The Relationship of Mutations in the MTHFR, Prothrombin, and PAI-1 Genes to Plasma Levels of Homocysteine, Prothrombin, and PAI-1 in Children and Adults

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Summary

Studies in adults have demonstrated that the genetic mutations C677T methylenetetrahydrofolate reductase (MTHFR), prothrombin 20210A, and the 4G polymorphism of the plasminogen activator inhibitor-1 (PAI-1) gene are associated with elevated plasma levels of homocysteine, prothrombin and PAI-1, respectively and with an increased risk of thrombosis. No similar data is available in children. Therefore, we assessed the relationship of plasma levels of homocysteine, prothrombin and PAI-1 with their respective mutations in 197 normal children, compared to 40 adults. By stepwise multiple regression, homocysteine was positively associated with age, PAI-1 activity was negatively associated with age, while PAI-1 antigen and prothrombin levels were associated with gender, being higher in girls than boys. When the genotypes were added to the regression model as additional explanatory variables, the MTHFR genotype accounted for 2.9% of the variance of homocysteine ($p = 0.024$), and the PAI-1 gene accounted for 2.7% of the variance of PAI-1 antigen levels ($p = 0.023$). Of children homozygous for the MTHFR mutation, 35% had homocysteine levels ≥ the age-specific 95th percentile, compared to 2% heterozygotes and 5% wild type normals ($p = 0.0001$). The mean homocysteine level was higher in children homozygous for the MTHFR gene (8.4 μmol/l) than in heterozygotes (5.5 μmol/l), $p < 0.05$. Of children homozygous for the 4G polymorphism of the PAI-1 gene, 19% had PAI-1 activity levels ≥ the age-specific 95th percentile, compared to 2% of heterozygotes and 3% of wild type normals ($p = 0.003$). Studies of the incidence of the MTHFR, prothrombin, and PAI-1 4G/5G genotypes in children with thrombosis, when compared to these healthy normals, will provide evidence as to which of these genes are associated with thrombophilia.

Supported in part by a Jewish Hospital Medical Research Council grant.

This work was carried out following an institutional research committee approved protocol with signed informed consent.

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Introduction

Thrombotic events are relatively uncommon in children compared to adults, but may be associated with significant morbidity and on occasion, death (1). The importance of inherited thrombophilic conditions predisposing to thrombosis in children has been well recognized (1-3). Recently, cDNA-polymerase chain reaction (PCR) based assays have been developed for the identification of three mutant or polymorphic genes which have been shown to be associated with inherited thrombophilia in adults. These genetic mutations and polymorphisms include the thermolabile (C677T) methylenetetrahydrofolate reductase (MTHFR) gene (4-6), the 20210 G to A transition in the 3'-untranslated (UT) region of the prothrombin gene (7-9) and a single base (guanosine) insertion/deletion, commonly called 4G/5G, in the promoter region of the plasminogen activator inhibitor-1 (PAI-1) gene (10-13).

Homozygosity for the C677T mutation of the MTHFR gene is associated with increased levels of plasma homocysteine, a recognized risk factor for thrombosis and atherosclerosis in adults (14, 15). Fasting total plasma homocysteine reference ranges in healthy adults have been well defined (16), and the association of elevated homocysteine levels in association with the MTHFR genotype has been studied (5). There is sparse data available in children with respect to fasting total plasma homocysteine levels (17) and there is no data, to our knowledge, correlating the MTHFR genotype with fasting total plasma homocysteine levels in children.

Carriers of the prothrombin 20210A polymorphism have been shown to have elevated plasma levels of prothrombin, and an increased risk of thrombosis, as well as cerebrovascular ischemic disease in young adulthood (7-9). Adults homozygous for the 4G allele in the PAI-1 gene have been shown to have higher plasma levels of PAI-1, compared to individuals with the 5G/5G genotype, with heterozygotes showing an intermediate level (11-13). The presence of the 4G allele has been shown to be associated with myocardial infarction at a young age (12) and with deep venous thrombosis (13). There are no studies, however, demonstrating the relationship between these 2 gene variants and plasma levels of prothrombin and PAI-1, respectively in children.

Our hypotheses were: (1) that the C677T MTHFR, prothrombin 20210A and the PAI-1 4G/4G gene variants are significant determinants of plasma levels of homocysteine, prothrombin and PAI-1 antigen and activity respectively, in children, as they are in adults, and (2) that the inclusion of individuals with the corresponding abnormal genotypes significantly influences the "normal" age-specific ranges for

Table 1 Prevalence of the methylenetetrahydrofolate reductase (MTHFR) C677T, prothrombin (Pro) 20210A, and plasminogen activator inhibitor-1 (PAI-1) 4G gene variants

		MTHFR C677T			Pro20210A		PAI-1 4G		
		NN	MN	MM	NN	MN	5G/5G	4G/5G	4G/4G
Adults	n	20	16	4	38	2	16	16	8
	%	50%	40%	10%	95%	5%	40%	40%	20%
Allele frequency		24/80 = 0.30			2/80 = 0.025		32/80 = 0.40		
Children	n	96	73	18	180	7	67	82	37
	%	51%	39%	10%	96%	4%	36%	44%	20%
Allele frequency		109/374 = 0.29			7/374 = 0.02		156/372 = 0.42		
All subjects	n	116	89	22	218	9	83	98	45
	%	51%	39%	10%	96%	4%	37%	43%	20%
Allele frequency		133/454 = 0.29			9/454 = 0.02		188/452 = 0.42		
African-Americans	n	26	5	1	32	0	18	11	3
	%	81%	16%	3%	100%	0%	56%	35%	9%
Allele frequency		7/64 = 0.11			0/64 = 0		17/64 = 0.26		
Whites	n	89	83	21	184	9	64	86	42
	%	46%	43%	11%	95%	5%	33%	45%	22%
Allele frequency		125/386 = 0.32			9/386 = 0.02		170/384 = 0.44		
African-Americans vs whites, differences:		χ^2=12.18, p=0.001			χ^2=1.52, p=0.2		χ^2=7.07, p=0.008		

NN=normal, "wild type"; MN=mutant, heterozygote; MM=mutant, homozygote.

the plasma levels of homocysteine, prothrombin and PAI-1. None of the previously published studies providing normal pediatric ranges for homocysteine, prothrombin and PAI-1 levels have excluded individuals who are carriers of the corresponding gene mutations (17-19). Therefore, the purpose of this study was (a) to determine the relationship between the C677T MTHFR mutation, the prothrombin 20210A transition, and the 4G polymorphism of the PAI-1 gene and plasma levels of homocysteine, prothrombin and PAI-1, respectively in 197 normal children, compared to 40 adults and (b) to provide pediatric normal values of homocysteine, prothrombin and PAI-1, uninfluenced by levels from individuals with the abnormal genotypes. The effects of age, sex, and race on these levels were also assessed.

Subjects, Materials and Methods

Subjects

The study was approved by the Children's Hospital Review Board on Investigation of Human Subjects. Appropriate informed consent was obtained from all parents or subjects. One hundred and ninety-seven children aged six months through 16 years (inclusive) admitted for elective same-day surgery provided a morning fasting blood sample. Study subjects were clinically well with no serious underlying illnesses, or family history of bleeding or thrombotic problems. Blood was collected after routine venipuncture, usually prior to anesthesia induction. At least 10 children at each year of age were selected. For comparison, 40 adults (17 men, 23 women) were also studied.

Blood Sampling and Plasma Preparation

Blood was collected in 3.2% buffered sodium citrate (1 part citrate : 9 parts blood). The samples were immediately transported and centrifuged at 2600 × g for 15 min to obtain platelet-poor plasma. The plasma was frozen in aliquots and stored at -70° C. Blood for PCR analysis was drawn in EDTA and the DNA extracted for subsequent analysis.

Assays

Total (free and protein-bound) plasma homocysteine levels were measured by high-pressure liquid chromatography (HPLC) according to the method of Ubbink et al. (20), with minor modification. Results were recorded as μM/l of total homocysteine. Plasma prothrombin levels were quantitatively determined (21) using Chromogenix chromogenic substrate S-2238 (Chromogenix AB, Taljegårdsgatan, Mölndal, Sweden) and *Echis carinatus* venom (Sigma Chemical Company, St. Louis, Missouri). Plasma PAI-1 antigen levels were quantitatively determined by an ELISA technique using TintElize PAI-1 kits (Biopool, Ontario, Canada), and plasma PAI-1 activity by the chromogenic assay kit, Spectrolyse/pL PAI-1 (Biopool).

Genomic DNA for the PCR assays was obtained by a simple salting out procedure (22) (by ethanol extraction). PCR for the enzyme MTHFR to determine the presence or absence of a point mutation at nucleotide 677 leading to a valine substitution for an alanine residue was performed using appropriate primers (4). The PCR product was digested with the restriction enzyme, *HinfI*. PCR for the prothrombin gene to determine the presence or absence of the point mutation at nucleotide 20210 leading to a G to A transition was performed using appropriate primers as previously described (7). The PCR product was digested with the restriction enzyme, *HindIII*. PCR for the identification of a sequence polymorphism in the promoter region of the PAI-1 gene 675 base-pairs upstream from the transcriptional start site resulting in two alleles containing either 4 or 5 guanosines in a row was performed using appropriate primers (11). After digestion, the products were electrophoresed on 3% agarose gel and stained with ethidium bromide.

Statistical Methods

First, the frequencies of genotype(s) of MTHFR, prothrombin, and PAI-1 in the whole group of children (Table 1) were determined. Relationships of age, race, sex, and gene polymorphisms for MTHFR, prothrombin, and PAI-1 to homocysteine, prothrombin, and PAI-1 activity and antigen levels were assessed by stepwise multiple regression (Table 2). Then, homozygotes for the MTHFR polymorphism, heterozygotes for the prothrombin gene polymorphism (there were no homozygotes), and homozygotes for the 4G/4G polymorphism of the PAI-1 gene were removed to allow establishment of "normal" plasma levels of homocysteine, prothrombin, and PAI-1 (Table 3). Since much of the data was not normally distributed by the Shapiro-Wilk test (23), Spearman correlations and Wilcoxon non-parametric tests of difference were used (Tables 3, 4). Percentiles for coagulation measures were obtained directly by ranking programs in SAS (Proc Univariate). Since age was correlated with plasma levels of homocysteine and PAI-1 activity in children, the cohort of children aged 6 months to 16 years (inclusive) was divided into 4 non-overlapping age groups (0.5-1, 1-5.5, 5.6-11.2, 11.3-16.9 years) (Table 3). Since gender was correlated with PAI-1 antigen and prothrombin levels, the cohort of children was divided into girls and boys (Table 3).

Table 2 Association of age, race, sex and genotypes with homocysteine, prothrombin, and PAI-1 levels

Stepwise Regression selection on explanatory variables : age, race, and sex

Dependent Variable	Significant explanatory variable	Sign	p value	partial R^2
Homocysteine	Age	+	0.0001	9.6%
PAI-1 activity	Age	-	0.0001	7.5%
PAI-1 antigen	Sex (female=0, male=1)	-	0.0018	4.9%
Prothrombin	Sex	-	0.023	2.6%

Stepwise Regression selection on explanatory variables : age, race, sex and genotype

Dependent Variable	Significant explanatory variable	Sign	p value	partial R^2
Homocysteine	Age	+	0.0001	9.5%
	MTHFR (NN=0, MN=1, MM=2)	+	0.024	2.9%
PAI-1 activity	Age	-	0.0001	12%
	PAIG (5G5G=0, 4G5G=1, 4G4G=2)	+	0.10	1.3%
PAI-1 antigen	Sex (female=0, male=1)	-	0.0015	5.1%
	PAIG	+	0.023	2.7%
Prothrombin	Sex	-	0.032	2.5%

NN=wild type normal, MN=heterozygote, MM=homozygote

Table 3 Plasma fasting total homocysteine, prothrombin, PAI-1 antigen and PAI-1 activity levels in 160 children and 38 adults after removing homozygotes for the MTHFR and PAI-1 gene polymorphisms, and heterozygotes for the prothrombin gene polymorphism

Group (age in years)	n	Median	Percentiles		Spearman Correlation (in children)
			90th	95th	
Homocysteine (μM/L)					
I (0.5-1 year)	10	5.6[a]	6.6	6.6	Age r= 0.53
II (1-5.5 years)	44	4.6[a]	6.9	7.0	p= 0.0001
III (5.6-11.2years)	46	5.3[a]	6.4	6.8	
IV (11.3-16.9 years)	53	6.6[b]	9.8	10.8	
Adults	35	7.0[b]	10.3	10.8	
Prothrombin (%)					
Girls	82	93[ab]	111	115	Sex r= -0.18
Boys	108	89[b]	105	112	p= 0.01
Adult women	21	94[ab]	105	111	
Adult men	17	102[a]	118	119	
PAI-1 antigen (ng/mL)					
Girls	71	14.9[a]	32.0	38.2	Sex r= -0.21
Boys	89	10.1[ab]	25.0	26.4	p= 0.007
Adult women	16	5.1[b]	29.5	29.5	
Adult men	16	9.3[ab]	32.0	32.0	
PAI-1 activity (U/mL)					
I (0.5-1 year)	17	25.9[a]	34.1	36.2	Age r= -0.20
II (1-5.5 years)	45	11.1[b]	16.4	16.8	p= 0.012
III (5.6-11.2years)	47	12.4[b]	20.4	20.8	
IV (11.3-16.9 years)	51	10.2[b]	20.2	23.7	
Adults	32	5.0[c]	12.2	14.4	

[a,b,c] If letters are common (within population group), variance transformed means do not differ; if letters differ, means differ, p≤0.05 (Bonferroni or Newman-Keuls test)

After performing variance stabilizing transformations (if needed), Bonferroni or Newman-Keuls comparisons were made between 5 age groups or 2 gender groups within each plasma component (Table 3), or between the 3 genotype groups for children or for adults (Table 4).

The sensitivity of the plasma assays for homocysteine, prothrombin and PAI-1 was determined by calculating the proportion of children with the abnormal genotype (C677T MTHFR, prothrombin 20210A, PAI-1 4G/4G) who had plasma levels of homocysteine, prothrombin or PAI-1 > 2 SD from the mean. Specificity of the plasma assays was determined by calculating the proportion of children with the normal genotype who had plasma levels of homocysteine, prothrombin or PAI-1 ≤ 97.5th percentile. The cutoff value (mean + 2 SD) was derived from the cohort of children determined to have the normal (wide-type) genotype.

Results

Subjects

Two hundred and thirty-seven subjects were enrolled in the study. This group consisted of 197 children aged 6 months to 16 years (87 girls, 110 boys) and 40 adults (17 males and 23 females). Of these, race was specified in 195 children, with 165 (85%) white, 29 (15%) African-American, 1 (0.5%) other. Of the 40 adults, 36 (90%) were white, 2 (5%) African-American, and 2 (5%) other. The MTHFR and prothrombin genotypes were determined in 227 subjects (187 children and 40 adults) and the PAI-1 genotype in 226 subjects (186 children and 40 adults) (Table 1). Fasting plasma total homocysteine levels were measured in 209 subjects (170 children and 39 adults). Plasma prothrombin, PAI-1 antigen and PAI-1 activity levels were determined in all 237 subjects (197 children and 40 adults). There were 161 children and 39 adults with both MTHFR genotype and homocysteine level determinations.

Prevalence of the MTHFR, Prothrombin, and PAI-1 Gene Mutations and Polymorphisms

The distributions of the three gene mutations were virtually identical in children and adults (Table 1). Whites were more likely than African-Americans to be carriers of the C677T MTHFR allele (χ^2 = 12.18, p = 0.001) and the 4G allele of the PAI-1 gene (χ^2 = 7.07, p = 0.008) (Table 1).

Table 4 Relationships between gene variants and plasma homocysteine, prothrombin, PAI-1 antigen and activity levels

	Children			Adults			Children versus adults p value
	# of subjects	# (%) with levels ≥ 95th percentile*	Mean ± SD	# of subjects	# (%) with levels ≥ 95th percentile*	Mean ± SD	
MTHFR gene C677T variant and homocysteine levels (μM/L)							
NN	83[†]	4 (5%)	5.9 ± 5.4[ab]	19	1 (5%)	7.0 ± 2.1[a]	0.018
MN	61[†]	1 (2%)	5.5 ± 1.7[a]	16	0 (0%)	7.6 ± 1.5[a]	0.0001
MM	17[†]	6 (35%)	8.4 ± 8.0[b]	4	3 (75%)	16.8 ± 12.9[b]	0.059
	Fisher's test p=0.0001			Fisher's test p=0.002			
Prothrombin gene 20210A variant and prothrombin levels (%)							
NN	180	10 (6%)	91 ± 14[a]	38	3 (8%)	97 ± 12[a]	0.011
MN	7	2 (29%)	98 ± 19[a]	2	1 (50%)	101 ± 21[a]	0.66
	Fisher's test p=0.07			Fisher's test p=0.19			
PAI-1 gene 4G/5G polymorphism and PAI-1 antigen levels (ng/mL)							
5G/5G	67[†]	1 (1%)	13.2 ± 7.8[a]	16	1 (6%)	11.1 ± 9.5[a]	0.094
4G/5G	82[†]	6 (7%)	15.0 ± 12.6[ab]	16[†]	1 (6%)	9.0 ± 7.7[a]	0.0015
4G/4G	37[†]	3 (8%)	17.1 ± 9.4[b]	8[†]	1 (13%)	14.0 ± 15.3[a]	0.17
	Fisher's test p=0.19			Fisher's test p=1.0			
PAI-1 gene 4G/5G polymorphism and PAI-1 activity levels (U/mL)							
5G/5G	67[†]	2 (3%)	12.5 ± 8.4[a]	16	1 (6%)	5.8 ± 4.8[a]	0.0021
4G/5G	82[†]	2 (2%)	12.2 ± 7.1[a]	16	0 (0%)	7.0 ± 3.8[a]	0.0024
4G/4G	37[†]	7 (19%)	15.6 ± 10.0[a]	8	2 (25%)	10.2 ± 8.4[a]	0.11
	Fisher's test p=0.003			Fisher's test p=0.096			

* Age -specific levels for homocysteine and PAI-1 activity (abnormal gene subjects removed), sex -specific levels for prothrombin and PAI-1 antigen (abnormal gene subjects removed). [†] Distribution not normal, variance stabilizing transformation done. SD=Standard Deviation. NN=wild type, 'normal'; MN=mutant, heterozygote; MM=mutant, homozygote. [a,b,c] If letters are common, means do not differ; if letters differ, means differ, p≤.05 (Bonferroni or Newman-Keuls test).

Associations of Age, Sex, Race and Genotypes with Homocysteine, Prothrombin and PAI-1 Levels

Race was not a significant independent determinant of prothrombin, PAI-1 or homocysteine levels (Table 2). This allowed pooling of these test results for whites and non-whites.

By stepwise multiple regression, homocysteine was positively associated with age, PAI-1 activity was negatively associated with age, while PAI-1 antigen and prothrombin levels were associated with gender, being higher in girls than boys (Table 2). When the genotypes were added to the regression model as additional explanatory variables, the MTHFR genotype accounted for 2.9% of the variance of homocysteine ($p = 0.024$), and the PAI-1 gene accounted for 2.7% of the variance of PAI-1 antigen levels ($p = 0.023$) (Table 2).

"Normal" Plasma Levels of Homocysteine, Prothrombin, PAI-1 Antigen and PAI-1 Activity

The median, 90th and 95th percentiles of homocysteine, prothrombin, PAI-1 antigen/activity in children and adults, excluding individuals with the abnormal genotypes are provided (Table 3). There were no differences ($p > 0.2$) noted in the medians (by Wilcoxon) upon inclusion or exclusion of individuals with abnormal genotypes both in children and in adults (data not shown).

Homocysteine was higher in 11.3-16.9 year-old children than in younger children. Adults' homocysteine levels were higher than all pediatric age groups excepting the 11.3-16.9 year-old children (Table 3). PAI-1 activity was higher in 0.5-1 year olds than in other pediatric age groups or in adults (Table 3). PAI-1 activity was higher in each pediatric age group than in adults (Table 3). Prothrombin and PAI-1 antigen levels were higher in girls than boys (Table 3).

Relationships between Gene Mutations and Homocysteine, Prothrombin and PAI-1 Levels

To further examine relationships between gene mutations and homocysteine, prothrombin, and PAI-1 levels, age-specific 95th percentile cutpoints for plasma homocysteine levels (C677T MTHFR homozygotes removed) and PAI-1 activity levels (4G allele homozygotes removed) were cross-tabulated against genotype (Table 4). Similar cross-tabulations were done using sex-specific 95th percentile cutpoints for prothrombin (heterozygotes for the prothrombin gene removed) and plasma PAI-1 antigen levels (4G allele homozygotes removed) (Table 4).

Homozygous carriers of the C677T MTHFR genotype were more likely to have plasma homocysteine levels ≥ the age-specific 95th percentile, compared to heterozygotes and wild type normals, both among children ($p = 0.0001$) and adults ($p = 0.002$) (Table 4). Mean homocysteine levels were higher in MTHFR homozygotes than in heterozygotes, both among children and adults ($p < 0.05$) (Table 4). Plasma homocysteine levels were progressively higher in heterozygous and homozygous carriers of the C677T MTHFR allele, compared to wild type normals among adults ($p = 0.003$). This increase, however, was not significant in children ($p = 0.075$). The slopes differed significantly ($p = 0.003$), with larger increases in adults with increasing polymorphism of the C677T MTHFR gene. Adults had higher plasma homocysteine than children in the wild type and heterozygous genotypes for the MTHFR gene (Table 4).

The percentage of children with prothrombin levels ≥ 95th percentile was 29% among those heterozygous for the prothrombin gene mutation and 6% among wild type normals; this difference, however, was not statistically significant ($p = 0.07$) (Table 4). When compared to children with the wild type normal prothrombin gene, adults had higher prothrombin levels (Table 4).

Of children homozygous for the 4G/4G mutation, 19% had PAI-1 activity levels ≥ the age-specific 95th percentile, compared to 2% of heterozygotes and 3% of wild type normals, $p = 0.003$ (Table 4). Within the 5G/5G or 4G/5G genotype, adults had lower PAI-1 activity levels than children (Table 4).

Children homozygous for the PAI-1 gene 4G/4G polymorphism had higher PAI-1 antigen levels than wild type normal children (Table 4). In adults, the PAI-1 genotype was not associated with PAI-1 antigen levels (Table 4). Adults with the 4G/5G genotype had lower PAI-1 antigen levels than children with the 4G/5G genotype (Table 4).

Sensitivity and Specificity of the Plasma Coagulation Tests

Only a very small percentage of children with heterozygous or homozygous genotypes had their respective levels of prothrombin, PAI-1 or homocysteine ≥ 2 SD above the mean for wild type normals (sensitivity) (Table 5). Almost all children with normal genotypes also had plasma levels which fell below the 97.5th percentile (specificity) (Table 5).

Table 5 Sensitivity and specificity of the tests used to determine the plasma levels of homocysteine, prothrombin and PAI-1 antigen and activity. Cutoff for normal plasma levels was + 2 SD from the mean as determined in children with normal genotypes

	Cut point (+ 2 SD)	Sensitivity	Specificity
MTHFR gene C677T variant and homocysteine levels (μM/L)			
NN MN MM	16.7	 0/61=0% 1/17=6%	83/83=100%
Prothrombin gene 20210A variant and prothrombin levels (%)			
NN MN	119	 1/7=14%	175/180=97%
PAI-1 gene 4G/5G polymorphism and PAI-1 antigen levels (ng/mL)			
5G/5G 4G/5G 4G/4G	28.8	 8/82=10% 5/37=14%	63/67=94%
PAI-1 gene 4G/5G polymorphism and PAI-1 activity levels (U/mL)			
5G/5G 4G/5G 4G/4G	29.3	 2/82=2% 4/37=11%	63/67=94%

Discussion

Mutations and polymorphisms in genes leading to an increased risk of thrombosis are common (1-13). Studies in adults have demonstrated that the gene variants, C677T MTHFR, prothrombin 20210A, and PAI-1 4G/4G are associated with elevated plasma levels of homocysteine (5, 6), prothrombin (7-9), and PAI-1 (10-13) respectively. Our study demonstrates these relationships, for the first time, in a cohort of healthy pediatric subjects.

In our study, the C677T polymorphism of the MTHFR gene was an independent, positive, significant determinant of homocysteine levels in children, accounting for 2.9% of their variance. Based on our data, the MTHFR genotype should be considered an important determinant of fasting plasma homocysteine levels in children, as it is in adults in whom the presence of this mutation appears to correlate with an increased risk of thrombosis (5, 6). Homozygosity for the thrombophilic C677T MTHFR gene variant was present in 11% of Caucasians and 3%

of African-Americans in our subject cohort. This prevalence is similar to previous reports in adults (6, 24).

In the current study, a significant relationship between the 20210A allele and elevated plasma prothrombin levels was not demonstrated among children. These results will have to be verified in future studies. Heterozygosity for the 20210A allele was found in 4% of subjects (allele frequency of 0.02). This is similar to the previously reported prevalence in adults (7-9).

In our study, the PAI-1 4G/4G polymorphism was associated with PAI-1 activity levels ≥ the 95th percentile of wild type normals in children. The PAI-1 genotype was an independent, significant determinant of PAI-1 antigen levels in children, as well. Children homozygous for the PAI-1 4G/4G polymorphism had higher PAI-1 antigen levels than wild type normal children. Adults homozygous for the 4G allele showed higher PAI-1 antigen and activity levels compared to the other genotypes. This trend however, was not statistically significant and may be a reflection of the small sample size of adults. Previously in adults, homozygosity for the 4G allele has been noted to be associated with elevated PAI-1 activity in some studies (25), elevated PAI-1 antigen levels in others (11, 26) and PAI-1 activity but not antigen levels in one study (13). The association of the 4G/4G genotype with arterial and venous thrombosis however, has not been proved conclusively (27). Studies demonstrating a relationship between the 4G/5G genotype and PAI-1 levels in patients have failed to show such a relationship in control subjects (13, 28). The association of the PAI-1 4G/4G genotype with plasma PAI-1 levels needs to be better defined by further studies. In our subjects, 20% were homozygous and 43% were heterozygous for the 4G allele in the PAI-1 gene, similar to previous reports (26).

Our data provide age-specific fasting plasma homocysteine levels in children. Reference ranges for total homocysteine in pediatric patients have been previously reported but these were not fasting levels (29, 30). It is important to measure fasting levels, since food intake can influence plasma homocysteine levels (31). The status of folate, vitamin B6, and vitamin B12 which are important cofactors in homocysteine metabolism (32) could not be determined in our subjects due to inadequate blood samples. Presence of C677T MTHFR was correlated with a more marked elevation in homocysteine levels in adults than in children. This difference and the variation in homocysteine levels with age noted in our study may be related to the markedly differing dietary patterns in these age groups, since plasma homocysteine levels are influenced by nutrition (33).

The low frequency of the three genetic variants in the general population may account for the insignificant influence of the abnormal genotypes on normal ranges of the plasma components noted in our study. While a direct comparison of data from two separate studies is not entirely valid due to the different subject characteristics and methodology utilized, the normal ranges obtained in our study are similar to previously published results (18, 19).

The racial distribution (85% Caucasian, 15% African-American) of the subjects in our study is identical to that of the general population in the United States. In addition, race was not found to be a significant determinant of any of the plasma levels. Studies with a larger number of African-American subjects are needed to accurately determine the racial differences in genotypes.

In summary, our study demonstrates that in a healthy pediatric population, the C677T MTHFR and the PAI-1 4G/4G variants are significant determinants of plasma homocysteine and PAI-1 activity levels respectively. In addition, it demonstrates that the normal ranges provided can be used for reference even when the genotype of the individual child is not known. Studies of the incidence of the C677T MTHFR, prothrombin 20210A, and PAI-1 4G/4G genotypes in children with thrombosis, when compared to these healthy normals, will provide evidence as to which of these genetic variants are associated with thrombophilia.

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Received June 30, 1998 Accepted after resubmission February 3, 1999