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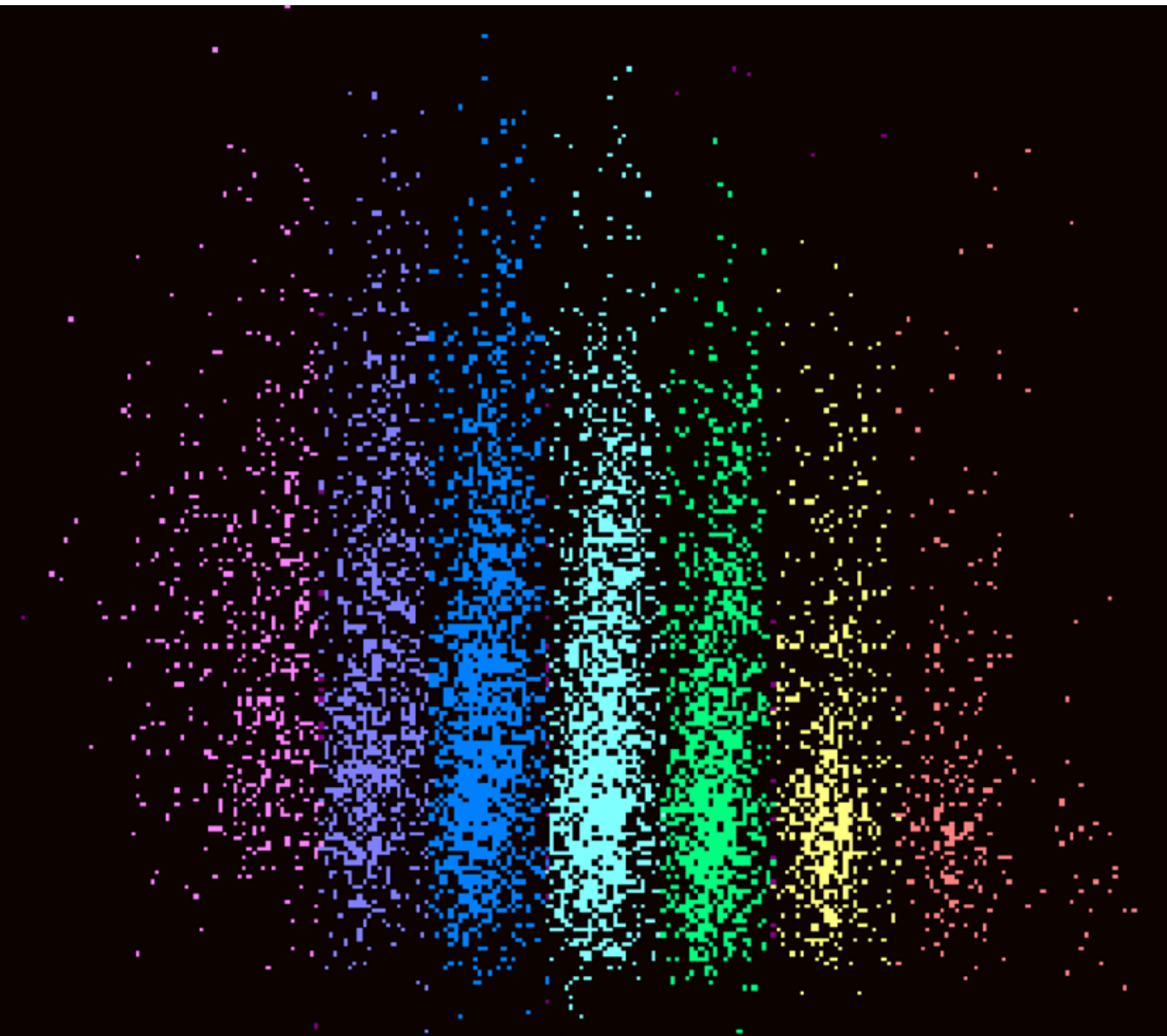

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Contents

European Atherosclerosis Journal is an international, peer-reviewed, fully open access, four-monthly journal covering all topics within atherosclerosis and cardiovascular disease areas. The European Atherosclerosis Journal is an official journal of SITeCS (Società Italiana di Terapia Clinica e Sperimentale - Italian Society for Experimental and Clinical Therapeutics).

European Atherosclerosis Journal

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Main text. The manuscript must contain the following sections: Introduction, Methods, Results, Discussion, Conflict of interest (mandatory), Funding (if applicable), Author contribution (mandatory), Acknowledgements (if applicable), References. Abbreviations must be defined when first used in the text.

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Evaluation of Clinical Features including the frequency of Familial Hypercholesterolemia, and 2-Year Cardiovascular Outcomes in Patients with Early Acute Coronary Syndrome: Real-Life Data from a Retrospective Cohort

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Keywords
Acute coronary syndrome; age of onset; familial hypercholesterolemia; mortality; patient outcomes; early cardiovascular disease

ABSTRACT

Objective: This retrospective study, based on real-world data, aimed to evaluate the clinical characteristics and 2-year cardiovascular outcomes in patients presenting with early acute coronary syndrome (ACS) in comparison with older patients in a tertiary healthcare center.

Methods: Information including at least 2-year endpoint data after the index ACS event was retrieved from hospital records. Patients without available follow-up data were contacted by phone-calls. Age limit for early cardiovascular disease was considered <55 years for men and <60 years for women.

Results: Of 985 consecutive ACS patients (770 men; age range, 21-93 years) 361 (36.6%) met the definition of early cardiovascular disease in terms of age at the index event. The following parameters were observed more frequently in the young-age group: smoking, a body mass index ≥30 kg/m², high total cholesterol level, high triglyceride level, low high-density lipoprotein cholesterol (HDL-C) level, and family history of coronary artery disease (CAD). The frequency of familial hypercholesterolemia (FH) was 7.6% and was higher in the young group (15.5%) than in the elderly group (3%) (p<0.001). During the follow-up period, the risk predictors for cardiovascular events were the index event (ST-segment elevation myocardial infarction or non-ST-segment elevation myocardial infarction) and the presence of hypertension, and the risk predictors for mortality were female sex, older age, in-hospital cardiovascular complications.

Conclusion: In this retrospective ACS cohort of a single center from Turkey, a very high rate of early ACS (36.6%) was observed. Compared to older patients, young ACS patients were more likely to be smokers, more obese, less diabetic and less hypertensive. High total cholesterol, high triglycerides, low HDL-C levels, high non-HDL-C, family history of CAD, and FH were also observed more frequently in the young ACS group. The high prevalence of FH may be a major factor in the high prevalence of premature ACS in this population. Both the in-hospital and 2-year follow-up mortality rates were significantly lower in the young group.

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Introduction

The incidence of coronary artery disease (CAD) increases with age, and generally atherosclerosis is considered a disease of old age. Acute myocardial infarction (MI) is not a common occurrence at young ages and its frequency has been reported between 5% and 13%, depending on the cut-off age used for defining early age [1–3]. Although data are limited, the incidence of early CAD has been reported to be unexpectedly high in Turkey. The EUROASPIRE-IV study reported a 12% overall incidence of MI in patients <50 years in Europe; however, in Turkish arm of the same study, this rate was reported to be 19% [4]. The disease course, complications and out-

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comes differ significantly in young as compared with older people. This retrospective study, based on real-life data, aimed to evaluate the clinical characteristics and 2-year cardiovascular (CV) outcomes in patients presenting with an early acute coronary syndrome (ACS) (<55 years for males and <60 years for females) compared with older patients in a tertiary healthcare center.

Methods

Patients

The present study was designed as a retrospective cohort study in a tertiary healthcare center. All consecutive patients admitted to the Cardiology Department of Ege University School of Medicine with a diagnosis of ACS [unstable angina pectoris (UAP), ST-segment elevation myocardial infarction (STEMI), and non-ST-segment elevation myocardial infarction (NSTEMI)] were included in a 6-month follow-up period. Patient information was retrospectively retrieved from archived medical records and from the electronic data system of the hospital. At least 2-year outcome data of patients after the index ACS event were obtained from the same medical data recording system of the hospital. Information of patients for whom follow-up data were not available was obtained via phone calls.

The study inclusion criteria were as follows:

i) hospital admission within 24 hours of the onset of symptoms related to the index event in female and male patients aged ≥18 years
ii) diagnosis of ACS (diagnosis code according to ICD-10 system; I20.0: UAP, I21: acute MI, I22: recurrent MI, I23: complications after acute MI) on the epicrisis (discharge) report form. In addition, the medical records of patients with diagnosis code of I25 (atherosclerotic CV disease) were reviewed to ascertain the presence of clinical signs of ACS. The diagnosis of ACS was verified based on the Universal Criteria for the Definition of Myocardial Infarction [5].

Data collection

The records of a total of 1,000 consecutive patients hospitalised for ACS within the specified study period were reviewed. A case report form was completed for each case, using the information retrieved from the hospital medical records. Patients' most recent medical record information was examined for mortality assessment. Post-discharge medical information of patients with no medical records after hospital discharge or in a 2-year period following hospital discharge was obtained via phone calls. Of the 1,000 patients, 15 were excluded because the diagnosis of ACS was not confirmed, and another 84 were excluded from the 2-year follow-up analysis because they could not be reached despite telephone calls (using the phone number recorded either in the electronic data system or in the patient's medical record) at least in two different days and at different times of the day.

The following information about ACS patients was retrieved from the patients' medical records: demographic characteristics, medical history, CV risk factors, physical examination findings, history of medication (during hospitalisation and recommended for use after hospital discharge), results of laboratory analysis, and in-hospital complications. CV outcomes in the 2-year follow-up period after hospital discharge were also retrospectively retrieved from hospital medical records, the hospital electronic laboratory and clinical recording system, or via phone calls for those with inadequately documented information or no readmission. All data collected were compared between the patients with early ACS and the rest of the study population (old-age group).

Definitions

ACS was divided into UAP (patients without ST-elevation on baseline electrocardiography [ECG], those not developing MI during follow-up, and those with no positive troponin values), STEMI (MI patients with ST-elevation on the baseline ECG and with positive troponin values), and NSTEMI (MI patients without ST-elevation on the baseline ECG and with positive troponin values during the follow-up). Early ACS was defined as age <55 years in men and <60 years in women at the time of the index event.

CV events that occurred during the follow-up period, elective coronary angioplasty, MI, UAP, congestive heart failure, cerebrovascular events and conditions requiring an implantable cardioverter defibrillator and cardiac resynchronization therapy were defined as CV events.

A systolic blood pressure of ≥140 mmHg (≥130 mmHg for diabetics) and/or a diastolic blood pressure of ≥90 mmHg (≥80 mmHg for diabetics) were considered high blood pressure (hypertension). Diabetes mellitus was diagnosed if the patient had a diabetes mellitus and had received anti-diabetic treatment and/or had a fasting plasma glucose level of ≥126 mg/dL during hospitalisation. CAD was defined as the presence of a previous MI, at least 50% stenosis of a coronary artery on coronary angiogram, coronary angioplasty and/or coronary artery bypass grafting.

A total cholesterol level of ≥174 mg/dL was considered a high total cholesterol level, a low-density lipoprotein cholesterol (LDL-C) level of ≥100 mg/dL was considered a high LDL-C level, a high-density lipoprotein cholesterol (HDL-C) level of <40 mg/dL in men and of <45 mg/dL in women was considered a low HDL-C level. A triglyceride level of ≥150 mg/dL was considered a high triglyceride level. Target LDL-cholesterol level with treatment was <70 mg/dL. Non-HDL-C level was calculated by subtracting the HDL-C level from the total cholesterol level.

The diagnostic scoring for familial hypercholesterolemia (FH) was performed using the Dutch Lipid Clinic Network diagnostic criteria [6–8]. FH scores were calculated according to the definition of De Backer et al [9]. In patients receiving lipid-lowering treatment at the time of hospitalisation, pre-treatment LDL-C level was calculated by multiplying the on-treatment LDL-C level by correction factors defined by Besseling et al. according to the type and dose of the drug used by the patient [10]. According to the total scores, FH was classified as follows: <3: unlikely FH, 3-5: possible FH, 6-8: probable FH, and >8: definite FH. The presence of xanthomas and corneal arcus could not be evaluated for the diagnosis of FH.

Statistical analysis

Data analyses were performed using Predictive Analytics SoftWare (PASW) version 18.0 (SPSS Inc., Chicago, IL USA for Windows program. Descriptive statistics were expressed as median and 25th percentile (interquartile 1 [Q1]) and 75th percentile (interquartile 3 [Q3]) for numerical variables and as number and percentage for categorical variables. Normality of numerical variables was analyzed by visual (histogram and probability graphics) and analytic methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). For non-normally distributed numerical variables, the comparison of two groups was performed using Mann-Whitney U test. For categorical variables, the chi-square test was used for the comparison of two groups; while Fischer's exact test statistic was used when the chi-square condition was not met. Logistic regression analysis and Cox regression analysis were performed to determine the risk factors for CV events in the follow-up period. The level of statistical significance was accepted as $p<0.05$.

Results

The median age of the ACS patients, of whom 770 (78.2%) were male, was 60 years (range, 21-93 years). The distribution of ACS patients according to the age group showed that the proportion of patients (29.7%) was highest in the 50-59 age group. There was a significant difference between female and male patients in terms of age distribution (p<0.001). While the proportion of males was higher in the <70-year age group, females were higher in the ≥70-year age group. The distribution of ACS patients according to sex and age groups is shown in **Figure 1**.

Evaluation of the study population in terms of age at the time of the index event showed that 361 (36.6%) of 985 patients met the definition of early CV disease. The general and clinical characteristics of ACS patients according to age at the index event are shown in **Table 1**. The most common index event in the young-age group was STEMI. The proportion of smokers and patients with a body mass index (BMI) of ≥30 kg/m^2 was higher in the young group; the presence of hypertension (based on both patient history and blood pressure measurement) and the presence of diabetes mellitus were more common in the old-age group. The proportion of patients having high total cholesterol, high triglycerides, and/or low HDL-C levels and a family history of CAD were higher in the young-age group.

Evaluation of the study population for the presence of FH revealed that 910 (92.4%) patients had unlikely FH, 68 (6.9%) patients had possible FH, 4 (0.4%) patients had probable FH and 3 (0.3%) patients had definite FH. The total number of patients diagnosed with FH was 75 (7.6%). The presence of FH was significantly higher in the young-age group than in the old-age group (15.5% and 3%, respectively; p<0.001). There were 50 possible (13.9%), 3 probable (0.8%) and 3 definite (0.8%) FH patients in the early ACS group, while there were 18 possible (2.9%), 1 probable (0.2%), and 0 definite (0%) FH in the old-age ACS group.

During the in-hospital period (6.27±4.37 days), 81 patients developed CV complications. There was no significant difference between the young- and old-age groups in the incidence of CV complications. The rates of infections and deaths during hospitalisation were higher in the old-age group (**Table 2**). Of the deaths during the in-hospital period, 33 were due to CV system and one was due to gastrointestinal haemorrhage.

The mean follow-up period after hospital discharge was 30.4±8.4 months for the whole study population. Post-discharge patient in-

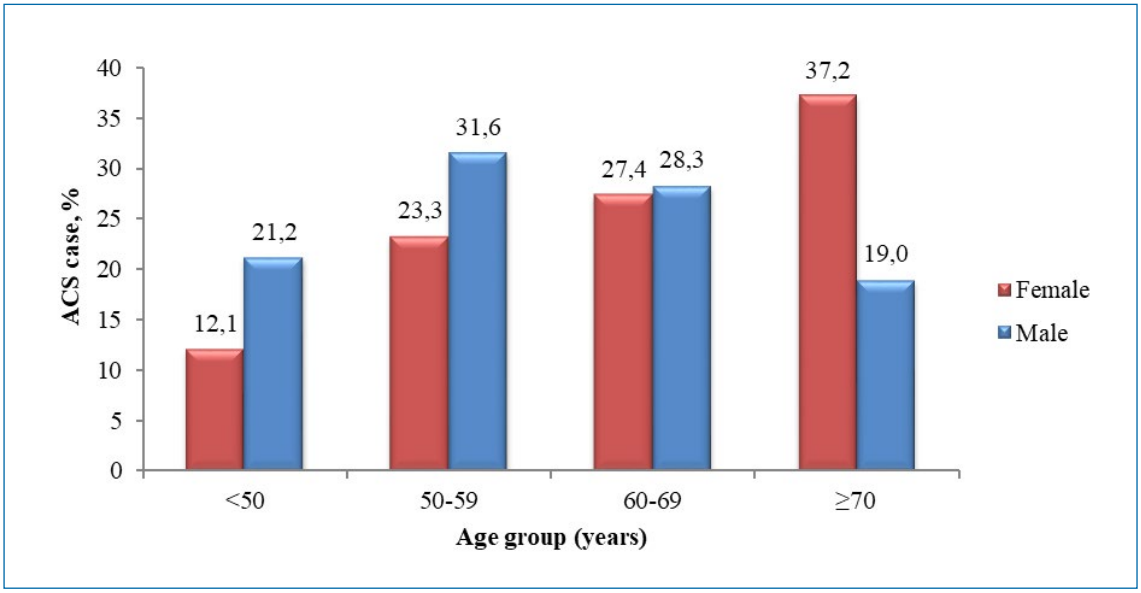

Figure 1 | Distribution of acute coronary syndrome patients according to sex and age groups.

Table 1 | General and clinical characteristics of acute coronary syndrome patients according to the age at index event.

	N	Young-Age Group (Females <60 years old Males <55 years old)	N	Old-Age Group (Females ≥60 years old Males ≥55 years old)	p
Age, year	361	49 (45-52)	624	66 (61-74)	**<0.001**
Sex	361		624		
Female		76 (21.1)		139 (22.3)	0.654
Index event	361		624		
UAS		116 (32.1)		254 (40.7)	
NSTEMI		68 (18.8)		168 (26.9)	**<0.001**
STEMI		177 (49)		202 (32.4)	

	N	Young-Age Group (Females <60 years old Males <55 years old)	N	Old-Age Group (Females ≥60 years old Males ≥55 years old)	p
Smoking	361		624		
Current		221 (61.2)		214 (34.3)	
Ex-smoker		52 (14.4)		147 (23.6)	**<0.001**
Never smoked		88 (24.4)		263 (42.1)	
History of obesity	361	152 (42.1)	624	266 (42.6)	0.873
BMI ≥30 kg/m²	94	28 (29.8)	158	25 (15.8)	**0.009**
History of hypertension	361	153 (42.4)	624	410 (65.7)	**<0.001**
Blood pressure ≥140/≥90 mmHg (≥130/≥80 mmHg in diabetics)	360	169 (46.9)	624	345 (55.3)	**0.012**
Total cholesterol, mg/dL	348	190 (165.5-217)	594	180 (155-213)	**0.002**
LDL-C, mg/dL	343	112 (91-135)	591	108 (86-133)	0.101
HDL-C, mg/dL	348	38 (33-46)	594	41 (34-49)	**0.006**
Non-HDL-C, mg/dL	348	151 (120.5-180)	594	139 (113-166)	**<0.001**
Triglyceride, mg/dL	348	173.5 (120.5-243.5)	594	138 (105-189)	**<0.001**
History of hyperlipidemia	361	183 (50.7)	624	308 (49.4)	0.687
High total cholesterol level (≥174 mg/dL)	348	233 (67.0)	594	337 (56.7)	**0.002**
High LDL-C level (≥100 mg/dL)	343	309 (90.1)	591	527 (89.2)	0.659
LDL-C level ≥70 mg/dL (not reaching the treatment goal)	343	225 (65.6)	591	359 (60.7)	0.140
Low HDL-C level (<40 mg/dL in males and <45 mg/dL in females)	348	196 (56.3)	594	283 (47.6)	**0.010**
High triglyceride level (≥150 mg/dL)	348	210 (60.3)	594	258 (43.4)	**<0.001**
Presence of diabetes mellitus	361	82 (22.7)	624	197 (31.6)	**0.003**
Family history of CAD	361	175 (48.5)	624	210 (33.7)	**<0.001**
Medication (at baseline)					
Statins	361	60 (16.6)	624	120 (19.2)	0.307
Fibrates	361	9 (2.5)	624	7 (1.1)	0.101
Ezetimibe	361	2 (0.6)	624	0 (0)	-
Aspirin	361	103 (28.5)	624	276 (44.2)	**<0.001**
Clopidogrel	361	22 (6.1)	624	43 (6.9)	0.627
Other anti-platelet agents	361	6 (1.7)	624	6 (1)	0.373
Warfarin	361	5 (1.4)	624	8 (1.3)	1.000
Beta blockers	361	84 (23.3)	622	198 (31.8)	**0.004**
Calcium channel blockers	361	24 (6.6)	622	92 (14.8)	**<0.001**
ARB/ACE-I	361	93 (25.8)	624	258 (41.3)	**<0.001**
Oral antidiabetic agents	361	42 (11.6)	624	117 (18.8)	**0.003**
Insulin	361	17 (4.7)	624	38 (6.1)	0.363
C-reactive protein level, mg/dL	40	1.24 (0.58-2.47)	45	0.68 (0.18-2.16)	0.096
Thyroid stimulating hormone level, mIU/L	49	1.23 (0.78-2.01)	80	1.28 (0.86-2.62)	0.662

The values are presented as median (Q1-Q3) or number (%), where appropriate.
ARB/ACE-I, angiotensin receptor blocker/angiotensin converting enzyme inhibitor; CAD, coronary artery disease; HDL, high-density lipoprotein; LDL, low density lipoprotein, UAP: Unstable angina pectoris, NSTEMI: Non-ST-elevation myocardial infarction, STEMI: ST-elevation myocardial infarction.

formation was obtained via phone calls in 46.6% of patients, from hospital medical records in 18.8% of patients, and by both in 34.6% of the patients. During the follow-up period, 50.6% of the patients experienced CV events and 8.1% (n=70) died. The cause of death was CV events in 51 patients; other causes of death included malignancy (n=10), renal failure (n=5), infections (n=3) and chronic obstructive pulmonary disease (n=1). The rates of CV events and death during the follow-up period were significantly higher in the old-age group (**Table 2**).

To determine the factors affecting the development of CV events during the follow-up period, a logistic regression analysis was performed by creating a model that included the variables of index event, age, hyperlipidaemia, hypertension, previous use of fibrates and previous use of beta blockers. Accordingly, the index event (STEMI or NSTEMI) and the presence of hypertension were found to be significant increasing risk factors. Being in the young-age group was a significant decreasing factor (**Table 3**). Cox regression analysis was performed to determine the factors affecting mortality during the follow-up period by creating a model including the following parameters: sex, age group, smoking, obesity, hypertension, diabetes mellitus, family history, use of angiotensin receptor blocker/angiotensin-converting enzyme inhibitor (ACE/ARB), use of insulin, in-hospital CV complications, hospital infections, LDL-C levels <70 mg/dL, non-HDL-C levels <130 mg/dL, and triglyceride levels ≥150 mg/dL. The significant associated factors for mortality were female sex, advanced age, in-hospital CV complications (**Table 4**).

Table 2 | In-hospital and post-discharge (follow-up) events according to the age.

	N	Young-Age Group (Females <60 years old Males <55 years old)	N	Old-Age Group (Females ≥60 years old Males ≥55 years old)	p
In-hospital					
Cardiovascular complications	327	24 (7.3)	574	57 (9.9)	0.191
Infection	327	11 (3.4)	574	37 (6.4)	**0.048**
Death	327	5 (1.5)	574	29 (5.1)	**0.008**
Follow-up					
Cardiovascular events	322	148 (46.0)	545	291 (53.4)	**0.034**
Death	322	13 (4.0)	545	57 (10.5)	**0.001**

Table 3 | Factors affecting the development of cardiovascular events during the follow-up period

	p	OR	95% CI
Index event (unstable angina pectoris) (Reference)	<0.001		
NSTEMI	**0.001**	1.858	1.30-2.65
STEMI	**<0.001**	2.349	1.71-3.23
Young age*	**0.006**	0.669	0.50-0.89
Presence of hypertension**	**0.047**	1.320	1.00-1.74

*<55 years in men and <60 years in women; **Blood pressure ≥140/≥90 mmHg (≥130/≥80 mmHg in diabetics).
CI, confidence interval; OR; Odds ratio; STEMI, ST-elevation myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction.

Table 4 | Factors affecting mortality during the follow-up period.

	p	OR	95% CI
Sex (Female)	**0.014**	3.061	1.25-7.48
Age group (<50 years) (Reference)	**<0.001**		
50-59 years	0.372	1.612	0.57-4.60
60-69 years	**0.036**	2.895	1.07-7.81
≥ 70 years	**<0.001**	6.452	2.45-17.01
Presence of obesity	0.051	1.606	1.00-2.59
Presence of hypertension	0.054	0.410	0.17-1.01
Presence of in-hospital cardiovascular complications	**0.042**	2.264	1.03-4.97
Presence of hypertension*Sex	**0.028**	3.359	1.14-9.92

CI, confidence interval; OR; Odds ratio.

Discussion

To the best of our knowledge, this retrospective cohort of 985 patients with ACS is the first study reporting the incidence of early ACS (36.6%) in Turkish patients. There are 3 large ACS registries in Turkey; the TUMAR study, the TURKMI study, and the Turkish arm of the EPICOR study [11–13]. The TUMAR registry enrolled 3,358 patients with a diagnosis of acute MI within 24 hours of symptom onset in the early years of 2000s. The Turkish arm of the EPICOR study, conducted in 2010 and 2011, reported data on 1,034 patients hospitalised for ACS within 24 hours of symptom onset who had a final diagnosis of UA, STEMI or NSTEMI and survived to discharge from 34 centers in Turkey [11]. TURKMI is a nationwide registry that was conducted to highlight the characteristics of patients admitted with acute MI within 48 hours of the onset of symptoms to the selected cardiology centers capable of PCI in Turkey [13]. The TURKMI study observed a 22.1% of early MI prevalence defined as < 50 years of age among patients with STEMI. The TUMAR study reported a 72% frequency of patients with early MI (defined as < 65 years of age with no sex criteria) and no further information was available on the clinical or laboratory data of the TUMAR population with early MI. The EPICOR study also did not provide information on the frequency and the clinical characteristics of patients with early ACS [11]. The only information on the frequency of early ACS for this population could be obtained from the EUROASPIRE studies, conducted in European countries, including Turkey. The incidence of young MI defined as an index event under the age of 50 years was overall 12% in the European countries as a whole, while it was 19% in the Turkish arm of the EUROASPIRE IV (78 centers from 24 countries) [4]. Moreover, the mean age of the study population was 64.0±9.6 years (with the lowest in Turkey [58.7±10.1 years] and the highest in Germany [67.4±8.9 years]). The mean age of the ACS patients in the TUMAR and EPICOR registries was 58±12 and 61.8±12 years, respectively. In the EUROASPIRE III study, the median age of CAD patients in Turkey was 60.5 years [14]. Likewise, in the present study, the median age of ACS patients was 60 years. All these data may suggest that people in Turkey suffer from coronary events at a younger age. This high rate of premature CV disease might be due to the overall high consanguinity rate (23.3%), which leads to increased genetic lipid and/or hereditary thrombotic disorders in Turkey [2,14].

In the present study, the most common index event in the young-age group was STEMI, whereas it was UAP in the old-age group. The proportion of smokers and those with a BMI ≥30 kg/m^2 was higher in the young-age group. However, hypertension (based on both history and blood pressure measurement) and diabetes mellitus were more commonly observed in the old-age group. A study from Japan reported similar results; 24.2% of 6,255 ACS patients were aged <60 years [15]. In this study, traditional CV risk factors such as history of MI, heart failure, diabetes mellitus, chronic kidney disease and hypertension were more prevalent among patients aged >60 years, whereas dyslipidemia, smoking and a family history of CAD were more prevalent among patients aged <60 years [15,16]. In a study from New Zealand, in which 1,199 patients presenting with MI were evaluated, 12.8% of patients were in the young-age group [16]. In this study, the rates of males, higher BMI, family history of premature CAD, and current smokers were reported to be higher in the young-age group than in the older age group. However, hypertension, dyslipidaemia and diabetes were observed less frequently in the young-age group.

Acute coronary syndromes occur approximately 10 years later in women than in men [17]. Hence, it has been reported that female ACS patients are generally older than male patients [17–20]. In a study from France, a 6.3% increase in ACS-related hospital visits was observed in women aged <65 years over a10-year period [21]. In the present study, distribution of age according to sex in ACS patients revealed that the rate of men was higher in young ages (<60 years), whereas the rate of women was higher in older ages (≥70 years), as expected.

Another important finding of this study is the higher prevalence of FH among young ACS patients. FH is an underdiagnosed condition that causes an increased risk of early CAD, and its prevalence in the general population has been reported to range from 0.01% to 0.58% [22,23]. The prevalence of FH among CAD patients has been reported to be higher, with a prevalence of potential FH (FH score ≥6) of 3.7%-8.3% [9,24]. The prevalence of FH has been reported to be 10-fold higher in patients hospitalised for ACS than in the general population [25]. The prevalence of FH in early ACS varies depending mainly on the cut-off age used for the definition of early onset; the lower the cut-off age, the higher the FH prevalence [2,25–32]. In the present study, FH prevalence was significantly higher in the young-age group than in old-age group (15.5% versus 3%). Our data are the first report of FH prevalence in an early ACS population from Turkey. In the Danish population, the prevalence of FH (probable+definite) is 6.9% among patients with premature MI (defined as MI before the age of 60 years) [29]. Another ACS cohort from Switzerland has shown a 4.8% prevalence of FH in patients with premature ACS [25,27]. In the present study, the rate of patients diagnosed with FH (possible+probable+definite) in ACS patients is 7.6%. The discrepancy between these early ACS/MI populations could also be due to inclusion criteria other than the defined age, i.e. in the EUROASPIRE IV study, as the patients were not enrolled consequently, a selection bias could exist [9].

The in-hospital mortality rate for ACS patients exceeds 5% [20]. In a large-scale study from China, the in-hospital mortality rate for ACS patients in two different hospitals was 2.5% and 3.6% [33]. A study from Japan reported higher mortality rates in older ACS patients (2.4% in those aged <60 years and 4.9% in those aged >60 years) [15]. In the present study, the in-hospital mortality rate was 3.8%, with higher rates in the older-age group than in the young-age group (5.1% vs. 1.5%).

In the present study, the frequency of CV events during a mean follow-up period of 30.4±8.4 months was 50.6% in patients discharged from hospital, while the mortality rate during the same period was 8.1%. The rates of CV events and death were higher in the old-age group than in the young-age group. During the follow-up period, the significant risk factors for CV events were the index event (STEMI or NSTEMI), the presence of hypertension and being in the young-age group was a negative risk factor. Moreover, the significant risk factors associated with mortality during the follow-up period were female sex, old age, and in-hospital CV complication.

Limitations of the Study

The retrospective nature of the study could be considered a limitation. However, retrospective data collection is valuable as it reflects the real-life setting. Nevertheless, physical signs of FH (xanthelasmas and xanthomas) and a detailed family history of high cholesterol levels and premature CAD, which are among the important diagnostic criteria of FH, could not be evaluated because of the retrospective design of this study. Therefore, the true prevalence of FH is likely underestimated. However, most of the previous studies harbor this limitation. On the other hand, the EUROASPIRE IV study, which demonstrated the prevalence of FH in ACS patients, was also a retrospective study in which the prevalence of FH was calculated using the same methodology as in the present study ([4,9]3,9). However,

in EUROASPIRE IV, patients were invited to participate in a face-to-face interview, which could also help to obtain more information on family history, etc. The lack of genetic analysis for the diagnosis of FH could also be regarded as a limitation.

Conclusion

In this retrospective ACS cohort from a single center in Turkey, a very high rate (36.6%) of early ACS was observed. Compared to older patients, young ACS patients were more likely to be smokers, more obese, less diabetic and less hypertensive. High total cholesterol levels, high triglyceride levels, high non-HDL cholesterol levels, low HDL-cholesterol levels, a family history of CAD and FH were also more frequently observed in the young ACS group. Both in-hospital and 2-year follow-up mortality rates were significantly lower in the old-age group.

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PCSK9 in extrahepatic tissues: What can we expect from its inhibition?

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Keywords
PCSK9;
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ABSTRACT

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an enzyme that belongs to the serine protease family and plays a key role in regulating low-density lipoprotein cholesterol (LDL-C) levels in the blood. PCSK9 binds to the LDL receptor (LDLR), targeting it for degradation, resulting in an increase in circulating LDL-C levels. Loss-of-function mutations in the PCSK9 gene are associated with lower LDL-C levels and lower cardiovascular risk; in contrast, gain-of-function mutations are a cause of familial hypercholesterolaemia. The identification of PCSK9 as a pharmacological target led to the development of inhibitors for the treatment of hypercholesterolaemia. To date, the monoclonal antibodies evolocumab and alirocumab (which target plasma PCSK9) and the small-interfering RNA inclisiran (which targets hepatic PCSK9 mRNA) have been approved for the treatment of hypercholesterolaemia. Although hepatic PCSK9 plays a central role in regulating plasma LDL-C levels, this protein is also expressed in other tissues, including the brain, pancreas, heart, kidney, intestine and adipose tissue. In extrahepatic tissues, the functions of PCSK9 are both dependent and independent of LDLR and not necessarily harmful. For this reason, it is essential to uncover any potentially harmful effects of therapies that inhibit PCSK9, beyond their known LDL-C-lowering and CV risk-reducing effects.

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Introduction

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an enzyme that belongs to the serine protease family and is an important regulator of low-density lipoprotein cholesterol (LDL-C) levels (1). PCSK9 binds to the LDL receptor (LDLR) and initiates endocytosis and subsequent lysosomal degradation of the LDLR, preventing the receptor from returning to the cell surface (1). This leads to an increase in circulating LDL-C levels. The *PCSK9* gene contains several polymorphisms, including gain-of-function and loss-of-function mutations, which significantly affect normal PCSK9 signalling and cholesterol metabolism (2). PCSK9 is predominantly expressed in the liver, which produces the bulk of circulating PCSK9, which in turn regulates plasma LDL-C levels.

The role of PCSK9 in determining plasma LDL-C levels and its association with cardiovascular disease has been suggested by the observation that loss-of-function mutations in the PCSK9 gene are associated with lower LDL-C levels and lower cardiovascular risk (3-6); in contrast, gain-of-function mutations have been identified as a cause of familial hypercholesterolaemia, with elevated LDL-C levels from birth and high cardiovascular risk (2, 7-11). Once PCSK9 was identified as a pharmacological target, research focused on the development of inhibitors to control hypercholesterolaemia. To date, two monoclonal antibodies (mAbs, evolocumab and alirocumab) targeting circulating PCSK9 and a small interfering ribonucleic acid (siRNA, inclisiran) targeting hepatic PCSK9 mRNA have been approved for the treatment of hypercholesterolaemia. Both approaches have

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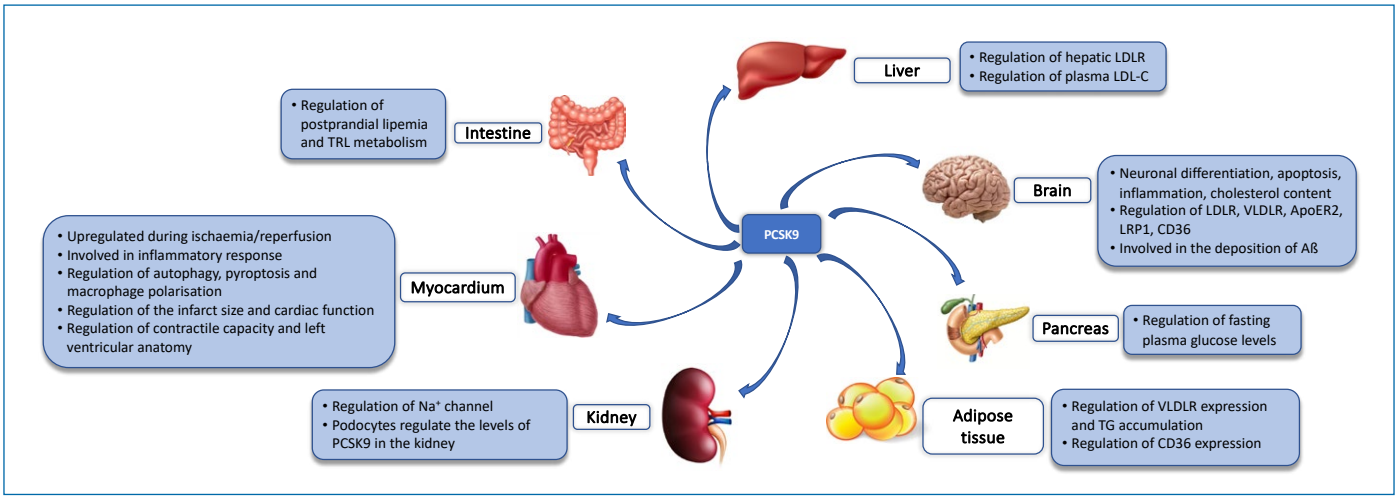

Figure 1 | Effects of PCSK9 in the liver and extra-hepatic tissues.

shown great efficacy in lowering LDL-C levels (~50-60% for mAbs and ~50% for siRNA); outcome trials have reported clinical benefit for both mAbs (12, 13); the ongoing ORION-4 is investigating the effect of inclisiran on clinical outcomes in patients with cardiovascular disease (NCT03705234).

Although PCSK9 has been studied primarily in the liver due to its important role in regulating plasma LDL-C levels, it is also expressed in other tissues, albeit to a lesser extent, such as the brain, pancreas, heart, kidneys, intestine, and adipose tissue. In these tissues, most of the effects exerted by PCSK9 are associated with metabolic pathways involving LDL-C, but relevant effects independent of LDLR metabolism have also been described (**Figure 1**). Based on this observation, questions have been raised about the possible effects of PCSK9 inhibitors in extrahepatic tissues. In this review, we aim to discuss the evidence available to date on this topic.

PCSK9 and the brain

PCSK9 was first discovered in neuronal cells undergoing apoptosis, and it appears to play a role in neuronal differentiation, cholesterol regulation, apoptosis, and inflammation in the brain (14). The brain is the most cholesterol-rich organ, but its cholesterol metabolism is uncoupled from peripheral tissues, as neither cholesterol nor PCSK9 can cross the blood-brain barrier under physiological conditions (15). In addition to the LDLR, PCSK9 regulates the levels of other receptors involved in the transport of cholesterol into neurons, including very low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2), as well as LDL receptor-related protein-1 (LRP1) and the scavenger receptor CD36 (16), which are highly expressed in the central nervous system (CNS). PCSK9 is present in cerebrospinal fluid at a low but constant level, in contrast to serum PCSK9 levels, which show large diurnal fluctuations (17), suggesting that the regulation of PCSK9 in cerebrospinal fluid may be different from that of PCSK9 in the bloodstream.

Although lowering LDL-C levels to very low levels is associated with clinical cardiovascular benefits, concerns have been raised about possible negative effects on cognitive function, as cholesterol is an essential component of myelin. This interaction between cholesterol homeostasis and cognition appears to be of particular importance in dementia.

Alzheimer's disease (AD), the most common cause of dementia, is a neurodegenerative disease characterised by continuous cognitive decline leading to poor quality of life (18). A body of evidence suggests that PCSK9 plays a role in AD, although our understanding of it is still incomplete. The accumulation of ß-amyloid (Aß) is a hallmark of AD; it arises from the amyloid precursor protein through the action of ß-site amyloid precursor protein-cleaving enzyme 1 (BACE1). Overexpression or inhibition of PCSK9 leads to decreased or increased expression of BACE1, respectively, and thus to higher or lower deposition of Aß (19). An indirect effect of PCSK9 has also been suggested, through the increase in systemic LDL-C levels, which may increase Aß deposition either by disturbing the balance of oxysterols (which can efficiently cross the blood-brain barrier) or by impairing Aß transport and degradation or by damaging the blood-brain barrier through an inflammatory process (20). Impaired Aß clearance in the brain promotes the onset and progression of AD. LRP1 and CD36 are two lipoprotein receptors that play a crucial role in Aß clearance by transporting Aß from the brain into the blood (21, 22). PCSK9 could therefore interfere with this clearance process by negatively regulating these receptors. In mice, mAbs against PCSK9 were able to reduce cerebral Aß burden, an effect that did not occur in mice lacking LRP1 (23). Another mechanism likely linking PCSK9 and AD is the induction of a pro-apoptotic effect in neuronal cells through the degradation of ApoER2 (24).

It is noteworthy that monoclonal antibodies, due to their size, cannot cross the intact blood-brain barrier under physiological conditions. However, under certain pathological conditions (such as ischaemic stroke) the permeability of the blood-brain barrier may be impaired. Short-term clinical trials with mAbs targeting PCSK9 found no association between very low LDL-C levels and cognitive impairment (13, 25-29). The EBBINGHAUS trial was specifically designed to investigate cognitive function in patients enrolled in FOURIER who received either evolocumab or placebo in addition to statins (25). No significant differences in cognitive function were observed between the two groups over a median of 19 months (25). In the open-label extension study (FOURIER-OLE), patients were followed up for a median of 5 years: there was no clear monotonic trend between a lower achieved LDL-C level and the risk of neurocognitive events (30). Genetic studies support the lack of association between PCSK9 inhibition and impaired cognitive function. PCSK9 LOF variants, which determine lifelong exposure to low LDL-C levels, were not found to be associated with neurocognitive abnormalities in blacks

participating in the REGARDS study (31). Mendelian randomisation analyses using data from a combined sample of ~740,000 participants showed no significant effects on cognition associated with genetic inhibition of PCSK9 (32), which was confirmed by another Mendelian randomisation analysis (33). In contrast, genetic HMGCR inhibition was associated with reduced cortical surface area, worsened reaction time, and impaired cognitive performance (32), which is consistent with the results of some studies on statins (34, 35). However, some studies have suggested a possible association between PCSK9 inhibition and cognitive impairment. The results of a Mendelian randomisation study have raised the possibility that exposure to PCSK9 inhibitors may predispose individuals to AD (36). A recent analysis of a large pharmacovigilance database found a disproportionality signal related to PCSK9 inhibitors (either as a class or as a single drug) and mental impairment (including memory impairment and amnesia) (37). However, it must be emphasised that this study was conducted with a database of spontaneous reports. Adequate long-term clinical trials could definitely shed more light on this topic.

PCSK9 and the pancreas

Cholesterol homeostasis appears to be essential for pancreatic ß-cell function (38). These cells express both LDLR and PCSK9, which may thus modulate LDLR expression and influence cell function. It is noteworthy that patients with familial hypercholesterolaemia who carry genetic defects leading to reduced LDLR expression/function are less likely to develop diabetes. On the other hand, statins have been shown to increase the risk of new-onset diabetes, especially in pre-diabetics or patients with established risk factors for diabetes (39), although the clinical benefit outweighs the risk. Based on its mechanism of action, PCSK9 inhibition has therefore been suspected of promoting the onset of diabetes, like statins.

PCSK9 loss-of-function variants have shown differential effects on glucose homeostasis, likely related to the genetic background of individuals and the type of the effect on PCSK9 (40). A Mendelian randomisation study showed that PCSK9 variants associated with lower LDL-C levels were also associated with higher fasting plasma glucose levels and increased risk of new-onset type 2 diabetes (41). This confirms a previous observation suggesting that exposure to LDL-C-lowering genetic variants is associated with a higher risk of type 2 diabetes (42). Variants in the *PCSK9* gene and variants in the *HMGCR* gene had approximately the same effect on diabetes risk per unit lower LDL-C level (43). However, pharmacological inhibition of PCSK9 does not appear to increase the risk of new-onset diabetes. A prespecified analysis of the FOURIER trial showed that evolocumab was effective in diabetic and non-diabetic patients and did not increase the risk of new-onset diabetes or worsen blood glucose levels during a median follow-up of 2.2 years (44). The FOURIER-OLE study showed that long-term LDL-C lowering with evolocumab was safe and well tolerated, and resulted in a further reduction in cardiovascular events compared with delayed treatment initiation (45). Interestingly, the rate of new-onset diabetes was not higher in patients who achieved very low LDL-C levels (<20 mg/dL) (30). A meta-analysis of 39 randomised clinical trials involving 66,748 patients treated with alirocumab or evolocumab showed that the use of these PCSK9 inhibitors was not associated with an increased risk of new-onset diabetes (27).

To better explore the potential impact of inhibiting PCSK9 on the development of diabetes, some studies have investigated the role of circulating versus locally produced PCSK9 in animal models. PCSK9 deficiency in mice has been shown to be associated with impaired glucose tolerance due to abnormalities in pancreatic islets, likely due to cholesterol overload of ß-cells and decreased pancreatic insulin secretion (46). However, this effect seems to be independent of circulating (liver-derived) PCSK9 but rather related to locally produced PCSK9 (46): liver-selective PCSK9 knockout mice, mimicking the condition of patients treated with a PCSK9 inhibitor, retain extrahepatic production of PCSK9 (in contrast to the condition of a loss-of-function mutation in the *PCSK9* gene, which affects all sites of production), with normal insulin production, LDLR expression and cholesterol levels in pancreatic islets (46). The ß-cell-specific knockout of PCSK9 resulted in unchanged circulating LDL-C levels with concomitant down-regulation of cholesterogenic genes, which should prevent cholesterol load and toxicity in ß-cells as well as alteration of glucose homeostasis (47). Of note, in this study PCSK9 was selectively inactivated only in mature ß-cells, resulting in residual PCSK9 expression in pancreatic islets (~30%) (47). Silencing PCSK9 expression in endocrine pancreas precursors and mature ß-cells and δ-cells resulted in a 90% reduction in PCSK9 expression in the pancreas (48). Circulating PCSK9 levels remained unchanged, but glucose intolerance was observed in mice due to defective insulin secretion (48). Increased LDLR expression and the resulting cholesterol accumulation were identified as the cause of the observed effect (48). Based on these observations, therapeutic inhibition of PCSK9 should not impair ß-cell function. Indeed, mAbs targeting PCSK9 act against circulating protein derived from the liver. Inclisiran, the siRNA approach that specifically targets PCSK9 mRNA in the liver (thanks to its structure that ensures specific recognition at the hepatic level), should not have diabetogenic properties.

PCSK9 and the heart

Adult differentiated cardiomyocytes constitutively express and release PCSK9 (49). PCSK9 expression is upregulated by inflammation and hypoxia (characteristic of an ischaemic heart) in cardiomyocytes (50, 51). Oxidised LDL, a marker of oxidative stress associated with reduced cardiac function, also increase the expression and release of PCSK9 (49), which appears to affect cardiomyocyte function in an autocrine manner, leading to reduced contraction and relaxation velocity, with the LOX-1 receptor being the most likely candidate to trigger the action of OxLDL (52). During ischaemia/reperfusion (such as acute myocardial infarction, MI), PCSK9 is also upregulated in immune cells, such as neutrophils, monocytes, and macrophages, which are immediately recruited to the ischaemic tissue. PCSK9 promotes the release of pro-inflammatory cytokines from macrophages, leading to a further reduction in cardiomyocyte viability and induction of cardiac cell apoptosis (51).

PCSK9 has been shown to be upregulated in the ischaemic heart of mice, with the zone adjacent to the infarcted areas showing the highest PCSK9 expression and intense autophagy activity, a self-degradation process activated during stress to promote cell survival and cardiac homeostasis (50). PCSK9 released by these cells contributes in determining the infarct size and cardiac function (50): mice lacking the PCSK9 gene or treated with a PCSK9 inhibitor showed better heart function and smaller infarct size (50). PCSK9 expression and release, as well as autophagy, were highest one week after the ischaemic event and then declined (50, 53). Analysis of heart sections from patients who had died of acute myocardial infarction showed PCSK9 and markers of autophagy being strongly expressed in the border zone between ischaemic and normal areas (50). It is likely that the release of PCSK9 early after the onset of myocardial ischaemia may be considered a protective response by stimulating autophagy, at least in the short term. It remains to be elucidated whether sustained PCSK9 release and autophagic response during hypoxia may have deleteri-

ous effects by inducing cell self-digestion and cell death. It is noteworthy that high expression of PCSK9 after myocardial infarction promotes pro-inflammatory M1 macrophage polarisation associated with poor myocardial repair, whereas PCSK9 deficiency promotes anti-inflammatory M2 macrophage polarisation and better protection against myocardial injury (54). Inhibition of PCSK9 has been shown to ameliorate myocardial injury after ischaemia/reperfusion by inhibiting autophagy and inflammation (55).

During acute cardiac ischaemia/reperfusion, high levels of circulating PCSK9 may trigger inflammatory and oxidative processes in ventricular cardiomyocytes, leading to cardiac dysfunction. Therefore, inhibition of PCSK9 could have a cardioprotective effect against ischaemia/reperfusion injury. Indeed, administration of a PCSK9 inhibitor prior to ischaemia resulted in a cardioprotective effect by inhibiting apoptosis, improving cardiac mitochondrial function, reducing infarct size and improving left ventricular function in rats (53, 56). When the PCSK9 inhibitor was administered during ischaemia or reperfusion, no benefits were observed (56). These results are not related to the lipid-lowering effect of the PCSK9 inhibitor, but rather to attenuated cardiac mitochondrial dysfunction and mitochondrial fission, and reduced apoptosis in the ischaemic myocardium (56). Another relevant observation is that PCSK9 regulates pyroptosis in cardiomyocytes during chronic myocardial ischaemia. Pyroptosis is a form of inflammatory programmed cell death that is closely associated with activation of the NLRP3 inflammasome (57). Both PCSK9 and pyroptosis-related proteins have been found to be highly expressed in the zone adjacent to the infarct (58). In *vitro*, PCSK9 activated the NLRP3 inflammasome and further enhanced pyroptosis in cardiomyocytes (58). Consistent with the results in mice, serum levels of PCSK9 and proteins related to pyroptosis were higher in patients with chronic myocardial ischaemia than in healthy subjects (58). Analysis of heart sections from patients who had died of acute MI showed that all these proteins were present in high concentrations in the zone adjacent to the infarcted area (58).

PCSK9 deficiency is associated with increased LDLR and CD36 expression in the heart, leading to lipid accumulation, and altered mitochondrial metabolism (59). These effects manifest as impaired cardiac function and heart failure with preserved ejection fraction (59). However, circulating PCSK9 does not affect cardiac metabolism: mice selectively lacking PCSK9 in the liver exhibit normal cardiac function (59). On the other hand, cardiomyocyte-specific deficiency of PCSK9 resulted in reduced contractile capacity, impaired cardiac function and left ventricular dilatation (60). Interestingly, individuals carrying a loss-of-function mutation in the *PCSK9* gene have increased epicardial fat accumulation and increased left ventricular mass index without alterations in the ejection fraction (59, 61). However, this finding could not be replicated in another study (62). Overall, these observations suggest that therapies targeting PCSK9 should not have negative effects on cardiac metabolism.

Accordingly, clinical trials have shown that therapy with the PCSK9 inhibitors evolocumab and alirocumab can significantly reduce the risk of myocardial infarction. The ODYSSEY OUTCOMES trial showed that alirocumab added to statin therapy reduced the overall incidence of MI, particularly the risk of type 1 (atherothrombotic, the most common form) and type 2 (myocardial oxygen supply-demand mismatch) MI (by 13% and 23%, respectively), but not type 4 (associated with percutaneous coronary intervention) MI (63). The benefit of alirocumab in reducing these types of MI was more pronounced when the increase in biomarkers (as a measure of infarct size), exceeded three times the upper normal limit (63). As an explanation for the effect on type 2 MI, the authors suggested that alirocumab may have improved myocardial oxygen supply (63). On the other hand, a prespecified analysis of the FOURIER trial showed that evolocumab significantly reduced the risk of the first MI by 27%; more specifically, type 1 MI was reduced by 32% and type 4 MI by 35%, while no effect was observed for type 2 MI (64). Evolocumab significantly reduced the risk of non-STEMI and STEMI (by 23% and 36%, respectively (64). A meta-analysis of data from 3 clinical trials of inclisiran failed to demonstrate difference in the risk of MI between patients randomised to inclisiran or placebo (65); the ongoing outcomes trial ORION-4 will shed light on this point.

A post-hoc analysis of the ODYSSEY OUTCOMES trial showed that, among post-ACS patients, alirocumab reduced the risk of MACE in patients without a history of heart failure, but not in patients with a history of heart failure, despite comparable reductions in LDL-C levels (66). In addition, there was a significant increase in non-fatal MI (66), and no effect of alirocumab on hospitalisations for HF, either overall or in the two subgroups (66). This finding is consistent with previous observations that statins do not reduce cardiovascular events in HF patients (67). This suggests the hypothesis that the clinical course of advanced heart failure does not appear to be influenced by anti-atherosclerotic therapies, as deterioration of myocardial function drives disease progression rather than atherosclerotic cardiovascular events (67). The limitations of this subgroup analysis do not allow any conclusions to be drawn, only the need to investigate the clinical efficacy of PCSK9 inhibitors in specific trials. The ongoing EVO-HF pilot trial is investigating whether evolocumab is effective in stable HF patients with reduced ejection fraction of ischaemic origin (NCT03791593).

PCSK9 and the kidney

Lipid and lipoprotein abnormalities are common features in patients with chronic kidney disease (CKD), in whom ASCVD is an important cause of mortality and morbidity. Each type of CKD has a typical phenotype, but overall, at least in the early stages, there are increased triglycerides, decreased high-density lipoproteins, and an excess of small, dense low-density lipoprotein particles. In the kidney, PCSK9 is involved in the regulation of the epithelial Na^+ channel (ENaC) by reducing its expression on the cell surface; this regulation appears to be independent of PCSK9 protease activity (68).

Podocytes (also known as visceral epithelial cells) are highly specialised cells lining the outer surface of the glomerular capillary. Dysfunction of these cells, as seen in patients with nephrotic syndrome, is associated with hypercholesterolaemia, mainly due to increased production and decreased clearance of apoB-containing lipoproteins. Studies in patients with kidney disease have shown that circulating PCSK9 levels are significantly increased compared to healthy subjects, but decrease during remission of the disease (69-72). This finding has also been confirmed in animal models of nephrotic syndrome (71). Injection of a nephrotoxic serum into C57BL/6J mice resulted in an increase in plasma PCSK9 and hypercholesterolaemia associated with a decrease in LDLR (71); this increase in PCSK9 is due to increased expression, increased secretion and decreased clearance (71). Interestingly, PCSK9 clearance was only reduced twofold after podocyte injury, and PCSK9 mRNA was generally not increased, suggesting a post-transcriptional mechanism by which damaged podocytes trigger a signal to the liver that leads to increased PCSK9 secretion (71). On the other hand, mice lacking PCSK9 show a reduced response to the treatment with nephrotoxic serum, with hypercholesterolaemia induced to a lesser extent, suggesting that multiple mechanisms are likely involved in the dyslipidaemia associated with nephrotic syndrome (71).

It is noteworthy that CKD patients receiving haemodialysis have

lower blood LDL-C and PCSK9 levels than healthy people, but those receiving statin therapy have comparable PCSK9 levels to healthy people (73).

Statins are widely prescribed to treat hypercholesterolaemia in patients with CKD. However, it must be emphasised that the clearance of most statins is affected by renal function, leading to excess of drug-drug interactions. In addition, statin therapy is effective in patients with mild-to-moderate CKD, while patients with advanced CKD benefit less (74-76). Evolocumab and alirocumab have been tested for efficacy in CKD patients and provided consistent results in both patients with preserved and impaired renal function (77). An analysis of the efficacy of evolocumab according to the renal function in the FOURIER trial showed that the reduction in LDL-C levels and relative risk reduction were similar for both primary and secondary endpoints in all stages of CKD (including patients with preserved function, stage 2 CKD and ≥stage 3 CKD) (78). The absolute risk reduction for the composite of cardiovascular death, MI, or stroke with evolocumab was numerically greater in patients with more advanced CKD (78). In an analysis of data from ODYSSEY phase 3 trials, alirocumab was shown to lower LDL-C levels independent of the presence or absence of impaired renal function (79). A pre-specified analysis of the ODYSSEY OUTCOMES trial of alirocumab found that alirocumab was effective in reducing LDL-C levels, major cardiovascular events and death across the range of renal function evaluated in patients with recent ACS and dyslipidaemia despite intensive statin therapy (80). However, it should be emphasised that eGFR <30 mL/min/1.73 m^2 was an exclusion criterion in this trial, and eGFR <20 mL/min/1.73 m^2 was an exclusion criterion in FOURIER. Based on this last observation, it is clear that further specifically designed trials are needed to assess whether therapy with mAbs against PCSK9 can have a negative impact on kidney disease and whether it is as effective in patients with advanced kidney disease.

PCSK9 and the intestine

The gut is involved in maintaining cholesterol homeostasis in the body through balanced metabolic cross-talk with the liver (81). PCSK9 is expressed in the small intestine of mice and humans (82). In mice, PCSK9 is expressed throughout the digestive tract and in the colon at levels similar to the liver. In the human intestine, PCSK9 is localised in the cytoplasm and accumulates in the subapical and basolateral compartments of the enterocytes. The cellular distribution of PCSK9 appears to be heterogeneous depending on the intestinal tract: in the duodenum, PCSK9 is expressed at both the apical and basolateral poles, whereas in the ileum it is mainly expressed at the apical pole. This heterogeneity is probably related to the function of PCSK9: in the upper part of the small intestine, PCSK9 is expressed at both poles of the enterocyte according to the absorption process and lipoprotein secretion, whereas in the ileum, which secretes less lipoproteins, PCSK9 is expressed only at the apical side. Furthermore, PCSK9 is not secreted from mature enterocytes in vitro and does not contribute to circulating PCSK9 levels (83), although this remains to be confirmed in vivo. PCSK9 appears to play a critical role in postprandial lipaemia. Indeed, PCSK9-deficient mice are protected from postprandial lipaemia (82). However, in mice, circulating PCSK9 rather than intestinal PCSK9 regulates postprandial lipaemia: both PCSK9-deficient and wild-type mice treated with alirocumab showed reduced postprandial lipaemia (via an LDLR-dependent pathway), an effect not observed in mice specifically lacking intestinal PCSK9 (84). Accordingly, subjects carrying PCSK9 loss-of-function variants had a more favourable lipid profile on fasting and attenuated levels of postprandial TG, apoB48, and total apoB (85), suggesting a role for PCSK9 in regulating TG-rich lipoproteins (TRLs) metabolism. In addition, treatment with evolocumab significantly reduced the postprandial lipaemic response to a mixed high-fat meal in type 2 diabetics, although the production and release of chylomicrons from intestine were not affected (86). Similarly, alirocumab reduced fasting plasma levels of TG and apoB48 and postprandial plasma response of TG and apoB48 in patients with type 2 diabetes on intensive insulin treatment (87). Of note, two studies found no effect of PCSK9 inhibition on the postprandial response in healthy normolipidaemic subjects (88, 89), suggesting that inhibition of PCSK9 may be of particular importance in diabetics who have increased production and impaired clearance of TRLs.

Treatment of human enterocytes with recombinant human PCSK9 markedly increased intestinal production and secretion of apoB and apoB48 by 50%; this effect was due to both an increase in apoB mRNA and an enhanced post-transcriptional apoB protein stability (90). Considering that there is one apoB molecule per TRL particle, this increase in TRL-apoB by PCSK9 suggests a potential doubling of the amount of pro-atherogenic TRL intestinal remnant particles in the circulation after a meal. LDLR expression in enterocytes was reduced by 50%, with concomitant increases in NPC1L1 protein levels, MTP protein levels and lipid transfer activity (90). Of note, treatment of enterocytes with PCSK9 siRNA reversed all observed effects (90). Accordingly, delivery of wild-type PCSK9 or a gain-of-function mutant to epithelial cells in the basolateral medium reduced LDLR at the basolateral membrane and caused marked perturbations in cholesterol homeostasis, including increased cholesterol uptake from the apical membrane via upregulation of NPC1L1, CD36 and ACAT2 and downregulation of HMG-CoAR activity (91).

In vivo, PCSK9 expression significantly reduced LDLR levels in the small intestine, but not in the large intestine in transgenic mice expressing human PCSK9 in multiple tissues (90); MTP expression and activity were increased in the small intestine (where chylomicrons are assembled), but were not detected in the large intestine (90). Similarly, plasma PCSK9 levels correlated positively with the pool size and production rate of intestinal TG-rich lipoproteins (containing apoB48), but not the fractional catabolic rate in men with varying degrees of insulin resistance (92). In addition, intestinal expression of the *PCSK9* gene was positively associated with genes involved in de novo cholesterol synthesis (*HMGCR* and *ACAT2*) and lipoprotein uptake (*LDLR*) (92).

Altogether, these observations suggest that pharmacological inhibition of PCSK9 may have a beneficial effect on postprandial lipaemia, an effect that may be particularly relevant in diabetic patients who have excessive postprandial lipaemia.

PCSK9 and the adipose tissue

Adipose tissue plays a central role in energy balance and storage, but is also involved in the metabolism of TG-rich lipoproteins (93). Adipose tissue also contains a very large pool of free cholesterol and promotes the transfer of cholesterol to HDL (94). Previous studies have shown that circulating PCSK9 regulates VLDLR expression and TG accumulation in visceral adipose tissue: $PCSK9^{-/-}$ mice exhibited significant visceral adipose tissue accumulation compared to wild-type mice, which was associated with adipocyte hypertrophy and increased fatty acid uptake as well as greater cell surface expression of VLDLR, with a mechanism being independent of LDLR (61, 95). Similar effects were observed following specific inactivation of hepatic PCSK9 in wild-type animals (95). In addition, carriers of the PCSK9 R46L loss-of-function variant had higher body mass index and increased percentage of total and android fat mass compared to

non-carriers (61). Circulating PCSK9 is also involved in the degradation of CD36, an important receptor participating in the metabolism of fatty acids and triglycerides in the liver and visceral adipose tissue: PCSK9$^{-/-}$ mice showed high expression of CD36 in adipose tissue, whereas adipocytes treated with PCSK9 showed a strong reduction in cell surface expression of CD36 (96).

PCSK9 is also abundantly expressed in the visceral adipose tissue (97). Of note, statins upregulate PCSK9 expression (98), an effect that has also been demonstrated in adipose tissue (99). PCSK9 expression in adipose tissue is positively correlated with body mass index in humans, suggesting that obesity and adiposity promote PCSK9 expression (97). Insulin and LDL upregulated the expression of PCSK9, LDLR, SREBP-1c and SREBP2 in human adipocytes, and atrial natriuretic peptide partially reversed these effects; this latter observation should be of interest in patients with obesity and hypertension (97). An analysis in overweight/obese individuals with normal LDL-C levels showed that individuals with lower than median plasma levels of PCSK9 had higher expression of LDLR in their white adipose tissue, accompanied by increased expression of CD36, IL-1ß secretion, postprandial hypertriglyceridaemia, lower white adipose tissue function and a lower disposition index, indicating a predisposition to type 2 diabetes (100). Increased LDL uptake is thought to impair adipocyte differentiation and consequently lead to white adipose tissue dysfunction (101), with a concomitant upregulation of MCP-1 expression that promotes the cross-talk between adipocytes and macrophages (100).

Among the various body fat depots, visceral epicardial adipose tissue (EAT) is a proxy of total visceral adiposity and a reliable marker of cardiovascular risk (102, 103). Under physiological conditions, it provides mechanical protection and also functions as an energy supplier, thermoregulator, and endocrine organ. However, under pathological conditions, EAT dysfunction can be detrimental and promote CVD progression (103). EAT is a source of PCSK9; local PCSK9 levels (but not plasma PCSK9 levels) correlated with EAT thickness and local inflammation (104); conversely, PCSK9 R46L carriers have higher EAT thickness compared to non-carriers (61). Further studies are needed to better define the role of PCSK9 in this specific tissue and the potential consequences of PCSK9 inhibition.

Conclusion

Since its discovery, PCSK9 has been shown to be a major determinant of circulating LDL-C levels through its main function in the liver, but also plays a key role in other tissues and organs. The rapid development and approval of PCSK9 inhibitors for the treatment of hypercholesterolaemia has paved the way for many unanswered questions related to the potential adverse effects of inhibiting PCSK9 in tissues and organs other than the liver. Many studies have attempted to answer these questions and provide evidence that inhibition of PCSK9 has not adverse effects, but many questions remain unresolved. Although PCSK9 inhibitors have been shown to be beneficial (the results for inclisiran are awaited), particularly in the context of cardiovascular health and metabolic disorders, the use of PCSK9 inhibitors could have effects on several other organs and tissues, particularly in the context of neurocognitive disorders, ß-cell function and diabetes, cardiac metabolism, heart failure and chronic kidney disease. To date, there is no evidence that PCSK9 inhibitors have negative effects on neurocognitive disorders or ß-cell function. In addition to the proven benefit for ASCVD, PCSK9 inhibition does not appear to negatively affect cardiac metabolism or have a negative impact on the clinical course of advanced heart failure. Monoclonal antibodies targeting circulating PCSK9 have been shown to be effective in reducing the risk of ASCVD in both patients with preserved and impaired renal function, although there are no specifically designed trials to assess their efficacy in patients with advanced kidney disease.

Results from experimental models are critical to understanding the underlying molecular mechanisms, but have limited translatability to humans. Similarly, observations in individuals carrying genetic variants of PCSK9 do not always translate to clinical trials of pharmacological inhibition of PCSK9, although they are critical in defining the precise role of this protein. Notwithstanding the role that PCSK9 inhibitors play in controlling hypercholesterolaemia, specific studies are needed to understand the long-term effects of PCSK9 inhibition.

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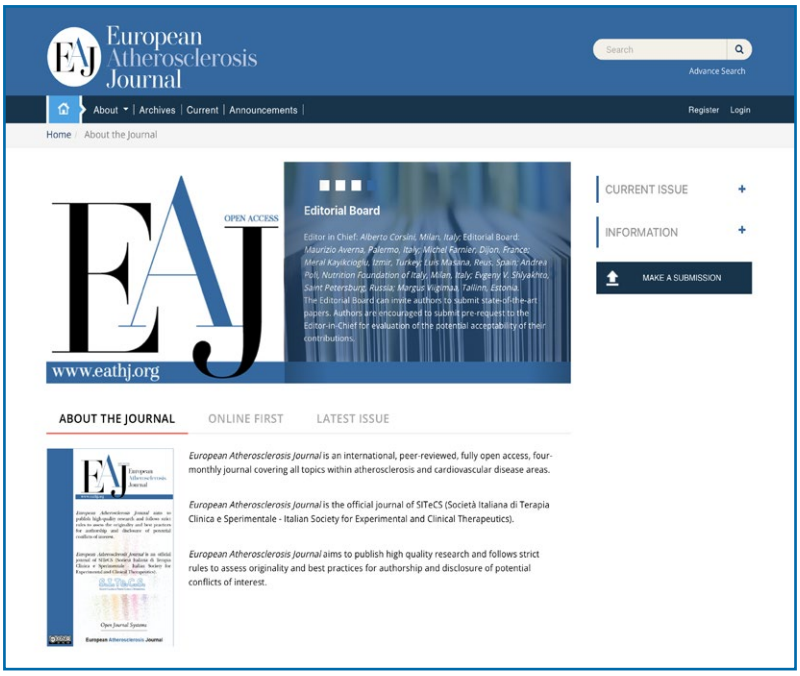

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