

The Thr92Ala Deiodinase Type 2 (*DIO2*) Variant Is Not Associated with Type 2 Diabetes or Indices of Insulin Resistance in the Old Order of Amish

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A common polymorphism of the type 2 deiodinase gene (Thr92Ala *DIO2*) was found to be associated with insulin resistance in a mixed Caucasian population. The aim of this study was to investigate the association of the Thr92Ala *DIO2* variant to indices of insulin resistance in the Old Order Amish. A genotype–phenotype association study was performed at the research clinic in Strasburg, Pennsylvania, and the molecular genetics laboratory at the University of Maryland, Baltimore, Maryland, and the National Institutes of Health, Bethesda, Maryland. A total of 1,268 subjects participated in the Amish Family Diabetes Study. An association among the Thr92Ala *DIO2* variant and type 2 diabetes, indices of insulin resistance (HOMA-IR), insulin secretion, free thyroid hormones, and thyrotropin (TSH) was found. No association was found among the Thr92Ala *DIO2* variant and type 2 diabetes, impaired glucose tolerance, or body mass index (BMI) in the Amish. In nondiabetics ($n = 747$), the Ala92 allele tended to be associated with decreased rather than increased insulin secretion. No differences were observed in thyroid hormones or TSH. Contrary to prior findings, the Thr92Ala *DIO2* variant tends to be associated with increased rather than decreased insulin sensitivity in the Amish. These findings could be secondary to a different genetic background or to environmental factors specific for this population.

Introduction

THYROID HORMONE HOMEOSTASIS is maintained by a multi-step, redundant system aimed to assure the signal specificity at the various target organs (1). In this context, the peripheral metabolism of thyroid hormones represents an important step in the modulation of the hormonal signaling. Specifically type 2 deiodinase (D2) regulates the conversion of the pro-hormone thyroxine (T4) into its active metabolite triiodothyronine (T3) in tissues that are extremely sensitive to thyroid hormone action (2). In humans, the distribution of D2 activity is wider than in rodents and includes thyroid, skeletal muscle, and adipose tissue (3,4). Previously, we identified a common missense mutation in the human D2 gene (Thr92Ala *DIO2*), which associated with insulin resistance during a euglycemic hyperinsulinemic clamp in a Caucasian population (5). We speculated that the Thr92Ala *DIO2* variant encodes a defective enzyme, decreasing the intracellular conversion of T4 to T3, leading to a reduction in

transcription of thyroid hormone-regulated genes such as GLUT4 in skeletal muscle and fat, and ultimately causing insulin resistance.

To gain further knowledge of the role of the Thr92Ala *DIO2* variant on glucose metabolism, we performed a large genotype–phenotype association study on diabetes and diabetes-related traits and circulating levels of thyroid hormones in a large, well-characterized genetically homogeneous founder population, the Old Order Amish.

Materials and Methods

Study subjects

The study population consisted of participants in the Amish Family Diabetes Study, a family-based study started in 1995 with the goal of identifying susceptibility genes for type 2 diabetes (6). This report is restricted to the 1,268 study subjects who were both genotyped for the Thr92Ala *DIO2*

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Part of the data presented in this manuscript was obtained while D.M., G.C., and F.S.C. worked at the University of Maryland School of Medicine, Baltimore, Maryland. Part of the data has been presented in abstract form at the 86th Annual Meeting of the Endocrine Society, New Orleans, Louisiana.

variant and characterized for one or more diabetes-related traits. Nearly all of the enrolled individuals are descendants of a small number of Amish who settled in Lancaster County, Pennsylvania, in the mid-eighteenth century (6,7). The large kindred and the availability of almost complete genealogy records make this population ideal for genetic studies (8,9). The study protocol was approved by the Institutional Review Board of the University of Maryland School of Medicine, and informed consent was obtained from each study participant.

Clinical and laboratory measurements

Study subjects received a standardized examination at the study clinic in Strasburg, Pennsylvania. Fasting blood samples were collected for measurements of glucose, insulin, thyrotropin (TSH), free T3, and free T4; total, high-density lipoprotein (HDL)-, and low-density lipoprotein (LDL)-cholesterol (by calculation), and triglycerides. A 3-hour 75-gram oral glucose tolerance test (OGTT) was performed with measurement of glucose and insulin levels at 30-minute intervals. Plasma glucose concentration was assayed with a Beckman glucose analyzer (interassay variation = 1.52%). Insulin levels were determined by radioimmunoassay (Linco, St. Louis, MO) (interassay variation = 4.42%). Total glucose and insulin areas under the curve (AUC) during the 3-hour OGTT were determined with the trapezoid method (10). HbA_{1c} levels were measured by high-performance liquid chromatography (HPLC) (interassay variation = 4.3%). Free thyroid hormones levels were determined in a subsample of 319 subjects. Lipid profile, free T3, and free T4 were measured by Quest Diagnostics (Baltimore, MD) (interassay variation = 1.6% for total cholesterol, 5.0% for HDL cholesterol, 1.6% for triglycerides, 3.1% for free T3, and 5.0% for free T4). TSH was evaluated by radioimmunoassay (interassay variation = 6.0%) (Nichols Institute Diagnostics, San Juan Capistrano, CA). Type 2 diabetes was defined by an age of diagnosis greater than age 35 years and by fasting plasma glucose level (≥ 7 mmol/L), 2-hour OGTT plasma glucose (≥ 11 mmol/L), random plasma glucose (≥ 11 mmol/L), the use of insulin or oral glucose-lowering agents, or a diagnosis of diabetes documented by a physician. Insulin secretion was defined as the insulin value measured 30 minutes after the 75-gram oral glucose challenge minus the fasting insulin value. The Thr92Ala *DIO2* variant was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (5), and genotypes were called independently by two observers. In case of discrepancy, which occurred less than 1% of the time, a repeated PCR-RFLP was performed. The error rate based upon blind replicates was 0.5%.

Statistical methods

We initially evaluated the association between type 2 diabetes and the Thr92Ala *DIO2* variant. Then we excluded subjects with diabetes ($n = 117$), those taking antilipid medications ($n = 43$), and those taking thyroid replacement or antithyroid medications ($n = 17$) and compared mean levels of glucose, lipids, and thyroid hormones across genotypes ($n = 165$ total exclusions). Of the remaining 1,103 subjects, glucose measurements were available on 747 subjects and TSH values were available in 803 subjects. Free thyroid hormone levels were assessed in a subgroup of 319 study sub-

jects. When appropriate, we conducted the analysis using log transformation of the data. We evaluated the effects of genotype on the dependent variable (diabetes or diabetes-related quantitative traits) adjusting for the effects of age and sex. We accomplished this using a variance component approach, which also allowed us to condition the covariate effects (including genotype) on the residual correlations between related family members. We considered additive genetic models in our analysis, in which the genotype effect was coded as 0, 1, or 2, depending on the number of Ala alleles present. These analyses were carried out using the SOLAR software program (11).

Results

The clinical characteristics of the 1,268 study subjects are described in Table 1. The mean age was 45.5 years, and the mean BMI was 26.4 and 27.8 kg/m² in males and females, respectively. The prevalence of type 2 diabetes in this family study was 10.5% and 17.1% in males and females, respectively.

Glucose metabolism parameters

The frequency of the Ala92 allele in the Amish population was 0.30, similar to that reported previously in a Caucasian population (5), and the genotype frequency distribution was in Hardy-Weinberg equilibrium. The prevalence of type 2 diabetes was 13.4% among those with the Thr/Thr genotype, 13.7% among those with the Thr/Ala genotype, and 19.5% among those with the Ala/Ala genotype ($p = 0.27$). Subsequent analyses were carried out only in nondiabetic individuals. Table 2 shows the mean levels of quantitative traits according to genotype. The Thr92Ala *DIO2* variant was not associated with fasting glucose or HOMA IR values, but was associated with decreased insulin secretion 1.56 ± 0.05 Thr/Thr versus 1.49 ± 0.04 Thr/Ala versus 1.36 ± 0.08 Ala/Ala LN pmol/L, $p = 0.03$. However, no statistical difference was observed in the glucose or insulin AUC (Table 2). Subanalyses conducted among subjects below and above the median BMI of the population suggests that this modest effect of the Thr92Ala *DIO2* variant on insulin secretion was more evident in lean individuals (Table 3).

Thyroid hormones and lipid profile values

The results of association studies between the Thr92Ala *DIO2* variant and circulating free thyroid hormones, TSH, and lipid profile values and the Thr92Ala *DIO2* variant are shown in Table 2. No association was found between the Thr92Ala *DIO2* variant and TSH or free T4 and free T3 val-

TABLE 1. CHARACTERISTICS OF THE STUDY POPULATION (MEANS \pm SE)

	Total (n = 1,268)	Male (n = 561)	Female (n = 707)
Age (years)	45.5 \pm 0.6	45.4 \pm 0.8	45.6 \pm 0.8
BMI (kg/m ²)	27.2 \pm 0.2	26.4 \pm 0.2	27.8 \pm 0.2
Diabetes (%)	14.1%	10.5%	17.1%
IGT (%)	19.9%	12.4%	26.4%
HbA _{1c} (%)	5.2 \pm 0.03	5.2 \pm 0.05	5.1 \pm 0.04

TABLE 2. AGE- AND SEX-ADJUSTED MEAN (\pm SE) GLUCOSE, INSULIN, THYROID HORMONE, AND LIPID MEASURES IN NONDIABETIC SUBJECTS ACCORDING TO *DIO2* GENOTYPE

	<i>Thr/Thr</i> (<i>n</i> = 367)	<i>Thr/Ala</i> (<i>n</i> = 309)	<i>Ala/Ala</i> (<i>n</i> = 71)	Age- and sex-adjusted <i>p</i> value
Fasting glucose (mmol/L)	5.03 \pm 0.03	5.06 \pm 0.04	4.97 \pm 0.06	0.67
Glucose 120' (mmol/L)	5.61 \pm 0.12	5.58 \pm 0.13	5.54 \pm 0.22	0.74
Fasting insulin (pmol/L)	64.53 \pm 1.47	69.67 \pm 2.65	65.38 \pm 3.15	0.34
Insulin 120' (pmol/L)	258.99 \pm 13.35	250.54 \pm 12.21	215.49 \pm 21.20	0.53
HOMA IR	2.44 \pm 0.11	2.64 \pm 0.12	2.44 \pm 0.20	0.40
HbA1c (%)	4.89 \pm 0.03	4.92 \pm 0.03	4.94 \pm 0.05	0.13
Insulin secretion ^a	1.56 \pm 0.05	1.49 \pm 0.04	1.36 \pm 0.08	0.03
Insulin AUC	633.9 \pm 31.7	620.8 \pm 36.4	567.4 \pm 62.1	0.35
Glucose AUC	19.2 \pm 0.3	19.0 \pm 0.3	18.7 \pm 0.5	0.34
TSH (mIU/L)	2.66 \pm 0.52	2.48 \pm 0.60	2.01 \pm 1.02	0.37
Free T3 (pg/dL) ^b	337.5 \pm 5.8	332.1 \pm 6.6	336.7 \pm 10.8	0.22
Free T4 (ng/dL) ^b	1.03 \pm 0.03	1.04 \pm 0.03	1.00 \pm 0.05	0.10
Total cholesterol (mmol/L)	5.63 \pm 0.11	5.71 \pm 0.10	5.99 \pm 0.18	0.067
HDL (mmol/L)	1.22 \pm 0.02	1.21 \pm 0.03	1.39 \pm 0.05	0.30
LDL (mmol/L)	3.81 \pm 0.08	3.85 \pm 0.09	4.05 \pm 0.15	0.21
Triglycerides (mmol/L)	0.933 \pm 0.040	0.917 \pm 0.044	0.928 \pm 0.074	0.52

^aInsulin secretion (LN pmol/L): LN insulin value measured 30 minutes after the 75-gram oral glucose challenge minus LN fasting insulin value.

^bFree thyroid hormones values were measured in a subset of 163 Thr/Thr, 125 Thr/Ala, and 31 Ala/Ala subjects.

ues. Finally, Ala92 carriers had marginally greater total cholesterol levels (5.99 \pm 0.18 Ala/Ala vs. 5.71 \pm 0.10 Thr/Ala vs. 5.63 \pm 0.11 Thr/Thr mmol/L, *p* = 0.067). The Thr92Ala *DIO2* variant was not associated with variation in HDL cholesterol or triglycerides levels.

Discussion

Previous studies performed in a mixed Caucasian population showed that, as compared Thr92 *DIO2* homozygotes,

carriers of the Ala92 allele had significantly lower glucose disposal assessed by hyperinsulinemic euglycemic clamp. Furthermore, in the presence of the inactivating *ADRB3* variant, the Ala92 *DIO2* allele was associated with a small but significant increase in BMI (5). Recently, Peeters et al. showed that the Thr92Ala *DIO2* variant associates with increased fasting levels of insulin and glucose, as well as increased HbA1c levels in a northern European population (12). Very recently, Canani and colleagues confirmed in a population of type 2 diabetes patients (13) our previous observation. We

TABLE 3. AGE- AND SEX-ADJUSTED MEAN (\pm SE) VALUES OF SELECTED TRAITS AMONG NONDIABETIC SUBJECTS ACCORDING TO MEDIAN BMI

	<i>Thr/Thr</i>	<i>Thr/Ala</i>	<i>Ala/Ala</i>	Age- and sex-adjusted <i>p</i> value
Insulin at 30 min (pmol/L)				
BMI \leq 26.03 (<i>n</i> = 331)	259.00 \pm 10.02	250.33 \pm 11.47	206.66 \pm 14.25	0.03
BMI > 26.03 (<i>n</i> = 339)	402.45 \pm 19.77	372.59 \pm 19.42	363.71 \pm 27.64	0.27
Insulin secretion				
BMI \leq 26.03	1.40 \pm 0.05	1.35 \pm 0.05	1.20 \pm 0.09	0.02
BMI > 26.03	1.60 \pm 0.05	1.51 \pm 0.06	1.56 \pm 0.11	0.23
Glucose 30 min				
BMI \leq 26.03	8.20 \pm 0.17	8.01 \pm 0.19	7.68 \pm 0.32	0.05
BMI > 26.03	8.85 \pm 0.15	8.69 \pm 0.17	8.71 \pm 0.32	0.42
Total cholesterol (mmol/L)				
BMI \leq 26.03	5.27 \pm 0.12	5.20 \pm 0.13	5.72 \pm 0.22	0.29
BMI > 26.03	5.65 \pm 0.12	5.73 \pm 0.14	5.70 \pm 0.24	0.69
LDL (mmol/L)				
BMI \leq 26.03	3.62 \pm 0.10	3.60 \pm 0.11	3.98 \pm 0.20	0.26
BMI > 26.03	4.01 \pm 0.11	4.09 \pm 0.12	4.08 \pm 0.22	0.62

speculated that the mechanism whereby the Thr92Ala *DIO2* variant leads to insulin resistance is through a decrease in function thus leads to a decrease in intracellular muscular concentrations of T3, a known regulator of GLUT4 transcription (14).

In the present study, we evaluated the effects of the Thr92Ala *DIO2* variant on diabetes-related traits in the Old Order Amish. In this population, we did not find any association between the Thr92Ala *DIO2* variant and type 2 diabetes or diabetes-related traits, including fasting or post-challenge glucose or insulin levels or in BMI. The reason for our inability to replicate the prior three positive studies is not clear. The prior positive studies were independent of one another, showed consistency in phenotype associations, i.e., associations with measures of insulin resistance, and contained moderately large numbers of well-characterized subjects, suggesting that it is unlikely that the observed associations were false positives due to type 2 error or population stratification. A limitation of the present study is that insulin resistance was assessed indirectly by fasting and post-challenge insulin levels and by calculation of the HOMA index.

These surrogate measures of insulin resistance are not nearly as precise as those obtained by hyperinsulinemic euglycemic clamp (15), as was performed in our prior study (5). It is also possible that the discrepancies are secondary to differences in genetic background between populations. For example, the Trp64Arg variant in *ADRB3* is quite low among the Amish (Hsueh and Shuldiner, unpublished). Alternatively, differences in environmental exposures (and interactions with *DIO2* genotype) might account for the differences observed between populations. For example, the Amish are known to be extremely physically active, which might have an overriding effect on insulin resistance (16,17). This would be in keeping with the observation that the effect of the Thr92Ala *DIO2* variant on insulin secretion was more evident in lean individuals, presumably more physically active. By contrast, the other Caucasian group we studied was sedentary (5,18). Therefore, physical activity may have a prevailing effect on insulin resistance in the current study population. In sedentary individuals, low intracellular levels of T3 would reduce GLUT4 transcription, leading to decreased glucose disposal. Conversely, physically active individuals (Old Order Amish) would override the decrease in GLUT4 transcription by the exercise-induced increase translocation of GLUT4 to the cell membranes (19). Although very speculative, this explanation could reconcile the apparently contrasting findings. This hypothesis is also supported by the marginally increase in plasma total cholesterol observed in Ala92 *DIO2* carriers, consistent with a decreased peripheral conversion of T4. A similar gene-environment interaction has been characterized in the apparent paradoxical effects of the Pro12Ala *PPAR γ* polymorphism on BMI in relation to different macronutrient dietary content (20).

Finally, the Thr92Ala *DIO2* variant may be in linkage disequilibrium with a yet-to-be-discovered "true pathogenic" variant, and the presence or frequency of the linked pathogenic variant may differ between populations.

Conflicting data have been published regarding *in vitro* functional characterization of the Thr92Ala *DIO2* variant. Although one group found no changes in the enzymatic activity (21), we observed that the Ala92 variant encodes an enzyme with a decreased affinity for its substrate (22). Very

recently, Canani and coworkers failed to demonstrate *in vitro* any difference between the two alleles, but showed with *ex vivo* experiments that the Ala 92 variant has a decreased enzyme velocity in thyroid and skeletal muscle (13).

We did not observe any differences in peripheral free T3 or free T4 levels in our population. However, it is likely that local alterations in deiodinase activity in muscle would not result in altered circulating levels of thyroid hormones. Hypercholesterolemia is well known to be associated with hypothyroidism and thus the marginal association of the Ala92 *DIO2* allele with increased cholesterol levels is consistent with a functional consequence of this variant.

In conclusion, the common Thr92Ala *DIO2* variant is not associated with diabetes or diabetes-related traits in the Old Order Amish. In light of three prior positive studies and functional data that suggests a functional consequence of the amino acid substitution, it is likely that interactions among environmental and/or other genetic determinants are important modulators of this variant's effects. Further studies will be necessary to identify these modulating factors and to better understand the clinical significance of the Thr92Ala *DIO2* variant in the development of insulin resistance.

Acknowledgments

We gratefully acknowledge our Amish liaisons and field workers and the extraordinary cooperation and support of the Amish community, without whom these studies would not be possible. The comments and suggestions of Drs. Marvin C. Gershengorn and Aaron Chidakel are highly appreciated. The study was supported by research grants from the American Federation of Aging Research (F.S.C.), NIH R01 DK54261 (A.R.S.) the American Diabetes Association (A.R.S.), the Baltimore Veterans Administration Geriatric Research and Education Clinical Center (GRECC), and the University of Maryland General Clinical Research Center, Grant M01 RR 16500, the General Clinical Research Centers Program, National Center for Research Resources (NCRR), NIH.

References

1. Yen PM 2001 Physiological and molecular basis of thyroid hormone action. *Physiol Rev* **81**:1097-1142.
2. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR 2002 Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev* **23**:38-89.
3. Croteau W, Davey JC, Galton VA, St Germain DL 1996 Cloning of the mammalian type II iodothyronine deiodinase. A selenoprotein differentially expressed and regulated in human and rat brain and other tissues. *J Clin Invest* **98**:405-417.
4. Salvatore D, Tu H, Harney JW, Larsen PR 1996 Type 2 iodothyronine deiodinase is highly expressed in human thyroid. *J Clin Invest* **98**:962-968.
5. Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET, Shuldiner AR, Celi FS 2002 Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3-adrenergic receptor. *Diabetes* **51**:880-883.
6. Hsueh WC, Mitchell BD, Aburomia R, Pollin T, Sakul H, Gelder Ehm M, Michelsen BK, Wagner MJ, St Jean PL, Knowler WC, Burns DK, Bell CJ, Shuldiner AR 2000 Dia-

- betes in the Old Order Amish: characterization and heritability analysis of the Amish Family Diabetes Study. *Diabetes Care* **23**:595–601.
7. Agarwala R, Biesecker LG, Hopkins KA, Francomano CA, Schaffer AA 1998 Software for constructing and verifying pedigrees within large genealogies and an application to the Old Order Amish of Lancaster County. *Genome Res* **8**:211–221.
 8. McKusick VA 1978 *Medical Genetic Studies of the Amish*. The Johns Hopkins University Press, Baltimore, MD.
 9. Cross HE 1976 Population studies and the Old Order Amish. *Nature* **262**:17–20.
 10. Le Floch JP, Escuyer P, Baudin E, Baudon D, Perlemuter L 1990 Blood glucose area under the curve. Methodological aspects. *Diabetes Care* **13**:172–175.
 11. Almasy L, Blangero J 1998 Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* **62**:1198–1211.
 12. Peeters RP, Attalki H, van den Beld AW, van Toor H, Janssen JAMJL, Kuiper GGJM, Uitterlinden SW, Lamberts SWJ, Visser TJ 2004 Polymorphisms in the type 2 deiodinase (D2) gene are associated with serum thyroid hormone parameters, insulin resistance and/or body composition. Program of the 76th Annual Meeting of The American Thyroid Association, Vancouver, BC 2004, p 734.
 13. Canani LH, Capp C, Dora JM, Souza Meyer EL, Wagner MS, Harney JW, Larsen PR, Gross JL, Bianco AC, Maia AL 2005 The Type 2 Deiodinase A/G (Thr92Ala) Polymorphism is associated with Decreased Enzyme Velocity and Increased Insulin Resistance in Patients with Type 2 Diabetes Mellitus. *J Clin Endocrinol Metab* **90**:3472–3478.
 14. Torrance CJ, Usala SJ, Pessin JE, Dohm GL 1997 Characterization of a low affinity thyroid hormone receptor binding site within the rat GLUT4 gene promoter. *Endocrinology* **138**:1215–1223.
 15. Matsuda M, DeFronzo RA 1999. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* **22**:1462–1470.
 16. Bassett DR, Schneider PS, Huntington GE 2004 Physical activity in an Old Order Amish community. *Med Sci Sports Exerc* **36**:79–85.
 17. Snitker S, Shuldiner AR 2004 BMI in the Old Order Amish (letter). *Med Sci Sports Exerc* **36**:1447.
 18. Toth MJ, Tchernof A, Rosen CJ, Matthews DE, Poehlman ET 2001 Regulation of protein metabolism in middle-aged, premenopausal women: roles of adiposity and estradiol. *J Clin Endocrinol Metab* **85**:1382–1387.
 19. Hayashi, T Wojtaszewski JF, Goodyear LJ 1997 Exercise regulation of glucose transport in skeletal muscle. *Am J Physiol* **273**:E1039–E1051.
 20. Luan J, Browne PO, Harding AH, Halsall DJ, O'Rahilly S, Chatterjee VK, Wareham NJ. 2001 Evidence for gene-nutrient interaction at the PPARgamma locus. *Diabetes* **50**:686–689.
 21. Peeters RP, van Toor H, Klootwijk W, de Rijke YB, Kuiper GG, Uitterlinden AG, Visser TJ 2003 Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. *J Clin Endocrinol Metab* **88**:2880–2888.
 22. Mentuccia D, Pollin T, Proietti-Pannunzi L, Frajese G, Tanner K, Celi FS 2003 Functional characterization of the Thr92Ala variant of the human type 2 deiodinase gene. Program of the 85th Annual Meeting of The Endocrine Society, Philadelphia, PA 2003, p 244.

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