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High prevalence of major cardiovascular risk factors in first-degree relatives of individuals with familial premature coronary artery disease The GENECARD project

THESE

préparée sous la direction du Professeur Gérard Waeber
avec la collaboration du Docteur Vincent Mooser
et présentée à la Faculté de biologie et de médecine de l'Université de Lausanne
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par

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High prevalence of major cardiovascular risk factors in first-degree relatives of individuals with familial premature coronary artery disease - The GENECARD project

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Rapport de synthèse

La prévalence de l'hypertension artérielle, d'une dyslipidémie, d'une obésité et d'un tabagisme est élevée chez les patients qui souffrent d' une maladie coronarienne familiale précoce (MC-FP). Le but de cette étude fut d'investiguer la prévalence de ces facteurs de risque cardiovasculaires au sein des membres d'une famille dont un patient est affecté d'une MC-FP.

Nous avons étudié 108 familles différentes dont au minimum 2 frères/sœurs ont survécu à une maladie coronarienne précoce. Cette dernière fut définie par la survenue d'un événement coronarien avant l'âge de 51 ans pour les hommes et 56 ans pour les femmes. Au total, nous avons identifié 222 patients atteints de MC-FP chez qui 158 frères/ sœurs, 197 enfants et 94 époux/ épouses ne souffraient pas de maladie coronarienne. Ces parents proches furent comparés à un collectif d'individus « contrôles » issus de la population générale.

Les frères/sœurs non affectés avaient une prévalence plus élevée d'hypertension artérielle (49% versus 24%, $p<0.001$), d'hypercholestérolémie (47% versus 34%, $p=0.002$), d'obésité abdominale (35% versus 24%, $p=0.006$) et de tabagisme (39% versus 24%, $p=0.001$) par rapport aux individus issus de la population générale.
Parmi les enfants, une prévalence plus élevée d'hypertension artérielle fut identifiée chez les femmes, et une prévalence plus élevée d'hypercholestérolémie et d'obésité abdominale dans les deux sexes par rapport aux contrôles de la population générale.
Aucune différence parmi les facteurs de risque cardiovasculaire n'a été observée entre les époux/ épouses et les contrôles.
Les frères/ sœurs affectés et non affectés par la MC-FP ont également été comparés entre eux. La prévalence des facteurs de risque était similaire dans les 2 groupes, sauf pour le tabagisme, qui avait une prévalence plus élevée chez les frères/sœurs affectés (76% versus 39%, $p=0.008$).

La prévalence de l'hypertension artérielle, de l'obésité, et de la dyslipidémie est également élevée chez les parents de premier degré de patients atteints de MC-FP, mais pas chez leurs époux/épouses. Ces personnes-là requièrent donc une attention médicale particulière en raison d'une vulnérabilité familiale et/ou génétique augmentée aux anomalies métaboliques athérogènes. Dans ces familles, le tabagisme pourrait être le facteur déclenchant de la MC-FP.

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High prevalence of major cardiovascular risk factors in first-degree relatives of individuals with familial premature coronary artery disease—The GENECARD project

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Abstract

Background: Hypertension, hypercholesterolemia, obesity and smoking are highly prevalent among patients with familial premature coronary artery disease (FP-CAD). Whether these risk factors equally affect other family members remains unknown.
Methods: We examined 222 FP-CAD patients, 158 unaffected sibs, 197 offspring and 94 spouses in 108 FP-CAD families (≥2 sibs having survived CAD diagnosed before age 51 (M)/56 (F)), and compared them to population controls.
Results: Unaffected sibs had a higher prevalence of hypertension (49% versus 24%, $p<0.001$), hypercholesterolemia (47% versus 34%, $p=0.002$), abdominal obesity (35% versus 24%, $p=0.006$) and smoking (39% versus 24%, $p=0.001$) than population controls. Offspring had a higher prevalence of hypertension (females), hypercholesterolemia and abdominal obesity than population controls. No difference was observed between spouses and controls. Compared to unaffected sibs, FP-CAD affected sibs had a similar risk factor profile, except for smoking, which was more prevalent (76% versus 39%, $p=0.008$).
Conclusions: Hypertension, obesity and hypercholesterolemia are highly prevalent among first-degree relatives, but not spouses, of patients with FP-CAD. These persons deserve special medical attention due to their familial/genetic susceptibility to atherogenic metabolic abnormalities. In these families, smoking may be the trigger for FP-CAD.

Keywords: Coronary artery disease; Risk factors; Epidemiology; Genetics; Family; Population controls

1. Introduction

Coronary artery disease (CAD) has a familial component, and first-degree relatives of CAD patients are at higher risk for cardiovascular diseases than the general population [1–4]. Twin studies indicate that this familial component is partly genetic, and that the genetic contribution to CAD is mostly apparent in premature forms of the disease [5]. Mendelian forms of dyslipidemia, such as familial hypercholesterolemia [6] or rare genetic mutations like the one reported in the MEF2A gene [7], only account for a small fraction of premature CAD cases, so that genes contribut-

Abbreviations: BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; FP-CAD, familial premature coronary artery disease; OR, odds ratio

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ing to common forms of premature CAD remain to be identified.

GENECARD is a family-based study designed to identify susceptibility genes to familial premature CAD (FP-CAD). In GENECARD, a total of 850 sib-pairs who had survived premature CAD diagnosed before the age of 51 years for males and 56 years for females have been recruited in six different centres located in North America and Europe [8], including sib-pairs from 108 families from the Lausanne accrual centre in Western Switzerland [9]. Given the young age cut-offs and the fact that at least two sibs were affected in these families, it was anticipated that the genetic contribution to the disease should be particularly pronounced in the GENECARD collection.

Detailed analysis of the cardiovascular risk factor profile in FP-CAD affected sib-pairs recruited in Lausanne established that the prevalence of hypertension, hypercholesterolemia, obesity and smoking was approximately twice as high in these individuals as compared to the general population. Moreover, it revealed a strong concordance between affected sibs for these conditions, suggesting that FP-CAD is, at least partly, due to an increased familial, possibly genetic, susceptibility to atherogenic metabolic disorders, and to smoking [9]. Because these risk factors are remediable, their detection in apparently healthy family members was deemed necessary. Accordingly, we designed the present study to investigate the prevalence of major cardiovascular risk factors in first-degree relatives (i.e. sibs and offspring) and spouses of FP-CAD patients.

2. Methods

The design of the GENECARD project [8] and the way sib-pairs who had survived FP-CAD were ascertained in Lausanne, Switzerland, area for this project have previously been described [9]. Briefly, a total of 103 patients with one sib who had equally survived premature CAD, four patients with two affected sibs and one patient with three affected sibs were recruited in the Lausanne area, totalizing 222 (180 M/42 F) FP-CAD affected persons from 108 sibships.

These persons were asked permission to contact their spouse, their offspring aged $\geq$ 18 years and their sibs who did not meet the affection status for GENECARD, i.e. they did not meet clinical criteria for FP-CAD. These individuals were contacted and were asked to participate in the project after having been fully informed about the study. The protocol was approved by the local Ethics Committee. Participation rate for unaffected sibs and offspring was approximately 70%, the remaining individuals declining to participate mostly due to geographical reasons and, more rarely, for lack of interest in this project. Spouses were only asked to participate in the study if they were of Caucasian origin and if they had children eligible for the study (i.e. aged 18 or older). Participation rate for spouses was 79%. Participants were recruited during a visit by the investigators to their home where they were asked to fill in a detailed questionnaire and they underwent a limited physical examination. Blood pressure was measured in sitting position after a 10-min rest using the Intellisense automatic sphygmomanometer (OMRON, Steinhausen, Switzerland) which had been previously validated [10]. Blood was collected after a >12 h fast and was immediately put on ice and stored refrigerated until processed. Total cholesterol levels were measured in these samples using the enzymatic CHOD-PAP method (Sorachim, Le Mans, France). Participants in the present study were all recruited between 1999 and 2001.

Each participant within the 25–74 age range was matched to three individuals of same sex and age randomly selected among 3616 individuals who participated in two population-based health examination surveys conducted in Western Switzerland (Cantons Vaud and Fribourg) in 1988–1989 and 1992–1993 [11] within the framework of the international multi-center MONICA project [12]. An allowance for a limited difference in age (maximum 3 years) was introduced in the matching criteria in order to achieve a fixed case-control ratio of 1:3. In MONICA, blood pressure had been measured using a manual sphygmomanometer, and total cholesterol levels had been measured using the enzymatic method CHOD-PAP (Boehringer, Mannheim, Germany). To allow proper comparison of blood pressure measurements in the local MONICA and the present study, blood pressure was measured in 67 participants of the present study using both a manual and the Omron sphygmomanometer. A high correlation was observed between the two types of measurements for both systolic and diastolic blood pressure ($r^2 = 0.91$ and 0.78, respectively), and linear regression was used to adjust the MONICA pressure readings for differences in the measurement methods. Similarly, 30 samples were randomly drawn from the MONICA serum repository and re-assayed for total cholesterol using the same laboratory and method as in the GENECARD project. A $r^2 = 0.65$ correlation was observed between these values, and again a linear regression was used to adjust the MONICA total cholesterol values. Body mass index (BMI) were not used in the present comparison, as height was self-reported (i.e. not measured) in GENECARD. However, we used waist circumference as a marker for abdominal obesity, as this parameter had been measured similarly in both projects. The presence of diabetes had not been recorded in the MONICA survey, so this variable was not included in the present analysis.

FP-CAD affected sibs were included in part of the analyses. Because some of these individuals had substantially changed their habits after they had been diagnosed with FP-CAD (for example, 55% of those who were smokers when diagnosed with CAD had stopped smoking by the time they were ascertained in GENECARD), data recorded in medical records at the time the diagnosis was originally made were used in these analyses, as described previously [9].

A substantial proportion of participants and controls, mostly in the parental generation (sibs and spouses), were receiving antihypertensive or lipid-lowering drugs, limiting the use of continuous traits and prompting us to use cat-

egorical variables. Because blood pressure, waist circumference and plasma cholesterol levels increase with age, we used separate definitions for hypertension, abdominal obesity and hypercholesterolemia for the parental and the offspring generations. Hypertension in the parental generation was operationally defined by the presence of systolic and/or diastolic blood pressure $\geq$ 160/95 mmHg and/or prescription of antihypertensive agents whereas lower thresholds ($\geq$140/90 mmHg) were applied to the offspring generation. Similarly, plasma total cholesterol levels $\geq$ 6.5 mmol/L, or prescription of lipid lowering agents, were used to define hypercholesterolemia in the parental generation, whereas $\geq$5.2 mmol/L was used as a threshold for the offspring. Abdominal obesity was defined as waist circumference $\geq$102 cm for males and $\geq$88 cm for females in the parental generation, and $\geq$94 cm for males and $\geq$80 cm for females in the offspring generation. Because this value was frequently missing for FP-CAD affected sibs at the time of diagnosis, abdominal obesity was defined as BMI $\geq$ 25 kg/m^2 for this particular group. Smoking was defined for both generations as active cigarette smoking at the time of recruitment, or at the time of event for FP-CAD individuals, or within the 5 preceding years.

The GENECARD project was initiated and sponsored by GlaxoSmithKline, which funded the recruitment and the collection of data and samples from FP-CAD affected sib-pairs. GlaxoSmithKline was not involved in the recruitment of unaffected family members or in the design of the present study. The corresponding author of this manuscript (VM) joined GlaxoSmithKline as a full-time employee after the recruitment was completed and during the analysis of the data. Submission of this manuscript was approved by GlaxoSmithKline.

Data are expressed as mean $\pm$ S.D. Conditional logistic regression for case-control studies [13] was used to calculate the odds ratios (ORs) and their confidence intervals for the various cardiovascular risk factors. Concordance for the presence of risk factors in FP-CAD affected persons and their family members was examined using kappa tests. Statistical analyses were carried out using STATA (release 6.0, Stata Corp, College Station, TX) and SPSS (release 4.0, SPSS, Inc., Chicago, IL).

3. Results

A total of 222 FP-CAD affected sibs, 158 unaffected sibs, 94 spouses and 197 offspring of FP-CAD individuals were ascertained in the present study. A full description of these individuals and the pedigrees are provided in Appendix A. The clinical characteristics of the participants, as well as those of the age- and sex-matched controls from the local MONICA population, are described in Table 1.

3.1. Risk factor profile in unaffected sibs

Sibs of FP-CAD individuals had, compared to their respective population controls, a similar systolic, but a higher diastolic blood pressure. A larger proportion of these sibs received anti-hypertensive agents, so that blood pressure levels in these individuals were probably underestimated. Overall, the prevalence of hypertension was twice as high in unaffected sibs compared to their controls. Similarly, total cholesterol levels did not differ between the two groups. However, the proportion of sibs receiving lipid-lowering agents was significantly larger than for controls, so that the prevalence of hypercholesterolemia was higher in unaffected sibs. Moreover, these sibs had a higher waist circumference, and the proportion of individuals with abdominal obesity was significantly higher in this group. Finally, smoking was also more prevalent in sibs than in controls. Overall, the cumulative number of the four risk factors under consideration was significantly higher in sibs than in controls [1.7 $\pm$ 1.0 (S.D.) versus 1.1 $\pm$ 0.9, $p < 0.001$]. Conversely, the proportion of individuals with

Table 1
Clinical characteristics of the participants and their controls

	Unaffected sibs	Controls	p	Spouses	Controls	p	Offspring	Controls	p
n	158	474		94	282		197	591	
Gender, M/F (%M)	69/89 (44)	207/267 (44)	NS[a]	15/79 (16)	45/237 (16)	NS[a]	100/97 (51)	300/291 (51)	NS[a]
Age, years (S.D.)	54.5 (8.1)	54.5 (8.1)	NS[a]	55.6 (5.7)	55.7 (5.7)	NS[a]	32.3 (5.3)	32.3 (5.3)	NS[a]
Systolic blood pressure, mmHg (S.D.)	138.8 (23.4)	137.2 (20.1)	NS	132.3 (20.0)	137.7 (18.9)	0.023	122.2 (13.6)	123.8 (14.9)	0.164
Diastolic blood pressure, mmHg (S.D.)	86.6 (12.5)	82.5 (12.2)	<0.001	83.3 (11.7)	82.9 (12.1)	NS	79.1 (11.1)	75.9 (11.5)	<0.001
Antihypertensive treatment (%)	36 (23)	47 (10)	<0.001	20 (21)	34 (12)	0.043	2 (1)	6 (1)	NS
Hypertension (%)[b]	77 (49)	114 (24)	<0.001	31 (33)	76 (27)	NS	39 (20)	112 (19)	NS
Total cholesterol, mmol/L (S.D.)	5.69 (1.05)	5.69 (1.39)	NS	5.72 (0.93)	5.77 (1.39)	NS	5.21 (1.09)	4.76 (1.31)	<0.001
Lipid lowering therapy (%)	46 (29)	38 (8)	<0.001	7 (7)	23 (8)	NS	10 (5)	6 (1)	0.001
Hypercholesterolemia (%)[b]	74 (47)	161 (34)	0.002	24 (25)	99 (35)	0.091	99 (50)	207 (35)	<0.001
Waist circumference, cm	91.4 (12.1)	88.5 (10.2)	0.008	87.4 (14.3)	85.0 (9.6)	0.143	83.6 (11.4)	81.0 (9.0)	0.005
Abdominal obesity (%)[b]	55 (35)	114 (24)	0.006	31 (33)	90 (32)	NS	56 (28)	88 (15)	<0.001
Smoking (%)[b]	61 (39)	115 (24)	0.001	30 (32)	68 (24)	0.127	83 (42)	260 (44)	NS

NS, not significant, $p > 0.20$.
[a] Criteria used for matching.
[b] As operationally defined in Section 2.

Table 2
Concordance of cardiovascular risk factors between participants in the study

Risk factor	Agreement	Between FP-CAD affected sib and		
		Unaffected sib (202)[a]	Spouse (69)[a]	Child (134)[a]
Hypertension	Observed agreement (%)	57.9	53.6	57.5
	Expected agreement (%)[b]	50.4	51.5	52.5
	Kappa coefficient (*p*)	0.15 (0.015)	0.04 (0.35)	0.11 (0.082)
Hypercholesterolemia	Observed agreement (%)	60.9	43.5	62.7
	Expected agreement (%)[b]	50.1	43.0	49.7
	Kappa coefficient (*p*)	0.22 (0.001)	0.01 (0.46)	0.26 (0.001)
Abdominal obesity	Observed agreement (%)	45.1	49.2	47.8
	Expected agreement (%)[b]	43.2	44.1	42.1
	Kappa coefficient (*p*)	0.03 (0.26)	0.09 (0.16)	0.10 (0.06)
Smoking	Observed agreement (%)	49.5	58.0	50.0
	Expected agreement (%)[b]	45.4	44.6	46.7
	Kappa coefficient (*p*)	0.08 (0.10)	0.24 (0.006)	0.06 (0.22)

[a] Number of pairs.
[b] Expected agreement obtained when assuming independence of risk factor distribution between FP-CAD affected sib and their family members. This analysis was restricted to family members of FP-CAD individuals for whom the cardiovascular risk profile was known at event.

none of these conditions was twice as low among sibs (15% versus 31%, $p<0.001$). Using conditional logistic regression analysis, the risk was significantly increased for unaffected sibs compared to population controls of having hypertension [OR 3.6 (95% CI 2.3–5.4)], hypercholesterolemia [1.8 (1.3–2.6)], abdominal obesity [1.6 (1.2–2.6)] and smoking [2.0 (1.4–3.0)]. These ORs were similar to those previously observed when comparing FP-CAD individuals to matched controls from the general population for hypertension [4.6 (3.0–7.0), $p=0.18$] or hypercholesterolemia [2.7 (1.9–3.9), $p=0.09$]. In contrast, the OR for smoking was significantly higher for FP-CAD individuals [4.2 (2.8–6.3)] than for their unaffected sibs ($p=0.008$). Direct comparison of ORs was not possible for obesity because of different methods of measurement (see Section 2).

To further explore the architecture of cardiovascular risk factors in these families, we next examined the concordance of major risk factors between FP-CAD affected patients, their unaffected sibs, their offspring and their spouses using kappa tests (Table 2). In this analysis, the agreement observed between FP-CAD patients and their sibs was higher than expected for hypertension and hypercholesterolemia, but was similar to the expected one for abdominal obesity and smoking.

The difference in the prevalence of risk factors between unaffected sibs and their controls was partly age-dependent

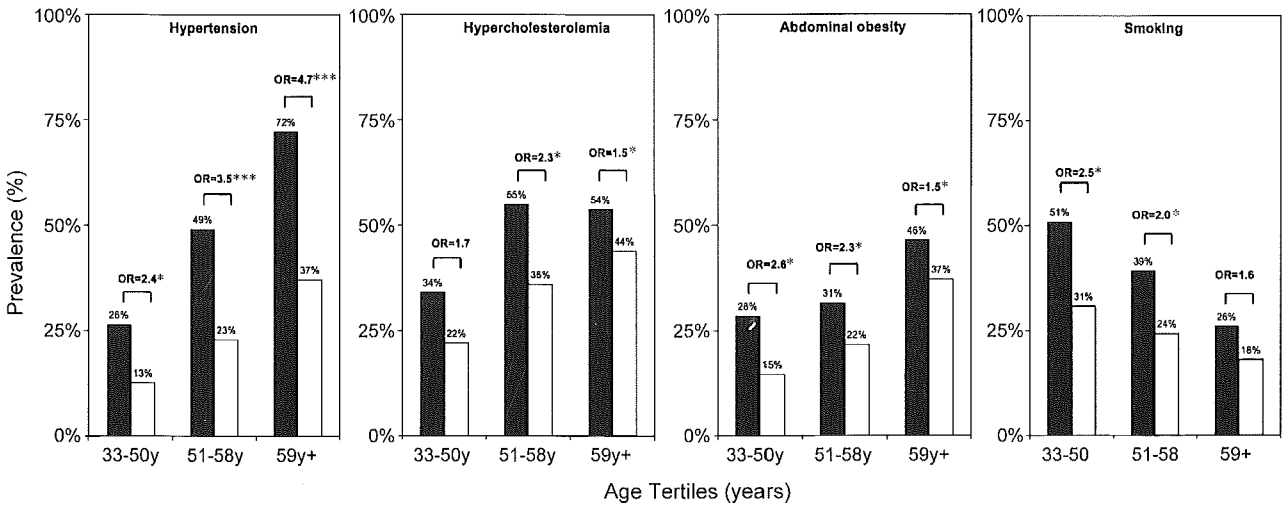


Fig. 1. Prevalence rates and odds ratios (OR) for hypertension, hypercholesterolemia, abdominal obesity and smoking among unaffected sibs of FP-CAD patients (solid bars) and their age- and gender-matched population controls (open bars), according to age tertile. Hypertension, hypercholesterolemia and abdominal obesity were defined as described in Section 2. Total number of unaffected sibs and controls was 158 and 474, respectively. Number on top of columns indicate OR. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. population controls.

(Fig. 1). The prevalence of hypertension was approximately two-fold higher in unaffected sibs than in the general population for the three age tertiles. In contrast, the difference between unaffected sibs and controls was only significant in the second and third age tertiles for hypercholesterolemia, and in the first and second tertiles for smoking. Abdominal obesity was more prevalent in unaffected sibs than in controls for the three age tertiles, but the difference was particularly striking for the first tertile.

3.2. Risk factor profile in spouses

Because FP-CAD is much more frequent in men, most spouses were females. Systolic blood pressure was lower in spouses than in their respective controls, but this difference may have been accounted for by the higher proportion of spouses who were treated for high blood pressure, so that the overall prevalence of hypertension was similar in spouses and in population controls (Table 1). Plasma levels of total cholesterol and waist circumference were similar in the two groups, and so was the prevalence of hypercholesterolemia, abdominal obesity and smoking. However, the prevalence of smoking was higher in individuals whose FP-CAD spouse was a smoker than in their respective controls [18/44 (42%) versus 23/132 (17%), OR 3.0 (1.5–6.4)]. These observations were reinforced by concordance analysis (Table 2). In this analysis, the concordance observed between FP-CAD patients and their spouses was similar to the one expected for hypertension, hypercholesterolemia and abdominal obesity, but was higher than expected for smoking.

3.3. Risk factor profile in offspring

The average age of the offspring was 32.3 ± 5.3 years (Table 1). Offspring presented a similar systolic, but a higher diastolic blood pressure than their population controls. The prevalence of blood pressure $\geq$ 160/95 mmHg was low in the offspring group and their controls (8% versus 6%, NS), and no difference was observed either when a lower threshold (140/90 mmHg) was used to define hypertension in this generation. When offspring were stratified based on gender, however, daughters, but not sons, had a higher prevalence of hypertension than their controls [9/48 (19%) versus 3/144 (2%), OR 9.0 (2.4–33.2)]. Plasma total cholesterol levels were higher in offspring than in controls. Overall, 12% of offspring and 10% of controls had plasma total cholesterol levels $\geq$ 6.5 mmol/L (NS). However, when using 5.2 mmol/L as a threshold to define hypercholesterolemia in this generation, the prevalence of hypercholesterolemia was significantly higher in offspring. Similarly, waist circumference was higher in offspring, and the proportion of offspring with abdominal obesity was twice as high as the one observed among controls. Smoking was equally prevalent in both groups. Overall, the intra-individual sum of risk factors was 1.9 ± 1.1 in offspring and 1.6 ± 1.1 in controls ($p < 0.001$).

In an attempt to identify which is the first detectable risk factor that differs between offspring of FP-CAD individuals and the general population, we stratified the offspring based on age tertile (Fig. 2). The prevalence of hypertension and smoking was similar between offspring and controls for the three age groups. In contrast, the prevalence of hypercholesterolemia and abdominal obesity was significantly higher in the three age groups.

We next examined the transmission of the risk factors from parents to offspring. A total of 121 offspring had both parents recruited in the present study. The risk of being hypertensive, hypercholesterolemic or smoker was not increased if none of the parents were carriers. In contrast, if both parents suffered from hypertension, hypercholesterolemia or obesity, the risk for the offspring was markedly increased. No such

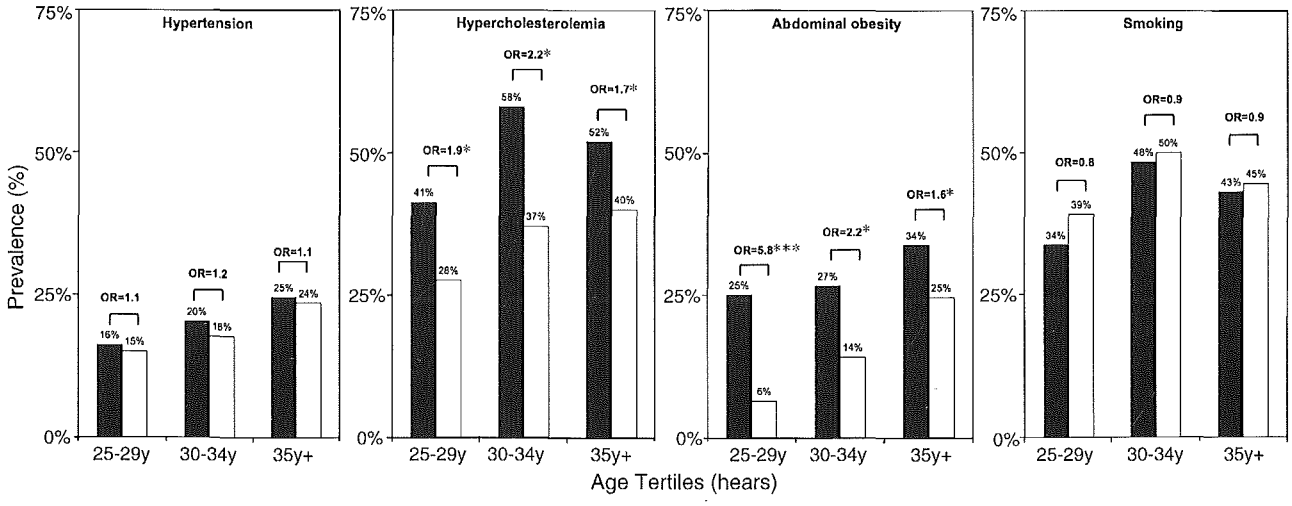


Fig. 2. Prevalence rates and odds ratios (OR) for hypertension, hypercholesterolemia, abdominal obesity and smoking among offspring of FP-CAD patients (solid bars) and their age- and gender-matched population controls (open bars), according to age tertile. Hypertension, hypercholesterolemia and abdominal obesity were defined as described in Section 2. Total number of offspring and population controls was 197 and 591, respectively. Number on top of columns indicate OR. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. population controls.

trend was observed for smoking, though (data not shown). In concordance analysis (Table 2), a strong and a borderline concordance was detected between FP-CAD affected parent and offspring for hypercholesterolemia and abdominal obesity, respectively. Overall, the concordance observed for hypertension did not reach significance. However, after stratification by gender, it appeared that the concordance was much higher for parent-daughters (kappa = 0.18, $p = 0.028$) than for parent-sons (kappa = 0.07, $p = 0.297$). No concordance was observed for smoking.

4. Discussion

Using a matching procedure with a reference population, we have previously shown that hypertension, hypercholesterolemia, obesity and smoking are highly prevalent among patients diagnosed with FP-CAD [9]. In the present study, we applied the same approach to investigate their first-degree relatives and spouses. We demonstrate that the prevalence of these four risk factors is equally high among unaffected sibs. We also show that the risk for hypercholesterolemia and hypertension was similarly elevated among FP-CAD patients and their unaffected sibs, but the prevalence of smoking was significantly higher in the former group. Moreover, we show that offspring, but not spouses, of FP-CAD patients are at higher risk for hypercholesterolemia, abdominal obesity and hypertension (for daughters) than the general population. In addition, we have detected a significant concordance for hypertension and hypercholesterolemia, but not smoking, between FP-CAD affected individuals and their first-degree relatives. Reciprocally, the only factor that was concordant between FP-CAD patients and their spouses was smoking.

Altogether, these data are indicative of the presence of a strong and complex familial, in part genetic, susceptibility to abdominal obesity, hypertension and hypercholesterolemia in families with FP-CAD. In the presence of smoking, which in these families does not seem to be genetically determined as concordance was only observed between spouses, these conditions may then lead to the clinical manifestation of the disease (i.e. CAD).

This data has immediate relevance for the prevention and clinical management of FP-CAD patients and their family members, and for our efforts in identifying the genetic basis of FP-CAD. Indeed, the data demonstrates that first-degree relatives of FP-CAD affected individuals are themselves at higher risk for CAD than the general population. The risk may be particularly high, as a positive family history may even amplify the risk associated with the conditions examined here [4]. Importantly enough, the four conditions under consideration are remediable, and interventions to correct them have been shown to protect patients from the development of CAD. Accordingly, proper detection, evaluation and management of major cardiovascular risk factors is warranted in persons who have two sibs known to have premature CAD, and intervention should be particularly aggressive considering the fact that siblings of patients with CAD are frequently overlooked in primary prevention of CAD [15]. Moreover, similar interventions are needed for individuals who have one parent and one uncle/aunt diagnosed with premature CAD. Considering the fact that these offspring already have an increased waist circumference, and the association between obesity and coronary lesions in the youth [16], indications for interventions should be extensive in this group. Conversely, our data does not indicate that spouses of FP-CAD affected individuals are at higher risk for CAD than the general population, unless they smoke, obviously, which they are at higher risk for if their affected spouse is himself/herself a smoker.

From a genetic perspective, this study indicates that a major contribution of genes acting independently from traditional risk factors to trigger the development of FP-CAD is unlikely. This does not rule out, however, the presence of rare gene variants responsible for the development of CAD in selected families, such as the MEF2A gene [7]. Moreover, it is conceivable that some susceptibility genes for FP-CAD develop their potential to trigger the occurrence of the disease in the presence of traditional risk factors. A series of linkage peaks, including peaks on chromosomes 1, 3, 5 and 7 identified in a recent whole genome scan performed on the GENECARD collection [14], supports this hypothesis. Conversely, it is also possible that unaffected sibs carry 'protective' genes that prevent the occurrence of CAD in presence of atherogenic metabolic abnormalities.

This study has some limitations. One is that only families of individuals who had survived FP-CAD were recruited, so that the data may be specific to less deadly forms of CAD. Another limitation is the fact that blood pressure and plasma total cholesterol levels had not been measured using the same methods in GENECARD and MONICA, and that appropriate corrections had to be made to the MONICA data. Finally, one cannot formally rule out a secular trend, as the MONICA measures used for controls had been taken between 1988 and 1993, whereas the GENECARD families were recruited between 1999 and 2001. In this respect, the fact that spouses had a very similar risk factor profile as the general population is very reassuring.

In conclusion, this data demonstrates a high prevalence of hypertension, hypercholesterolemia, obesity and smoking in families with FP-CAD, and the importance of a proper detection and management of these remediable conditions. Avoiding smoking may be particularly beneficial as smoking may trigger the clinical expression of CAD in these susceptible families.

Acknowledgements

This manuscript is dedicated to the memory of our friend and colleague Vincent Wietlisbach, who made a major contribution to this project, and who passed away during the final steps of manuscript preparation. The authors are very grateful to the participants in the study. They also want to thank Alice

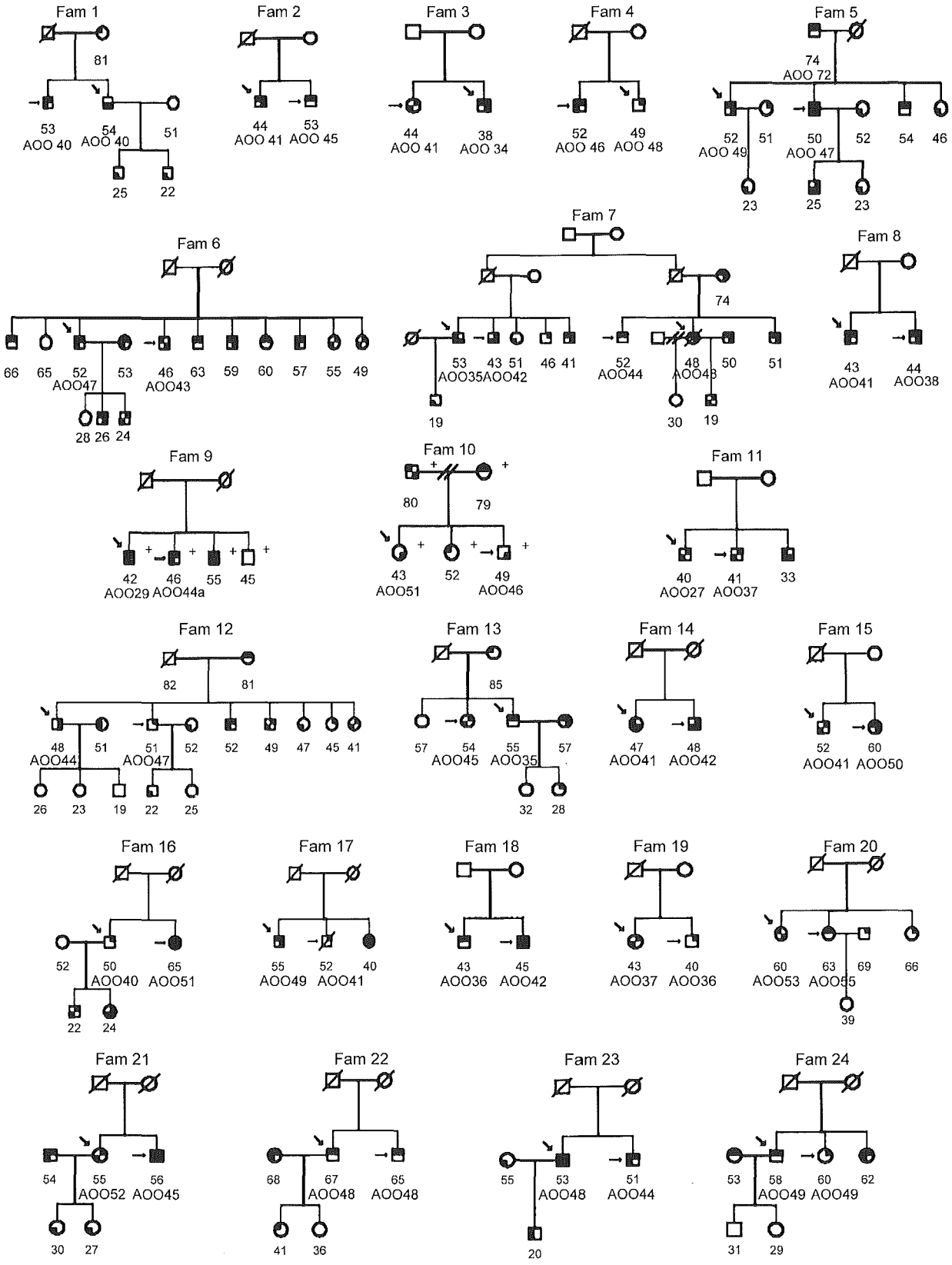


Fig. A1. Complete description of the 108 families (Fam) who were included in the present data set. Men are depicted by squares, females by circles and age at the time of recruitment (in years) is given below symbols. Deceased parents are depicted using crossed symbols. Closed upper left corner indicates hypertension, upper right corner hypercholesterolemia, lower left corner smoking and lower right corner abdominal obesity, as defined in Section 2. Arrows indicate FP-CAD affected sibs. AOO denotes age of onset for FP-CAD affected sibs. Genders have been scrambled to protect privacy.

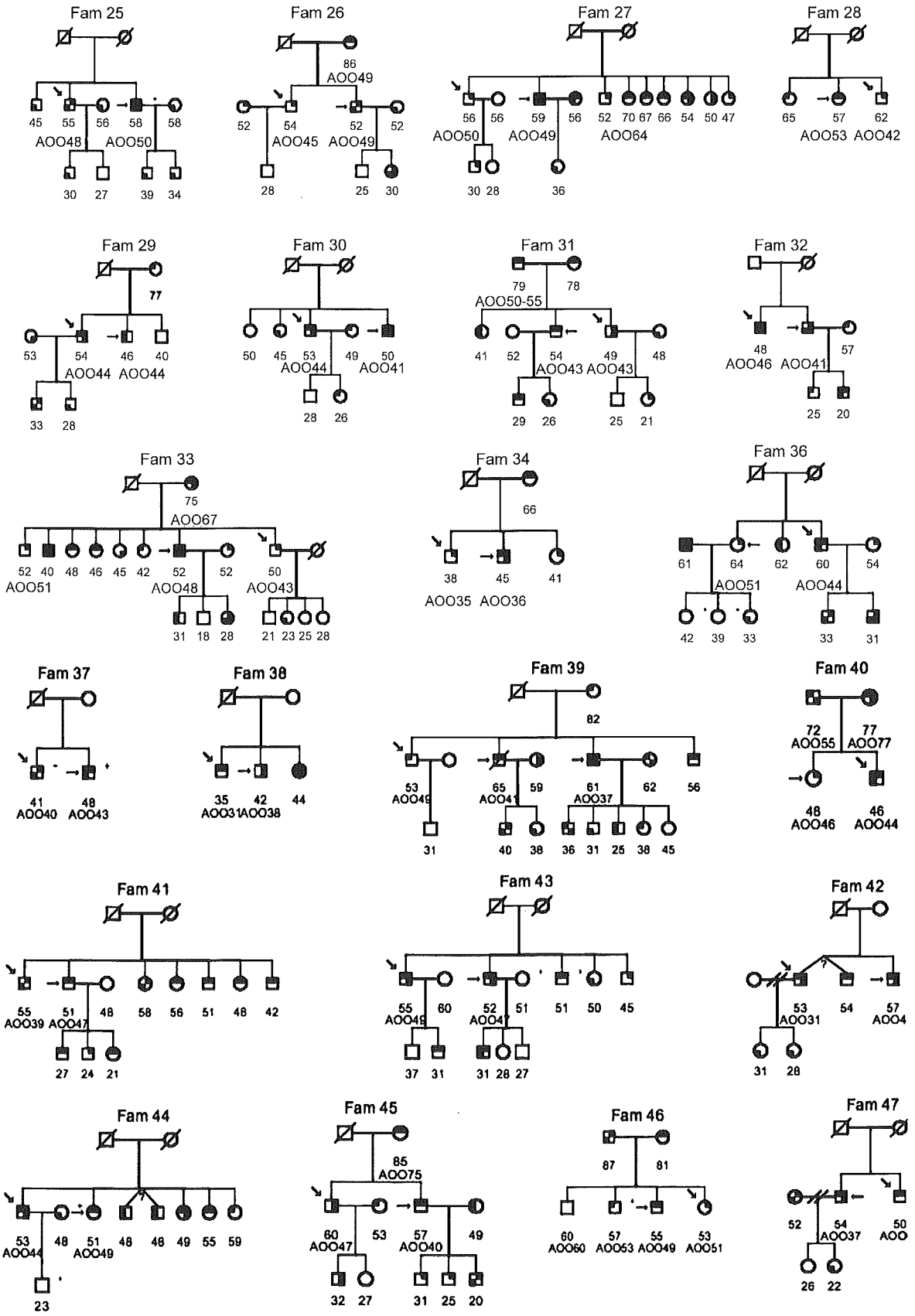
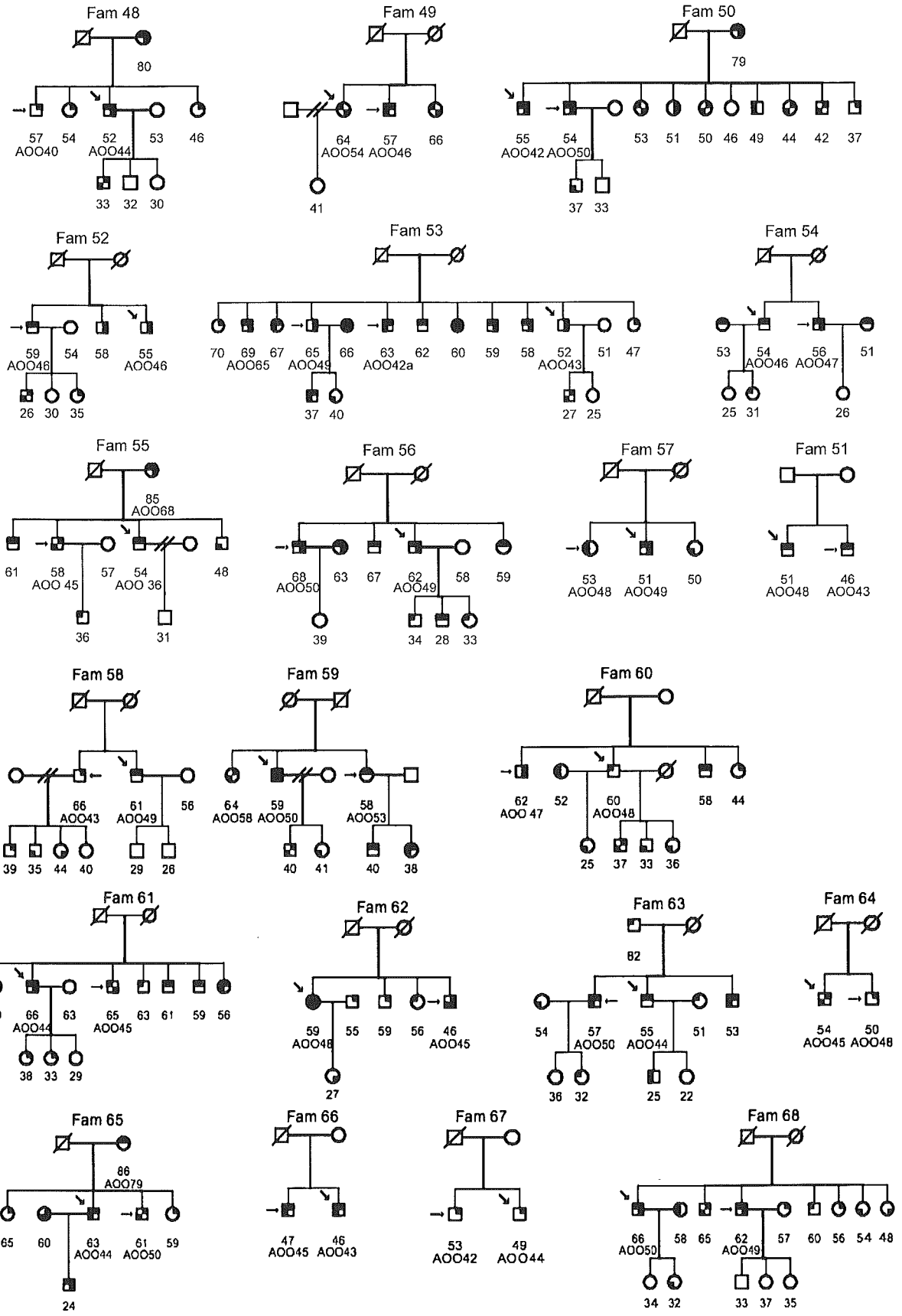


Fig. A1. (*Continued*).



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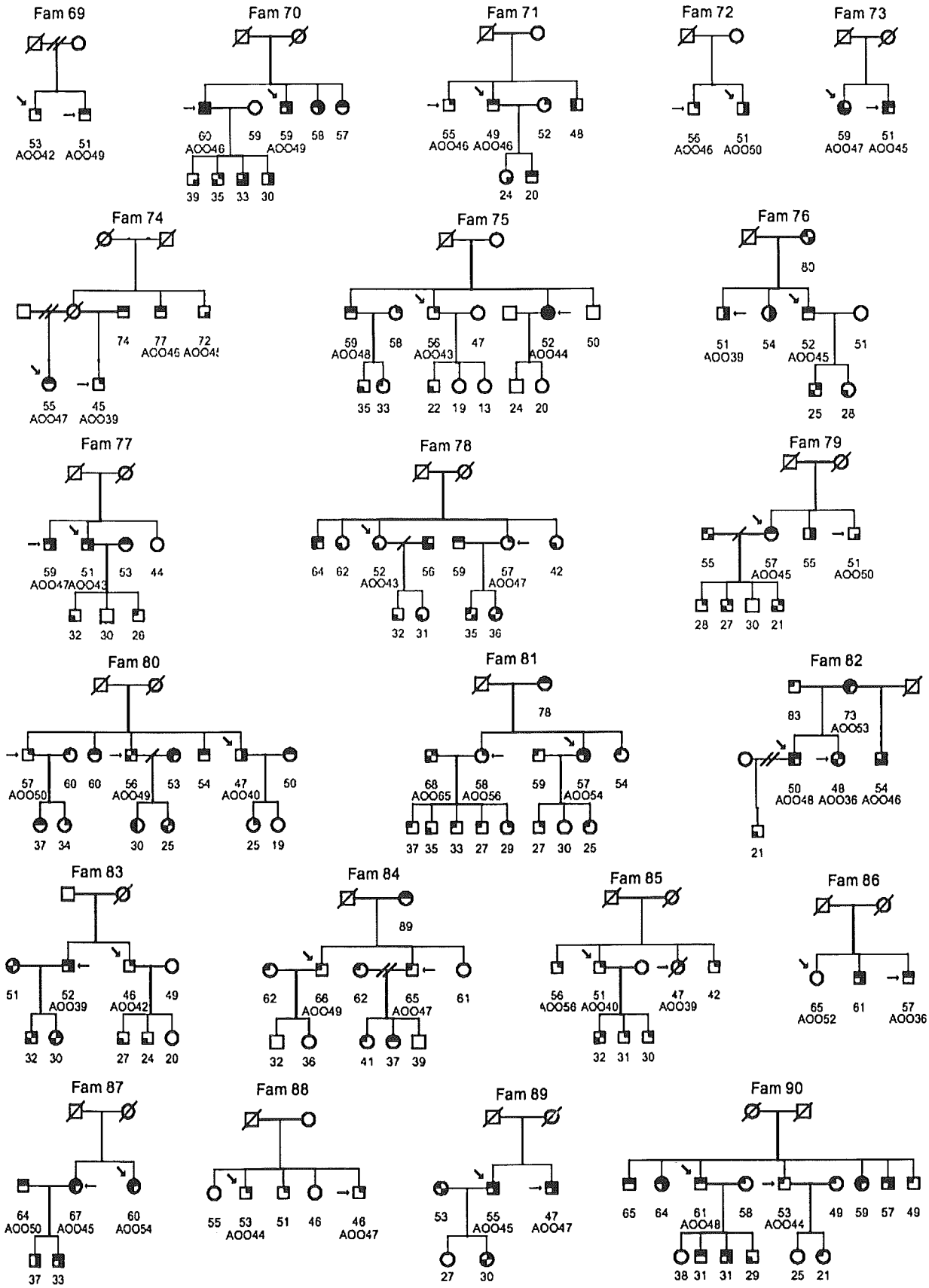

Fig. A1. (*Continued*).

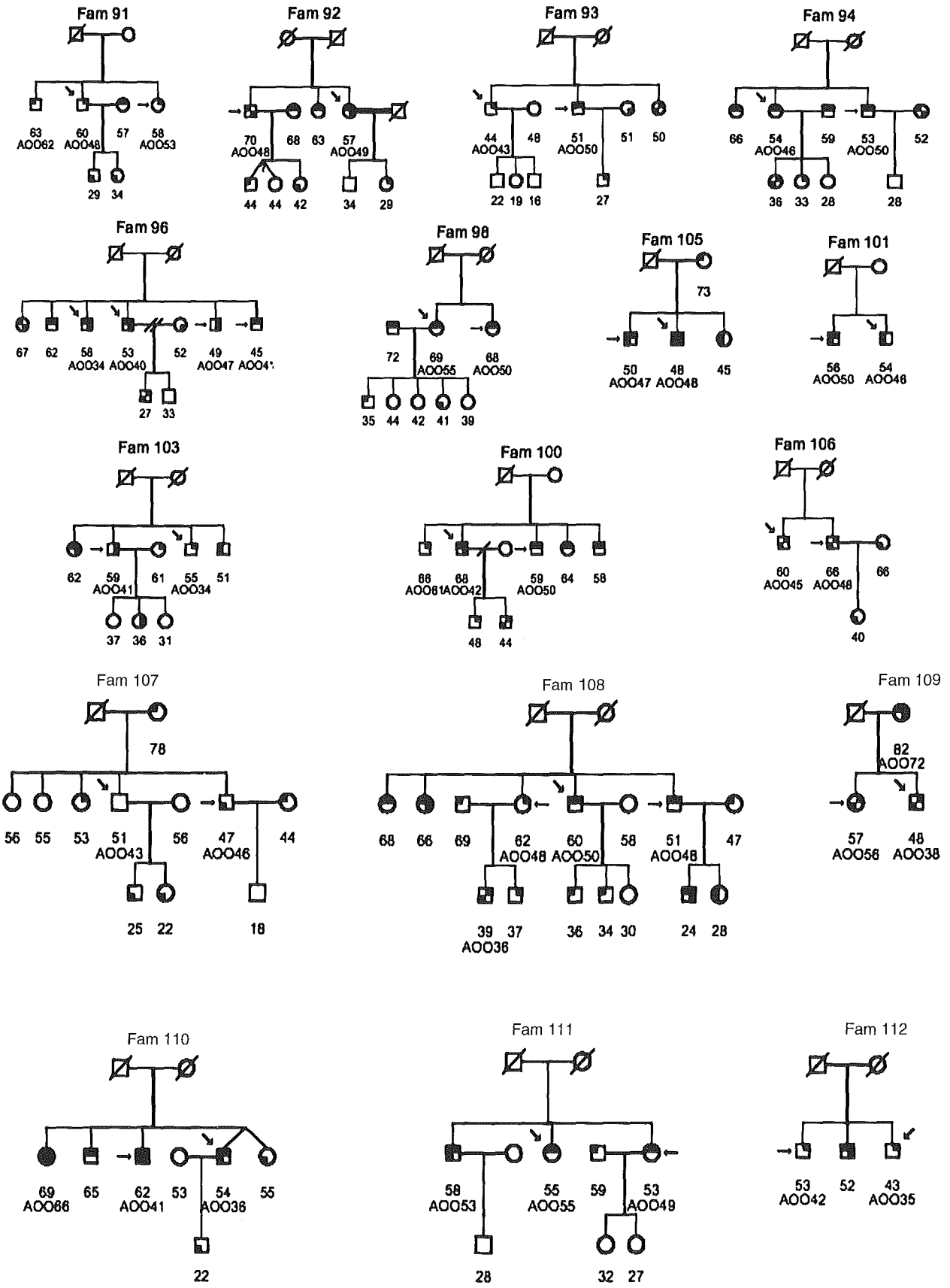

Fig. A1. (*Continued*).

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Appendix A

Complete description of the 108 families (Fam) who were included in the present data set are shown in Fig. A1.

References

[1] Williams RR, Hunt SC, Heiss G, et al. Usefulness of cardiovascular family history data for population-based preventive medicine and medical research (the Health Family Tree Study and the NHLBI Family Heart Study). Am J Cardiol 2001;87:129–35.
[2] Sesso HD, Lee IM, Gaziano JM, et al. Maternal and paternal history of myocardial infarction and risk of cardiovascular disease in men and women. Circulation 2001;104:393–8.
[3] Schildkraut JM, Myers RH, Cupples LA, Kiely DK, Kannel WB. Coronary risk associated with age and sex of parental heart disease in the Framingham Study. Am J Cardiol 1989;64:555–9.
[4] Jousilahti P, Puska P, Vartiainen E, Pekkanen J, Tuomilehto J. Parental history of premature coronary heart disease: an independent risk factor of myocardial infarction. J Clin Epidemiol 1996;49:497–503.
[5] Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. N Engl J Med 1994;330:1041–6.
[6] Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. J Clin Invest 1973;52:1544–68.
[7] Wang L, Fan C, Topol SE, Topol EJ, Wang Q. Mutation of MEF2A in an inherited disorder with features of coronary artery disease. Science 2003;302:1578–81.
[8] Hauser ER, Mooser V, Crossman DC, et al. Design of the Genetics of Early Onset Cardiovascular Disease (GENECARD) study. Am Heart J 2003;145:602–13.
[9] Jomini V, Oppliger-Pasquali S, Wietlisbach V, et al. Contribution of major cardiovascular risk factors to familial premature coronary artery disease: the GENECARD project. J Am Coll Cardiol 2002;40:676–84.
[10] Anwar YA, Giacco S, McCabe EJ, Tendler BE, White WB. Evaluation of the efficacy of the Omron HEM-737 IntelliSense device for use on adults according to the recommendations of the Association for the Advancement of Medical Instrumentation. Blood Press Monit 1998;3:261–5.
[11] Wietlisbach V, Paccaud F, Rickenbach M, Gutzwiller F. Trends in cardiovascular risk factors (1984–1993) in a Swiss region: results of three population surveys. Prev Med 1997;26:523–33.
[12] Tunstall-Pedoe H, Kuulasmaa K, Mahonen M, et al. Contribution of trends in survival and coronary-event rates to changes in coronary heart disease mortality: 10-year results from 37 WHO MONICA project populations. Monitoring trends and determinants in cardiovascular disease. Lancet 1999;353:1547–57.
[13] Hosmer DWLS. Applied logistic regression. New York, NY: John Wiley & Sons; 1989.
[14] Hauser ER, Crossman DC, Granger CB, et al. A genomewide scan for early-onset coronary artery disease in 438 families: the GENECARD Study. Am J Hum Genet 2004;75:436–47.
[15] Hengstenberg C, Holmer SR, Mayer B, et al. Siblings of myocardial infarction patients are overlooked in primary prevention of cardiovascular disease. Eur Heart J 2001;22:926–33.
[16] McGill Jr HC, McMahan CA, Zieske AW, et al. Effects of nonlipid risk factors on atherosclerosis in youth with a favorable lipoprotein profile. Circulation 2001;103:1546–50.