PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Impact of Genetic Testing on Low-density Lipoprotein Cholesterol in Patients with Familial Hypercholesterolemia (GenTLe-FH): A Randomised Waiting List Controlled Open-label Study Protocol
AUTHORS	Nomura, Akihiro; Tada, Hayato; Okada, Hirofumi; Nohara, Atsushi; Ishikawa, Hideki; Yoshimura, Kenichi; Kawashiri, Masa-aki

VERSION 1 – REVIEW

REVIEWER	Liam Brunham University of British Columbia Canada
REVIEW RETURNED	09-Aug-2018

GENERAL COMMENTS	This is a study protocol manuscript. The authors describe an open label trial of genetic testing in 100 patients with clinical diagnosed FH, using the JAS criteria. Genetic testing will be performed by targeted resequencing using the MiSeq. Patients will be randomized to receive genetic counselling plus FH patient education, or FH patient education only. Follow up is 48 weeks. The primary outcomes is change in LDL-C at 24 weeks. Secondary outcomes include blood tests, smoking, changes in lipid lowering therapy use and patient satisfaction at 24 and 48 weeks. Overall this is a sensible, small trial that will add to the growing knowledge of genetic testing for FH. My comments are aimed at clarifying aspects of the study design and rationale. 1. A few points could be better explained in the text. What counselling will be provided to the causal variant - patients in the intervention group? From the supplemental materials provided (which are in Japanese), it appears that data from Khera et al. JACC will be used to counsel patients on risk of CVD based on presence or absence of a FH-causing variant. However, the concern is that this may underestimate risk in the patients in whom an FH-causing variant is not found. In Khera et al, patients were identified by LDL levels, with no information on family history of clinical stigmata. Risk would be expected to be higher in patients with tendon xanthomas and strong family histories. How will this be taken into account in the counselling provided? 2. Lp(a) levels are not mentioned in the protocol. As these are an important determinant of risk in FH, they should be measured and incorporated into the counselling. 3. Similar to comment #1, how will "genetically estimated future cardiovascular risk based on the result of genetic testing" be determined?

	4. The rationale for the study design could be better explained. If the goal is to investigate the "efficacy of genetic testing" for FH, why not randomize patients to genetic testing vs no genetic testing, rather than perform genetic testing in all patients and randomize to counselling or no counselling? 5. Will patients in the control group have access to their genetic test result without counselling? 6. Table 1 and Figure 1 allude to a cross over period but this is not described in the text. 7. How will adherence to prescribed medication be measured? 8. Will lipid targets or choice of therapy (eg PCSK9 inhibitor) differ between patients with or without an FH-causing mutation? Will the treatment be part of the study protocol?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Liam Brunham
Institution and Country: University of British Columbia, Canada
Please state any competing interests or state 'None declared': None

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Overall this is a sensible, small trial that will add to the growing knowledge of genetic testing for FH. My comments are aimed at clarifying aspects of the study design and rationale.

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Author reply: Thank you for your comment. As you pointed out above, tendon xanthomas and family history are also key components to evaluate future risks in FH patients. In our previous study, we found that FH patients (both positive and negative genetic testing results) with clinical signs (xanthomas and/or family history) had higher risk for coronary artery disease compared to those without (Tada, et al. EHJ 2016). Third slide in supplementary data 1 shows the results and we certainly provide the information at each counseling. We corrected a sentence to clarify it in the text.

Changes to Text (Bold) (page 9, line 22-23): In addition, we will inform odds ratios of future cardiovascular risk, based on the presence or absence of **1)** a causal genetic variant **and 2) a clinical sign (xanthomas and/or family history of FH)** by using the original Japanese documents (Supplemental Data 1).

2. Lp(a) levels are not mentioned in the protocol. As these are an important determinant of risk in FH, they should be measured and incorporated into the counselling.

Author reply: Thank you for your suggestion. We will check Lp(a) levels to inform the results to the participants.

Changes to Text (Bold) (page 11, line 1): We will check patients' background profiles, height, body weight, blood pressure (BP), heart rate (HR), subjective/objective symptoms, complete blood counts, chemistry, lipid profile, **Lp(a)**, fasting glucose, hemoglobin A1C, lipid-lowering agents' regimen, PSQ-18, smoking status, and genetic testing.

3. Similar to comment #1, how will "genetically estimated future cardiovascular risk based on the result of genetic testing" be determined?

Author reply: We appreciate your advice. We will mainly provide odds ratios of FH-mutation carrier status (and/or presence of FH clinical signs) compared with noncarriers (and/or no clinical signs) for cardiovascular diseases(CVD) according to our previous studies. Although diagnosis of clinical FH itself is a strong risk factor for CVD compared to non-FH, we will let them know how CVD risks differ due to presence / absence of FH mutation by specific numbers and which risk categories they belong. We clarified what was genetically estimated future cardiovascular risk in the Methods section.

Changes to Text (Bold) (page 9, line 21): In addition, we will inform **odds ratios of** future cardiovascular risk, based on the presence or absence of 1) a causal genetic variant and 2) a clinical sign (xanthomas and/or family history of FH) by using the original Japanese documents (Supplemental Data 1).

4. The rationale for the study design could be better explained. If the goal is to investigate the "efficacy of genetic testing" for FH, why not randomize patients to genetic testing vs no genetic testing, rather than perform genetic testing in all patients and randomize to counselling or no counselling?

Author reply: Thank you for your important comment. As you pointed out, the goal of this study is to assess the "efficacy of informing future cardiovascular risk based on genetic testing". We corrected all the relevant sentences in the manuscript.

Changes to Text (Bold):
(Abstract: page 2, line 8)
Therefore, this study intends to evaluate the efficacy of **counseling future cardiovascular risk based on genetic testing** in addition to standard patients' education program in patients with FH.

(Strengths and limitations of this study 1: page 3, line 9)
・This would be the first randomised, waiting-list controlled study to assess whether **disclosing the risk for future cardiovascular diseases based on genetic testing results** correlates with reduced LDL cholesterol levels in patients with FH over standard FH education.

(Introduction: page5, line 6-7)
Thus, this study aims to evaluate whether **informing genetically estimated future cardiovascular risk based on** genetic testing besides usual patient education correlates with reduced LDL cholesterol levels in patients with FH.

(Discussion: page 13, line 1-4)

For this study to demonstrate the clinical efficacy of **informing cardiovascular risk based on genetic testing results**, it is imperative not only to confirm the FH diagnosis by genetic testing but also to provide thoughtfulevidence that genetic testing**-based cardiovascular risk disclosure** could enhance the prognosis of patients with FH.

(Discussion: page 14, line 21, 24)
This study would be the first randomized controlled trial investigating the clinical efficacy of the disclosure of future CHD risk **based on genetic testing results** in patients with FH.
We hypothesize that the intervention group has a lower plasma LDL cholesterol level than the control group at six months after randomization. Finally, this study will provide insights into the importance of genetic testing and its **disclosure of cardiovascular risk** in patients with FH.

5. Will patients in the control group have access to their genetic test result without counselling?

Author reply: No, they won't. The genetic testing results in the control group and their future cardiovascular risks will be informed via counseling after 6 months of randomisation as the intervention group received. However, if the participant do wants to open one's result without counselling, we would show it without counseling and will report the case as a protocol deviation.

6. Table 1 and Figure 1 allude to a cross over period but this is not described in the text.

Author reply: Thank you for pointing it out. We corrected the spelling inconsistency at text body, Table 1 and Figure 1 (observational period > main period, crossover period > observational period) and added sentences in the Methods section.

Changes to Text (Bold) (page 10, line 5-8):
Regarding the control group, we will only disseminate standard FH patient education using the Japanese booklet for FH patient education according to the FH management guideline (Supplemental Data 2);[1] this education will be provided by a clinical cardiology physician specialist. After the education, we will set time to answer the queries of patients adequately. **After evaluating primary endpoint (24th week from randomisation), the control group will receive their genetic testing results and future cardiovascular risks via counseling as the intervention group receives.**
Both groups will be followed-up until 48th weeks from randomisation. Furthermore, it will be possible to receive additional counseling and/or outpatient visit when patients want or are afraid of genetic testing regardless of the intervention or control groups during and after the trial.

7. How will adherence to prescribed medication be measured?

Author reply: We will check the regimen of prescribed medication and will ask participants adherence to them by self-report manner in every outpatient-clinic visit. We added the sentence in the Methods section.

Changes to Text (Bold) (page 11, line 7): We expect to arrange regular follow-up visits at eight weeks (±14 days), 16 weeks (±14 days), 24 weeks (±14 days), 32 weeks (±14 days), 40 weeks (±14 days), and 48 weeks (±14 days) to evaluate patients' body weight, BP/HR, symptoms, blood test results, prescription, **adherance to prescribed medication by self-report**, PSQ-18, and smoking status. Furthermore, we will evaluate the primary outcome at 24 weeks and recorded adverse events and concomitant medication throughout the trial.

8. Will lipid targets or choice of therapy (eg PCSK9 inhibitor) differ between patients with or without an FH-causing mutation? Will the treatment be part of the study protocol?

Author reply: Thank you for your comment. This clinical question is also what we would like to disclose through this study. Based on the current guideline, target LDL-C level for clinically diagnosed FH patients is the same regardless of FH-causing mutation carrier status. However, participants who receive their future cardiovascular risk may change their mediation (e.g. more PCSK9 inhibitor for relatively high-risk participants, or less lipid-lowering medications for relatively low-risk participants). Treatment regimen itself is not a part of this study protocol, but we surely check their medication, change of medication. If there is a huge difference between intervention and control groups regarding treatment regimen, we will use the medication as an adjustment / stratification factor as an exploratory analysis.

VERSION 2 – REVIEW

REVIEWER	Liam Brunham UBC Canada
REVIEW RETURNED	27-Oct-2018

GENERAL COMMENTS	The study protocol is improved and the authors have adequately responded to the issues raised during the prior review. The authors are to be commended for this important trial.