

# Association Between the $\alpha$ -Adducin Gly460Trp Polymorphism and Systolic Blood Pressure in Familial Combined Hyperlipidemia

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**Background:** In a genome scan for familial combined hyperlipidemia (FCHL), a locus contributing to systolic blood pressure (SBP) has been identified on chromosome 4, containing the  $\alpha$ -adducin gene (*ADD1*). In previous studies, an association has been found between the  $\alpha$ -adducin Gly460Trp polymorphism and salt-sensitive hypertension. In this study, we investigated the association between the  $\alpha$ -adducin Gly460Trp polymorphism and blood pressure in FCHL patients.

**Methods:** A total of 79 unrelated patients with FCHL and 121 unrelated controls (spouses) were recruited for the study. Blood pressure was measured in a standardized fashion, with the subject in sitting position after 10 min of rest. The  $\alpha$ -adducin Gly460Trp polymorphism was detected by mutagenically separated polymerase chain reaction.

**Results:** The genotype frequencies of both FCHL patients and controls were in Hardy-Weinberg equilibrium.

The  $\alpha$ -adducin Gly460Trp polymorphism showed a significant association with FCHL, the number of subjects carrying a 460Trp allele was significantly higher in patients compared with controls (53% v 33%,  $\chi^2 = 8.0$ ,  $P = .018$ ). In FCHL patients carrying at least one 460Trp allele, SBP was significantly higher compared with patients homozygous for the 460Gly allele (140 mm Hg and 130 mm Hg respectively,  $P = .015$ ).

**Conclusions:** This study shows that the 460Trp allele is associated with FCHL. Furthermore, SBP is increased in patients carrying the 460Trp allele. Am J Hypertens 2001;14:1185-1190 © 2001 American Journal of Hypertension, Ltd.

**Key Words:** Blood pressure,  $\alpha$ -adducin, polymorphism, genetic markers, familial combined hyperlipidemia.

**F**amilial combined hyperlipidemia (FCHL) is a common lipid disorder characterized by elevated plasma cholesterol and triglyceride levels with segregation in first-degree relatives.<sup>1</sup> In a previous study, about one-third of families with FCHL and 27% of FCHL relatives appeared to have familial dyslipidemic hypertension.<sup>2</sup> Because insulin resistance is a frequent finding in FCHL<sup>1,3,4</sup> and because insulin can increase renal tubular sodium reabsorption,<sup>5</sup> it is plausible that hyperinsulinemia could contribute to the pathogenesis of hypertension in FCHL by rendering blood pressure more sodium-dependent.<sup>6</sup> Although the mechanisms that cause sodium sensitivity in blood pressure are still unknown, several genes are thought to play a role in this process. Interestingly, the

$\alpha$ -adducin Gly460Trp polymorphism is associated with enhanced renal tubular sodium reabsorption and can account for a sodium-sensitive rise in blood pressure.<sup>7,8</sup> Recently, several genome scans have confirmed the multigenic nature of blood pressure<sup>9,10</sup> and hypertension<sup>11</sup> in humans. A locus that contributes to systolic blood pressure (SBP) in families with FCHL was identified on chromosome 4 (LOD score of 3.9), which contains the  $\alpha$ -adducin gene (*ADD1*).<sup>10</sup> In Milan hypertensive rats, *ADD1* is a candidate gene for SBP, showing a LOD score of 3.2 with SBP.<sup>12</sup> Based on these data, we set out to investigate the association between the  $\alpha$ -adducin Gly460Trp polymorphism and FCHL and to determine the relationship of different genotypes with blood pressure in patients with FCHL.

Received February 12, 2001. Accepted June 11, 2001.

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## Methods

### Study Population

A total of 79 unrelated white FCHL patients were identified at our Lipid Clinic. The FCHL patients met each of the following criteria: 1) a primary hyperlipidemia including fasting total plasma cholesterol >250 mg/dL (6.5 mmol/L) and/or fasting plasma triglyceride concentration >200 mg/dL (2.3 mmol/L); 2) at least one first-degree relative with a different hyperlipidemic phenotype (Fredrickson Classification IIa, IIb, or IV); 3) a positive family history of premature coronary artery disease. Premature coronary artery disease was defined as the occurrence of a myocardial infarction or other cardiovascular disease before the age of 60 years in at least one first-degree relative of the patient, or the patient him/herself. Secondary causes of hyperlipidemia (renal or hepatic insufficiency, hypothyroidism, and medication), presence of the apo E2/E2 genotype, and subjects with tendon xanthomas or a diagnosis matching familial hypercholesterolemia were excluded. The control group, which consisted of 121 spouses, was age-matched. These 121 spouses were married either with one of the 79 patients or with another family member with FCHL who was not included in this study. The study was approved by the hospital's Medical Ethical Committee. Informed consent was obtained from each individual recruited.

### Measurements

All measurements were performed at the Clinical Research Unit in the morning (8 AM to 10 AM) after an overnight fast. Subjects had refrained from smoking and were only allowed to drink water in the morning. Participants also had abstained from alcohol for at least 72 h. All participants were weighed in underwear, their height was determined, and their body mass index (BMI) was subsequently calculated as weight in kilograms/(height in meters)<sup>2</sup>. The waist was measured at the level of the umbilicus, the hip circumference was measured at the level of the trochanter major and the waist-to-hip ratio was calculated from these figures. Blood pressure was measured twice in a standardized fashion, with the subject in sitting position after 10 min of rest. Cuff size was adjusted to the circumference of the arm, and the arm was placed with the cuff at heart-level. The average of two measurements was used for analysis.

### Biochemical Analysis

Any lipid-lowering medication had been withdrawn during the 2 weeks before blood samples were collected. After an overnight fast, venous blood was collected in precooled EDTA (1 mg/mL) vacutainer tubes for measurement of lipids and insulin. Total cholesterol and fasting triglyceride concentrations were measured in duplicate by a commercially available colorimetric assay (Monotest Cholesterol kit, Boehringer Mannheim 1442350 and GPO-PAP, Boehringer Mannheim, 701912, respectively). Fast-

ing insulin concentration was determined using an ELISA (Mercodia AB, Uppsala, Sweden), with a cross-reactivity with proinsulin of <0.01%.

### Genetic Analysis

DNA was extracted from whole blood using the Wizard Genomic DNA Purification Kit (Promega, Leiden, the Netherlands). The  $\alpha$ -adducin Gly460Trp polymorphism was detected by mutagenically separated polymerase chain reaction (PCR).<sup>13,14</sup> Briefly, two allele-specific primers (FP614G: 5'-GGG GCG ACG AAG CTT CCG AGG TAG-3'; FP614T: 5'-GCT GAA CTC TGG CCC AGG CGA CGA AGC TTC CGA GGA TT-3') and their nonselective complementary strand primer (RP614: 5'-CCT CCG AAG CCC CAG CTA CCC A-3') were mixed and used for the PCR amplification in a single reaction. Deliberate differences were introduced into the allele-specific primers in addition to the base substitution, to drastically reduce cross-reactions between two allelic PCR in a mixed reaction. The mutagenically separated PCR products (corresponding with the Gly460 and 460Trp alleles), varying 14 bp in size, were resolved on a 3% agarose gel and visualized using ethidium bromide and ultraviolet light. Genotypes were scored independently by two experienced researchers.

### Statistical Analysis

The calculation of allele frequencies to test for Hardy-Weinberg equilibrium was carried out using  $\chi^2$  analysis comparing expected against observed frequencies. To test for associations, genotype frequencies (Gly460Gly, Gly460Trp, and Trp460Trp) were compared by  $\chi^2$  analysis. Mann-Whitney U test and  $\chi^2$  analysis were used to determine differences in clinical characteristics between FCHL patients and their controls and between the two genotype groups (Gly460Gly versus 460Trp allele) of the  $\alpha$ -adducin Gly460Trp polymorphism. Analysis of variance (ANOVA) was done to determine the combined effect of the  $\alpha$ -adducin Gly460Trp polymorphism and FCHL on SBP. A total of 43 patients with FCHL (55%) were not using antihypertensive medication. To exclude the effect of antihypertensive treatment on blood pressure values, analyses on the genotype level were done only with patients who were not using antihypertensive medication. Data are presented as medians with interquartile ranges. A *P* value of <.05 was considered statistically significant. Statistical analyses were performed using SPSS for Windows, version 10.0 (SPSS, Chicago, IL).

## Results

The clinical characteristics of the 79 unrelated FCHL patients and the 121 spouse controls are summarized in Table 1. The SBP was significantly higher in the FCHL patients compared with controls; age and diastolic blood pressure (DBP) were not significantly different between patients and controls. As expected, differences were found

**Table 1.** Clinical characteristics of all FCHL patients and spouse controls

	FCHL	Spouses
Number	79	121
Age (y)	54 (46, 59)	50 (43, 60)
Sex (male/female)	48/31	50/71†
BMI (kg/m <sup>2</sup> )	27.3 (25.0, 29.7)	24.7 (22.5, 28.1)*
WHR	0.94 (0.88, 0.98)	0.85 (0.79, 0.92)*
TC (mmol/L)	6.71 (5.90, 7.77)	5.45 (4.93, 6.02)*
TG (mmol/L)	2.78 (2.06, 4.31)	1.17 (0.92, 1.55)*
Insulin (mU/L)	10.0 (6.0, 14.1)	5.0 (2.0, 8.0)*
SBP (mm Hg)	138 (125, 145)	128 (118, 140)†
DBP (mm Hg)	86 (80, 92)	83 (76, 92)

FCHL = familial combined hyperlipidemia; BMI = body mass index; WHR = waist/hip ratio; TC = total cholesterol; TG = plasma triglyceride; SBP = systolic blood pressure; DBP = diastolic blood pressure.

Values are medians (interquartile ranges).

\*  $P < .001$ ; †  $P < .01$ .

between FCHL patients and spouse controls for gender, BMI, waist–hip ratio, total plasma cholesterol and plasma triglyceride concentration, and insulin levels. Genotypes were obtained from 79 patients and 121 controls. Both the groups of FCHL patients and spouse controls were in Hardy-Weinberg equilibrium. The  $\alpha$ -adducin Gly460Trp polymorphism showed a significant association with FCHL. Notably, the number of 460Trp allele carriers was significantly higher in the patients (42/79, 53%) compared with spouse controls (40/121, 33%,  $\chi^2 = 8.0$ ,  $P = .018$ , Fig. 1). Because it is known that the G to T substitution in the  $\alpha$ -adducin gene has functional consequences,<sup>7</sup> genotype-phenotype relationships were studied. When FCHL patients without antihypertensive treatment ( $N = 43$ ) were considered separately, SBP was significantly higher in the 460Trp allele carriers compared with 460Gly allele homozygotes (140 mm Hg (interquartile range 130 to 152) and 130 mm Hg (interquartile range 115 to 139) respectively, Fig. 2A). No differences were found between 460Trp allele carriers and 460Gly homozygotes in DBP, age, BMI, waist–hip ratio, insulin levels, and lipids (Table 2). In spouse controls without antihypertensive medication

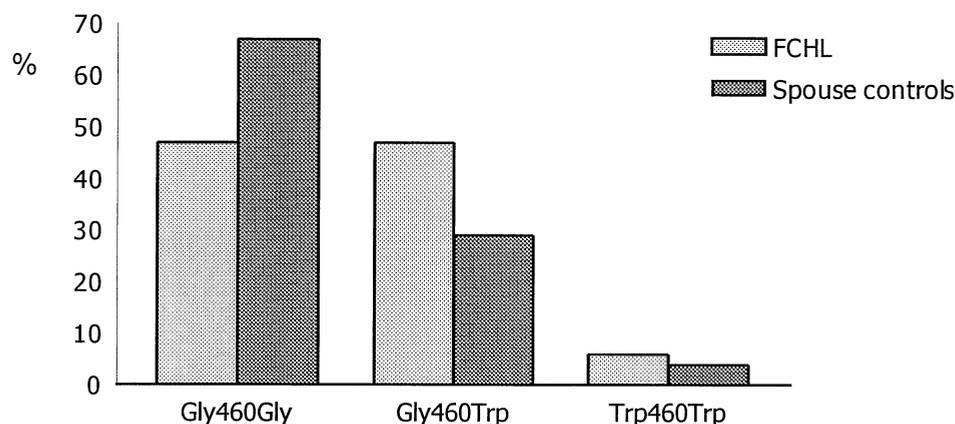
no significant differences were found between the two genotype groups (Fig. 2B).

Table 3 shows the effect of the  $\alpha$ -adducin Gly460Trp polymorphism and FCHL on SBP and DBP. Although in univariate analysis the combination of FCHL and the 460Trp allele is associated with elevated SBP, ANOVA just failed to reach statistical significance ( $F = 2.577$ ,  $P = .056$ ).

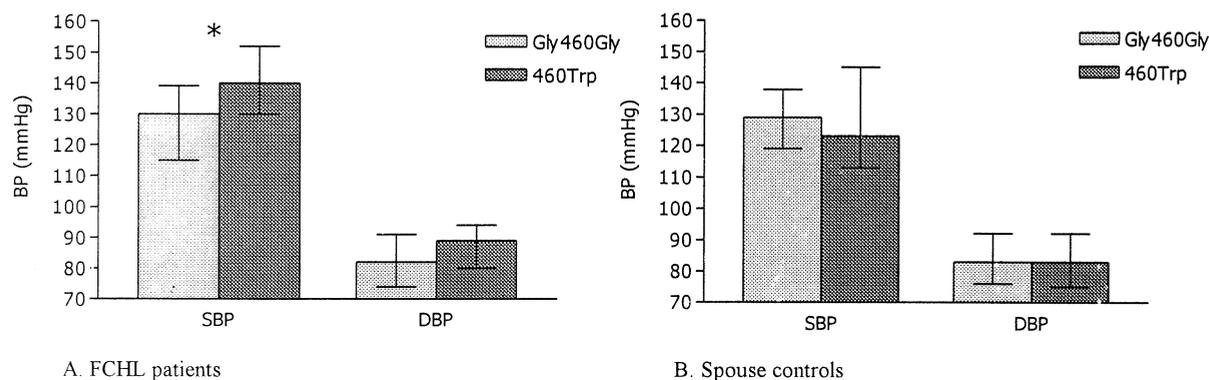
## Discussion

The present study shows that the 460Trp allele of the  $\alpha$ -adducin gene is associated with FCHL. In addition, we found that SBP is increased in FCHL patients carrying the 460Trp allele.

The frequency of the 460Trp allele varies in different populations. In this study, 53% of the FCHL patients carried the 460Trp allele, whereas 33% of the spouse controls were 460Trp allele carriers. The genotype distribution in our control group is comparable to that in a Scottish population<sup>15</sup> with low-to-normal blood pressure and to the distribution in a normotensive Finnish popula-



**FIG. 1.**  $\alpha$ -Adducin Gly460Trp polymorphism genotype distribution in 79 unrelated familial combined hyperlipidemia (FCHL) patients and 121 spouse controls. In all, 42 of 79 FCHL patients were 460Trp allele carriers (53%), and 40 of 121 spouses carried the 460Trp allele (33%).  $\chi^2 = 8.0$ ;  $P = .018$ .



**FIG. 2. A)** Systolic blood pressure (SBP) and diastolic blood pressure (DBP) by genotype groups of the  $\alpha$ -adducin Gly460Trp polymorphism in patients with FCHL without antihypertensive medication. SBP in Gly460Gly genotype ( $n = 22$ ): 130 (115, 139) mm Hg, SBP in 460Trp allele carriers ( $n = 21$ ): 140 (130, 152) mm Hg,  $P = .015$ . **B)** SBP and DBP by genotype groups of the  $\alpha$ -adducin Gly460Trp polymorphism in spouse controls without antihypertensive medication. SBP in Gly460Gly genotype: 129 (119, 138) mm Hg, SBP in 460Trp allele carriers: 123 (113, 145) mm Hg,  $P = .700$ . Data are presented as medians (interquartile ranges).

tion.<sup>16</sup> In Italians, lower frequencies of the 460Trp allele were found,<sup>8</sup> whereas in a Japanese study<sup>14</sup> and in an Anglo-Celtic population<sup>17</sup> the frequency of subjects with this genotype was much higher. At present, genotype frequencies of the  $\alpha$ -adducin Gly460Trp polymorphism in FCHL populations have not been reported.

Our Dutch FCHL population comprised more men than women, which resulted in an expected significant gender difference between FCHL patients and spouse controls. The  $\alpha$ -adducin polymorphism was equally distributed between men and women. Because risk factors for elevated SBP such as gender, age, waist-hip ratio, and BMI were not significantly different between patients carrying the 460Trp allele and patients homozygous for the 460Gly allele, in univariate analysis the 460Trp allele emerged as a new risk factor for elevation of SBP in FCHL.

Analyses on the genotype level were performed only in patients without antihypertensive medication (54% of all patients with FCHL) to exclude an effect of treatment on the association between the  $\alpha$ -adducin polymorphism and blood pressure. Only 8% of the spouse controls were using antihypertensive medication. Even when all FCHL patients were considered ( $N = 79$ ), including those on antihypertensive treatment, SBP was still higher in the 460Trp

allele carriers, but this difference just failed to reach statistical significance (140 mm Hg *v* 130 mm Hg,  $P = .058$ ).

The mechanism by which  $\alpha$ -adducin could increase blood pressure is not fully known.  $\alpha$ -Adducin is thought to regulate ion transport through changes in the actin cytoskeleton.<sup>18,19</sup> The heterodimer protein is present in many tissues, including the kidney.<sup>19</sup> Adducin is thought to stimulate  $\text{Na}^+/\text{K}^+$ -ATPase, thus promoting sodium reabsorption by renal tubular cells. Indeed, there is evidence to show that adducin polymorphisms are involved in genetic alterations of cell  $\text{Na}^+$  transport and the pathogenesis of primary hypertension in rats and humans.<sup>20</sup> Our present data suggest that there may be an interaction between FCHL, the  $\alpha$ -adducin polymorphism and blood pressure. Theoretically, this could be related to obesity, insulin resistance,<sup>21</sup> and salt sensitivity.<sup>22</sup> Essential hypertensive patients who are salt sensitive are relatively insulin resistant compared with essential hypertensive patients who are less salt sensitive, independently of confounding factors such as age, obesity, and glucose tolerance. Furthermore, there is a significant correlation between salt-induced changes in blood pressure and fasting insulin levels, suggesting a relationship between hyperinsulinemia and salt sensitivity.<sup>6</sup> In our study, the 460Trp allele of the  $\alpha$ -ad-

**Table 2.** Clinical characteristics in FCHL patients without antihypertensive treatment by genotype groups

	Gly460Gly	460Trp
Number	22	21
Age (y)	51 (39, 57)	51 (47, 60)
Sex (male/female)	15/7	13/8
BMI ( $\text{kg}/\text{m}^2$ )	27.5 (25.5, 29.6)	27.8 (25.6, 29.9)
WHR	0.94 (0.89, 0.98)	0.98 (0.89, 1.01)
TC (mmol/L)	7.11 (6.30, 8.51)	7.15 (6.47, 8.07)
TG (mmol/L)	3.80 (2.22, 5.83)	2.77 (2.09, 4.46)
Insulin (mU/L)	9.5 (5.3, 16.5)	11.5 (8.3, 14.0)

Abbreviations as in Table 1.

Values are medians (interquartile ranges).

**Table 3.** Association between the  $\alpha$ -adducin Gly460Trp polymorphism, FCHL, and blood pressure

	FCHL	Spouses
Gly460Gly	<i>n</i> = 22	<i>n</i> = 76
SBP	130 (115, 139)	129 (119, 138)
DBP	82 (74, 91)	83 (76, 92)
460Trp	<i>n</i> = 21	<i>n</i> = 37
SBP	140 (130, 152)	123 (113, 145)
DBP	89 (80, 94)	83 (75, 92)

Abbreviations as in Tables 1 and 2.

Subjects without antihypertensive medication; data are presented as medians (interquartile ranges).

ducin polymorphism that can induce a sodium-dependent rise in blood pressure is associated with a rise in SBP in patients with FCHL but not in normal subjects. A possible explanation is that FCHL patients have significantly higher plasma insulin levels compared to controls. The hyperinsulinemia is a key background feature to explain both the expression of hypertension in FCHL and the association.

Another possible explanation for our present results is the fact that patients with FCHL have impaired vascular function.<sup>23</sup> In hypercholesterolemia and hypertension, impaired endothelium dependent vasodilation has been documented<sup>24</sup> and vasodilation to acetylcholine is blunted in sodium-sensitive hypertensive patients even on restricted sodium diets. This abnormality may contribute to blood pressure elevation when sodium intake is increased.<sup>25</sup> However, other studies suggest that patients with hypercholesterolemia who have impaired endothelially dependent relaxation remain normotensive.<sup>26</sup> Therefore, decreased NO availability in itself does not necessarily result in systemic hypertension, but it may enhance the individual's sensitivity to the hypertensinogenic effect of dietary sodium.<sup>27</sup> In this theory, endothelial dysfunction mediates, in part, the expression of higher SBP and hypertension, especially in FCHL.

In conclusion, the present study shows that the 460Trp allele of the  $\alpha$ -adducin polymorphism is associated with FCHL. Furthermore, systolic blood pressure is increased in patients carrying the 460Trp allele. However, it is still possible that another gene in this chromosomal region in linkage disequilibrium with the 460Trp allele is the true cause of hypertension in FCHL. Future work should include functional studies to reveal whether the  $\alpha$ -adducin Gly460Trp polymorphism is a marker for functional changes associated with blood pressure elevation in FCHL.

## Acknowledgments

We thank the patients and spouses for participating in this study. This study has been supported by NWO grant 900-95-297 (TdB) and a grant from the University Hospital Maastricht.

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