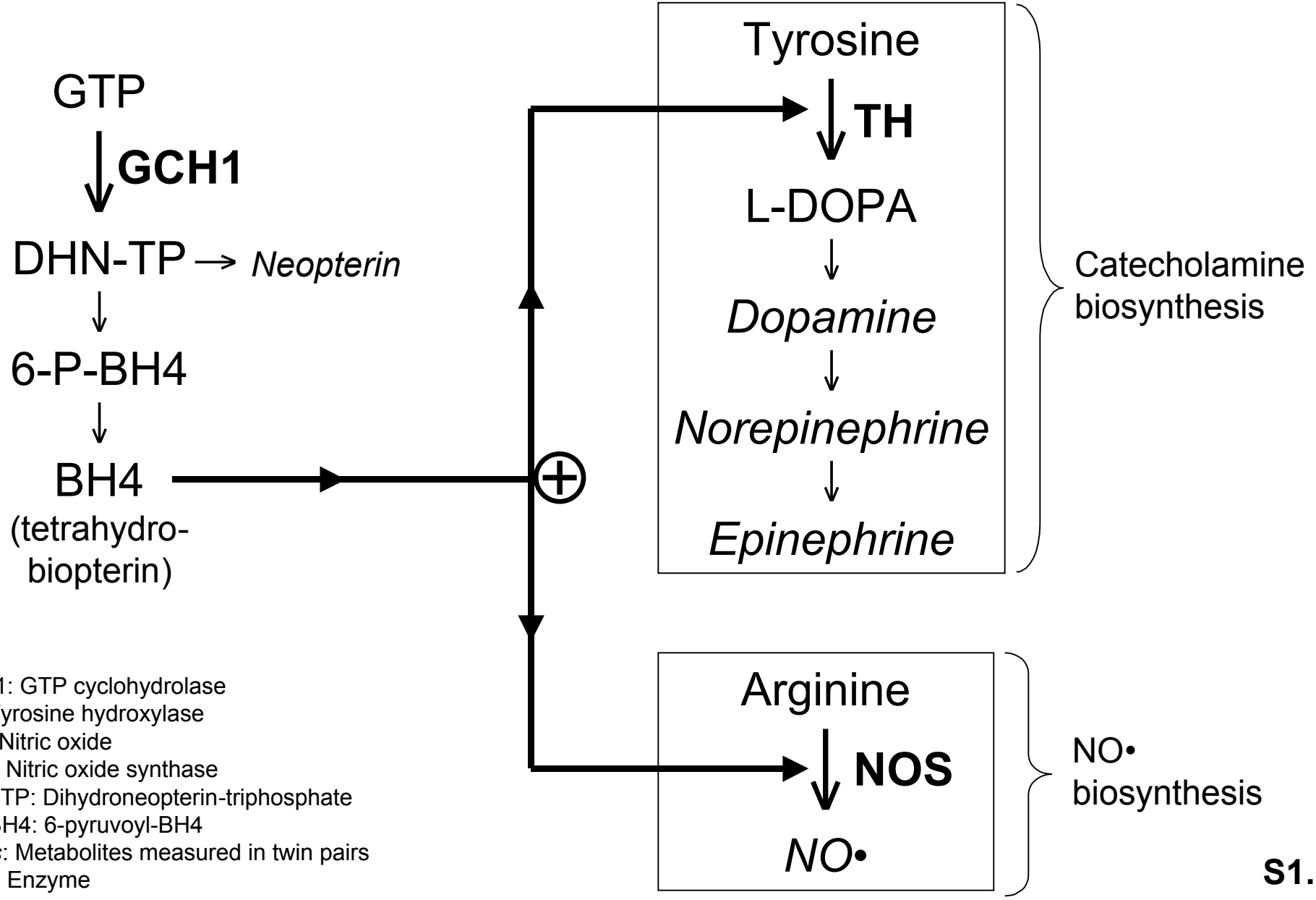
GTP cyclohydrolase (*GCH1*): Role in formation of catecholamines and nitric oxide (NO•)

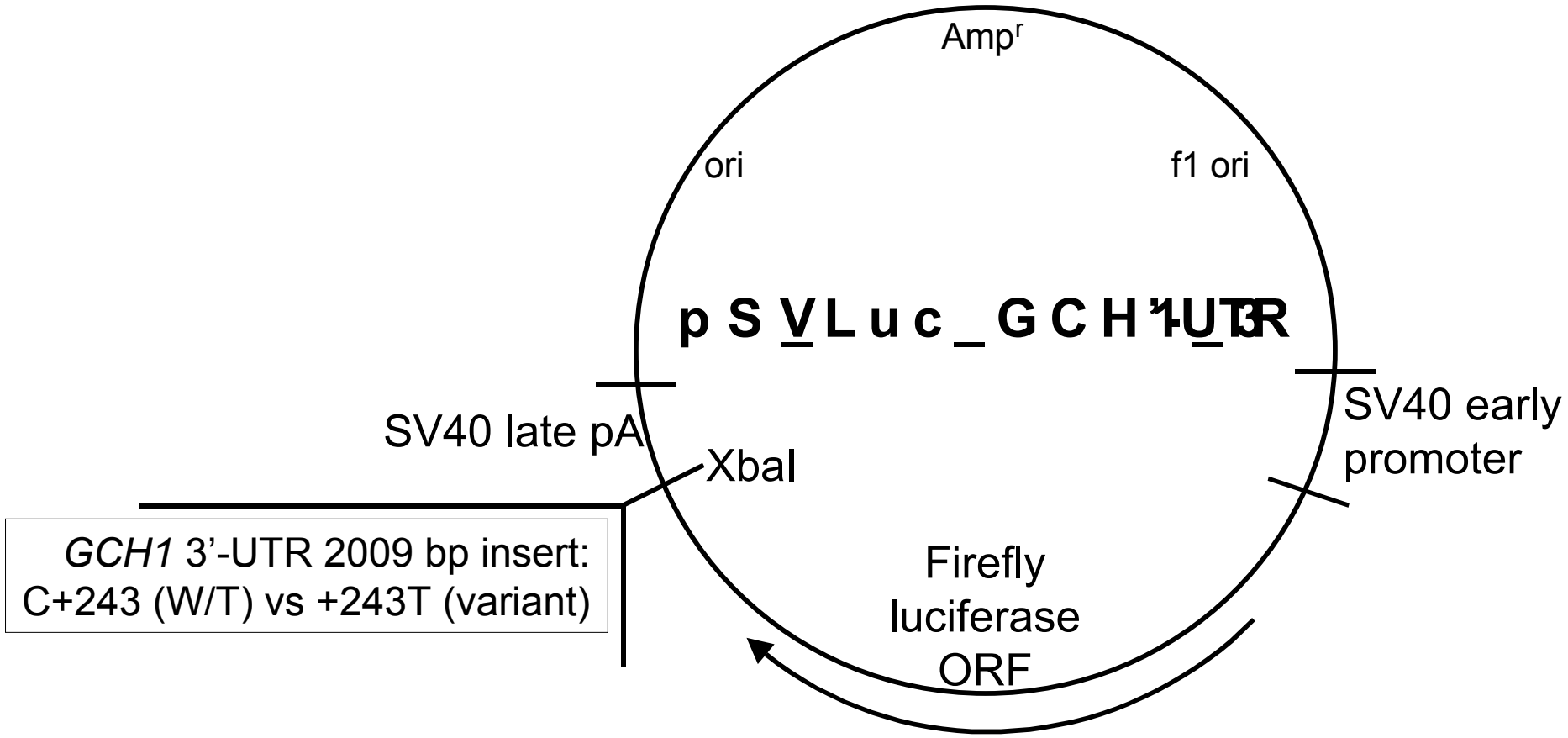


GCH1: GTP cyclohydrolase
TH: Tyrosine hydroxylase
NO•: Nitric oxide
NOS: Nitric oxide synthase
DHN-TP: Dihydroneopterin-triphosphate
6-P-BH4: 6-pyruvoyl-BH4
Italics: Metabolites measured in twin pairs
Bold: Enzyme

S1.

GCH1 3'-UTR variant C+243T:

Effect on transfected luciferase reporter expression in chromaffin cells



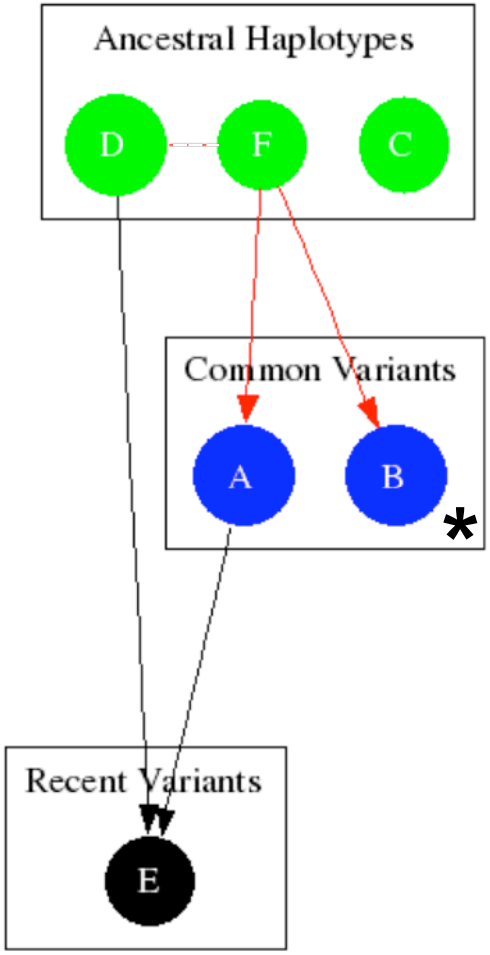
GCH1 3'-haplotype block: Human phylogeny.

Ancestral Haplotypes		Num
C	A C M M M C	8
D	C C T T T T	7
F	A ? T T T C	(112)

Common Variants		Num
A	A C T T T C	85
B	A T T T T C	27

3'-UTR +243**T** (C59038**T**)

Recent Variants		Num
E	C C T T T C	1



SUPPLEMENTAL FIGURES AND LEGENDS.

Supplemental Figure 1. GTP cyclohydrolase (*GCH1*): Role in formation of catecholamines and nitric oxide (NO•). The diagram illustrates enzymatic pathways subserving formation of tetrahydrobiopterin (BH4), an essential cofactor for the neurotransmitter biosynthetic enzymes tyrosine hydroxylase (*TH*) and nitric oxide synthase (*NOS*). *GCH1* is the rate-limiting enzyme in the formation of BH4. BH4 is also a cofactor for phenylalanine hydroxylase and tryptophan hydroxylase.

Supplemental Figure 2. Effect of *GCH1* 3'-UTR polymorphism on gene expression *in vitro*. Construction of a 3'-UTR reporter plasmid. The vector was pGL3-Promoter (Promega). Wild-type (C+243) versus variant (+243T) 3'-UTRs were subcloned into the XbaI site downstream (3') of the firefly luciferase open reading frame.

Supplemental Figure 3. *GCH1* haplotype phylogeny. The HAP algorithm analyzed common SNPs (each minor allele frequency >10% in white or black subjects) across *GCH1*, to impute 2 haplotype blocks, the more 3' of which is displayed here, containing the 3'-UTR C+243T (C59038T). Block 2 is composed of the following polymorphisms: A37633C (intron 2), C59038T (exon 6; 3'-UTR), T59694A (exon 6; 3'-UTR), T59695A (exon 6; 3'-UTR), T59700A (exon 6; 3'-UTR), C60025T (exon 6; 3'-UTR). The resulting haplotypes are here designated A through F. Haplotype B uniquely bears the 59038T (+243T) variant, and is derived uniquely from Ancestral haplotype F. "Ancestral" (green disk), "Common" (blue disk), and "Recent" (black disk) haplotypes are defined in the Methods section. Red lines: single mutation events in a lineage. Black lines: recombination events in a lineage (necessarily two sources for each resulting daughter haplotype). "?" in an ancestral haplotype represents the precursor to a mutation. For example, we know that B and F differ by one mutation. The ancestral haplotype F contains a question mark where that mutation occurs. In fact, one of

the variants (C or T) was the original and the other is the mutation, but we cannot conclusively determine which one arose first. The "?" allows us to present the information that the two haplotypes are related, knowing where the mutation occurred, but not which haplotype is the parent. Ancestral haplotypes with "?" have their numbers in parentheses, since these ancestors do not occur in the modern experimental data; the number in parentheses is the sum of the two haplotypes that are possible given the uncertainty (“?”) at that base position. For example, the count for F is (112), which is the sum of the counts for A and B. The sum of all of the numbers not in parentheses is the total number of haplotypes in the sample (or 2 times the number of individuals studied). Haplotypes were imputed from resequencing data in n=64 individuals (Num), 30 African American (Afr), and 34 white (Eur).

Supplemental Table 1: GTP cyclohydrolase (*GCH1*): Systematic human polymorphism discovery. Polymorphisms with minor allele frequencies >5% are indicated in **bold** type.

GTP cyclohydrolase (*GCH1*): Systematic human polymorphism discovery

SNP #	Polymorphism[a]	Position[b]	Amino acid change	Minor allele frequency in population (2n=number of chromosomes) Black (2n=60)	White (2n=68)	Total (2n=128)
1	A/G	-1009	N/A	0.10	0	0.047
2	**G/A**	**-811**	**N/A**	**0.617**	**0.25**	**0.42**
3	**T/C**	**-756**	**N/A**	**0.617**	**0.25**	**0.42**
4	G/T	-689	N/A	0.033	0	0.016
5	G/C	-688	N/A	0.033	0	0.016
6	A 1-bp deletion	-685	N/A	0.033	0	0.016
7	**G/A**	**-592**	**N/A**	**0.033**	**0.074**	**0.055**
8	G/A	-528	N/A	0.017	0	0.008
9	T/G	-229	N/A	0.083	0	0.039
10	C/G	-185	N/A	0.05	0	0.023
11	G/C	-164	N/A	0	0.029	0.016
12	G/A	-145	N/A	0.017	0.015	0.016
13	**C/T**	**18**	**N/A**	**0.183**	**0**	**0.086**
14	A/C	24	N/A	0.017	0	0.008
15	C/T	28	N/A	0.067	0	0.031
16	G/C	80	N/A	0.017	0	0.008
17	**C/T**	**109**	**N/A**	**0.183**	**0**	**0.086**
18	C/T	359	Leu71Leu	0.017	0	0.008
19	C/G	544	N/A	0.017	0.059	0.039
20	**C/T**	**635**	**N/A**	**0.15**	**0**	**0.07**
21	A/C	37558	N/A	0.033	0	0.016
22	**A/C**	**37633**	**N/A**	**0**	**0.059**	**0.063**
23	G/A	43146	N/A	0.017	0	0.008
24	G/C	56024	N/A	0	0.015	0.008
25	C/T	56968	Arg184Cys	0	0.015	0.008
26	G/T	57053	N/A	0.017	0	0.008
27	G/A	57133	N/A	0.017	0	0.008
28	**C/T**	**59038**	**N/A**	**0.20**	**0.221**	**0.211**
29	C/T	59407	N/A	0	0.015	0.008
30	G/A	59472	N/A	0.017	0	0.008
31	A/G	59527	N/A	0.017	0.088	0.047
32	T/C	59645	N/A	0.067	0	0.031
33	T/C	59646	N/A	0.067	0	0.031
34	(TTC)4/(TTC)3	59685	N/A	0.067	0	0.031
35	**T/A**	**59694**	**N/A**	**0.133**	**0**	**0.063**
36	**T/A**	**59695**	**N/A**	**0.133**	**0**	**0.063**
37	**T/A**	**59700**	**N/A**	**0.133**	**0**	**0.063**
38	**AT 2-bp insertion**	**59852**	**N/A**	**0.133**	**0**	**0.063**
39	C/T	59978	N/A	0.083	0	0.039
40	**C/T**	**60025**	**N/A**	**0**	**0.103**	**0.055**
41	C/A	60361	N/A	0.017	0	0.008
42	T/C	60710	N/A	0.017	0	0.008

a: Major allele/Minor allele.
b: The NCBI source clones are NM_00016 (mRNA isoform 1), NP_000152 (protein), and NT_0-26437 (genomic DNA). Base positions are numbered downstream (+) or upstream (-) of exon 1 (cap site).

Supplemental Table 2. Shared genetic determination (genetic covariance, RhoG [ρ_G], pleiotropy) and environmental determination (environmental covariance, RhoE [ρ_E]) for traits correlated with nitric oxide excretion. RhoG and RhoE were determined in SOLAR. Both RhoG and Rho E are fractions, scaled from –1 to +1. Spearman non-parametric trait-on-trait correlations are also shown. Significance (p) values for correlations refer to differences from zero (i.e., no correlation).

	Spearman correlation of that trait with urine nitric oxide/creatinine			**Heritability**		**Shared determination by environment (RhoE, environmental covariance; fraction)**			**Shared determination by heredity (Pleiotropy; RhoG, genetic covariance; fraction)**		
	Correlation coefficient	**P**	**N**	**h^2**	**SEM**	**RhoE [ρ_E]**	**SEM**	**P**	**RhoG [ρ_G]**	**SEM**	**P**
Physical.											
Body mass index, kg/m^2	-0.056	0.305	306	0.822	0.0297	-0.0477	0.0908	0.599	-0.0774	0.114	0.501
Hemodynamic.											
Systolic BP mmHg	0.018	0.561	306	0.444	0.0706	0.154	0.0886	0.0874	-0.127	0.153	0.398
Diastolic BP, mmHg	0.032	0.733	306	0.514	0.0619	-0.0597	0.0870	0.494	0.00915	0.136	0.946
Heart rate, bpm	**-0.112**	**0.041**	306	0.303	0.0781	-0.0752	0.0890	0.401	-0.128	0.176	0.469
Autonomic.											
Baroreflex coupling at 0.05-0.15 Hz, msec/mmHg	-0.029	0.592	306	0.339	0.126	0.0198	0.0940	0.833	-0.053	0.194	0.785
Pulse (R-R) interval, msec/beat											
Maximum (log10)	0.061	0.263	306	0.0958	0.100	-0.144	0.0851	0.0970	0.603	0.449	0.0607
Mean	**0.119**	**0.0305**	330	0.618	0.0574	0.0624	0.0923	0.501	0.133	0.131	0.314
Minimum (log10)	0.085	0.118	306	0.277	0.0864	0.0737	0.0883	0.406	0.0297	0.189	0.876
Standard deviation (log10)	-0.011	0.839	306	0.141	0.0876	-0.0449	0.0867	0.605	0.303	0.273	0.242
Biochemical.											
Urine neopterin, µmol/mol creatinine	**0.419**	**0.000001**	338	0.382	0.0734	**0.326**	**0.0754**	**0.000069**	**0.493**	**0.128**	**0.00183**
Urine dopamine, ng/gm creatinine (log10)	**0.267**	**0.0000015**	316	0.355	0.0692	0.123	0.0801	0.128	0.244	0.1496	0.119
Urine epinephrine, ng/gm creatinine	**0.344**	**0.0000010**	315	0.681	0.0486	**0.266**	**0.0801**	**0.00165**	0.227	0.116	0.0647
Urine norepinephrine, ng/gm creatinine	**0.233**	**0.0000295**	315	0.465	0.0649	0.0879	0.0823	0.288	0.0672	0.139	0.632