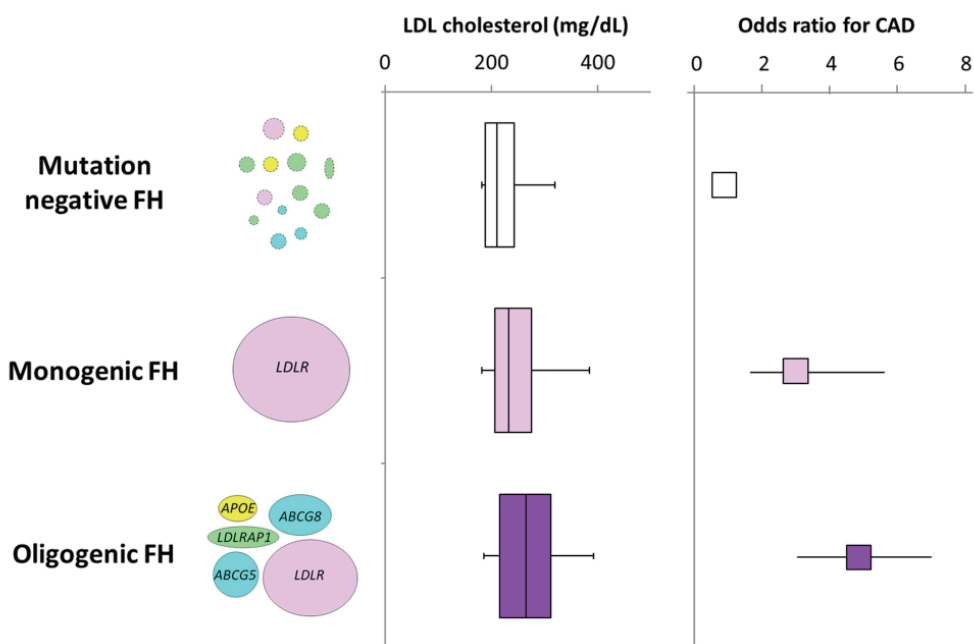
- Some patients with FH have additional rare mutations associated with LDL cholesterol elevation<sup>38</sup>. We have called this condition oligogenic FH; these patients have significantly higher LDL cholesterol levels than patients with pure monogenic FH (Fig. 4).

### Precision Medicine for FH

As already stated, FH can be diagnosed very early by cascade screening and universal screening. Accumulated evidence suggests that LDL cholesterol-lowering therapy may not need to be so intensive in patients who are diagnosed and start treatment at an early phase. In our experience, a homozygous FH patient whose LDL cholesterol level was not adequately lowered but who started treatment at 13 years of age had a better prognosis than his older brother, with nearly the same genetic and environmental background, who started his treatment at 23 years of age<sup>39</sup>. The patients' genetic status can help us to determine how and when to treat them. If a patient with FH has an ATP-binding cassette subfamily G member 5/8 (*ABCG5/ABCG8*) rare mutation besides an LDL receptor mutation, ezetimibe should be added to statin treatment, since there is a report that showed that dyslipidemic patients with a mutation in the *ABCG5/ABCG8* gene exhibited better response when adding ezetimibe on top of atorvastatin therapy<sup>40</sup> (Fig. 5). Imaging modalities, such as carotid ultrasound and coronary computed tomography (CT), as well as physiological function tests, such as pulse wave velocity, have been shown to be useful for further risk stratification<sup>41-43</sup>.

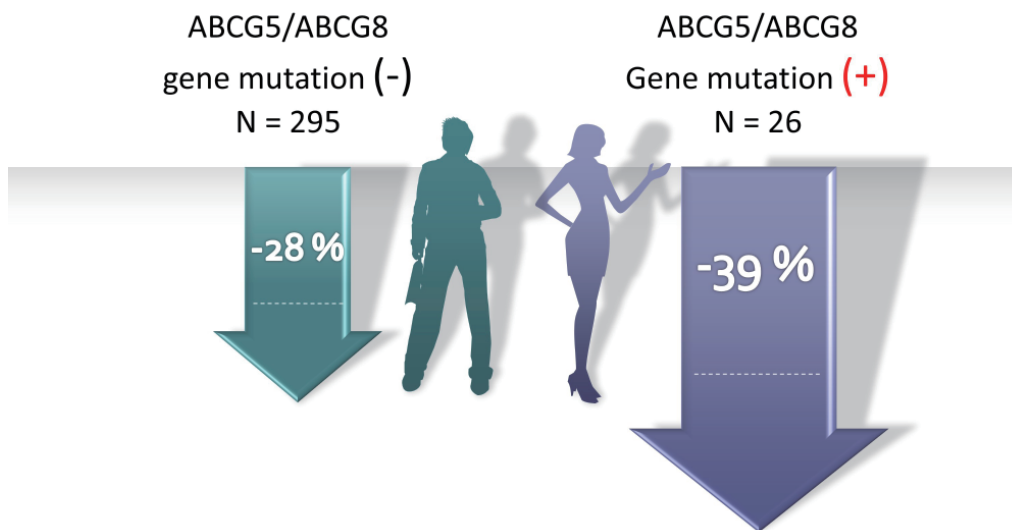
### Precision Medicine for ASCVD in the General Population

As stated above, genetic testing for FH, assuming this condition is caused by a rare genetic mutation or mutations appears to lead to precision medicine for ASCVD. How about ASCVD in the general population? The risk of ASCVD is highly heritable among the general population. To determine the “hidden” biomarkers of ASCVD, a number of genetic association studies have identified many loci and common genetic variations associated with inherited elevation in the risk of ASCVD<sup>44</sup>. Although a single common genetic variation does not have enough power to be used in precision medicine, combining multiple common genetic variations into a single score has been shown to be clinically useful for this purpose,



**Fig. 4.** Impact of genetic status of familial hypercholesterolemia (FH) and oligogenic FH

Compared with individuals with mutation-negative FH who are potentially affected by polygenic causes, individuals with monogenic FH have higher low-density lipoprotein (LDL) cholesterol and higher odds for coronary artery disease (CAD). Additionally, those with oligogenic FH appear to have even higher LDL cholesterol and higher odds for CAD. Reproduced with permission from Tada, *et al.* *Curr Opin Lipidol*, 2019; 30: 300-306.



**Fig. 5.** Personalized medicine for hyper-low-density lipoprotein (LDL) cholesterolemia according to mutation status of the ABCG5/ABCG8 gene

Vertical arrows indicate the degree of LDL cholesterol reduction when adding ezetimibe on top of atorvastatin 10 mg. Individuals who have an ABCG5 or ABCG8 genetic mutation have a better response to the addition of ezetimibe to atorvastatin treatment. Reproduced with permission from Tada, *et al.* *Lipids Health Dis*, 2020; 19: 3.

including ASCVD outcomes. The initial attempt was published in 2010, where a polygenic risk score based on 13 SNVs associated with ASCVD was moderately associated with actual ASCVD events. Although this

initial score could show a significant association with the events, it did not add useful information in terms of risk discrimination<sup>45</sup>. A few years later, we showed that a polygenic risk score using 50 SNVs was

significantly associated with ASCVD events, beyond family history information, and that it was as useful as other traditional risk factors for risk discrimination<sup>46</sup>). Furthermore, a polygenic risk score that comprised approximately 6.6 million SNVs appears to be much more powerful<sup>47</sup>). Interestingly, when we look at extreme phenotypes, such as early onset of myocardial infarction (<55 years), the contribution of such polygenic high risk appears to be much higher than that of FH caused by rare mutations<sup>48</sup>). Now that we know how to pinpoint a set of individuals at high risk, we should know how to lower their risk. There are two simple established ways to do this. The first is statin therapy. It has been shown that statin therapy is much more effective in patients with polygenic high risk than in those with polygenic low risk<sup>49</sup>). The results clearly suggest that statin therapy can reduce at least a portion of the high risk for ASCVD. The second is healthy lifestyle. Another study showed that a healthy lifestyle can reduce polygenic high risk<sup>50</sup>). Thus, DNA is not destiny; it is not deterministic of this disease. We can find other ways of lowering the risk if we have more data. Furthermore, there are different lifestyles in different parts of the world. Currently, most data come from groups investigating people of European ancestry. We have to find other strategies using data from people of other ethnicities.

### Phenome in Addition to Genome

Recently, so-called phenome-wide association studies (PheWAS) have become feasible based on the increased availability of phenotypic data from electronic health records. These studies can better characterize human genome–phenome relationships. Particularly, PheWAS has the advantage of identifying genetic variants with pleiotropic properties, either good or bad. Cai *et al.* showed that individuals with a particular SNV in the interleukin 6 receptor gene (*IL6R*) were at reduced risk for aortic aneurysms, besides known “on-target” effect of *IL6R* blockade from clinical trials (reduced risk of coronary heart disease) as well as “off-target” effect (higher hemoglobin level)<sup>51</sup>). Rao *et al.* showed that a *PCSK9* missense variant was associated with a reduced risk of ischemic stroke and was not associated with an increased risk of diabetes<sup>52</sup>). These results appear to be compatible with findings obtained in clinical trials using *PCSK9* inhibitors. Furthermore, a recent PheWAS focusing on Lipoprotein (a) [*LPA*] genotypes and thousands of phenotypes showed an inverse relationship between FH and elevated Lp(a)<sup>53</sup>). Lp(a) is elevated in patients with FH, the mechanism of which remains unclear. This study clearly showed that Lp(a) levels were elevated in patients with a clinical

diagnosis of FH, based on a higher frequency of *LPA* genotypes leading to elevated LDL cholesterol, premature cardiovascular disease, and family history of cardiovascular disease, thereby increasing the likelihood of being diagnosed with FH.

### Future Directions

As stated repeatedly, precision medicine for ASCVD based on human genome information is quite reasonable, and it is the way to go. We need several items and steps to pursue this goal:

- We need much more data regarding the human genome and ASCVD. The human genome, as well as environmental factors, is quite diverse across ethnicities. Thus, we need data from ethnicities globally to feel confident.

- We need more advanced genotyping technology. Although so-called next-generation sequencing has facilitated our research over the decades, it still requires several steps that consume time and effort, such as polymerase chain reaction (PCR). If we can determine DNA sequences without PCR, the time and cost of DNA sequencing will be dramatically reduced.

- We need a good educational program for the general public to accept such advanced technology. It is sometimes quite difficult to explain to people why genotyping is important. As stated above, DNA is not destiny, at least for ASCVD development. There are several simple ways to ease the risk of ASCVD. Appropriate recognition of precision medicine based on the human genome among the general population is a prerequisite for this goal.

- We need randomized, controlled trials of intervention with statins and/or healthy lifestyle based on genotype to show the benefits of precision medicine.

### Conclusion

We have repeatedly emphasized that precision medicine for ASCVD based on the human genome is already feasible. However, several more steps are required for it to be accepted as a common practice.

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### Conflict of Interest Disclosures

None.

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