Polymorphism of ABCG2 Gene in Hyperuricemia Patients of Han And Uygur Ethnicity with Phlegm/Non-Phlegm Block in Xinjiang, China

Background: This study investigated the relationship between hyperuricemia (with phlegm/non-phlegm block) and ABCG2 gene polymorphism in Han and Uygur people from Xinjiang, China.

Material/Methods: We recruited 600 hyperuricemia patients with phlegm/non-phlegm block. Genomic DNA was extracted from the whole blood. Gene polymorphism was classified by SnaPshot method.

Results: The SNP loci rs2725220 and rs2231137 of the ABCG2 gene, but not rs2231142, were significantly different between patients with non-phlegm block and phlegm block (P<0.05). The rs2231142 allele G was the protective factor in Uygur hyperuricemia patients. In both Han and hyperuricemia patients, the rs2725220 allele G was a protective factor and the rs2231137 allele C was a risk factor. For non-phlegm-block hyperuricemia, the rs2231142 and rs2231137 genotypes were significantly different between Uygur and Han patients (P<0.05). The rs2231142 allele G was 1.563 times higher in the Uygur patients compared with Han, and rs2231137 allele C was 1.673 times higher in the Uygur patients compared with the Han. For phlegm-block hyperuricemia, rs2231142 allele G was 1.397 times higher in the Uygur patients compared with the Han.

Conclusions: ABCG2 gene rs2231137 with more allele C tends to be phlegm-block type and rs2725220 with more allele G tends to be non-phlegm-block type. In the Uygur hyperuricemia patients, ABCG2 gene rs2231142 with more allele G tends to be non-phlegm-block type. Allele C of rs2231137 and allele G of rs2231142 in ABCG2 gene are more likely to be found in the Uygur people.

MeSH Keywords: Biochemistry • Genetics • Hyperuricemia • Polymorphism, Genetic

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Background

Hyperuricemia is a group of diseases caused by excess uric acid and/or decreased uric acid excretion by the kidneys, and it is a type of metabolic syndrome [1]. Hyperuricemia is diagnosed if the blood uric acid is higher than 420 μmol/l (7.0 mg/ml) in males and higher than 357 μmol/l (6 mg/ml) in premenopausal women [2,3]. Many studies have shown that hyperuricemia is the pathological basis of gouty arthritis, kidney stones, renal failure, and metabolic syndromes such as hypertension, atherosclerosis, coronary heart disease, glycolipid metabolic disorders, obesity, and insulin resistance disease [4,5].

There are 2 reasons for the high concentration of uric acid in the blood: one is the rate of synthesis of uric acid in the liver and the other is the rate of uric acid excretion from the kidneys [6]. The most common cause of abnormal uric acid excretion is the abnormal expression of urate transporter in the kidneys [7]. At present, the most studied genes related to primary hyperuricemia are mainly from the renal uric acid transport system [7]. The genetic factors of hyperuricemia account for 40–70% [8]. The ATP-binding cassette subfamily G member 2 (ABCG2) is an ATP-binding transporter protein that is widely distributed in tissues with secretion and excretion functions. Functional abnormalities in ABCG2 can lead to a decrease in uric acid excretion [7,9]. At present, it is thought that the single-nucleotide polymorphism (SNP) of ABCG2 gene plays an important role in abnormal uric acid excretion.

Xinjiang is located in the northwest corner of China, where Winter is cold and summer is hot. In Traditional Chinese Medicine (TCM), the severe cold weather hurts Yang qi; and the heat injures the body fluid and blood. Xinjiang is a multi-ethnic province and the majority of people there eat greasy, sweet, and spicy food, resulting in spleen and stomach damage. The factors of internal stagnation of fluid dampness, damp heat, endogenous phlegm, 6-month cold weather in the northwest, short days and long nights, as well as narrowed range of activities, all limit the outdoor activities of residents and result in sedentary behavior. Thus, the phlegm block or phlegm stasis mutual resistance syndrome is commonly seen. Therefore, it is of practical significance to study the molecular genetic mechanism of phlegm block and non-phlegm block types of hyperuricemia in different ethnic groups in Xinjiang.

In this study, the ABCG2 gene polymorphism in Xinjiang Han and Uygur hyperuricemia patients with phlegm block or non-phlegm block was investigated. The genetic polymorphism of ABCG2 is closely related to the pathogenesis of gout, with the common genetic variants of Q141K (rs2231142) and V12M (rs2231137) [10,11]. In this study, the polymorphic sites of ABCG2 (rs2725220, rs2231137, and rs2231142) were investigated and compared between the different ethnic groups with hyperuricemia with phlegm block or non-phlegm block. The polymorphisms at these sites may affect the expression of genes and influence disease occurrence. Our findings may be of great significance for early intervention in hyperuricemia with TCM.

Material and Methods

Clinical characteristics of patients

Patients with hyperuricemia were recruited from April 2014 to August 2015 in the cities of Urumqi and Kashgar of Xinjiang, China. Inclusion criteria were meeting the diagnostic criteria for hyperuricemia and age 30–70 years. There was no limitation on gender. Exclusion criteria: 1) cardiovascular, liver, kidney, hematopoietic diseases, or mental illness; and 2) secondary hyperuricemia caused by blood disease (such as leukemia), malignant tumors (such as myeloma), kidney disease (such as renal failure and polycystic kidney), certain endocrine diseases (such as hypothyroidism and hyperparathyroidism), or drug-induced hyperuricemia (such as diuretics, aspirin, anti-tuberculosis drugs). All patients were reviewed by 3 experienced physicians to determine the type of TCM syndromes. A total of 600 patients were included. There were 361 males (60.17%) and 239 females (39.83%) ages 32–70 years old, with a mean age of 55.54±7.50 years. Of the 600 patients, there were 300 Han people and 300 Uygur people. There were 301 patients with phlegm block and 299 patients with non-phlegm block. Fasting peripheral blood (5 ml) was collected from each patient. Prior written and informed consent was obtained from every patient and the study was approved by the Ethics Review Board of the Traditional Chinese Medicine Hospital of Xinjiang Uygur Autonomous Region.

Diagnostic criteria for hyperuricemia

Hyperuricemia was diagnosed based on the 1977 criteria from the American Rheumatology Association [12], with blood uric acid in males >420 μmol/L (7.0 mg/ml) and in females >357 μmol/L (6 mg/ml).

Diagnostic criteria for phlegm block [13]

The common symptoms of phlegm block included: feeling of heaviness in the head and body, heaviness in the head as if being wrapped, palpitations, dizziness, fatigue, chest tightness, forgetfulness, irritability, bitter taste, dry mouth and throat, sticky mouth, general edema, swelling pain of head and eyes, and insomnia. The phlegm block was diagnosed when a patient had feelings of heaviness in the head and body or heaviness in the head as if being wrapped with any of the other 6 above symptoms.
Gene polymorphism analysis by SNaPshot method

DNA was extracted from peripheral blood using the Easy Pure Blood Genomic DNA Kit (Cat# EE121; TransGen Biotech, Beijing, China). The primer sequences for Multiple PCR were as follows: rs2231142 upstream: GCCTTAAGGATGATGTTGTGAT; downstream: ATCAGAGTCATTTTATCCACACA; rs2725220 upstream: CACTACTTCTTAGCCTTCTTTT; downstream: ACATTAAATAACTCCATTCTGAAC; rs2231137 upstream: F: TTGCAATCTCATTTATCTGGAC downstream: R: CAAGGTAGAAAGCCACTCTTCAG. The final concentration of each primer in the multiple PCR primers was 1 μM. The HotStar HiFidelity Polymerase Kit (Cat# 202605; Qiagen, Valencia, CA, USA) was used for multiple PCR. The PCR system for multiple PCR consisted of DNA 1 μL, 10 * buffer 1.5 μL, MgCl2 (25 mmol) 1.5 μL, DNTP (10 mmol) 0.3 μL, primer 0.15 μL, polymerase 0.3 μL, and ddH2O to a total volume of 15 μL. Touch-down PCR was used in multiple PCR as follows: pre-denaturation for 3 min at 94°C, followed by (94°C for 15 s, 60°C for 30 s) –0.5°C/Cycles for 11 cycles, (94°C for 15 s, 54°C for 15 s, 72°C for 30 s) for 24 cycles, and 72°C for 3 min. The PCR product was purified with ExoI and FastAP. The reaction system contained PCR product 3 μL, Exol (Cat#EN0581, Thermo scientific, USA, 20 U/µL) 0.2 µL, FastAP (Cat#EF0654, Thermo scientific, USA, 1 U/µL) 0.8 µL, Exol buffer 0.7 µL, and ddH2O supplemented to 7.0 µL. The reaction was performed for 15 min at 37°C and 15 min at 80°C.

After purification, SNaPshot single-base extension was performed using the SNaPshot® Multiplex Kit (Cat#4323161; Applied Biosystems, Foster City, CA, USA). The primer sequences for extension reaction were: rs2231142 TTTTTTTTTTTTTTTGTTGCAAGCCGAAGAGCTGCTGAGAACT; rs2725220 TTTTTTTTTTTTTTCCCCTCAAAAATTAATTATCTGAGC; rs2231137 TTTTTTTTTTTTAAGCCATTGGTGTTTCCTTGTGACA. The reaction system was 6.0 µL in volume, which consisted of 2 µL PCR product, 1 µL Snapshot Mix, 0.2 µL each extension primer, and ddH2O. The PCR procedure was as follows: pre-denaturation at 96°C for 1 min, followed by 30 cycles of 96°C for 10 s, 52°C for 5 s, and 60°C for 30 s. The extension reaction product was purified and then sequenced on an ABI3730 XL DNA Analyzer (Applied Biosystems), with GeneScan™ 120 LIZ TM Size Standard (Cat #4324287; Applied Biosystems). The data were analyzed using GeneMapper version 4.1 (Applied Biosystems).

Table 1. Hardy-Weinberg equilibrium of rs2725220, rs2231137 and rs2231142 site on ABCG2 gene.

| Ethnicity  | SNP site | Allele (1/2) | Groups                       | Frequency of gene types, observed (expected) | HWP value |
|------------|----------|--------------|------------------------------|----------------------------------------------|-----------|
|            |          |              | Han (n=300)                  |                                              |           |
| rs2725220  | C/G      | Phlegm block type (n=151) | 82 (81.6) 58 (58.81) 11 (10.6) | 23 (22.58) | 0.884     |
| rs2231137  | C/T      | Non-phlegm block type (n=149) | 56 (55.58) 70 (70.85) 23 (22.58) | 23 (22.58) | 0.884     |

|            |          | Phlegm block type (n=150) | 71 (73.48) 43 (41.08) 0.530 |           |           |
|            |          | Non-phlegm block type     | 42 (45.13) 80 (73.74) 27 (30.13) | 0.300     |           |

|            |          | Phlegm block type (n=150) | 74 (73.92) 29 (29.04) 0.989 |           |           |
|            |          | Non-phlegm block type     | 61 (64.68) 75 (67.64) 14 (17.68) | 0.182     |           |

|            |          | Phlegm block type (n=150) | 49 (55.24) 12 (14.25) 1 (0.38) 0.283 |           |           |
|            |          | Non-phlegm block type     | 47 (42.67) 66 (74.67) 37 (32.67) 0.155 |           |           |

|            |          | Phlegm block type (n=150) | 36 (34.08) 71 (74.84) 43 (41.08) 0.530 |           |           |
|            |          | Non-phlegm block type     | 42 (45.13) 80 (73.74) 27 (30.13) 0.300 |           |           |

|            |          | Phlegm block type (n=150) | 82 (81.6) 58 (58.81) 11 (10.6) | 23 (22.58) | 0.884     |
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1 – common allele; 2 – rare allele. HWP – Hardy-Weinberg equilibrium test p value. * One patient of the phlegm block type in the Han group did not generate sequencing result at SNP site rs2231142.
Statistical analysis

SPSS17.0 software was used for data analysis. Hardy-Weinberg equilibrium was tested by chi-square goodness-of-fit test. The chi-square test was used to compare the differences in genotype and allele frequencies of the 2 groups. P<0.05 was considered as statistically significant.

Results

Comparison of genotype distribution between phlegm-block and non-phlegm block groups

The results showed that the rs2725220, rs2231142, and rs2231137 loci of ABCG2 gene met Hardy-Weinberg equilibrium in both Uygur and Han patients (Table 1). Therefore, the samples in this study were representative of the population and could be used for subsequent data analysis.

In Uygur hyperuricemia patients, there were significant differences in rs2725220, rs2231142, and rs2231137 loci of ABCG2 gene met Hardy-Weinberg equilibrium in both Uygur and Han patients (Table 1). Therefore, the samples in this study were representative of the population and could be used for subsequent data analysis.

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As shown in Table 3, rs2231137 genotype was CT type. C allele was found to be a risk factor for phlegm block in both Han and Uygur hyperuricemia patients (Han P<0.001, OR=8.428 and 95% CI [4.364, 16.27], Uygur P<0.001, OR=3.619, 95% CI [1.978, 6.621]). The OR value showed that among the Han and Uygur patients with hyperuricemia, the probability of gene G in the non-phlegm block group was 0.565 and 0.368 times higher than that in phlegm block group, indicating rs2725220 G allele is a protective factor in phlegm block group. The rs2231137 genotype was the GT type, but there was no significant difference in the Han hyperuricemia, and the allele G was the protective factor in the Uygur phlegm block group (P>0.05, OR=0.665, 95% CI [0.474, 0.925]).
### Table 3. Distribution of SNP alleles between non-phlegm block type and phlegm block type of Han and Uygur hyperuricemia patients.

| Ethnicity | SNP sites | Allele | Phlegm block type n=301 (%) | Non-phlegm block type n=299 (%) | \( \chi^2 \)  | P value | Bon OR value | 95% CI |
|-----------|-----------|--------|-----------------------------|--------------------------------|---------|---------|-------------|--------|
| Han (n=300) | rs2231142* | G      | 143 (0.477)                 | 164 (0.55)                      | 3.248   | 0.072  | 0.0125      | [0.536–1.026] |
|           |           | T      | 157 (0.523)                 | 134 (0.45)                      |         | 1       | ref         |        |
|           | rs2725220 | G      | 80 (0.265)                  | 116 (0.389)                     | 10.546  | 0.001  | 0.0125      | [0.409–0.798] |
|           |           | C      | 222 (0.735)                 | 182 (0.611)                     |         | 1       | ref         |        |
|           | rs2231137 | C      | 291 (0.964)                 | 226 (0.758)                     | 52.799  | 0.000  | 0.0125      | [4.364–16.27] |
|           |           | T      | 11 (0.036)                  | 72 (0.242)                      |         | 1       | ref         |        |
| Uygur (n=300) | rs2231142 | G      | 168 (0.56)                  | 197 (0.657)                     | 5.888   | 0.015  | 0.0125      | [0.474–0.925] |
|           |           | T      | 132 (0.44)                  | 103 (0.343)                     |         | 1       | ref         |        |
|           | rs2725220 | G      | 73 (0.243)                  | 140 (0.467)                     | 32.675  | 0.000  | 0.0125      | [0.250–0.520] |
|           |           | C      | 227 (0.757)                 | 160 (0.533)                     |         | 1       | ref         |        |
|           | rs2231137 | C      | 285 (0.95)                  | 252 (0.84)                      | 19.314  | 0.000  | 0.0125      | [1.978–6.621] |
|           |           | T      | 15 (0.05)                   | 48 (0.16)                       |         | 1       | ref         |        |

When \( P<0.05 \), OR >1 and 95% CI lower limit >1, it indicates a risk factor; when \( P<0.05 \), OR <1 and 95% CI upper limit <1, it indicates a protective factor; ref referred to a control factor. * One patient of the phlegm block type in the Han group did not generate sequencing result at SNP site rs2231142. Bon – p value in the step down Bonferroni test.

### Table 4. Genotype distribution of phlegm block type and non-phlegm block type between Han and Uygur.

| Groups | SNP sites | Allele (1/2) | Ethnicity | Genotype n (%) | \( \chi^2 \)  | P value | Bon |
|--------|-----------|--------------|-----------|----------------|---------|---------|-----|
|        | rs2231142* | T/G          | Uygur     | 29 (0.193) 74 (0.493) 47 (0.313) | 0.042  | 0.120  | 0.0125 |
|        |           |              | Han       | 43 (0.287) 71 (0.473) 36 (0.240) |         |         |       |
| Phlegm block type (n=301) | rs2725220 | C/G          | Uygur     | 89 (0.593) 49 (0.327) 12 (0.080) | 0.011  | 0.582  | 0.0125 |
|        |           |              | Han       | 82 (0.543) 58 (0.384) 11 (0.073) |         |         |       |
|        | rs2231137 | C/T          | Uygur     | 136 (0.907) 13 (0.087) 1 (0.007) | 0.012  | 0.543  | 0.0125 |
|        |           |              | Han       | 140 (0.927) 11 (0.073) 0 (0.000) |         |         |       |
| Non-phlegm block type (n=299) | rs2231142 | G/T          | Uygur     | 61 (0.407) 75 (0.500) 14 (0.093) | 0.078  | 0.020  | 0.0125 |
|        |           |              | Han       | 42 (0.282) 80 (0.537) 27 (0.181) |         |         |       |
|        | rs2725220 | C/G          | Uygur     | 47 (0.313) 66 (0.440) 37 (0.247) | 0.042  | 0.124  | 0.0125 |
|        |           |              | Han       | 56 (0.376) 70 (0.470) 23 (0.154) |         |         |       |
|        | rs2231137 | C/T          | Uygur     | 105 (0.700) 42 (0.280) 3 (0.020) | 0.065  | 0.039  | 0.0125 |
|        |           |              | Han       | 84 (0.564) 58 (0.389) 7 (0.047) |         |         |       |

* One patient of the phlegm block type in the Han group did not generate sequencing result at SNP site rs2231142. Bon – p value in the step down Bonferroni test.
Comparison of genotype distribution of phlegm block hyperuricemia between Han and Uygur

In the case of hyperuricemia with phlegm block type, rs2231142, rs2752220, and rs2231137 were not statistically different between Uygur and Han phlegm block groups (P>0.05). In non-phlegm block type, rs2231142 and rs2231137 were significantly different between Han and Uygur patients (P<0.05) (Table 4).

As shown in Table 5, the allele G of rs2231142 was a risk factor for the phlegm block group (P<0.05, OR=1.397 and 95% CI [1.013~1.927]) when comparing Han patients with Uygur patients with phlegm block hyperuricemia. We found that allele G was 1.397 times higher in Hans compared with Uygur patients. In the case of non-phlegm block hyperuricemia, the allele G (P<0.05, OR=1.563 and 95% CI [1.125~2.173]) and allele C (P<0.05, OR=1.673 and 95% CI [1.118~2.512]) were risk factors for Uygur patients when comparing Han with Uygur patients. The rs2231142 allele G in Uygur patients was 1.563 times higher compared with Han patients. The rs2231137 allele C was 1.397 times higher in Han patients compared with Uygur patients.

Discussion

In TCM, the cause of hyperuricemia is phlegm blood stasis, and is associated with the liver, spleen, and kidney [14,15], whereas modern Western medicine regards uric acid excretion as the main cause of hyperuricemia [16]. The uric acid transporter protein is an important part and key carrier of purine metabolism, and its abnormal expression and dysfunction is the leading cause of uric acid excretion [17]. ABCG2 is considered to be a high-capacity uric acid transporter, and its dysfunction is associated with serum uric acid levels and gout/hyperuricemia [18]. Genome-wide association studies have also reported that ABCG2 affects serum uric acid concentrations [19–22]. In this study, we analyzed the polymorphism of ABCG2 in phlegm-block hyperuricemia and non-phlegm-block hyperuricemia of different ethnic groups.

The SNPs of ABCG2 gene affect the expression and function of ABCG2 [9]. In this study, rs2231142, rs2752220 and rs2231137 SNPs of ABCG2 [8,23,24], which are potentially associated with hyperuricemia and gout, were investigated. For example, Qiu et al. [25] have shown that Q141K polymorphism of ABCG2 gene (rs2231142, allele A and genotype AA) increased the risk of gout. Functional studies of ABCG2 have revealed that homozygous individuals with variant Q141K (Gln141Lys; i.e., individuals with type A alleles) have significantly lower expression of the transporters, while heterozygotes have moderate expression levels. The base change from C to A on the ABCG2 gene rs2231142 site induces the transition from glutamine to lysine in the encoded peptide, and the mutation significantly decreases the protein expression in the homozygotes, seriously
affecting the uric acid excretion function, which is considered to be an important regulating factor for gout [26,27]. Studies have shown that the Q141K mutation may lead to a significant decrease in the stability of ABCG2, with no significant changes in the mRNA level. Degradation of the Q141K variant of ABCG2 is mediated by the ubiquitin-mediated proteasome. In vitro studies showed that when the proteasome-mediated degradation is inhibited by MG132, the levels of Q141K variants are restored [28–30]. Okada et al. [23] reported that rs2725220 is located in chromosome 4q22 and has a significant correlation with the level of uric acid. There have been few studies concerning the rs2725220 polymorphism. A previous study has shown that the gene polymorphisms are associated with hyperuricemia in Koreans [23]. The association of the SNP locus rs2231137 on ABCG2 gene with other diseases is widely studied [24,31,32]. However, there is no research on the association between the SNP locus rs2231137 on ABCG2 gene and hyperuricemia. However, bioinformatics analysis has shown that rs2231137 (G34A) is a polymorphic site with relative high frequency in humans. It is located on exon2 of the ABCG2 gene, which represents a variation from G to A, leading to the transition from valine to methionine. The variation of the A allele reduces ABCG2 transporter activity [26,33].

The present study showed that rs2725220, rs2231137, and rs2231142 loci of ABCG2 were different in the hyperuricemia phlegm block group and non-phlegm block group, indicating that the polymorphisms of these 3 SNP loci can significantly affect the occurrence of urinary phlegm block hyperuricemia, and rs2725220 and rs2231137 were different in hyperuricemia in Han patients. There was no significant difference in rs2231142 in Han patients, which indicates that the polymorphism of rs2725220 and rs2231137 in Han patients can significantly affect the pathogenesis of hyperuricemia.

The 3 SNP loci were also significantly different between Han and Uygur patients. Among them, rs2231137 and rs2231142 were significantly different between Han and Uygur patients with non-phlegm block hyperuricemia, indicating the SNP polymorphism of ABCG2 gene in non-phlegm block type hyperuricemia among different ethnic groups.

Last but not least, we found that rs2231142 allele G was a protective factor for the Han and Uighur patients with hyperuricemia, indicating that allele T increases the risk of hyperuricemia.

Other pathology studies have also reported that AA type impedes the protein expression of ABCG2. Mizuno et al. [34] have speculated that the incidence of hematologic toxicity for the AA genotype (ABCG2 421C>A) is higher than with other genotypes [34]. A similar study has shown that C421A induced conversion of glutamate at position 141 of the ABCG2 protein to lysine (Q141K), resulting in a 53% reduction in urinary acid transport rate [35] and leading to an increase in serum uric acid levels. About 10% of white people with gout were associated with C421A deletion mutations [21]. Dehghan et al. reported that the rs16890979, rs2231142, and rs1165205 SNPs of ABCG2 were associated with gout [21]. The present study showed that rs2725220 allele G was a protective factor. In 2014, Jae et al. also found, in the Korean population, the abnormal uric acid levels (≥7.0 mg/dL) caused by GC or CC genotype was 1.78 times higher than that caused by GG genotype [23]. In addition, our study showed that the allele C of rs2231137 was a risk factor for Han and Uighur patients with hyperuricemia. To the best of our knowledge, this is the study of the role of rs2231137 SNP of ABCG2 in hyperuricemia. The non-synonymous mutations at the 3 SNP sites of rs2231142, rs2231137, and rs2725220 of the ABCG2 gene, which could, at least to some extent, affect the gene transcriptional level, protein translation expression level, and the degradation pathways. The protein expression and activity would be affected, as well as the downstream protein interactions.

Conclusions

In conclusion, ABCG2 gene rs2231137 with more allele C is more likely to be associated with the phlegm block type and rs2725220 with more allele G is more likely to be associated with the non-phlegm block type. In the Uygur hyperuricemia patients, ABCG2 gene rs2231142 with more allele G tends to be non-phlegm block type. Allele C of rs2231137 and allele G of rs2231142 in ABCG2 gene are more likely to be found in Uygur people. Our findings may not only increase the genetic resources of ethnic minorities but also provide a genetic basis for investigating the pathogenesis of hyperuricemia in ethnic minorities in the future.

Conflict of interest

None.

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