Familial hypercholesterolaemia (FH) is a common autosomal codominant hereditary disease caused by defects in the LDL receptor (LDLR) gene, and one of the most common characteristics of affected subjects is premature coronary heart disease (CHD). In heterozygous FH patients, the clinical expression of FH is highly variable in terms of the severity of hypercholesterolaemia and the age of onset and severity of CHD. Identification of mutations in the ATP binding cassette transporter 1 (ABCA1) gene in patients with Tangier disease, who exhibit reduced HDL cholesterol and apolipoprotein A1 concentrations and premature coronary atherosclerosis, has led us to hypothesise that ABCA1 could play a key role in the onset of premature CHD in FH. In order to know if the presence of the R219K variant in the ABCA1 gene could be a protective factor for premature CHD in FH, we have determined the presence of this genetic variant by amplification by PCR and restriction analysis in a group of 374 FH subjects, with and without premature CHD. The K allele of the R219K variant was significantly more frequent in FH subjects without premature CHD (0.32, 95% CI 0.27 to 0.37) than in FH subjects with premature CHD (0.25, 95% CI 0.21 to 0.29) (p<0.05), suggesting that the genetic variant R219K in ABCA1 could influence the development and progression of atherosclerosis in FH subjects. Moreover, the K allele of the R219K polymorphism seems to modify CHD risk without important modification of plasma HDL-C levels, and it appears to be more protective for smokers than non-smokers.
was determined enzymatically in the supernatant after precipitation of apo B containing lipoproteins with dextran and magnesium sulphate. Plasma LDL-C was calculated according to the Friedewald formula. Lp(a) was determined according to the Friedewald formula.17 Lp(a) was determined according to the Friedewald formula.

Table 1 Clinical and biochemical characteristics of the FH subjects included in this study

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PCHD</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>66</td>
<td>144</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.3 (6.6)</td>
<td>51.1 (10.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2 (3.0)</td>
<td>27.0 (3.5)</td>
<td>NS</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>10.7 (1.8)</td>
<td>10.8 (1.9)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>8.7 (1.8)</td>
<td>8.9 (1.9)</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.55 (0.71)</td>
<td>1.57 (0.73)</td>
<td>NS</td>
</tr>
<tr>
<td>HDLC (mmol/l)</td>
<td>1.24 (0.36)</td>
<td>1.14 (0.30)</td>
<td>NS</td>
</tr>
<tr>
<td>Lp(a) (µmol/l)</td>
<td>1.50 (1.52)</td>
<td>2.11 (2.01)</td>
<td>0.049</td>
</tr>
<tr>
<td>Xanthommas</td>
<td>66.0%</td>
<td>61.1%</td>
<td>0.008</td>
</tr>
<tr>
<td>Smoking</td>
<td>77.8%</td>
<td>55.4%</td>
<td>0.002</td>
</tr>
<tr>
<td>Hyper tension</td>
<td>27.9%</td>
<td>18.0%</td>
<td>NS</td>
</tr>
<tr>
<td>DM2</td>
<td>11.9%</td>
<td>2.3%</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Values are mean (SD) for quantitative variables, and percentages for qualitative variables. PCHD, premature coronary heart disease; BMI, body mass index; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; HDLC, high density lipoprotein cholesterol; Lp(a), lipoprotein(a); Smoking, current and former smokers; DM2, diabetes mellitus type 2; NS, non-significant.

RESULTS

The clinical characteristics and the lipid levels of the 374 heterozygous FH subjects selected for this study are shown in table 1. There were no statistical differences between the premature CHD group and control group, concerning the body mass index (BMI), total cholesterol, LDL-C, triglycerides, HDL-C, presence of xanthomas, and hypertension. In contrast, Lp(a) levels were higher in the male premature CHD group than in the male control group (p<0.05). However, in females, no differences were observed for Lp(a) concentrations. FH subjects without premature CHD were older than the premature CHD group owing to the selection criteria. Arcus cornæalis and diabetes mellitus type 2 were more frequent in the control group than in the premature CHD group, probably because of the difference in age between the selected groups. There were more current or former smokers in the premature CHD group than in the control group for both genders.

The R219K polymorphism was the result of a nucleotide change G→A at position 1051 of the cDNA sequence and it results in the substitution of lysine for arginine at amino acid 219 of the ABCA1 protein. The genotype of this polymorphism for each of the 374 studied subjects was determined by amplification by PCR and restriction analysis with XhoI (fig 1). After digestion of the 166 bp fragment obtained by PCR, the three possible genotypes were distinguished: homozygous GG (166 bp), heterozygous GA (166, 101 and 65 bp), and homozygous AA (101 and 65 bp).

The R219K polymorphism was in Hardy-Weinberg equilibrium in the control and premature CHD groups. The genotype frequency distribution for the R219K polymorphism is shown in table 2. The frequency of the RK and KK genotypes and the frequency of K allele carriers (genotypes RK+KK) was significantly lower in the premature CHD group than in the control group (p<0.05). Similarly, the allele frequency distribution was significantly different between both groups. The allelic frequencies for the minor K allele of the R219K polymorphism were 0.32 (95% CI 0.27 to 0.37) and 0.25 (95% CI 0.21 to 0.29) for control and premature CHD groups, respectively (p<0.05). The presence of the K allele of the R219K polymorphism reduced the coronary event risk in the FH studied population (odds ratio 0.63, 95% CI 0.42 to 0.95).

The clinical and biochemical characteristics of carriers and non-carriers of the K allele for the R219K variant in the ABCA1

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Gene are shown in table 3. Male subjects with the RR genotype had higher LDL-C levels than K allele carriers of the R219K polymorphism (p=0.04). However, this difference was not observed in females or in all subjects as a whole. On the other hand, subjects not carrying the K allele of the R219K polymorphism had more xanthomas than subjects with the RK or KK genotypes (p=0.04). This difference was also observed when subjects were analysed by gender, although in this case the difference did not reach statistical significance.

The remaining variables, age, BMI, lipid and lipoprotein levels, and presence of arcus cornealis, did not show differences between carriers and non-carriers of the K allele of the R219K polymorphism.

To assess whether the presence of the K allele of the R219K variant has an effect on the age of onset of the first coronary event in the premature CHD group, we analysed the distribution of carriers and non-carriers of the K allele in subjects who suffered their first coronary event before 40 years old (first quartile) (PCHD<40 group, n=53), in subjects who suffered their first coronary event after 40 years old (PCHD≥40 group, n=163), and in the control group. The percentages of non-carriers and carriers of the K allele in each of the three groups of FH subjects analysed are shown in fig 2. A different distribution was observed, with fewer carriers of the K allele of the R219K polymorphism in the PCHD<40 group than in the PCHD≥40 and fewer than in the control group. The difference between the PCHD<40 and control groups was statistical significant (p=0.035). In this case, the odds ratio of carrying the K allele in PCHD<40 v the control group was 0.51 (95% CI 0.27 to 0.96).

The premature CHD group as a whole has a higher percentage of smoking subjects than the control group (59% v 27%, p<0.0001). In the FH population studied, smoking increases the coronary event risk to 3.88 (95% CI 2.44 to 6.19). However, when the age of onset of the first coronary event was taken into account and the two groups, PCHD<40 and PCHD≥40, were analysed separately, no statistical differences in the percentage of smoking subjects between the PCHD<40 and PCHD≥40 groups were observed (63% and 58%, respectively). The effect of the K allele of the R219K polymorphism on a premature event of CHD (before 40 years) was analysed in smokers and non-smokers separately. In smoking (current and former) subjects, the odds ratio of onset of premature CHD before 40 years old in carriers v non-carriers of the K allele was 0.45 (95% CI 0.16 to 1.25, p=0.123). In non-smoking subjects, the odds ratio of having a premature coronary event before 40 years old in carriers v non-carriers of the K allele was 0.76 (95% CI 0.28 to 2.08, p=0.595). In subjects with premature CHD before 40 years old, the odds ratio of carrying the K allele in smokers v non-smokers was 0.35 (95%
R219K polymorphism of 46% in the European population, way that subjects carrying the protective allele have a slower polymorphism could influence the phenotype of FH in such a way that HDL-C is a major independent factor involved in the development of premature CHD. The anti-atherogenic function of HDL has been attributed to its role in reverse cholesterol transport, where the protein ABCA1 plays a crucial role in its transport, where the protein ABCA1 plays a crucial role in its action of smoking with a determined genotype has already been described for other genes. Therefore, Humphries et al have also found differences in the risk of CHD without detectable changes in plasma lipid levels. However, another common variant in ABCA1, I823M, has been reported to be a significant source of variation in plasma HDL-C. These findings of different risk of CHD but no differences in lipid levels would suggest that modification in reverse cholesterol transport may vary the flux of cholesterol towards the liver without necessarily modifying the plasma lipid concentrations. Singaraja et al have shown that overexpression of ABCA1 induced the increase of cholesterol efflux from macrophages, the HDL particles being better acceptors of cholesterol, although the increase in plasma HDL-C levels was small. It is possible that the R219K variant increases the activity of ABCA1 in a similar way, although the precise mechanism underlying the functional effect of this variant will require further analyses.

In this series of FH patients studied, we have observed a correlation between the presence of the K allele of the R219K variant and the age of onset of the first coronary event. The younger FH subjects with early proven coronary events are less often carriers of the protective K allele. Comparison of the odds ratio of the control group >40 years old (PCHD>40), and premature coronary heart disease with first coronary event before 40 years old (PCHD<40); p indicates the difference between the control and PCHD<40 groups.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR</td>
<td>RK+KK</td>
<td>p</td>
</tr>
<tr>
<td>No</td>
<td>120</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>TG [mmol/l]</td>
<td>1.53 [0.65]</td>
<td>1.60 [0.81]</td>
<td></td>
</tr>
<tr>
<td>HDL-C [mmol/l]</td>
<td>1.17 [0.32]</td>
<td>1.19 [0.33]</td>
<td></td>
</tr>
<tr>
<td>Apo A1 [g/l]</td>
<td>47.4%</td>
<td>36.4%</td>
<td></td>
</tr>
<tr>
<td>Arcus cornealis</td>
<td>57.9%</td>
<td>68.2%</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean (SD) for quantitative variables, and percentages for qualitative variables. BMI, body mass index; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; lpa, lipoprotein(a); NS, non-significant.

Chi-squared test for trend (Cochran-Armitage test) with p=0.035. These results would suggest that the protective effect of the K allele of the R219K variant on premature CHD risk is more pronounced in smokers.
subjects carrying the K allele of the R219K variant would be more protected against lipoprotein oxidation and subsequent risk of atherosclerosis. Further studies will be necessary to confirm this hypothesis.

In this work we show that the R219K variant of ABCA1 influences premature CHD frequency in subjects with heterozygous familial hypercholesterolemia. FH subjects are a very high cardiovascular risk owing to their high LDL-C levels as a consequence of a mutation in the LDLR gene, but other genetic and/or environmental factors modify the disease expression and it is possible that one of them could be the variant R219K in ABCA1. Our results indicate the importance of considering other loci in the clinical consequences of FH. Although the ABCA1 locus, and specifically the polymorphism R219K, influences the early onset of CHD in FH, other differences are also implicated with small and/or large contributions, the disease expression being the result of the interaction of these loci. Further studies to that effect will help to identify FH subjects at high risk of premature CHD, which will be helpful for better prevention and management of cardiovascular disease in familial hypercholesterolemia.

ACKNOWLEDGEMENTS
This work was supported by grants from Fondo de Investigación Sanitaria (FIS 00/0992), Diputación General de Aragón (DGA 0016/99-BM), and Ministerio de Ciencia y Tecnología (SAF 2001-2466-C05). MA is a recipient of a fellowship from Fondo de Investigación Sanitaria.

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A common variant in the ABCA1 gene is associated with a lower risk for premature coronary heart disease in familial hypercholesterolaemia

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doi: 10.1136/jmg.40.3.163

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