

BMJ Open

Exploration into lipid management and persistent risk in patients hospitalised for acute coronary syndrome in Japan (EXPLORE-J): Rationale and design for a prospective observational study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2016-014427
Article Type:	Protocol
Date Submitted by the Author:	05-Oct-2016
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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	acute coronary syndrome, familial hypercholesterolaemia, Japan, lipid management, proprotein convertase subtilisin kexin 9

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3 **Exploration into lipid management and persistent risk in patients hospitalised for acute**
4 **coronary syndrome in Japan (EXPLORE-J): Rationale and design for a prospective**
5 **observational study**
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5 **Key words:** acute coronary syndrome, familial hypercholesterolaemia, Japan, lipid management,
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Abstract

Introduction The present study is the largest registry study ever conducted in Japan exploring the prevalence of familial hypercholesterolaemia (FH) among acute coronary syndrome (ACS) patients. Our study aims to (1) evaluate the status of lipid management and the subsequent risk of major cardiovascular events following hospitalisation of Japanese ACS patients in real-world clinical practice; (2) determine the proportion of Japanese ACS patients who achieve the lipid management goal and have a reduction of event risks with strict lipid management (low-density lipoprotein-cholesterol <1.81 mmol/L); (3) determine the prevalence of FH; and (4) investigate the clinical significance of proprotein convertase subtilisin kexin 9 (PCSK9) level.

Methods and analysis We will conduct a multicentre, prospective, observational study of approximately 2,000 Japanese ACS patients with/without FH hospitalised between April 2015 and August 2016. The primary endpoint is the incidence of major adverse cardiovascular events (MACEs) after initial hospitalisation. The secondary endpoints are (1) MACE developed from Visit 1 to Visit 2 (Day 30); (2) MACE developed from Visit 2 (Day 30) to Visit 5 (Day 730); (3) treatment rate by lipid-lowering therapies (any statin or intensive, PCSK9 inhibitor, fibrates, and ezetimibe); (4) incidence of events by the addition of the following outcomes to the primary endpoint: coronary revascularisation due to myocardial ischaemia, revascularisation other than coronary artery, inpatient treatment for occurrence or exacerbation of heart failure, transient ischaemic attack, acute arterial occlusion, central retinal artery occlusion, and other adverse events prolonging or requiring hospitalisation; and (5) proportion of subjects achieving target lipid levels.

Ethics and dissemination The study protocol was submitted to the ethical review committee of each participating centre for approval. Participation in the study is voluntary and anonymous.

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3 The study findings will be disseminated in international peer-reviewed journals and presented at
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5 relevant conferences.
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9 **Clinical trial registration:** UMIN000018946
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Strengths and limitations of this study

- This study will provide new insights into the relationship between acute coronary syndrome (ACS) and recurrent events and the relationship between recurrent events and serum low-density lipoprotein-cholesterol levels in Japanese ACS patients.
- This study will be the first to evaluate the familial hypercholesterolaemia (FH) to ACS ratio and will provide important information regarding PCSK9 concentrations, including (1) the dynamic change of PCSK9 concentrations during post-ACS medical management, (2) the relationship between PCSK9 concentrations and cardiovascular events, (3) the difference of PCSK9 concentrations between FH and non-FH, and (4) the relationship among PCSK9 concentrations, lipid parameters and lipid lowering therapies for both statin-naïve and statin-exposed patients.
- This study will be a prospective, large-scale, observational study of approximately 2,000 Japanese ACS patients from 59 participating centres.
- This study is the largest FH registry study in Japan targeting the high-risk population.
- This study will be limited by the inherent limitations of the observational study design (e.g., susceptibility to biases and confounders, and the inability to establish causality) and the small sample size; the generalisability of our findings will be limited to the Japanese population.

INTRODUCTION

The incidence of atherosclerotic disease, including heart disease, in Japan is increasing and is associated with a change towards a Western lifestyle and increase in dyslipidaemia.[1] There is a positive correlation between low-density lipoprotein-cholesterol (LDL-C) levels and the incidence of coronary artery disease.[2, 3] Therapy to lower LDL-C helps prevent cardiovascular events. Large statin trials have demonstrated the benefits of achieving an LDL-C level of 1.42–2.02 mmol/L, which has served as a basis for more aggressive European and US guidelines [4]. In particular, aggressive therapy to achieve LDL-C <1.81 mmol/L, the target value for lipid management (in patients for whom aggressive therapy is intended) in European and US guidelines,[5, 6] or the use of high-intensity statins according to American College of Cardiology/American Heart Association guidelines have helped to reduce cardiovascular events and the progression of atherosclerosis.[5-10]

However, the Japanese guidelines for the prevention of atherosclerosis published in 2012 set higher target values for LDL-C management at <3.11 mmol/L in primary prevention for high-risk patients and <2.59 mmol/L for secondary prevention because the evidence for benefit was suggested to be insufficient.[11] Previous Japanese studies (ESTABLISH and the follow-up Extended-ESTABLISH study) reported that aggressive LDL-C lowering reduced the incidence of death, the recurrence of acute coronary syndrome (ACS), and cerebral infarction compared with a control group.[12, 13] The MEGA study showed the benefit of lipid-lowering therapy in Japanese patients, but to values higher than 2.59 mmol/L.[14] While a number of imaging studies with intravascular ultrasound and other modalities have shown the benefits of aggressive LDL-C-lowering therapy in Japanese patients in terms of reduced plaque volume or coronary plaque regression,[15-19] there is no large-scale clinical study that shows the need for aggressive

lipid-lowering therapy in Japan, especially to LDL-C target values lower than 2.59 mmol/L. It is generally accepted that insufficient evidence is available to determine the benefit of such therapy. Therefore, investigations into this issue, and evaluation of a possible relationship between LDL-C and recurrent coronary events and coronary artery disease progression in Japan are needed.

Familial hypercholesterolaemia (FH) is an inherited, autosomal dominant disease resulting from abnormalities in the genes coding for the LDL receptor and related molecules. It is characterised by three major signs: (i) hyper-LDL-cholesterolaemia; (ii) early onset coronary artery diseases; and (iii) tendon/skin xanthoma. Untreated FH carries an extremely high risk of developing coronary artery diseases, particularly in men aged between 30 and 50 years and women aged between 40 and 70 years.[20, 21] Risk data from the Simon Broome study in 1991 showed that the risk of cardiovascular death was 99 times higher in patients with FH at ages 20–39 years.[22] Although heterozygous FH is present in an estimated 300,000 patients in Japan,[23] the diagnosis rate is only <1% of the estimated number of patients with FH in Japan.[21] According to an investigation by the Ministry of Health, Labour and Welfare in Japan,[20] the prevalence of FH is 4%–19% of patients presenting with ACS. FH may be under-diagnosed because of a masking affect by statin therapy, or the transient reduction of LDL-C associated with acute myocardial infarction (Figure 1). A prospective cohort observational study of 4,534 patients with ACS in Switzerland reported an FH prevalence of 1.6%–5.5%, and a higher adjusted risk of coronary death or myocardial infarction among patients with FH than without (hazard ratio, 2.46–3.53 after 1 year).[24] To date there have been no reports regarding the ACS recurrence rate in patients with FH in Japan. Therefore, it is important to clarify the prevalence of FH in patients with ACS, the recurrence rate of coronary artery disease in patients

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3 with FH, and risk factors for recurrence to provide the optimal treatment for Japanese patients
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5 with FH.
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9 Proprotein convertase subtilisin kexin 9 (PCSK9) is a protein that binds to LDL receptors to
10 induce their degradation in intracellular lysosomes and inhibit their recycling.[25] Interestingly,
11 patients with loss-of-function mutations in the *PCSK9* gene have low LDL-C levels and rarely
12 develop cardiovascular diseases compared with normal individuals. PCSK9 inhibitors are an
13 effective lipid-lowering therapy. PCSK9 and LDL-C levels show a positive correlation in
14 patients not treated with a lipid-lowering therapy, but this relationship disappears when lipid-
15 lowering therapies, particularly oral statins, are administered.[26,27] Statins may simultaneously
16 increase the concentration of PCSK9 and the expression of LDL receptors, while lowering LDL-
17 C.[26] However, despite our current knowledge of PCSK9, its clinical significance in ACS
18 patients remains unclear. The ODYSSEY Outcomes trial [28] will be the largest outcomes trial
19 examining the effect of PCSK9 inhibition on reducing cardiovascular morbidity and mortality in
20 ACS patients; however, until these results become available, the EXPLORE-J trial will reveal
21 important insights into this issue.
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40 Considering the above-mentioned gaps in the literature, the primary objective of this study is
41 to evaluate the status of lipid management and risk of major cardiovascular events in Japanese
42 ACS patients in real-world clinical practice. Secondary study objectives are to identify the
43 proportion of ACS patients in Japan who (i) achieve the target value of lipid management (LDL-
44 C \leq 2.59 mmol/L); (ii) have a reduction of event risks with strict lipid management (LDL-C
45 < 1.81 mmol/L); and (iii) who have FH and are hospitalised for treatment of ACS. The risk of
46 recurrent ACS in patients with FH compared with patients without FH will be determined using
47 the diagnostic criteria for FH as shown in **Table 1**. Other objectives are to investigate the clinical
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significance of PCSK9 concentrations by observing the time-course profile of PCSK9 concentrations in patients presenting with ACS and analysing the relationship between the concentration of PCSK9 and FH, lipid-lowering therapy, and lipid management levels.

Table 1^a Diagnostic criteria for heterozygous FH in adults (aged 15 years and older)

Hyper LDL cholesterolaemia (LDL-C \geq 180 mg/dL before treatment)
Tendon xanthoma (tendon xanthoma in the back of hand, elbow, knee, etc. or Achilles tendon thickening) or tuberous xanthoma
Family history (blood relatives within the second degree of kinship) of FH or early onset coronary artery disease

Notes

- *Diagnosis is established excluding secondary hyperlipidaemia.*
- *FH is diagnosed when two or more items are met. Diagnosis by genetic examination is advised if FH is suspected.*
- *Tuberous xanthoma does not include xanthelasma of the eyelid.*
- *Achilles tendon thickening is diagnosed by a thickness of \geq 9 mm on radiogram.*
- *FH is strongly suspected if LDL-C is \geq 250 mg/dL.*
- *If the patient is already on drug therapy, refer to the lipid level that triggered the treatment.*
- *Early onset coronary artery diseases are defined as those in which onset occurs at <55 years of age in men and <65 years of age in women.*

^aReproduced with modifications Harada-Shiba et al.[20] with permission from the *Journal of Atherosclerosis and Thrombosis*.

FH, familial hypercholesterolaemia; LDL, low-density lipoprotein.

METHODS AND ANALYSIS

Study design

This will be a multicentre, prospective, observational study of Japanese patients presenting with ACS. Sixty-five sites are planned to participate in this study (see **Appendix**). These sites were chosen based on their ability to provide the most advanced percutaneous coronary intervention in Japan for patients presenting with ACS. Consecutive patients requiring hospitalisation for ACS were registered in 59 sites, for a total of 2,016 patients, between April 2015 and August 2016.

After the patients provided written informed consent within 7 days after hospitalisation for ACS, the investigator at each study centre successively registered subjects who met the inclusion criteria. Successive registration of patients limits the selection bias by the investigator. The schedule for initial and follow-up examinations and the data to be collected are shown in **Figure 2** and **Table 2**.

Table 2 Observations and endpoints

Category	Method and materials	Observation and examination items
Demographic characteristics (subjects' background)	Interview	Age, sex, and smoking and drinking status
ACS	Medical examination and interview	Onset date of ACS, date of hospitalisation, disease type, description of treatment
History of present illness/previous	Interview	Particular previous history of cardiovascular diseases and cardiovascular risk-related diseases and history of

Category	Method and materials	Observation and examination items
history/therapies		their treatments (immediately before hospitalisation and at each visit).
Physical findings	Medical examination	Body height, body weight, and presence or absence of xanthoma
Reference LDL-C value	Interview	Value before treatment such as that obtained at a health examination
Family history	Interview	Coronary artery diseases, ischaemic cerebral infarction, and hypercholesterolaemia in relatives to the second degree of kinship.
Primary endpoints	Interview	Investigation of outcome (alive or dead), date of death, cause of death, and presence or absence of non-fatal ACS and non-fatal cerebrovascular diseases requiring in-hospital treatments
Secondary endpoints	Interview	Presence or absence of event
Haematological and biochemical examinations	Serum	<p>The following parameters will be measured from blood obtained in the sitting position when the symptoms are stable:</p> <p>Total cholesterol, HDL cholesterol, LDL cholesterol (automatic calculation), triglycerides, apoA1, apoB, Lp(a), creatinine, blood glucose, HbA_{1c}, hsCRP, haemoglobin, and haematocrit</p>

Category	Method and materials	Observation and examination items
PCSK9	Serum	Collective measurement at the central laboratory
FH gene examination	Whole blood	Collective measurement at the central laboratory
Radiography of the Achilles tendon	Radiography	Whenever possible, radiography of the Achilles tendon will be performed during index hospitalisation for registration, but radiography obtained by Visit 3 will also be acceptable

ACS, acute coronary syndrome; LDL-C, low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin kexin 9; apo, apolipoprotein; HDL, high-density lipoprotein; HbA_{1c} glycosylated haemoglobin; hsCRP, high sensitivity C-reactive protein; Lp(a), lipoprotein A; FH, familial hypercholesterolaemia.

Data are collected at Visit 1 (within 14 days after hospitalisation due to ACS), and Visits 2 to 5 during the 2-year observational period on Days 30 (± 7 days), 180 (± 30 days), 365 (± 30 days), and 730 (± 30 days). Data are collected using an electronic case report form. The information collected at each visit is shown in **Table 2**. During the 2-year observation period (Visits 2–5), if a subject is transferred to another hospital during this time, the institution will be asked to provide the following information for the follow-up: attendance/non-attendance; date of observation; primary endpoints (investigation of outcome [alive/dead]); secondary endpoints (presence or absence); physical examination; fasting haematological and biochemical examinations, including PCSK9 concentrations (Visits 2–4); and medications.

The samples collected in this study will be sent to a central laboratory (BML General Laboratory BML, INC., Saitama, Japan) under freezing conditions (-20°C) until completion of

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3 the study so that re-examination can be performed. All samples will be discarded after
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5 completion of the study.
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10 11 **Study subjects**

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14 The study subjects are Japanese patients presenting with ACS, hospitalised between April 2015
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16 and August 2016. In total, 2,016 subjects were registered.
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20 The key inclusion criteria are as follows: age ≥ 20 years; hospitalisation for any ACS
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22 including ST-segment elevation myocardial infarction (STEMI), non ST-segment elevation
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24 myocardial infarction (NSTEMI), and unstable angina; and ability to obtain written informed
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26 consent. STEMI is defined as the presence of chest symptoms such as pain or breathlessness
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28 suspected to be caused by myocardial ischaemia, persisting for ≥ 20 min; ST elevation of ≥ 1 mm
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30 on ≥ 2 contiguous leads or new left bundle branch block; and elevated troponin T ≥ 0.1 $\mu\text{g/L}$ or
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32 creatine phosphokinase-MB two times above the upper limit of normal. Acute NSTEMI is
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34 defined as the presence of chest symptoms such as pain or breathlessness suspected to be caused
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36 by myocardial ischaemia, persisting for ≥ 20 min ≤ 24 h before admission; not having ST-segment
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38 elevation ≥ 1 mm or new left bundle branch block; and the presence of elevated troponin T ≥ 1.0
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40 $\mu\text{g/L}$ or creatine phosphokinase-MB two times above the upper limit of normal. Unstable angina
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42 is defined as the presence of resting or nocturnal chest pain that may be persistent (≥ 20 min) and
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44 at least one of the following: ST depression ≥ 0.5 mm or T wave inversion ≥ 3 mm; troponin T
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46 ≥ 0.014 $\mu\text{g/L}$ or < 1.0 $\mu\text{g/L}$; confirmation of significant stenosis by diagnostic imaging; new
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48 decrease in wall motion detected by echocardiography; or reversible myocardial perfusion defect
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50 detected by myocardial perfusion imaging.
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3 The key exclusion criteria are as follows: patients with chest pain and coronary artery
4 diseases presenting with concomitant serious diseases, patients with in-stent thrombosis, patients
5 enrolled in other interventional studies that could affect lipid profile, and those judged as
6 inappropriate by the investigators or subinvestigators.
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12 13 14 15 16 **Primary endpoint**

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18 The primary endpoint is the incidence of major adverse cardiovascular events (MACE), defined
19 as death associated with myocardial infarction or other cardiovascular death, major non-fatal
20 coronary event (myocardial infarction or hospitalisation for unstable angina), or ischaemic
21 stroke. Incidences are monitored independently by the investigator at each local site. A time
22 window will be allowed for observations at Visit 2 (± 7 days) and Visits 3–5 (± 30 days).
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31 Deaths associated with myocardial infarction or other cardiovascular deaths are defined as
32 death secondary to acute myocardial infarction or any death with a clear relationship to
33 underlying coronary heart disease, sudden death, heart failure, complication of coronary
34 revascularisation procedure where the cause of death is clearly related to the procedure,
35 unobserved or unexpected death, or other death that cannot be definitively attributed to a non-
36 cardiovascular cause. Non-fatal myocardial infarction is defined in accordance with the
37 ACC/AHA/ESC Universal Definition of Myocardial Infarction.[29]
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48 Ischaemic stroke is characterised by an acute episode of focal cerebral, spinal, or retinal
49 dysfunction caused by infarction, defined by at least one of the following: pathological, imaging,
50 or other objective evidence of acute, focal cerebral, spinal, or retinal ischaemic injury in a
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3 defined vascular distribution; symptoms of acute cerebral, spinal, or retinal ischaemic injury
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5 persisting for ≥ 24 hours or until death, with other aetiologies excluded.
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10 11 **Secondary endpoints**

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14 The secondary endpoints are MACE developed from Visit 1 to Visit 2 (Day 30); MACE
15 developed from Visit 2 (Day 30) to Visit 5 (Day 730); treatment rate by the following lipid-
16 lowering therapies: any statin, intensive statin (e.g., atorvastatin ≥ 20 mg, rosuvastatin ≥ 10 mg,
17 and pitavastatin ≥ 4 mg), PCSK9 inhibitor, and other lipid-lowering therapies such as fibrates and
18 ezetimibe; incidence of events by the addition of the following outcomes to the primary
19 endpoints: coronary revascularisation due to myocardial ischaemia, revascularisation other than
20 in the heart, inpatient treatment for occurrence or exacerbation of heart failure, transient
21 ischaemic attack, acute arterial occlusion, central retinal artery occlusion, and other adverse
22 events prolonging or requiring hospitalisation; and proportion of subjects achieving target lipid
23 levels per study visit. The treatment rate will be determined based on written prescriptions. Other
24 endpoints include the prevalence rates of FH in patients with ACS, comparison of PCSK9
25 concentration between patients with or without FH, and comparison between clinical diagnosis
26 of FH based on the guidelines vs. genetic analysis.
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49 **Discontinuation from study**

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51 The criteria used to allow discontinuation from the study include the withdrawal of consent by
52 the subject or his/her legal representative, patient ineligibility (violation of the study contract),
53 death of a subject, or removal by the judgement of the investigator or subinvestigators.
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Safety protocol

Because this is an observational study (i.e., non-interventional) there will be no adverse events caused by a study drug. Patients will receive drugs as normally prescribed in daily medical practice.

Serious adverse drug reactions caused by a drug will be the subject for application of relief under the Relief System for Sufferers from Adverse Drug Reactions similar to that in daily medical practice. Treatments for other adverse drug reactions will be covered by the national insurance scheme.

Statistical analyses

The sample size was calculated to assess the persistent cardiovascular risk (defined as MACE) from the index event to 2 years. Based on the PACIFIC registry, in which the incidence of MACE was 6.4% at 2 years, a sample size of 2,000 has a precision of $\pm 1\%$ in the incidence of MACE with a 95% confidence interval (CI) of 0.053–0.074.[30] With this sample size, the subgroup analysis (the comparison of MACE in patients with LDL-C <1.81 mmol/L and ≥ 1.81 mmol/L) will be performed. Because 409 of 1827 (22.3%) Japanese ACS patients in the PACIFIC study and 305 of 1145 (26.6%) Japanese ACS patients in an ongoing database study reached an LDL-C level <1.81 mmol/L, we expect to have 446–532 patients with an LDL-C level <1.81 mmol/L in this registry. All patients meeting the inclusion criteria will be analysed. The demographic data will be presented as the mean, median, standard deviation, and range for continuous data, and number and proportion of subjects in each category for categorical data.

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3 The analysis of the primary endpoint will be as follows: for the incidence of MACE at 2
4 years, Kaplan–Meier analysis will be used to estimate the event observed first after registration
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6 in the whole population. The Greenwood formula will determine the 95% CI.
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11 Subgroup analysis will investigate the association of factors with the incidence of MACE,
12 LDL-C <1.81 mmol/L vs \geq 1.81 mmol/L at registration, and the presence or absence of FH. The
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14 Cox proportional hazard model and subgroup analysis will compare demographic factors (age,
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16 sex, smoking history, body mass index, and underlying diseases) using a two-sided significance
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18 level of 5% (not considering multiplicity, as the study is exploratory). Subgroup analysis will be
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20 performed for LDL-C (<1.81 mmol/L or \geq 1.81 mmol/L) at the time when the event has occurred.
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26 Analysis of the secondary endpoints (MACE developed by Visit 2 [Day 30]; MACE
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28 developed between Visit 2 [Day 30] and Visit 5 [Day 730]; and treatment rate by lipid-lowering
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30 therapy) will be assessed by determining the ratio of subjects on each lipid-lowering therapy
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32 administered during observation and the 95% CI using the full analysis set. The incidences of
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34 events at Visit 2 and between Visits 2–5 or from the time of registration to the first event, and the
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36 associations of factors with each event will be determined by the same procedures as for the
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38 primary endpoints. For the other endpoints, the prevalence rate of FH in patients with ACS will
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40 be assessed and its 95% CI from the diagnosis of FH will be determined. Logistic regression
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42 analysis and subgroup analysis of the background factors that are associated with the prevalence
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44 of FH for exploratory investigation will be determined. The summary statistics for the
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46 concentration of PCSK9 at each measurement point will be calculated and a trend diagram
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48 (individual and mean/standard deviation) will be developed. In addition, the presence or absence
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50 of the influence of FH by subgroup analysis will be determined.
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In the survival analysis, missing values will be censored while continuous data and discrete data will be analysed on the basis of measured values without performing any special processing. Furthermore, we will also perform a sensitivity analysis where continuous data are handled using the last observation carried forward imputation, and discrete data are included only in the denominator but not in the numerator. No special processing will be performed for outliers. For the statistical analysis, SAS version 9.4 software (SAS Institute Inc., NC, USA) will be used.

Quality assurance measures

The study investigators will ensure compliance to the study protocol. Any change in factors that affect the safety of subjects or the scientific quality of this study (study design, endpoints, number of patients, and criteria for registration) will require a revision of the protocol, which must be approved in advance by the ethical review committee. The study records will be stored and information made available to auditors, ethical review committees, or regulatory authorities on request.

ETHICS AND DISSEMINATION

This study is conducted in compliance with the Declaration of Helsinki (amended in October 2013) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects (enacted on December 22, 2014). Prior to the study initiation, the investigator or subinvestigators submitted the protocol and informed consent form to the ethical review committee of each study centre and obtained their approval. Patient anonymity will be protected by the use of subject identification codes. A cooperation fee of 5,000 Japanese yen (about \$42 or €37 at Oct 2015) for

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3 study participation will be provided for each patient upon request from the study centre. All
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5 patients were required to provide written informed consent.
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9 The results of this study will be presented at major cardiovascular-related congresses. The data
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11 regarding FH will be presented at atherosclerotic-related congresses.
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14 15 16 17 **CONCLUSION**

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19 The EXPLORE-J study will provide new insights into the relationship between ACS and
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21 recurrent events, the relationship between recurrent events and serum LDL-C levels, the FH to
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23 ACS ratio, and the PCSK9 concentration in ACS patients in a Japanese population. As a large-
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25 scale study including 2,016 patients from 59 centres, this study will be the first ACS registry to
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27 seek insight into FH in Japan to target the high-risk population.
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35 **Acknowledgements**

36
37 The authors thank Marion Barnett, J. Ludovic Croxford, PhD, and Michelle Belanger, MD, of
38
39 Edanz Group for providing medical writing support; Tamio Teramoto of Teikyo University
40
41 (Tokyo, Japan), Shun Ishibashi of Jichi Medical University (Tochigi, Japan), Kotaro Yokote of
42
43 Chiba University (Chiba, Japan), Tomonori Okamura of Keio University (Tokyo, Japan), and
44
45 Hiroyuki Daida of Juntendo University (Tokyo, Japan) for collaboration and advice regarding
46
47 planning of the study; Yosuke Ujike and Yasuyoshi Nakahigashi, of Sanofi (Tokyo, Japan) for
48
49 providing writing and editing support; Mebix, Inc. (Tokyo, Japan) for assistance with study
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51 implementation/operation; BML, Inc. (Tokyo, Japan) for PCSK9- and genome-related analysis;
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60 CTD, Inc. (Tokyo, Japan) for consulting; and Shizuya Yamamashita of Rinku General Medical

Center (Osaka, Japan), Toru Yoshizumi of Kawasaki Hospital (Hyogo, Japan), Micron, Inc. (Tokyo, Japan) for radiography of the Achilles tendon.

Competing interests

Kiyoko Uno is an employee of Sanofi. Masato Nakamura, Atsushi Hirayama, Junya Ako, Atsushi Nohara, Hidenori Arai, and Mariko Harada-Shiba have received consultation fees from Sanofi.

Funding

This work was supported by Sanofi and Regeneron Pharmaceuticals, Inc.

Authors' contributions

M Nakamura, K Uno, A Hirayama, J Ako, A Nohara, H Arai, and M Harada-Shiba all served on the steering committee as principal investigators and equally contributed to conception and design of the study, protocol development, acquisition of data, analysis and interpretation of the data, and drafting and revising the publication for important intellectual content. M Nakamura, K Uno, A Hirayama, J Ako, A Nohara, H Arai, and M Harada-Shiba all approved the final version of the manuscript and have agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data sharing statement

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No additional unpublished is available.

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Figure legends

Figure 1 Number of patients diagnosed with familial hypercholesterolaemia by country. FH, familial hypercholesterolaemia. Reproduced from Nordestgaard et al.[15] with permission from the European Heart Journal.

Figure 2 Study flow and design. ^aFor FH gene examination, blood will be collected once from the patients who provide consent, at a visit made after registration. ^bRadiographs of the Achilles tendon will be obtained during hospitalisation for registration as a rule, but a radiography obtained by Visit 3 is acceptable. FH, familial hypercholesterolaemia; PCSK9, proprotein convertase subtilisin kexin 9

Appendix: EXPLORE-J Study Centres

Almeida Memorial Hospital

Anjo Kosei Hospital

Chiba Emergency Medical Center

Chiba University Graduate School of Medicine

Fukui Cardiovascular Center

Fukuoka Tokushukai Hospital

Gifu Heart Center

Gifu Prefectural General Medical Center

Gunma Prefectural Cardiovascular Center

Hakodate Municipal Hospital

Higashi Takarazuka Satoh Hospital

Higashiyamato Hospital

Hiroshima City Hospital

Hyogo Brain and Heart Center

Iwaki Kyoritsu General Hospital

Iwatsuki Minami Hospital

Jichi Medical University School of Medicine

Juntendo University Graduate School of Medicine

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3 Juntendo University Shizuoka Hospital
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6 Kanazawa Cardiovascular Hospital
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9 Kikuna Memorial Hospital
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12 Kishiwada Tokushukai Hospital
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15 Kitasato University School of Medicine
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18 KKR Takamatsu Hospital
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21 Kokura Memorial Hospital
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24 Kumamoto University Hospital
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27 Kurashiki Central Hospital
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30 Kurume University School of Medicine
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33 Kyorin University School of Medicine
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36 Mitsui Memorial Hospital
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39 Nagoya Daini Red Cross Hospital
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42 National Cerebral and Cardiovascular Center
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45 National Hospital Organization Kure Medical Center and Chugoku Cancer Center
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48 Nihon University Itabashi Hospital
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51 Nihon University School of Medicine
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54 Nippon Medical School
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57 Nippon Medical School Musashi-Kosugi Hospital
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3 Ome Municipal General Hospital
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6 Osaka General Medical Center
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21 Sakakibara Heart Institute
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27 Sekishinkai Kawasaki Saiwai Hospital
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30 Sendai Kousei Hospital
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33 Showa University Northern Yokohama Hospital
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36 St. Mary's Hospital
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39 Teikyo University School of Medicine
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42 The Sakakibara Heart Institute of Okayama
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45 Toho University School of Medicine
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51 Tokorozawa Heart Center
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57 Tokuyama Central Hospital
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Tokyo Women's Medical University

Yokohama City University Medical Center

Yokohama Rosai Hospital

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Figure 1

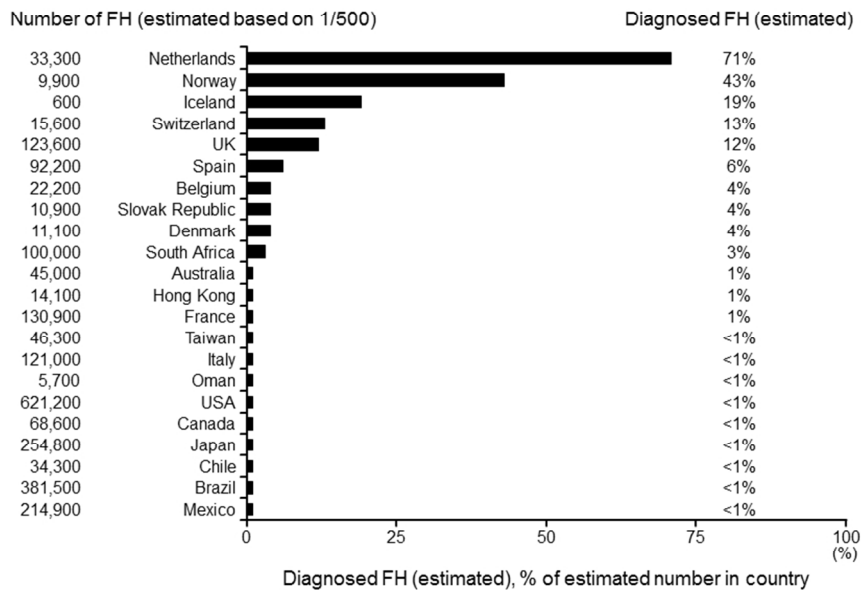


Figure 1

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Figure 2

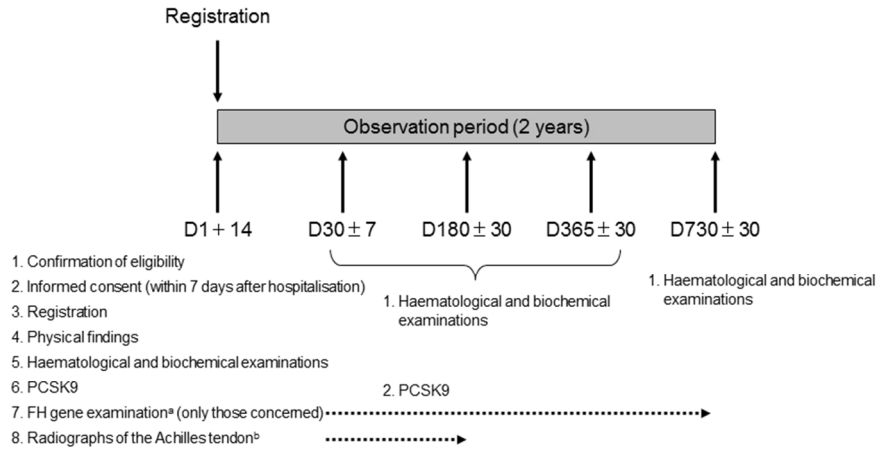


Figure 2

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BMJ Open

Exploration into lipid management and persistent risk in patients hospitalised for acute coronary syndrome in Japan (EXPLORE-J): Protocol for a prospective observational study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2016-014427.R1
Article Type:	Protocol
Date Submitted by the Author:	11-Mar-2017
Complete List of Authors:	Nakamura, Masato; Toho University Ohashi Medical Center, Division of Cardiovascular Medicine Uno, Kiyoko; Sanofi Hirayama, Atsushi ; Nihon University School of Medicine, Division of Cardiology Ako, Junya; Kitasato University, Department of Cardiovascular Medicine Nohara, Atsushi; Kanazawa University of Graduate School of Medical Sciences, Department of Lipidology Arai, Hidenori; National Center for Geriatrics and Gerontology Harada-Shiba, Mariko ; National Cerebral & Cardiovascular Center Research Institute, Department of Molecular Innovation in Lipidology
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	acute coronary syndrome, familial hypercholesterolaemia, Japan, lipid management, proprotein convertase subtilisin kexin 9

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13 5 Masato Nakamura¹, Kiyoko Uno², Atsushi Hirayama³, Junya Ako⁴, Atsushi Nohara⁵, Hidenori
14 6 Arai⁶, and Mariko Harada-Shiba⁷
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Key words: acute coronary syndrome, familial hypercholesterolaemia, Japan, lipid management, proprotein convertase subtilisin kexin 9

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3 **27 Abstract**
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6 **28 Introduction** The present study is the largest registry study ever conducted in Japan exploring
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the prevalence of familial hypercholesterolaemia (FH) among acute coronary syndrome (ACS)
patients. Our study aims to (1) evaluate the status of lipid management and the subsequent risk of
major cardiovascular events following hospitalisation of Japanese ACS patients in real-world
clinical practice; (2) determine the proportion of Japanese ACS patients who achieve the lipid
management goal and have a reduction of event risks with strict lipid management (low-density
lipoprotein-cholesterol <1.81 mmol/L); (3) determine the prevalence of FH; and (4) investigate
the clinical significance of proprotein convertase subtilisin kexin 9 (PCSK9) level.

36 **Methods and analysis** We will conduct a multicentre, prospective, observational study of
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approximately 2,000 Japanese ACS patients with/without FH hospitalised between April 2015
and August 2016. The primary endpoint is the incidence of major adverse cardiovascular events
(MACEs) after initial hospitalisation. The secondary endpoints are (1) MACE developed from
Visit 1 to Visit 2 (Day 30); (2) MACE developed from Visit 2 (Day 30) to Visit 5 (Day 730); (3)
treatment rate by lipid-lowering therapies (any statin or intensive, PCSK9 inhibitor, fibrates, and
ezetimibe); (4) incidence of events by the addition of the following outcomes to the primary
endpoint: coronary revascularisation due to myocardial ischaemia, revascularisation other than
coronary artery, inpatient treatment for occurrence or exacerbation of heart failure, transient
ischaemic attack, acute arterial occlusion, central retinal artery occlusion, and other adverse
events prolonging or requiring hospitalisation; and (5) proportion of subjects achieving target
lipid levels.

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Ethics and dissemination The study protocol was submitted to the ethical review committee of
each participating centre for approval. Participation in the study is voluntary and anonymous.

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3 50 The study findings will be disseminated in international peer-reviewed journals and presented at
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6 51 relevant conferences.
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9 52 **Clinical trial registration:** UMIN000018946
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55 **Strengths and limitations of this study**

- 56 • This study will provide new insights into the relationship between acute coronary syndrome
57 (ACS) and recurrent events and the relationship between recurrent events and serum low-
58 density lipoprotein-cholesterol levels in Japanese ACS patients.
- 59 • This study will be the first to evaluate the familial hypercholesterolaemia (FH) to ACS ratio
60 and will provide important information regarding PCSK9 concentrations, including (1) the
61 dynamic change of PCSK9 concentrations during post-ACS medical management, (2) the
62 relationship between PCSK9 concentrations and cardiovascular events, (3) the difference of
63 PCSK9 concentrations between FH and non-FH, and (4) the relationship among PCSK9
64 concentrations, lipid parameters and lipid lowering therapies for both statin-naïve and statin-
65 exposed patients.
- 66 • This study will be a prospective, large-scale, observational study of approximately 2,000
67 Japanese ACS patients from 59 participating centres.
- 68 • This study is the largest FH registry study in Japan targeting the high-risk population.
- 69 • This study will be limited by the inherent limitations of the observational study design (e.g.,
70 susceptibility to biases and confounders, and the inability to establish causality) and the small
71 sample size; the generalisability of our findings will be limited to the Japanese population.

76 INTRODUCTION

77 The incidence of atherosclerotic disease, including heart disease, in Japan is increasing and is
78 associated with a change towards a Western lifestyle and increase in dyslipidaemia.[1] There is a
79 positive correlation between low-density lipoprotein-cholesterol (LDL-C) levels and the
80 incidence of coronary artery disease.[2, 3] Therapy to lower LDL-C helps prevent cardiovascular
81 events. Large statin trials have demonstrated the benefits of achieving an LDL-C level of 1.42–
82 2.02 mmol/L, which has served as a basis for more aggressive European and US guidelines [4].
83 In particular, aggressive therapy to achieve LDL-C <1.81 mmol/L, the target value for lipid
84 management (in patients for whom aggressive therapy is intended) in European and US
85 guidelines,[5-7] or the use of high-intensity statins according to American College of
86 Cardiology/American Heart Association guidelines have helped to reduce cardiovascular events
87 and the progression of atherosclerosis.[5-11]

88 However, the Japanese guidelines for the prevention of atherosclerosis published in 2012 set
89 higher target values for LDL-C management at <3.11 mmol/L in primary prevention for high-
90 risk patients and <2.59 mmol/L for secondary prevention because the evidence for benefit was
91 suggested to be insufficient.[12] Previous Japanese studies (ESTABLISH and the follow-up
92 Extended-ESTABLISH study) reported that aggressive LDL-C lowering reduced the incidence
93 of death, the recurrence of acute coronary syndrome (ACS), and cerebral infarction compared
94 with a control group.[13, 14] The MEGA study showed the benefit of lipid-lowering therapy in
95 Japanese patients, but to values higher than 2.59 mmol/L.[15] While a number of imaging
96 studies with intravascular ultrasound and other modalities have shown the benefits of aggressive
97 LDL-C-lowering therapy in Japanese patients in terms of reduced plaque volume or coronary
98 plaque regression,[16-20] there is no large-scale clinical study that shows the need for aggressive

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3 99 lipid-lowering therapy in Japan, especially to LDL-C target values lower than 2.59 mmol/L. It is
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6 100 generally accepted that insufficient evidence is available to determine the benefit of such therapy.
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8 101 Therefore, investigations into this issue, and evaluation of a possible relationship between LDL-
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10 102 C and recurrent coronary events and coronary artery disease progression in Japan are needed.

103 Familial hypercholesterolaemia (FH) is an inherited, autosomal dominant disease resulting
104 from abnormalities in the genes coding for the LDL receptor and related molecules. It is
105 characterised by three major signs: (i) hyper-LDL-cholesterolaemia; (ii) early onset coronary
106 artery diseases; and (iii) tendon/skin xanthoma. Untreated FH carries an extremely high risk of
107 developing coronary artery diseases, particularly in men aged between 30 and 50 years and
108 women aged between 40 and 70 years.[21, 22] Risk data from the Simon Broome study in 1991
109 showed that the risk of cardiovascular death was 99 times higher in patients with FH at ages 20–
110 39 years.[23] Although heterozygous FH is present in an estimated 300,000 patients in
111 Japan,[24] the diagnosis rate is only <1% of the estimated number of patients with FH in
112 Japan.[22] According to an investigation by the Ministry of Health, Labour and Welfare in
113 Japan,[21] the prevalence of FH is 4%–19% of patients presenting with ACS. FH may be under-
114 diagnosed because of a masking affect by statin therapy, or the transient reduction of LDL-C
115 associated with acute myocardial infarction. A prospective cohort observational study of 4,534
116 patients with ACS in Switzerland reported an FH prevalence of 1.6%–5.5%, and a higher
117 adjusted risk of coronary death or myocardial infarction among patients with FH than without
118 (hazard ratio, 2.46–3.53 after 1 year).[25] To date there have been no reports regarding the ACS
119 recurrence rate in patients with FH in Japan. Therefore, it is important to clarify the prevalence
120 of FH in patients with ACS, the recurrence rate of cardiovascular events in patients with FH, and
121 risk factors for recurrence to provide the optimal treatment for Japanese patients with FH.

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3 122 Proprotein convertase subtilisin kexin 9 (PCSK9) is a protein that binds to LDL receptors to
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6 123 induce their degradation in intracellular lysosomes and inhibit their recycling.[26] Interestingly,
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8 124 patients with loss-of-function mutations in the *PCSK9* gene have low LDL-C levels and rarely
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10 125 develop cardiovascular diseases compared with normal individuals. PCSK9 inhibitors are an
11
12 126 effective lipid-lowering therapy. PCSK9 and LDL-C levels show a positive correlation in
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14 127 patients not treated with a lipid-lowering therapy, but this relationship disappears when lipid-
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16 128 lowering therapies, particularly oral statins, are administered.[27,28] Statins may simultaneously
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18 129 increase the concentration of PCSK9 and the expression of LDL receptors, while lowering LDL-
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20 130 C.[27] However, despite our current knowledge of PCSK9, its clinical significance in ACS
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22 131 patients remains unclear. Considering the above-mentioned gaps in the literature, the primary
23
24 132 objective of this study is to evaluate the status of lipid management and risk of major
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26 133 cardiovascular events in Japanese ACS patients in real-world clinical practice. Secondary study
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28 134 objectives are to identify the proportion of ACS patients in Japan who (i) achieve the target value
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30 135 of lipid management (LDL-C \leq 2.59 mmol/L); (ii) have a reduction of event risks with strict lipid
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32 136 management (LDL-C $<$ 1.81 mmol/L); and (iii) who have FH. The risk of recurrent ACS in
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34 137 patients with FH compared with patients without FH will be determined using the diagnostic
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36 138 criteria for FH as shown in **Table 1**. Other objectives are to investigate the clinical significance
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38 139 of PCSK9 concentrations by observing the time-course profile of PCSK9 concentrations in
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40 140 patients presenting with ACS and analysing the relationship between the concentration of PCSK9
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42 141 and FH, lipid-lowering therapy, and lipid management levels.
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54 143 **Table 1^a** Diagnostic criteria for heterozygous FH in adults (aged 15 years and older)
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Hyper LDL cholesterolaemia (LDL-C \geq 180 mg/dL before treatment)
Tendon xanthoma (tendon xanthoma in the back of hand, elbow, knee, etc. or Achilles tendon hypertrophy) or xanthoma tuberosum
Family history (blood relatives within the second degree of kinship) of FH or early onset coronary artery disease

Notes

- *Diagnosis is established excluding secondary hyperlipidaemia.*
 - *FH is diagnosed when two or more items are met. Diagnosis by genetic examination is advised if FH is suspected.*
 - *Tuberous xanthoma does not include xanthelasma of the eyelid.*
 - *Achilles tendon hypertrophy is diagnosed by a thickness of \geq 9 mm on soft-X-ray imaging.*
 - *FH is strongly suspected if LDL-C is \geq 250 mg/dL.*
 - *If the patient is already on drug therapy, refer to the lipid level that triggered the treatment.*
 - *Early onset coronary artery diseases are defined as those in which onset occurs at <55 years of age in men and <65 years of age in women.*
- ^aReproduced with modifications Harada-Shiba et al.[21] with permission from the *Journal of Atherosclerosis and Thrombosis*.
- FH, familial hypercholesterolaemia; LDL, low-density lipoprotein.

METHODS AND ANALYSIS

Study design

This will be a multicentre, prospective, observational study of Japanese patients presenting with ACS. Sixty-five sites are planned to participate in this study (see **Appendix**). These sites were chosen based on their ability to provide the most advanced percutaneous coronary intervention in Japan for patients presenting with ACS. Consecutive patients requiring hospitalisation for ACS were registered in 59 sites, for a total of 2,016 patients, between April 2015 and August 2016.

After the patients provided written informed consent within 7 days after hospitalisation for ACS, the investigator at each study centre successively registered subjects who met the inclusion

168 criteria. Successive registration of patients limits the selection bias by the investigator. The
 169 schedule for initial and follow-up examinations and the data to be collected are shown in **Figure**
 170 **1** and **Table 2**.

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172 **Table 2** Observations and endpoints

Category	Method and materials	Observation and examination items
Demographic characteristics (subjects' background)	Interview	Age, sex, and smoking and drinking status
ACS	Medical examination and interview	Onset date of ACS, date of hospitalisation, disease type, description of treatment
History of present illness/previous history/therapies	Interview	Particular previous history of cardiovascular diseases and cardiovascular risk-related diseases and history of their treatments (immediately before hospitalisation and at each visit).
Physical findings	Medical examination	Body height, body weight, and presence or absence of xanthoma
Reference LDL-C value	Interview	Value before treatment such as that obtained at a health examination
Family history	Interview	Coronary artery diseases, ischaemic cerebral infarction, and hypercholesterolaemia in relatives to the second

Category	Method and materials	Observation and examination items
		degree of kinship.
Primary endpoints	Interview	Investigation of outcome (alive or dead), date of death, cause of death, and presence or absence of non-fatal ACS and non-fatal cerebrovascular diseases requiring in-hospital treatments
Secondary endpoints	Interview	Presence or absence of event
Haematological and biochemical examinations	Serum	The following parameters will be measured from blood obtained in the sitting position when the symptoms are stable: Total cholesterol, HDL cholesterol, LDL cholesterol (automatic calculation), triglycerides, apoA1, apoB, Lp(a), creatinine, blood glucose, HbA _{1c} , hsCRP, haemoglobin, and haematocrit
PCSK9	Serum	Collective measurement at the central laboratory
FH gene examination	Whole blood	Collective measurement at the central laboratory
Radiography of the Achilles tendon ^a	Radiography	Whenever possible, radiography of the Achilles tendon will be performed during index hospitalisation for registration, but radiography obtained by Visit 3 will also be acceptable

173 ^aRadiography of the Achilles tendon was performed based on the recommendation of the Japan
 174 Atherosclerosis Society FH guidelines.[21]
 175 ACS, acute coronary syndrome; LDL-C, low-density lipoprotein cholesterol; PCSK9, proprotein
 176 convertase subtilisin kexin 9; apo, apolipoprotein; HDL, high-density lipoprotein; HbA_{1c}

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3 177 glycosylated haemoglobin; hsCRP, high sensitivity C-reactive protein; Lp(a), lipoprotein A; FH,
4 178 familial hypercholesterolaemia.
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9 180 Data are collected at Visit 1 (within 14 days after hospitalisation due to ACS), and Visits 2 to
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11 181 5 during the 2-year observational period on Days 30 (± 7 days), 180 (± 30 days), 365 (± 30 days),
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13 182 and 730 (± 30 days). Data are collected using an electronic case report form. The information
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15 183 collected at each visit is shown in **Table 2**. During the 2-year observation period (Visits 2–5), if a
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17 184 subject is transferred to another hospital during this time, the institution will be asked to provide
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19 185 the following information for the follow-up: attendance/non-attendance; date of observation;
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21 186 primary endpoints (investigation of outcome [alive/dead]); secondary endpoints (presence or
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23 187 absence); physical examination; fasting haematological and biochemical examinations, including
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25 188 PCSK9 concentrations (Visits 2–4); and medications.
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30 189 The samples collected in this study will be sent to a central laboratory (BML General
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32 190 Laboratory BML, INC., Saitama, Japan) under freezing conditions (-20°C) until completion of
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34 191 the study so that re-examination can be performed. All samples will be discarded after
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36 192 completion of the study.
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41 42 43 194 **Study subjects**

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46 195 The study subjects are Japanese patients presenting with ACS, hospitalised between April 2015
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48 196 and August 2016. In total, 2,016 subjects were registered.
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51 197 The key inclusion criteria are as follows: age ≥ 20 years; hospitalisation for any ACS
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53 198 including ST-segment elevation myocardial infarction (STEMI), non ST-segment elevation
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55 199 myocardial infarction (NSTEMI), and unstable angina; and ability to obtain written informed
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3 200 consent. STEMI is defined as the presence of chest symptoms such as pain or breathlessness
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5 201 suspected to be caused by myocardial ischaemia, persisting for ≥ 20 min; ST elevation of ≥ 1 mm
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8 202 on ≥ 2 contiguous leads or new left bundle branch block; and elevated troponin T ≥ 0.1 $\mu\text{g/L}$ or
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10 203 creatine phosphokinase-MB two times above the upper limit of normal. Acute NSTEMI is
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12 204 defined as the presence of chest symptoms such as pain or breathlessness suspected to be caused
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14 205 by myocardial ischaemia, persisting for ≥ 20 min ≤ 24 h before admission; not having ST-segment
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16 206 elevation ≥ 1 mm or new left bundle branch block; and the presence of elevated troponin T ≥ 1.0
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18 207 $\mu\text{g/L}$ or creatine phosphokinase-MB two times above the upper limit of normal. Unstable angina
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20 208 is defined as the presence of chest pain that may be persistent (≥ 20 min) and at least one of the
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22 209 following: ST depression ≥ 0.5 mm or T wave inversion ≥ 3 mm; troponin T ≥ 0.014 $\mu\text{g/L}$ or < 1.0
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24 210 $\mu\text{g/L}$; confirmation of significant stenosis by diagnostic imaging; new decrease in wall motion
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26 211 detected by echocardiography; or reversible myocardial perfusion defect detected by myocardial
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28 212 perfusion imaging.
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34 213 The key exclusion criteria are as follows: patients with chest pain and coronary artery
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36 214 diseases presenting with concomitant serious diseases, patients with in-stent thrombosis, patients
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38 215 enrolled in other interventional studies that could affect lipid profile, and those judged as
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40 216 inappropriate by the investigators or subinvestigators.
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47 218 **Primary endpoint**

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50 219 The primary endpoint is the incidence of major adverse cardiovascular events (MACE), defined
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52 220 as death associated with myocardial infarction or other cardiovascular death, major non-fatal
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54 221 coronary event (myocardial infarction or hospitalisation for unstable angina), or stroke.
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3 222 Incidences are monitored independently by the investigator at each local site. A time window
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5 223 will be allowed for observations at Visit 2 (± 7 days) and Visits 3–5 (± 30 days).
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8 224 Deaths associated with myocardial infarction or other cardiovascular deaths are defined as
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10 225 death secondary to acute myocardial infarction or any death with a clear relationship to
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12 226 underlying coronary heart disease, sudden death, heart failure, complication of coronary
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14 227 revascularisation procedure where the cause of death is clearly related to the procedure,
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16 228 unobserved or unexpected death, or other death that cannot be definitively attributed to a non-
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18 229 cardiovascular cause. Non-fatal myocardial infarction is defined in accordance with the
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21 230 ACC/AHA/ESC Universal Definition of Myocardial Infarction.[29]
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25 231 Ischaemic stroke is characterised by an acute episode of focal cerebral, spinal, or retinal
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27 232 dysfunction caused by infarction, defined by at least one of the following: pathological, imaging,
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29 233 or other objective evidence of acute, focal cerebral, spinal, or retinal ischaemic injury in a
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31 234 defined vascular distribution; symptoms of acute cerebral, spinal, or retinal ischaemic injury
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33 235 persisting for ≥ 24 hours or until death, with other aetiologies excluded.
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39 40 41 237 **Secondary endpoints**

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43 238 The secondary endpoints are MACE developed from Visit 1 to Visit 2 (Day 30); MACE
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45 239 developed from Visit 2 (Day 30) to Visit 5 (Day 730); treatment rate by the following lipid-
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47 240 lowering therapies: any statin, intensive statin (e.g., atorvastatin ≥ 20 mg, rosuvastatin ≥ 10 mg,
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49 241 and pitavastatin ≥ 4 mg), PCSK9 inhibitor, and other lipid-lowering therapies such as fibrates and
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51 242 ezetimibe; incidence of events by the addition of the following outcomes to the primary
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53 243 endpoints: coronary revascularisation due to myocardial ischaemia, revascularisation other than
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3 244 in the heart, inpatient treatment for occurrence or exacerbation of heart failure, transient
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5 245 ischaemic attack, acute arterial occlusion, central retinal artery occlusion, and other adverse
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8 246 events prolonging or requiring hospitalisation; and proportion of subjects achieving target lipid
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11 247 levels per study visit. The treatment rate will be determined based on written prescriptions. Other
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13 248 endpoints include the prevalence of FH in patients with ACS, comparison of PCSK9
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15 249 concentration between patients with or without FH, and comparison between clinical diagnosis
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18 250 of FH based on the guidelines vs. genetic analysis.
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22 23 252 **Discontinuation from study**

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26 253 The criteria used to allow discontinuation from the study include the withdrawal of consent by
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28 254 the subject or his/her legal representative, patient ineligibility (violation of the study contract),
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31 255 death of a subject, or removal by the judgement of the investigator or subinvestigators.
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35 36 257 **Safety protocol**

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39 258 Because this is an observational study (i.e., non-interventional) there will be no adverse events
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42 259 caused by a study drug. Patients will receive drugs as normally prescribed in daily medical
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45 260 practice.
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47 261 Serious adverse drug reactions caused by a drug will be the subject for application of relief
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49 262 under the Relief System for Sufferers from Adverse Drug Reactions similar to that in daily
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52 263 medical practice. Treatments for other adverse drug reactions will be covered by the national
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55 264 insurance scheme.
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266 Statistical analyses

267 The sample size was calculated to assess the persistent cardiovascular risk (defined as MACE)
268 from the index event to 2 years. Based on the PACIFIC registry, in which the incidence of
269 MACE was 6.4% at 2 years, a sample size of 2,000 has a precision of $\pm 1\%$ in the incidence of
270 MACE with a 95% confidence interval (CI) of 0.053–0.074.[30] With this sample size, the
271 subgroup analysis (the comparison of MACE in patients with LDL-C < 1.81 mmol/L and ≥ 1.81
272 mmol/L) will be performed. Because 409 of 1827 (22.3%) Japanese ACS patients in the
273 PACIFIC study and 305 of 1145 (26.6%) Japanese ACS patients in an ongoing database study
274 reached an LDL-C level < 1.81 mmol/L, we expect to have 446–532 patients with an LDL-C
275 level < 1.81 mmol/L in this registry. All patients meeting the inclusion criteria will be analysed.
276 The demographic data will be presented as the mean, median, standard deviation, and range for
277 continuous data, and number and proportion of subjects in each category for categorical data.

278 The analysis of the primary endpoint will be as follows: for the incidence of MACE at 2
279 years, Kaplan–Meier analysis will be used to estimate the event observed first after registration
280 in the whole population. The Greenwood formula will determine the 95% CI.

281 Subgroup analysis will investigate the association of factors with the incidence of MACE,
282 LDL-C < 1.81 mmol/L vs ≥ 1.81 mmol/L at registration, and the presence or absence of FH. The
283 Cox proportional hazard model and subgroup analysis will compare demographic factors (age,
284 sex, smoking history, body mass index, and underlying diseases) using a two-sided significance
285 level of 5% (not considering multiplicity, as the study is exploratory). Subgroup analysis will be
286 performed for LDL-C (< 1.81 mmol/L or ≥ 1.81 mmol/L) at the time when the event has occurred.

287 Analysis of the secondary endpoints (MACE developed by Visit 2 [Day 30]; MACE
288 developed between Visit 2 [Day 30] and Visit 5 [Day 730]; and treatment rate by lipid-lowering

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3 289 therapy) will be assessed by determining the ratio of subjects on each lipid-lowering therapy
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5 290 administered during observation and the 95% CI using the full analysis set. The incidences of
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8 291 events at Visit 2 and between Visits 2–5 or from the time of registration to the first event, and the
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10 292 associations of factors with each event will be determined by the same procedures as for the
11
12 293 primary endpoints. For the other endpoints, the prevalence rate of FH in patients with ACS will
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14 294 be assessed and its 95% CI from the diagnosis of FH will be determined. Logistic regression
15
16 295 analysis and subgroup analysis of the background factors that are associated with the prevalence
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18 296 of FH for exploratory investigation will be determined. The summary statistics for the
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20 297 concentration of PCSK9 at each measurement point will be calculated and a trend diagram
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22 298 (median values) will be developed. In addition, the presence or absence of the influence of FH by
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24 299 subgroup analysis will be determined.
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30 300 In the survival analysis, missing values will be censored while continuous data and discrete
31
32 301 data will be analysed on the basis of measured values without performing any special processing.
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34 302 Furthermore, we will also perform a sensitivity analysis where continuous data are handled using
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36 303 the last observation carried forward imputation, and discrete data are included only in the
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38 304 denominator but not in the numerator. No special processing will be performed for outliers. For
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40 305 the statistical analysis, SAS version 9.4 software (SAS Institute Inc., NC, USA) will be used.
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47 307 **Quality assurance measures**

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50 308 The study investigators will ensure compliance to the study protocol. Any change in factors that
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52 309 affect the safety of subjects or the scientific quality of this study (study design, endpoints,
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54 310 number of patients, and criteria for registration) will require a revision of the protocol, which
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56 311 must be approved in advance by the ethical review committee. The study records will be stored
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3 312 and information made available to auditors, ethical review committees, or regulatory authorities
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11 315 **ETHICS AND DISSEMINATION**

14 316 This study is conducted in compliance with the Declaration of Helsinki (amended in October
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16 317 2013) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects
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18 318 (enacted on December 22, 2014). Prior to the study initiation, the investigator or subinvestigators
19
20 319 submitted the protocol and informed consent form to the ethical review committee of each study
21
22 320 centre and obtained their approval. Patient anonymity will be protected by the use of subject
23
24 321 identification codes. A cooperation fee of 5,000 Japanese yen (about \$42 or €37 at Oct 2015) for
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26 322 study participation will be provided for each patient upon request from the study centre. All
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28 323 patients were required to provide written informed consent.
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33 324 The results of this study will be presented at major cardiovascular-related congresses. The data
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35 325 regarding FH will be presented at atherosclerotic-related congresses.
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41 327 **CONCLUSION**

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44 328 The EXPLORE-J study will provide new insights into the relationship between ACS and
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46 329 recurrent events, the relationship between recurrent events and serum LDL-C levels, the FH to
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48 330 ACS ratio, and the PCSK9 concentration in ACS patients in a Japanese population. As a large-
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50 331 scale study including 2,016 patients from 59 centres, this study will be the first ACS registry to
51
52 332 seek insight into FH in Japan to target the high-risk population.
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334 **Acknowledgements**

335 The authors thank Marion Barnett, J. Ludovic Croxford, PhD, and Michelle Belanger, MD, of
336 Edanz Group for providing medical writing support; Tamio Teramoto of Teikyo University
337 (Tokyo, Japan), Shun Ishibashi of Jichi Medical University (Tochigi, Japan), Kotaro Yokote of
338 Chiba University (Chiba, Japan), Tomonori Okamura of Keio University (Tokyo, Japan), and
339 Hiroyuki Daida of Juntendo University (Tokyo, Japan) for collaboration and advice regarding
340 planning of the study; Yosuke Ujike and Yasuyoshi Nakahigashi, of Sanofi (Tokyo, Japan) for
341 providing writing and editing support; Mebix, Inc. (Tokyo, Japan) for assistance with study
342 implementation/operation; BML, Inc. (Tokyo, Japan) for PCSK9- and genome-related analysis;
343 CTD, Inc. (Tokyo, Japan) for consulting; and Shizuya Yamamashita of Rinku General Medical
344 Center (Osaka, Japan), Toru Yoshizumi of Kawasaki Hospital (Hyogo, Japan), Micron, Inc.
345 (Tokyo, Japan) for radiography of the Achilles tendon.

346

347 **Competing interests**

348 Kiyoko Uno is an employee of Sanofi. Masato Nakamura, Atsushi Hirayama, Junya Ako, Atsushi
349 Nohara, Hidenori Arai, and Mariko Harada-Shiba have received consultation fees from Sanofi.

350

351 **Funding**

352 This work was supported by Sanofi and Regeneron Pharmaceuticals, Inc.

353

354 **Authors' contributions**

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3 355 M Nakamura, K Uno, A Hirayama, J Ako, A Nohara, H Arai, and M Harada-Shiba all served on
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6 356 the steering committee as principal investigators and equally contributed to conception and
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8 357 design of the study, protocol development, acquisition of data, analysis and interpretation of the
9
10 358 data, and drafting and revising the publication for important intellectual content. M Nakamura, K
11
12
13 359 Uno, A Hirayama, J Ako, A Nohara, H Arai, and M Harada-Shiba all approved the final version
14
15 360 of the manuscript and have agreed to be accountable for all aspects of the work in ensuring that
16
17 361 questions related to the accuracy or integrity of any part of the work are appropriately
18
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20 362 investigated and resolved.
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26 364 **Data sharing statement**
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28 365 No additional unpublished is available.
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Data sharing statement

No additional unpublished is available.

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3 460 **Figure legends**
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6 461 **Figure 1** Study flow and design. ^aFor FH gene examination, blood will be collected once from
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8 462 the patients who provide consent, at a visit made after registration. ^bRadiographs of the Achilles
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10 463 tendon will be obtained during hospitalisation for registration as a rule, but a radiography
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12 464 obtained by Visit 3 is acceptable. FH, familial hypercholesterolaemia; PCSK9, proprotein
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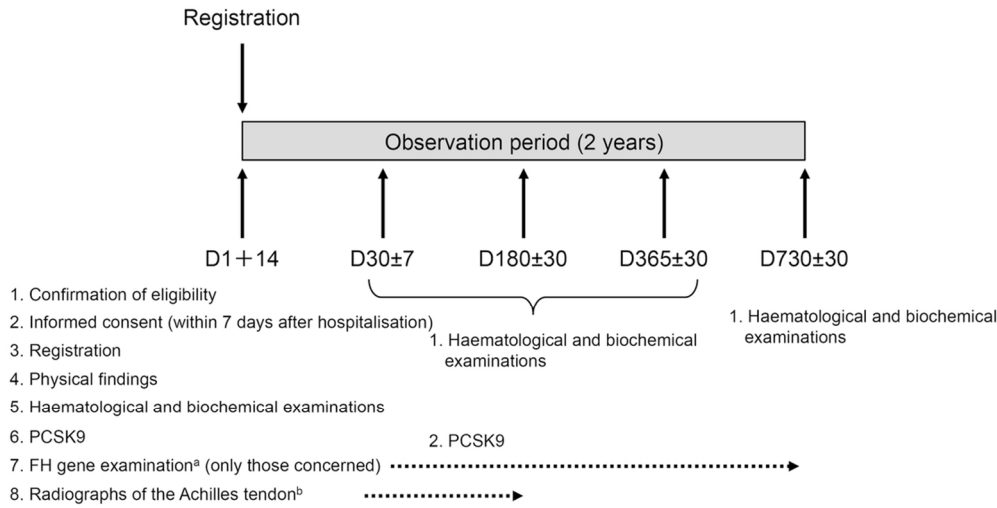


Figure 1

102x52mm (300 x 300 DPI)

review only

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Appendix: EXPLORE-J Study Centres

Almeida Memorial Hospital

Anjo Kosei Hospital

Chiba Emergency Medical Center

Chiba University Graduate School of Medicine

Fukui Cardiovascular Center

Fukuoka Tokushukai Hospital

Gifu Heart Center

Gifu Prefectural General Medical Center

Gunma Prefectural Cardiovascular Center

Hakodate Municipal Hospital

Higashi Takarazuka Satoh Hospital

Higashiyamato Hospital

Hiroshima City Hospital

Hyogo Brain and Heart Center

Iwaki Kyoritsu General Hospital

Iwatsuki Minami Hospital

Jichi Medical University School of Medicine

Juntendo University Graduate School of Medicine

Juntendo University Shizuoka Hospital

1
2
3 Kanazawa Cardiovascular Hospital
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6 Kikuna Memorial Hospital
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9 Kishiwada Tokushukai Hospital
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12 Kitasato University School of Medicine
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15 KKR Takamatsu Hospital
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18 Kokura Memorial Hospital
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21 Kumamoto University Hospital
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24 Kurashiki Central Hospital
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27 Kurume University School of Medicine
28
29

30 Kyorin University School of Medicine
31
32

33 Mitsui Memorial Hospital
34
35

36 Nagoya Daini Red Cross Hospital
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39 National Cerebral and Cardiovascular Center
40
41

42 National Hospital Organization Kure Medical Center and Chugoku Cancer Center
43
44

45 Nihon University Itabashi Hospital
46
47

48 Nihon University School of Medicine
49
50

51 Nippon Medical School
52
53

54 Nippon Medical School Musashi-Kosugi Hospital
55
56

57 Ome Municipal General Hospital
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1
2
3 Osaka General Medical Center
4

5
6 Rinku General Hospital
7

8
9 Saga-Ken Medical Centre Koseikan
10

11
12 Saiseikai Fukuoka General Hospital
13

14
15 Saiseikai Kumamoto Hospital
16

17
18 Sakakibara Heart Institute
19

20
21 Sakurabashi Watanabe Hospital
22

23
24 Sekishinkai Kawasaki Saiwai Hospital
25

26
27 Sendai Kousei Hospital
28

29
30 Showa University Northern Yokohama Hospital
31

32
33 St. Mary's Hospital
34

35
36 Teikyo University School of Medicine
37

38
39 The Sakakibara Heart Institute of Okayama
40

41
42 Toho University School of Medicine
43

44
45 Tokai University School of Medicine
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47
48 Tokorozawa Heart Center
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50
51 Tokushima Prefectural Central Hospital
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54 Tokuyama Central Hospital
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57 Tokyo Women's Medical University
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Yokohama City University Medical Center

Yokohama Rosai Hospital

For peer review only