PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	The Hokuriku-plus Familial Hypercholesterolemia Registry Study: Rationale and study design
AUTHORS	Tada, Hayato; Okada, Hirofumi; Yoshida, Shohei; Shimojima, Masaya; Nomura, Akihiro; Tsuda, Toyonobu; Mori, Mika; Takashima, Shin-ichiro; Kato, Takeshi; Usui, Soichiro; Sakata, Kenji; Hayashi, Kenshi; Fujino, Noboru; Inazu, Akihiro; Takahara, Shizuko; Imai, Yasuhito; Matsubara, Takao; Nohara, Atsushi; Miwa, Kenji; Namura, Masanobu; Terai, Hidenobu; Yoshida, Taiji; Araki, Tsutomu; Minamoto, Masahiro; Aburao, Toru; Ito, Yuji; Nakanishi, Chiaki; Kawasaki, Suguru; Todo, Yasuhiro; Koizumi, Junji; Kita, Yoshihito; Matsumoto, Hiroshi; Shintaku, Hiroaki; Hodatsu, Akihiko; Ino, Hidekazu; Higashikata, Toshinori; Takata, Mutsuko; Misawa, Katsushi; Yamaguchi, Masato; Noji, Yoshihiro; Osato, Kazuo; Mabuchi, Tomohito; Ichise, Taro; Kaku, Bunji; Katsuda, Shoji; Fujimoto, Manabu; Uchiyama, Katsuharu; Fujioka, Kensuke; Nakahashi, Takuya; Nozue, Tsuyoshi; Michishita, Ichiro; Usuda, Kazuo; Otowa, Kanichi; Okeie, Kazuyasu; Hirota, Satoshi; Aburadani, Isao; Kurokawa, Keisuke; Takatori, Osamu; Hondo, Shunichiro; Oda, Hiroyuki; Takata, Shigeo; Murai, Hisayoshi; Kinoshita, Masaki; Nagai, Hideo; Sekiguchi, Yoshiteru; Sakagami, Satoru; Omi, Wataru; Fujita, Chikara; Katsuki, Tatsuo; Ootsuji, Hiroshi; Igarashi, Atsushi; Nakano, Manabu; Okura, Seiichiro; Maeno, Koji; Mitamura, Yasuhito; Sugimoto, Naoki; Yamamoto, Masakazu; Akao, Hironobu; Kajinami, Kouji; Takamura, Masayuki; Kawashiri, Masa-aki

VERSION 1 – REVIEW

REVIEWER	Liam Brunham University of British Columbia, Canada
REVIEW RETURNED	11-Apr-2020

GENERAL COMMENTS	This article describes the design of new registry of patients with Familial Hypercholesterolemia in the Hokuriku district of Japan. Overall this was a clearly written description of the registry. National registries of FH are a recognized priority for this under-diagnosed and under-treated condition and I applaud the authors for this effort. Below are a few items the authors may wish to consider to further improve this work: 1) The statement "The diagnostic rate of this disease among European nations are quite high" is not quite accurate. The diagnostic rate is high only in specific European countries that have implemented national screening programs, such as The

	Netherlands, Norway, and more recently Slovenia. In many other European countries the diagnostic rate is very low. 2) Are there other FH registries in Japan, and can the authors link their initiative with these to develop a registry that is more representative of the entire country? 3) The absence of patient involvement in the design of the registry is a missed opportunity. It would be valuable to obtain input from patient representatives of FH patient groups to inform the design of the registry based on what they see as the most important outcomes. 4) The genetic screening strategy is a strength, but could be improved. It is not clear if this is being done as part of research or clinical care (in a clinical laboratory). There is no strategy mentioned regarding return of results to participants. In addition, the approach to identify missense variants ("missense variants that 5 in silico damaging scores (SIFT, PolyPhen-2 HDIV, PolyPhen-2 HVAR, MutationTaster2, LRT), all predicted as pathogenic") may work for LDLR variants, but is not appropriate for identifying FH-causing variants in either APOB or PCSK9. 5) Page 7, line 11. This is confusing. Achilles thickness if mentioned twice, and x-ray is referred to but this is apparently physical exam collection? 6) The definition of prior ASCVD is "coronary atherosclerosis (≥ 75% stenotic lesion)". Does this imply that patients with stable angina with <75% stenosis would not be classified as ASCVD? 7) The authors may wish to consider some form of routine screening for subclinical atherosclerosis in patients without prior ASCVD history, eg CAC.

REVIEWER	Dario Rahelic Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Merkur University Hospital University of Zagreb School of Medicine Croatia
REVIEW RETURNED	21-Apr-2020

GENERAL COMMENTS	Familial hypercholesterolemia (FH) is an autosomal dominant inherited genetic disease, that has an extremely elevated cardiovascular risk associated with significantly elevated LDL cholesterol. The prevalence of FH is underestimated worldwide. Although there are several European registries on FH it would be of importance to have an FH registry for Japanese population. The study protocol was clearly written. However, some minor revisions should be considered. I would consider to follow changes in body weight and waist circumference. Furthermore, dietary habits (Food diary and FFQ), intake of dietary supplements and physical activity of participants should be monitored during the study. If not, it should be listed in Study limitations. It would be necessary to perform language editing.

REVIEWER	Carlos A Aguilar Salinas Instituto Nacional de Ciencias Medicas y Nutricion Mexico City Mexico
REVIEW RETURNED	29-May-2020

GENERAL COMMENTS	The main limitations of the study are: Critical information is missing in the paper: -Definition of outcomes -Quality control of the lab assessments -Approach to protect privacy -Other ongoing FH registries that includes Japanese patients -Interaction between the registry information and the physician on charge of the patient care -Primary source of the information -Statistical analyses plan including multivariate models to control confounding variable Authors should disclose if the cascade screening results will be included and the affected relatives considered in the registry -The follow up program is poorly described

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

Answers to Reviewer 1
Thank you very much for your favorable and constructive comments. We revised our manuscript according to your suggestions point by point.

Please leave your comments for the authors below
This article describes the design of new registry of patients with Familial Hypercholesterolemia in the Hokuriku district of Japan. Overall this was a clearly written description of the registry.

National registries of FH are a recognized priority for this under-diagnosed and under-treated condition and I applaud the authors for this effort.

Below are a few items the authors may wish to consider to further improve this work:
1) The statement "The diagnostic rate of this disease among European nations are quite high" is not quite accurate. The diagnostic rate is high only in specific European countries that have implemented national screening programs, such as The Netherlands, Norway, and more recently Slovenia. In many other European countries the diagnostic rate is very low.

Our response: Thank you for your pointing out this matter. We revised the descriptions according to this comment.

2) Are there other FH registries in Japan, and can the authors link their initiative with these to develop a registry that is more representative of the entire country?

Our response: In fact, there is another FH registries in Japan named PROLIPID-FH registry (They do not have an official website. Also they do not publish protocol paper). We can link our initiative with that registry in the future, which should be more representative of the entire country. We added descriptions regarding this point in Discussion section.

3) The absence of patient involvement in the design of the registry is a missed opportunity. It would be valuable to obtain input from patient representatives of FH patient groups to inform the design of the registry based on what they see as the most important outcomes.

Our response: Thank you again for your important comment. We agree that patients involvement in the design of the registry may provide useful information to us; however, it is quite difficult to contact to patients for research purpose in Japan. We added descriptions regarding this point as a limitation of this study.

4) The genetic screening strategy is a strength, but could be improved. It is not clear if this is being done as part of research or clinical care (in a clinical laboratory). There is no strategy mentioned regarding return of results to participants. In addition, the approach to identify missense variants ("missense variants that 5 in silico damaging scores (SIFT, PolyPhen-2 HDIV, PolyPhen-2 HVAR, MutationTaster2, LRT), all predicted as pathogenic") may work for LDLR variants, but is not appropriate for identifying FH-causing variants in either APOB or PCSK9.

Our response: Thank you so much for your great comment. In this study, genetic analyses will be performed as a part of research; however, results will be returned to the patients with genetic counseling upon patients' request. We added descriptions regarding this point in the texts. In addition, we fully agree with the reviewer that the current descriptions about determination of pathogenic mutations are inadequate. In fact, it is true that the framework sometimes does not work well, especially regarding the missense variants in *PCSK9* and *APOB* genes. We actually considered only several established mutations in *PCSK9* or *APOB* gene as pathogenic mutations of FH (registered in ClinVar). We revised the texts accordingly.

5) Page 7, line 11. This is confusing. Achilles thickness if mentioned twice, and x-ray is referred to but this is apparently physical exam collection?

Our response: This is a mistake. We corrected the text. We defined Achilles tendon thickness as ≥9.0 mm by X-ray according to Japanese guideline (J Atheroscler Thromb. 2018 Jun 1;25(6):539-553).

6) The definition of prior ASCVD is "coronary atherosclerosis (≥ 75% stenotic lesion)". Does this imply that patients with stable angina with <75% stenosis would not be classified as ASCVD?

Our response: Yes, the patents with stable angina with <75% stenosis are NOT classified as ASCVD in this study. It is true that some studies consider >50% stenosis as threshold of ASCVD; however, we would like to use rather strict definition in this study.

7) The authors may wish to consider some form of routine screening for subclinical atherosclerosis in patients without prior ASCVD history, eg CAC.

Our response: Thank you so much again for your important comment. It is true that "routine" screening for subclinical atherosclerosis is not mandatory in this study; however, such screening using carotid ultrasound and/or computed tomography for FH in a regular basis (every 2 to 3 years) is recommended in our clinical practice, thus, we believe that we can detect the development of subclinical as well as clinical atherosclerosis in FH in this study. We added the descriptions on this issue in the texts.

Reviewer: 2
Reviewer Name: Dario Rahelic
Institution and Country: Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Merkur University Hospital
University of Zagreb School of Medicine
Croatia

Please state any competing interests or state 'None declared': None declared

Answers to Reviewer 2
Thank you very much for your favorable comments. We revised our manuscript according to your suggestions point by point.

Please leave your comments for the authors below
Familial hypercholesterolemia (FH) is an autosomal dominant inherited genetic disease, that has an extremely elevated cardiovascular risk associated with significantly elevated LDL cholesterol. The prevalence of FH is underestimated worldwide. Although there are several European registries on FH it would be of importance to have an FH registry for Japanese population.

The study protocol was clearly written. However, some minor revisions should be considered.

I would consider to follow changes in body weight and waist circumference. Furthermore, dietary habits (Food diary and FFQ), intake of dietary supplements and physical activity of participants should be monitored during the study. If not, it should be listed in Study limitations.

Our response: Thank you for your great comment. We can follow changes in body weight and waist circumference. However, it is quite unfortunate that we cannot collect information about dietary habits and physical activity in this study. We added descriptions regarding this issue as a limitation.

It would be necessary to perform language editing.

Our response: We have made our revision language-edited through commercial service upon this suggestion.

Reviewer: 3
Reviewer Name: Carlos A Aguilar Salinas
Institution and Country: Instituto Nacional de Ciencias Medicas y Nutricion
Mexico City
Mexico
Please state any competing interests or state 'None declared': None declared

Answers to Reviewer 3
Thank you very much for your constructive comments. We revised our manuscript according to your suggestions point by point.

Please leave your comments for the authors below
The main limitations of the study are:
Critical information is missing in the paper:
-Definition of outcomes

Our response: We added descriptions regarding this point.

-Quality control of the lab assessments

Our response: In this study, quality control of the lab measurements are responsible for each institution. We added description regarding this issue.

-Approach to protect privacy

Our response: Thank you for your pointing out this important issue. To protect privacy, all the information will be anonymized in each institution before data entry. We added descriptions regarding this issue.

-Other ongoing FH registries that includes Japanese patients

Our response: There is another FH registries in Japan named PROLIPID-FH registry (They do not have an official website. Also they do not publish protocol paper). We can link our initiative with that registry in the future, which should be more representative of the entire country. We added descriptions regarding this point in Discussion section.

-Interaction between the registry information and the physician on charge of the patient care

Our response: Thank you for your great comment. Actually, there is no interaction between the registry information and physicians until the initial report is officially published. One of the rationale for this is because we wanted to observe the current status/situations of the treatments and prognoses of FH in Japan in an unbiassed manner. We will be giving useful feedbacks from this registry not only to physicians on charge of the patient care, but also to all over the world through scientific papers once the determined study period over. However, we added descriptions regarding this issue in limitation section.

-Primary source of the information

Our response: The primary source of the information will be those obtained through electronic health records in each institute.

-Statistical analyses plan including multivariate models to control confounding variable

Our response: We added confounding variables to consider.

Authors should disclose if the cascade screening results will be included and the affected relatives considered in the registry

Our response: Thank you again for your important comment. In this study, there is no exclusion criteria regarding the condition of the referral to our hospitals. In addition, cascade screening to find FH patients are recommended by the Japanese guideline (J Atheroscler Thromb. 2018 Jun 1;25(6):539-553). Accordingly, we anticipate many patients with FH identified through cascade screening will be included in this study. Such inclusion of different generations, especially young generations through cascade screening will help us to estimate what age is the starting point of development of atherosclerosis among patients with FH. We added texts describing this point in Discussion section.

-The follow up program is poorly described

Our response: We added descriptions regarding this issue in the texts.