Worldwide Ethnic Distribution of the G Protein β3 Subunit 825T Allele and Its Association with Obesity in Caucasian, Chinese, and Black African Individuals

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Abstract. Recently, it was demonstrated that one allele (825T) of the gene encoding the G protein β3 subunit (*GNB3*) is associated with hypertension in Germans. This study investigates a possible association with obesity in young male Germans, Chinese, and black South Africans with low, intermediate, and high 825T allele frequencies, respectively. In each of these three distinct cohorts, the 825T allele frequency was increased significantly in overweight (body mass index [BMI] ≥25 kg/m^2) and obese individuals (BMI >27 kg/m^2) compared to those with normal weight. The 825T allele frequencies in these three BMI groups were, respectively, 29.5, 39.3, and 47.7% in Germans, 46.8, 53.9, and 58.6% in Chinese, and 83.1, 87.7, and 90.9% in South Africans. In each of these three distinct groups, the 825T allele was significantly associated with obesity with odds ratios between 2 and 3. More urban than rural black Africans were overweight despite similar 825T allele frequencies in both populations, which underscores the role of both genetic and environmental factors. BP values in young male whites increased significantly with increasing BMI values but were independent of the C825T polymorphism, suggesting that hypertension associated with the 825T allele could be a consequence of obesity. Genotyping of 5254 individuals from 55 native population samples from Africa, the Americas, Europe, Asia, Australia, and New Guinea demonstrated highest 825T allele frequencies in black Africans (82%) and intermediate values in east Asians (47%). It is anticipated that high frequencies of the 825T allele in Africans and Asians may contribute to an obesity and hypertension epidemic if Westernization of lifestyles continues.

Currently available data suggest a worldwide continuous increase in obesity prevalence, which is recently also being observed in developing countries (1). This prompts some authors to predict an "obesity epidemic" (2) with an increased prevalence of hypertension, stroke, coronary artery disease, and type 2 diabetes mellitus, for which obesity is a major risk factor (3). A considerable part of obesity is due to environmental factors and lifestyle, but between 40 and 70% of the variation of body mass index (BMI) is estimated to be heritable (4).

We recently described a C825T polymorphism in the gene *GNB3* encoding the β3 subunit of heterotrimeric G proteins, which are key components of intracellular signal transduction in all cells of the body (5). The 825T allele is associated with the occurrence of a splice variant, termed Gβ3-s, which, despite a deletion of 41 amino acids, is functionally active in a reconstituted system. The *GNB3* 825T allele is also associated with enhanced G protein activation, resulting in increased cell

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proliferation (6,7) and hypertension in Caucasians (5,8,9). The frequency of the 825T allele (fT) was markedly increased to 50% in young Canadian Oji-Cree Indians compared to 25 to 30% in Caucasians and, unexpectedly, the 825T allele was associated with lower rather than increased BP values (10). Nevertheless, fT was increased (57%) in subjects under antihypertensive therapy, although this was not statistically increased from control subjects potentially due to the small number of hypertensive individuals in this population. Similarly, fT amounted to 49% in Japanese, but no association of the 825T allele with hypertension was detected (11). Taken together, these findings suggested significant ethnic differences in 825T allele distributions and, possibly, inter-ethnic differences in associations with hypertension.

The present study was designed to investigate two main topics. First, we searched for a potential association between the 825T allele and obesity in individuals of different ethnicity and living habits in their native country. Second, we determined the worldwide ethnic distribution and the presumed ancestral state of the *GNB3* 825 polymorphism.

Our analyses were based on some underlying considerations: First, Na^+/H^+ exchanger activity, *i.e.*, the intermediate phenotype leading to the detection of the C825T polymorphism (5,12), is elevated in obese hypertensive and normotensive subjects but not in lean individuals (13). Thus, hypertension associated with enhanced G protein activation as indicated by the 825T allele could develop secondary to obesity. Furthermore, intracellular signal transduction *via* pertussis toxin (PTX)-sensitive G proteins is of major importance in adipogenesis (14). Transgenic mice lacking the PTX-sensitive Giα2 subunit are lean and deficient in fat mass (15). On the other hand, increased expression of Giα2 or expression of constitutively active Giα2 subunits in fibroblasts results in lipid accumulation and adipogenic conversion of cells (16). Thus, we tested for an association of the *GNB3* 825T polymorphism and obesity in several ethnic groups.

Materials and Methods

This study was conducted in agreement with the Declaration of Helsinki, and informed consent was obtained from all enrolled individuals.

BMI Study

In addition to nutritional status and physical activity, BMI strongly depends on age, gender, social and ethnic background, as well as country of residence (17). Furthermore, existing disorders and medication can exert uncontrollable effects on body weight. Therefore, all measures were taken to render the involved study groups as similar as possible. In the present study, only men ages 18 to 30 were included. No attempt was made to specifically enrich the sample with underweight or overweight individuals. Thus, the enrolled cohorts roughly resemble cross-sectional samples of the respective gender and age groups of the respective countries. Body weight was determined in light clothing, and body height was measured without shoes, heels together, with back to the wall. Overweight was defined as BMI $\geq$25.0 kg/m^2 and obesity as $>$27.0 kg/m^2 (3).

German Cohort. We recruited 277 healthy male Caucasoid individuals ages 18 to 30 yr at the Department of Blood Transfusion Medicine, University Hospital of Essen. These individuals attend the blood bank usually every 3 mo for blood donation and are under close health surveillance to guarantee high-quality blood products. According to German law, blood donors must be free of any medication and acute or chronic infectious diseases, to mention but a few requirements. Thus, these individuals represent a cross-sectional sample of young healthy men of the German population. BP is measured regularly in these individuals at every visit in the sitting position after a rest of 10 min using standard sphygmomanometry.

Chinese Population Sample. Male healthy Chinese subjects ages 18 to 30 yr were recruited at the Jinan Technical College and the Tongji Medical University at Wuhan and the samples were combined.

African Populations. Black male, healthy individuals ages 18 to 30 yr were recruited by the National Blood Transfusion Service Zimbabwe (Harare; $n = 450$) and The South African Blood Transfusion Service (Johannesburg; $n = 256$).

Ethnic Distribution Study

DNA samples were obtained from previously established, anonymous human diversity collections or from newly recruited volunteers. No further information regarding health status is available. Thus, reported values are estimates of 825T allele frequencies in the different populations studied and cannot be used as reference values for disease-related studies. DNA samples from the West Pygmies were purchased from the Coriell Institute for Medical Research (Camden, NJ). DNA samples from nonhuman primates were prepared from mouth swabs and obtained from different zoos in Germany.

DNA Preparation and Genotyping

DNA was prepared and PCR-based genotyping was done as described previously (5). In the BMI studies, all genotyping was performed in a blinded manner, *i.e.*, investigators were unaware of the individuals' data. PCR products of nonhuman primate DNA samples were directly sequenced using standard procedures.

Statistical Analyses

Allele frequencies were compared using Cochran–Mantel–Haenszel test for trend over the three genotypes. Other frequency comparisons were done using the χ^2 test or the χ^2 test for trend. Wherever continuous variables were compared, *t* test (in case of two groups) or one-way ANOVA (in case of more than two groups) was applied. When odds ratios (OR) adjusted for other terms (age or sample collective) were calculated, logistic regression was used. All confidence intervals are calculated at the 95% level.

Results

Characterization of Enrolled Individuals

Anthropometric data of the enrolled individuals are summarized in Table 1. 825T allele frequencies differed significantly between different ethnic groups and were lowest in Germans (31.9%), intermediate in Chinese (47.7%), and highest in Africans (81.4 to 84.1%). BMI values were significantly higher in Germans compared to all other ethnic groups (Figure 1). Remarkably, BMI was higher in urban compared to rural Zimbabweans. Using a definition for underweight of BMI $<$18.5 kg/m^2, a marked difference between Germany, a completely industrialized country with a largely uniform lifestyle, and the developing countries was observed. Conversely, the risk of being overweight (BMI $\geq$25.0 kg/m^2 *versus* $<$25.0 kg/m^2) was

Table 1. Characteristics of study populations[a]

Characteristic	Germany (Essen)	China (Wuhan/Jinan)	South Africa (Johannesburg)	Urban Zimbabwe (Harare)	Rural Zimbabwe (Bulawayo)
n	277	960	254	223	236
Genotypes (TT/TC/CC)	28/121/128	215/486/259	178/71/5	148/67/8	159/69/8
Age (yr)	23.9 (3.4)	24.6 (4.4)	22.2 (4.5)	22.4 (3.9)	19.0 (2.0)
Weight (kg)	76.5 (9.8)	64.4 (9.1)[b]	65.9 (10.5)[b]	67.3 (8.4)[b]	64.2 (9.2)[b,c]
Height (cm)	180.4 (7.3)	170.8 (5.1)[b]	171.8 (8.3)[b]	173.6 (6.3)[b]	173.2 (6.6)[b]
BMI (kg/m^2)	23.5 (2.5)	22.0 (2.7)[b]	22.2 (3.3)[b]	22.4 (2.7)[b]	21.5 (2.3)[b,c]
Underweight (BMI <18.5 kg/m^2)	3 (1.1)	56 (5.8)[b]	23 (9.1)[b]	8 (3.6)[b]	16 (6.8)[b,c]
Normal weight (BMI 18.5 to 24.9 kg/m^2)	204 (73.6)	776 (80.8)	178 (70.1)	176 (78.9)	208 (88.1)
Overweight (BMI ≥25.0 kg/m^2)	70 (25.3)	128 (13.3)	53 (20.9)	39 (17.4)	12 (5.1)
Obese (BMI >27 kg/m^2)	22 (7.9)	58 (6.0)	22 (8.7)	11 (4.9)	6 (2.5)

[a] Categorical variables are given as *n* (%), continuous variables are means (SD). BMI, body mass index.
[b] $P < 0.01$ *versus* Germany.
[c] $P < 0.01$ *versus* urban Zimbabwe.

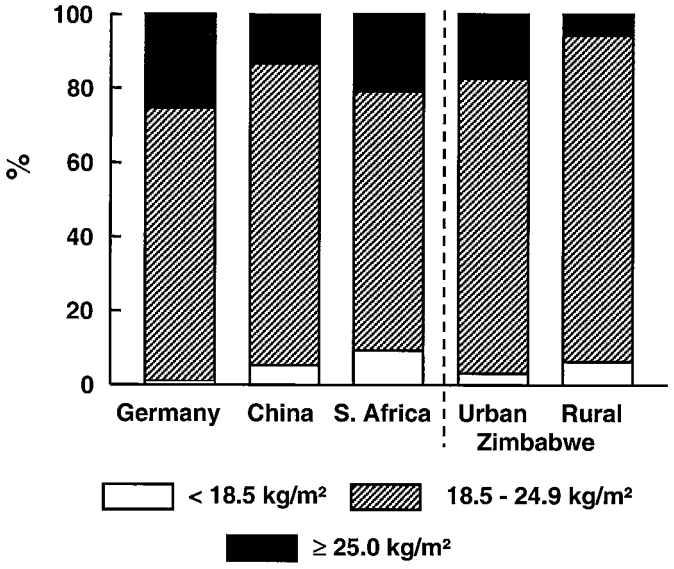

Figure 1. Distribution of body mass index (BMI) values in the different study groups. S. Africa, South Africa. For details, see the text and Table 1.

Table 2. GNB3 genotype and BMI in young, male Germans[a]

Parameter	BMI (kg/m^2)		
	<25.0	≥25.0	>27
TT	17 (8)	11 (16)	5 (23)
TC	88 (43)	33 (47)	11 (50)
CC	102 (49)	26 (37)[b]	6 (27)[c]
Σ	207	70	22
fT (%)	29.5	39.3	47.7
Height (cm)	180.6 (7.3)	179.6 (7.4)	181.0 (5.9)
Weight (kg)	73.2 (7.8)	86.2 (8.7)[d]	93.5 (6.9)[d]
SBP (mmHg)	128.7 (10.2)	133.1 (12.9)[d]	135.9 (10.6)[d]
DBP (mmHg)	78.2 (7.2)	81.9 (9.2)[d]	84.3 (9.7)[d]

[a] Genotypes are given as *n* (%) and continuous variables as means (SD). fT, 825T allele frequency; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[b] $P = 0.03$, genotype distribution significantly different from BMI <25 kg/m^2 (χ^2 test for trend).
[c] $P = 0.01$, genotype distribution significantly different from BMI <25 kg/m^2 (χ^2 test for trend).
[d] $P < 0.001$, significantly different from group with BMI <25 kg/m^2.

increased in Germany compared to China, South Africa, and Harare (Figure 1 and Table 1). Interestingly, within Zimbabwe a significantly increased OR of 3.4 (1.8 to 6.7; $P = 0.0002$) for overweight was found upon comparison of the urban with the rural sample, which again shows the impact of lifestyle on BMI. Having confirmed this expected relationship between BMI and country of residence, we subsequently investigated the potential association between the 825T allele and BMI within the specified populations.

825T Allele, BMI, and BP in German Men

BMI was significantly different between genotypes (TT = 24.9 ± 2.3 kg/m^2; TC = 23.6 ± 2.4 kg/m^2; CC = 23.1 ± 2.5 kg/m^2; $P = 0.003$) (Table 2). This difference was also seen when the TT and TC genotypes were combined (23.8 ± 2.4 kg/m^2) and compared with the CC genotype ($P = 0.01$). BMI was correlated with weight but not height. Age was not significantly different between genotypes (TT = 26.4 ± 2.8; TC = 25.3 ± 3.5; CC = 25.7 ± 3.3 yr; $P = 0.2$). 825T allele frequency was 29.5, 39.3, and 47.7% in normal weight, overweight, and obesity, respectively (Table 2 and Figure 2). Genotype distribution of the overweight and obese group was significantly different from the group with normal weight ($P < 0.05$). For overweight (BMI ≥25 kg/m^2 *versus* <25 kg/m^2), OR (TT/CC) was 2.5 (1.1 to 6.1; $P = 0.03$) and OR (TC/CC) was 1.5 (0.8 to 2.6; $P = 0.2$). OR (TT/CC) for BMI >27 kg/m^2 *versus* < 25 kg/m^2 was 5.0 (1.4 to 18.3; $P = 0.0083$) and OR

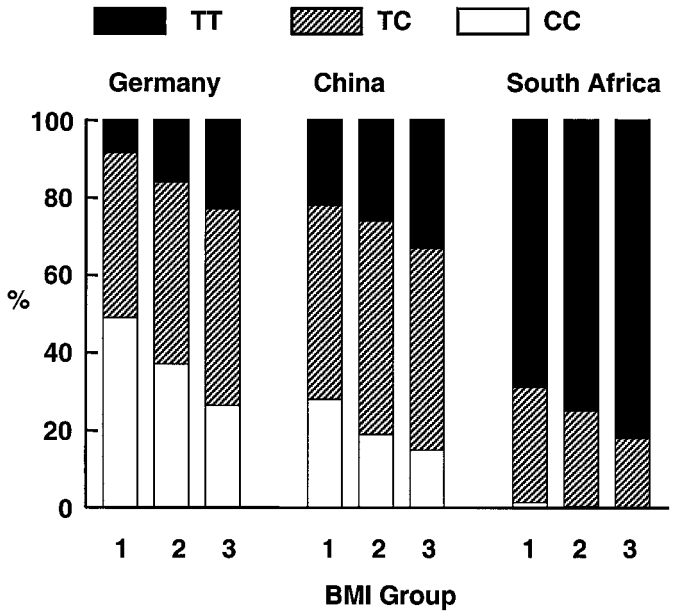

Figure 2. Genotype distribution according to BMI values in the German, Chinese, and South African sample. 1, BMI <25 kg/m^2; 2, BMI ≥25 kg/m^2; 3, BMI >27 kg/m^2. Genotypes are given as % of total.

(TC/CC) was 2.2 (0.8 to 6.3; $P = 0.13$). Systolic BP values were not significantly different between genotypes (TT: 129.6 ± 79.6 mmHg; TC: 129.3 ± 78.6 mmHg; CC: 130.4 ± 11.0 mmHg; $P = 0.86$), but significantly increased in the overweight and obese group compared to that with BMI <25 ($P = 0.003$) (Table 2). Systolic BP increased linearly by 0.5 mmHg per BMI unit ($r^2 = 0.05$; $P = 0.0002$). Likewise, diastolic BP values were independent of genotype (TT: 79.6 ± 6.1 mmHg; TC: 78.6 ± 8.4 mmHg; CC: 79.5 ± 7.9 mmHg; $P = 0.78$), but again significantly increased with BMI ($P < 0.001$) (Table 2) with a mean slope of 0.6 mmHg per BMI unit ($r^2 = 0.09$; $P < 0.0001$). Hence, the C825T polymorphism is apparently not directly associated with BP values in this group of young normotensive individuals, whereas BP values increase significantly with increased BMI values.

825T Allele and BMI in Chinese Men

Age was not significantly different between genotypes in the Chinese cohort (TT = 24.8 ± 4.5; TC = 24.6 ± 4.4; CC = 24.2 ± 4.3 yr). When compared by one-way ANOVA, BMI values were not significantly different between genotypes (TT = 22.0 ± 3.0 kg/m^2; TC = 22.2 ± 2.7 kg/m^2; CC = 21.8 ± 2.4 kg/m^2; $P = 0.2$). However, when the values of TT and TC genotypes were combined (BMI = 22.1 ± 2.7 kg/m^2), a statistically nonsignificant trend ($P = 0.1$) for increased BMI compared to CC genotype was noticed. 825T allele frequency was 46.8, 53.9, and 58.6% in normal weight, overweight, and obese individuals, respectively (Table 3 and Figure 2). OR (TT/CC) for overweight *versus* normal weight was 1.8 (1.0 to 3.1; $P = 0.03$) and OR (TC/CC) was 1.7 (1.0 to 2.7; $P = 0.04$). For BMI >27 kg/m^2 *versus* <25 kg/m^2, OR (TT/CC) was 2.7 (1.2 to 6.2; $P = 0.01$) and OR (TC/CC) was 1.8 (0.9 to 4.0; $P = 0.09$).

Table 3. GNB3 825T allele and BMI in young, male Chinese[a]

Parameter	BMI (kg/m^2)		
	<25	≥25	>27
TT	181 (22)	34 (26)	19 (33)
TC	416 (50)	70 (55)	30 (52)
CC	235 (28)	24 (19)	9 (15)
Σ	832	128	58
fT (%)	46.8	53.9[b]	58.6[c]
Height (cm)	170.6 (5.1)	172.2 (5.4)	172.2 (5.4)
Weight (kg)	62.0 (6.4)	80.5 (7.1)[d]	85.2 (6.3)[d]

[a] Genotypes are given as *n* (%) and continuous variables as means (SD). Abbreviations as in Tables 1 and 2.
[b] $P = 0.03$ for difference in genotype distribution *versus* normal weight from χ^2 test for trend.
[c] $P = 0.01$ for difference in genotype distribution *versus* normal weight from χ^2 test for trend.
[d] $P < 0.001$ *versus* group with BMI <25.0 kg/m^2.

825T Allele and BMI in Black African Men

825T allele frequency and genotype distributions were similar in individuals from South Africa and Zimbabwe regardless of rural or urban origin (Table 4). The rural African sample had significantly lower age and body weight compared to the urban African sample ($P < 0.01$). The prevalence of overweight in rural Africans (5%) was significantly lower than in Africans from Harare (17.4%) and Johannesburg (20.9%). Using these latter figures, we calculate a crude OR for overweight (combined urban *versus* rural) of 3.8 (2.1 to 7.1; $P < 0.0001$), age-adjusted OR of 2.7 (1.4 to 5.3), indicating a strong effect of environmental influences on BMI. The risk for overweight associated with the 825T allele was difficult to estimate within the respective African samples due to the relative lack of individuals with the CC genotype. Only a common logistic model, therefore, was calculated for the samples pooled from the Johannesburg and Harare populations. Crude OR (TT/CC) for overweight in the combined urban Johannesburg and Harare samples was 3.8 (0.5 to 28.9; $P = 0.16$), and crude OR (TC/CC) is 3.1 (0.4 to 24.3; $P = 0.25$). Although these values did not reach statistical significance due to the low number of homozygous 825C allele carriers, this effect was qualitatively similar to that observed in Germans and Chinese. Age-adjusted OR (TC/TT) was 0.7 (0.3 to 1.7), and OR (CC/TT) was calculated at 0.6 (0.1 to 5.3). In the Johannesburg sample, 825T allele frequency increased to 90.9% in individuals with BMI >27 kg/m^2 (Table 4 and Figure 2). There was no correlation between age and genotype in the African samples.

Finally, the impact of the 825T allele on overweight in "lean" populations was estimated after combining the Chinese (Table 3) and African (Table 4) samples. After adjustment for age and sample collective, OR (TT/CC) was 1.8 (1.1 to 3.0; $P < 0.02$) and OR (TC/CC) was 1.6 (0.97 to 2.5; $P = 0.06$).

Table 4. GNB3 825T allele and BMI in young, male black Africans[a]

Parameter	South Africa			Zimbabwe			
	BMI, Urban (Johannesburg)			BMI, Urban (Harare)		BMI, Rural	
	<25	≥25	>27	<25	≥25	<25	≥25
TT	138 (69)	40 (75)	18 (82)	119 (65)	29 (73)	151 (68)	7 (58)
TC	58 (29)	13 (25)	4 (18)	57 (31)	10 (25)	64 (29)	5 (42)
CC	5 (2)	0	0 (0)	7 (4)	1 (2)	8 (3)	0
Σ	201	53	22	183	40	223	12
fT (%)	83.1	87.7	90.9	80.6	85.0	79.2	82.1
Height (cm)	172.5 (8.1)	168.9 (8.6)	168.7 (9.4)	174.2 (5.9)	171.1 (7.2)	173.2 (6.5)	173.4 (9.2)
Weight (kg)	62.5 (7.8)	78.1 (10.1)[b]	84.0 (10.5)[b]	64.9 (6.2)	78.2 (8.6)[b]	63.6 (7.1)	82.5 (9.9)[b]

[a] Genotypes are given as *n* (%) and continuous variables as means (SD). BMI values are kg/m^2.
[b] $P < 0.05$, significantly different from groups with normal BMI.

Thus, the effect of the 825T allele on overweight was remarkably similar in "lean" and fully Westernized populations.

Ethnic Distribution of the GNB3 825T Allele

A total of 5254 DNA samples of unrelated individuals was analyzed. The ethnic distribution of the 825T allele is displayed in Table 5 and Figure 3. All populations with $n > 40$ were in Hardy–Weinberg equilibrium (HWE). This suggests that preferential selection of genotypes is not (or is no longer) detectable. The geographic frequency distribution of the 825T allele displays marked frequency intervals that correspond to the subdivision into major human ethnic groups, with black Africans (excluding the admixed American blacks, and the small Guinean sample) ranging from 74 to 91%, Mongoloids from 42 to 52%, and Caucasoid (except the Middle East) from 21 to 38%. In the Middle East and North Africa, the frequencies are transitional compared to Africans, ranging from 45 to 56%. The Amerindians have a wide frequency range (11 to 42%). Interestingly, the Khoisanoids and Pygmies, isolated descendants of the most ancient African populations (18,19), have lower values than other Africans, between 66 and 72%. Similarly, 825T allele frequencies were high in North Australian aborigines and individuals from Highland Papua New Guinea, but still lower than in Africans. In Table 5, the overall allele frequency of each major ethnic group (*e.g.*, 82% for black Africans) is also given, averaging over subpopulation averages rather than over individuals, to avoid distortion due to variable sample sizes. To test whether the frequency differences between ethnic groups might be artifacts of inadequate sample sizes, the nine major ethnic groupings in Table 5 were submitted to 2 by 2 comparisons (20). Most pairwise differences were found to be statistically significant at the $P < 0.05$ level, except for comparisons involving the Pygmies and the Papuans, for whom the sample sizes are smallest. Motivated by the uniformity of 825T allele frequencies among Europeans (Germans approximately 30%) and black Africans (approximately 82%), we estimated the proportion of European admixture in U.S. blacks (72% 825T allele) at 21%, in good agreement with previous estimates of 15 to 20% (21).

We also genotyped and sequenced DNA from unrelated animals of the species *Pan troglodytes* (common chimpanzee; $n = 4$), *Pan paniscus* (pygmy/Bonobo chimpanzee; $n = 2$), *Gorilla gorilla* (lowland gorilla; $n = 3$), *Pongo pygmaeus* (orangutan; $n = 3$), *Cercopithecus aethiops* (African green monkey; $n = 1$), *Macaca mulatta* (rhesus monkey; $n = 3$), and *Sanguinus oedipus* (cotton top tamarin; $n = 1$). All of these primates were typed homozygous carriers of the C825 allele in the *GNB3,* indicating the stability of this position for at least 30 million years, which in turn suggests that the C allele is the evolutionary ancestral allele in primates.

Discussion

825T Allele and BMI

The worldwide increasing prevalence of obesity prompts many researchers to determine modifiable and nonmodifiable (genetic) factors underlying this epidemic (22). We have investigated here whether the *GNB3* 825T allele, which predisposes for hypertension (5,8,9), potentially predisposes for obesity. This approach was prompted by the observation that enhanced signaling *via* PTX-sensitive G proteins enhances adipogenesis (16), whereas animals lacking the G protein Gαi2 subunit are runted due to deficiency in fat mass (15). Thus, intracellular signal transduction *via* Gαi2 could play a major role in the pathogenesis of obesity (14). Interestingly, the splice variant Gβ3-s associated with the 825T allele can interact with Gαi2 (5), and recent studies have confirmed that activation of heterotrimeric G proteins is enhanced in the presence of this splice variant (23). Thus, the 825T allele is not a nonfunctional genetic marker. Instead, it can be used for the prediction of enhanced intracellular signal transduction in humans. It should be noted, however, that linkage equilibrium with a yet unidentified alteration in *GNB3* cannot be excluded. The complete coding region of the G protein β3 subunit cDNA has been sequenced, and no changes apart from the C825T polymorphism have been identified (5). However, potential relevant mutations in introns of *GNB3* cannot be excluded. The *GNB3* locus located on chromosome *12p13* is flanked by the gene encoding CD4 and genes of unknown identity (24). Therefore,

Table 5. Worldwide ethnic distribution of the *GNB3* 825T allele

	n	TT	TC	CC	%T
Black Africans	1015	625	349	41	79
Guinea	13	4	9	0	65
Ivory Coast	33	22	10	1	82
Ghana	11	9	2	0	91
Nigeria	25	17	7	1	82
Cameroon	15	10	5	0	83
Congo (former Zaire)	44	28	15	1	81
Namibia (Ovambo)	30	22	8	0	87
Zimbabwe (Shona)	299	198	90	11	81
Zambia	42	22	18	2	74
South Africa (!Xhosa)	99	67	29	3	82
Kenya	22	17	5	0	89
Uganda	34	27	6	1	88
Tanzania	55	32	22	1	78
West Indies	10	7	3	0	85
United States (blacks)	283	143	120	20	72
East Pygmies					68
Kongo/ex-Zaire (Efe)	19	7	12	0	68
West Pygmies					72
C. African Republic (Biaka)	9	4	5	0	72
Khoisanoids					66
Namibia (Bushmen)	97	41	47	9	66
Caucasoids	3289	348	1443	1498	33
Germany	1855	162	795	898	30
Spain	30	3	12	15	30
Italy	31	4	14	13	35
Greece	16	1	8	7	31
Finland (Oulu)	100	3	41	56	24
Russia	28	1	10	17	21
Poland	60	2	22	36	22
Croatia	30	3	10	17	27
S. Hungary	27	1	16	10	32
Saudi Arabia (Riyadh)	200	51	103	46	51
Egypt (Cairo)	55	17	28	10	56
Morocco	29	7	12	10	45
Turkey	138	19	66	53	38
Iran	29	2	17	10	36
Uzbekistan	48	7	21	20	36
Pakistan (Karachi)	189	27	82	80	36
India (New Delhi, Lucknow)	240	25	114	101	34
India (Dravidians)	30	1	14	15	27
Sri Lanka (Singhalese)	154	12	58	84	27
Mongoloids	520	100	276	144	46
N. China (Han)	30	3	20	7	43
S. China (Wuhan)	222	40	108	74	42
S. China (Bai)	29	7	15	7	50
S. Korea	31	3	21	7	44
Japan (Shiga, Gifu)	146	29	84	33	49
N. Thailand	62	18	28	16	52
Amerindians					26
W. Canada (Musqueam)	27	3	12	12	33
N. Brazil (Cayapo)	25	1	12	12	28
N. Brazil (Yanomami)	25	3	10	12	32
N. Brazil (Araro do Laranjal)	17	0	10	7	29
N. Brazil (Awa Guaja)	23	2	9	12	28
N. Brazil (Parakanan)	17	2	1	14	15
N. Brazil (Katuena)	24	4	12	8	42
N. Brazil (Arara do Iriri)	27	0	6	21	11
N. Chile (Atacamenos)	30	1	17	12	32
S. Chile (Araucanians)	30	0	9	21	15
Australoids					72
N. Australia	30	13	17	0	72
New Guineans					50
Highland Papua NG	30	10	20	0	50

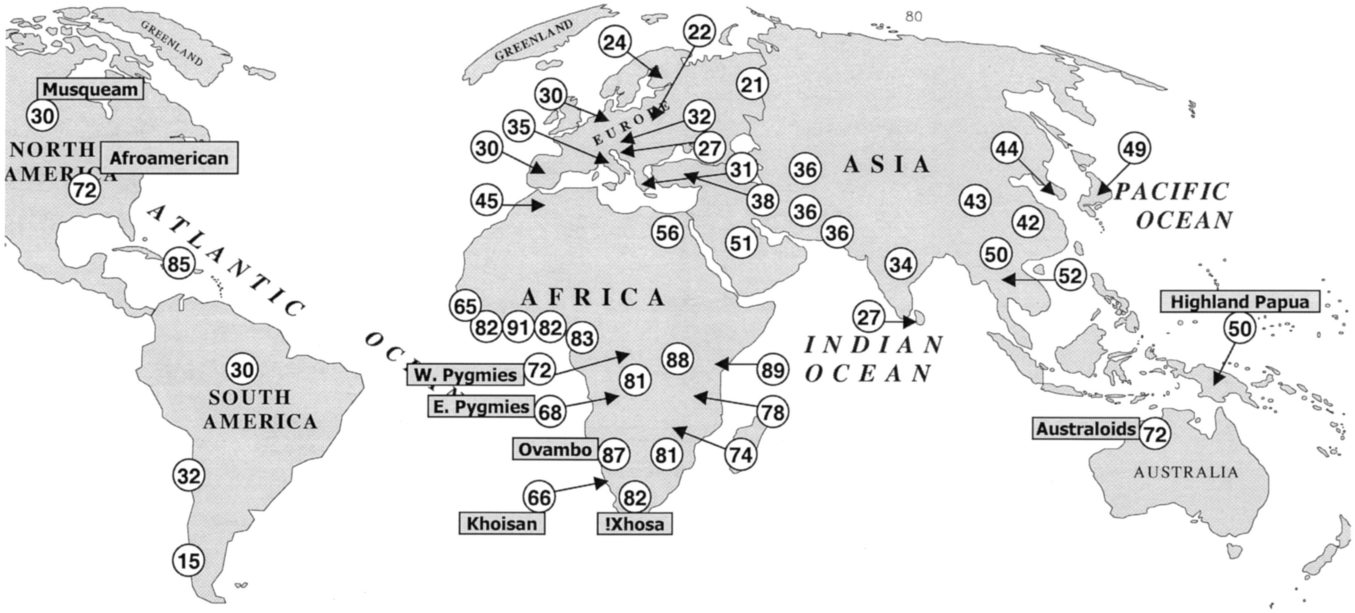

Figure 3. Worldwide distribution of the *GNB3* 825T allele. The figures are 825T allele frequencies; the small South American samples are combined.

linkage disequilibrium of the *GNB3* 825T allele with polymorphisms in these genes cannot be excluded. However, this latter possibility appears rather unlikely since the 825T allele is closely associated with enhanced G protein signaling (5), and no sequences have been identified in the vicinity of *12p13* that encode for G protein subunits or regulators of G protein function.

To investigate the effect of the 825T allele on BMI and BP in ethnically and culturally diverse populations, we set up the population cohorts as homogeneously as possible by including only men in the age range of 18 to 30 yr. Although individuals were selected at random to resemble a healthy, *quasi* cross-sectional sample from the considered age groups, they obviously did not constitute a random sample of the respective populations. This makes selection effects a possible disadvantage of the study. However, whereas selection may act on phenotype, despite all cautionary measures taken, there is no rationale for over- or undersampling a certain genotype given the phenotype. Furthermore, the prevalences of underweight and overweight in the sample are in good accordance with what is known of these traits in the general populations. As an example, the prevalences of underweight (5.8%) and overweight (13.3%) in our Chinese sample are similar to those reported by others, which were 11.6 and 9.8%, respectively, with an increasing trend for obesity as a result of lifestyle changes (25,26). Likewise, higher BMI in urban compared to rural areas of Zimbabwe has been documented (27).

We have used BMI as a measure of body fat mass, although we are aware of the fact that BMI is also influenced by muscle and bone mass. However, more sophisticated techniques for direct assessment of body fat, *e.g.*, densitometry or bioelectrical impedance techniques, were not available in China and in Africa. On the other hand, most investigators agree that BMI values can be used to compare body composition across ethnic groups (28,29). The BMI cutoff point of ≥ 25 kg/m^2 used here for defining overweight is commonly accepted. Obesity, on the other hand, is frequently defined as a BMI value of ≥ 30 kg/m^2, although others have used a cutoff value of >27 kg/m^2 in recent studies (30). Since most of our samples were taken from developing/transitional countries in which overt obesity is relatively rare in the studied age groups, we here defined obesity as a BMI >27 kg/m^2.

A consistent and significant effect of the 825T allele on BMI is evident in three ethnically and culturally diverse populations. This effect was most pronounced in Germans, whose lifestyle is characterized by high-caloric, fat-enriched nutrition and low physical activity. A similar effect of the 825T allele on BMI was found in the Chinese cohort, despite the fact that nutrition is poor in fat but rich in rice and vegetables, thus being quite different from that of Germans. Interestingly, the OR (TT/CC) for overweight were comparable in the Chinese and the German samples. The precise risk for overweight associated with the 825T allele is difficult to estimate in the African samples due to the relative lack of individuals with the CC genotype. However, only one (= 4.8%) out of a total of 21 CC genotypes was found in the overweight group, suggesting a qualitatively similar effect. In general, we estimate the OR (TT/CC) for overweight to be approximately 2 to 3 for all populations studied, a rather impressive value for such a highly variable phenotype, and our finding of a consistent association across different ethnic groups makes a fortuitous association very unlikely.

On the basis of the available data, it is difficult to say whether the 825T allele exerts a codominant or a recessive influence on BMI, the latter being a weak phenotype and a quantitative trait strongly influenced by environmental factors. On the cellular level, one 825T allele suffices to generate Gβ3-s via alternative splicing and to establish a phenotype of

enhanced G protein reactivity (5). Anecdotal evidence suggests that G protein activation is further enhanced in cells from individuals with the TT genotype (W. Siffert, unpublished observations). Thus, the 825T allele appears to exert a codominant effect on G protein signaling on the cellular level, which may be explained by an increased expression of the splice variant Gβ3-s in the presence of two affected alleles. Previous studies on hypertension suggested at least a gene–dose effect regarding BP with an increased risk for homozygous compared to heterozygous individuals (5). A similar trend was observed here, as heterozygous 825T allele had increased OR for overweight and obesity. This effect was statistically significant in the Chinese group. At present, we cautiously propose a codominant effect of the 825T allele on overweight, this hypothesis being, however, largely based on previously made observations on the cellular level (5). A definite answer to this pending question requires a different study setup, for example a case-control study specifically designed to compare genotype distribution in overtly obese (BMI >30 kg/m^2) and normal weight individuals. Furthermore, studies are required to clarify the molecular mechanisms that may be responsible for the tendency to gain weight in individuals carrying an 825T allele. Although studies on transgenic animals and transfected cell lines suggest a major role of PTX-sensitive G proteins in adipogenesis (15,16), such findings do not explain the associations made here. Additional studies on transgenic animals expressing Gβ3-s are urgently required to establish a potential causal relationship between this splice variant, enhanced G protein activation, and obesity. In addition, characterization of signal transduction in adipocytes from homozygous 825T and C825 allele carriers is essential to determine whether obesity actually results from increased lipid accumulation.

The present study also demonstrates that obesity is not an inherited disease, but obviously requires the interaction of multiple susceptibility genes with environmental factors. This is illustrated by the large difference in overweight prevalence in the urban Harare and Johannesburg *versus* the rural Zimbabwe sample. The lifestyle of the rural sample is drastically different from the urban one with regard to two major aspects: The fat content of the diet is low and it consists mainly of maize meal and vegetables. Due to the lack of private cars and public transportation facilities, these individuals are accustomed to walking long distances. Thus, the level of physical activity can be considered high. In contrast, individuals from Johannesburg and Harare are largely Westernized with a high level of fat-rich nutrition and alcohol consumption. Thus, in the presence of an almost identical frequency of the 825T allele in the African rural and urban samples, only individuals exposed to an "unhealthy" Western lifestyle appear at risk for overweight. It is tempting to speculate that the 825T allele might be neutral as long as lifestyle resembles that of our hunter-gatherer ancestors but may become detrimental upon Westernization. Thus, presence of the 825T allele can only increase the risk for obesity in concert with certain behavorial or environmental factors, but clearly does not cause obesity by its sole presence. This would also be in accordance with the observation that the prevalence of overweight was highest in Germans despite lowest 825T allele frequency of the characterized samples. As an alternative hypothesis, one could propose that the genetic background of the different ethnic groups studied differs with regard to many other genes that induce or prevent obesity, thereby potentially enhancing or reducing the effect of the 825T allele in Chinese and black Africans. Although the genetic background of Caucasoid, Asian, and black populations is certainly different, we do not believe that this is a major argument to be considered here for two reasons. First, we found a consistent association of the 825T allele with BMI in individuals of different ethnic origin. Second, it is rather unlikely that urban and rural black individuals from Zimbabwe, all belonging to the Zimbabwean Shona tribe, differ substantially with regard to their genetic background. Nevertheless, overweight in the urban Zimbabwean sample was significantly more frequent compared to the rural sample. Hence, the gene effect is seen only when certain environmental factors come into play. Such a scenario is also compatible with the "thrifty genotype hypothesis" proposed by Neel (31). This hypothesis suggests that "ancestral," body fat-conserving genes may still be operative in modern man and may contribute to diabetes in conjunction with a sedentary lifestyle.

825T Allele and BP

Three independent case-control studies (5,9,32) and one association study in a WHO MONICA cohort (8) have confirmed an association of the *GNB3* 825T allele with hypertension in Caucasians. However, the mechanism by which enhanced G protein activation contributes to high BP has remained obscure. Based on theoretical considerations, increased vasoconstriction can be ruled out as most vasoactive hormones activate receptors coupled to PTX-insensitive G proteins. Homozygous and heterozygous 825T allele carriers do not respond with an exaggerated BP increase upon infusion of an α2-adrenergic receptor-activating compound (33). Thus, hypertension in 825T allele carriers appears to result from slow-acting mechanisms. The findings presented here for the German cohort apparently support this theoretical concept. Whereas BP was virtually independent of the C825T polymorphism, both systolic and diastolic BP were significantly dependent on BMI, and it is commonly known that overweight and obesity are main risk factors for hypertension. As a working hypothesis, therefore, we propose that the 825T allele may predominantly increase the risk for obesity, which, over years, could then precipitate in hypertension. A definite answer to this pending question requires additional studies specifically designed to address this problem.

Worldwide Distribution of the 825T Allele

The *GNB3* 825T allele frequencies reflect ethnic subdivisions (34,35).The data, therefore, are compatible with (but they do not necessarily prove) a scenario in which the allele frequencies were subjected to random drift during racial differentiation. To investigate the alternative scenario of drift through selection, we have calculated the HWE for the larger samples ($n > 40$). As is seen in Table 5, there is no significant deviation from HWE, indicating that any recent selection on the genotypes is not detectable in our data. This does not

exclude the possibility that selection is at work, but is not significantly affecting HWE. Also, significant selection may have acted during earlier times, but it seems unlikely that the selective forces would have desisted by today across all of the diverse ethnic groups and cultures that we tested for HWE. Furthermore, there are no correlations between 825T allele frequency and climate or lifestyle of the sampled populations, which include hunter-gatherers, nomadic pastoralists, sedentary agriculturalists, and industrialized Westerners. There remains the hypothesis that disease was involved in producing the current frequency distribution, which may be investigated when sufficient data on prehistoric diseases become available. It should be noted here that immune cell activation was found enhanced in Caucasoid 825T allele carriers as inferred from increased neutrophil chemotaxis (36). The 825T allele was absent in all of the primates we typed thus far, including the closest relatives of humans, the common chimp, pygmy chimp/Bonobo, lowland gorilla, and orangutan, suggesting that C is the ancestral state at nucleotide position 825, and that the mutation of C to T probably occurred since the human-chimpanzee split approximately 5 million years ago. A lower bound for the 825C to T mutation may be postulated on the basis of its present distribution in human populations: The hunter-gatherer West Pygmies and the Khoisan, who have been genetically isolated since an early African founder event about 100,000 years ago (18,19), harbor both the 825C and 825T alleles at appreciable frequencies (Table 5). Accordingly, the C/T polymorphism would already have been present at the time of the subsequent major east African expansion 60,000 to 80,000 years ago, which probably populated Eurasia and repopulated most of Africa (18,37,38). Because agriculture did not commence until after climatic stabilization at the end of the Younger Dryas glacial phase 11,400 years ago (39), it is unlikely that agricultural lifestyle was involved in shaping the early 825C/T distribution.

Conclusions and Perspectives

Our findings may explain part of the significant ethnic differences regarding susceptibility to obesity and obesity-related disorders (40–42). For example, black Americans display a higher prevalence of obesity, hypertension, and type 2 diabetes mellitus compared to whites in the United States (43,44). Black Americans still share much of their genetic makeup with their ancestors from West Africa, and many studies have been conducted to investigate the relationship between environmental factors, BMI, hypertension, and type 2 diabetes. The prevalence of hypertension and type 2 diabetes was consistently found to be low in African communities, but strongly increased across the Caribbean toward the United States (41,44). Even within the African community, lifestyle changes (urbanization) contribute significantly to the prevalence of obesity and hypertension as illustrated by an increased prevalence of hypertension and obesity in urban Nigeria compared to rural farmers in the same country (41,44–46). Compatible observations have been made in Australian aborigines who, upon adapting a "Western" lifestyle, show exceedingly high rates of obesity-related disorders, this process being apparently reversible if aborigines reverted temporarily to traditional hunter-gatherer diets and lifestyles (47). General lack of obesity may explain why the !Kung bushmen do not display an age-dependent BP rise (48) despite a high 825T allele frequency. We have shown here that black Americans as well as Australian aborigines display high frequencies of the 825T allele (Table 5). Assuming that the effect of the 825T allele on BMI described here for Germans, Chinese, and black Africans is similar in black Americans and Australian aborigines, we speculate that the high number of 825T allele carriers could be a major determinant for the observed difference in the prevalence of obesity and obesity-related disorders in specific ethnic groups living in countries with largely uniform lifestyle conditions. Finally, if Westernization of lifestyle continues at the present rate, the high prevalence of the 825T allele may be predictive of an obesity and hypertension epidemic in developing countries.

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