Homozygous p.Ser267Phe in SLC10A1 is associated with a new type of hypercholanemia and implications for personalized medicine

Ruihong Liu, Chuming Chen, Xuefeng Xia, Qijun Liao, Qiong Wang, Paul J. Newcombe, Shuhua Xu, Minghui Chen, Yue Ding, Xiaoying Li, Zhihong Liao, Fucheng Li, Minlian Du, Huaiqiu Huang, Ruimin Dong, Weiping Deng, Ye Wang, Binghui Zeng, Qihao Pan, Dazhuang Jiang, Hao Zeng, Pak Sham, Yingnan Cao, Patrick H. Maxwell, Zhi-liang Gao, Liang Peng & Yiming Wang

SLC10A1 codes for the sodium-taurocholate cotransporting polypeptide (NTCP), which is a hepatocellular transporter for bile acids (BAs) and the receptor for hepatitis B and D viruses. NTCP is also a target of multiple drugs. We aimed to evaluate the medical consequences of the loss of function mutation p.Ser267Phe in SLC10A1. We identified eight individuals with homozygous p.Ser267Phe mutation in SLC10A1 and followed up for 8–90 months. We compared their total...
Bile acids (BAs) are synthesized from, and thereby regulate the levels of, cholesterol, which is the parent molecule of steroid hormones including sex hormones and adrenocortical hormones. BAs are essential for the absorption of lipids and lipid-soluble nutrients from the intestine.

Sodium-taurocholate cotransporting polypeptide (NTCP), which is encoded by the SLC10A1 gene (solute carrier family 10 member 1; GenBank accession no. 6554), transports conjugated BAs and, to a less extent, unconjugated BAs into hepatocytes from the plasma. NTCP has recently been identified as a hepatocellular receptor for hepatitis B virus (HBV) and hepatitis D virus (HDV). It also transports multiple drugs into hepatocytes, where they are metabolized. Hence, NTCP is a target, or one of the targets, for several FDA-approved drugs, including ezetimibe (for lowering blood lipids) and fusidic acid (antibacterial infection) used in Europe and Australia, as well as drugs that are currently in clinical trials, such as mycophenolate B (anti-HBV/HDV infection).

It has been known that mutations in at least 10 genes involved in BA metabolism can cause serious diseases associated with hypercholanemia (such as progressive intrahepatic cholestasis) (Online Mendelian Inheritance in Man, OMIM http://www.ncbi.nlm.nih.gov/omim and literature). However, whether mutations in SLC10A1 cause any clear phenotype has been a riddle.

To date there are only four cases with deleterious mutations in SLC10A1 have been reported. One paper reported a patient with congenital hypercholanemia, hypotonia, growth retardation, and cognitive deficiency, who was homozygous for a p.Arg252His mutation in SLC10A1. However, because the genetic analysis in this case was restricted to SLC10A1, the causal gene for the patient's phenotypes is still open for investigation. The p.Ser267Phe mutation in SLC10A1 (rs2296651) is one of the most prevalent deleterious variations in East Asia among genes that are involved in BA metabolism, with an allele frequency of 8–12% in individuals in Southern China (http://browser.1000genomes.org/Homo_sapiens/Variant/Population?db=core;r=14:70244693–70245693;v=rs2296651;vdb=variation;vf=1673765). This mutation has also been identified in African and Latino populations (http://exac.broadinstitute.org/variant/14-70245193-G-A). In vitro experiments have shown that the p.Ser267Phe mutation loses most of its function of bile acid uptake and the ability to support HBV or HDV infection.

Another paper reported a pediatric patient who was homozygous for a p.Ser267Phe mutation in SLC10A1 and had increased blood BA levels. However, this case had complications of liver disease and dermatitis, and the infant was delivered by cesarean section due to entanglement of the umbilical cord. These complications make it difficult to interpret the medical consequence of the mutation p.Ser267Phe in SLC10A1. Very recently, the fourth single case was reported, in whom the deleterious mutations of p.Ser267Phe and the truncating mutation p.Ser206Profs*12 in SLC10A1 were identified. Apart from hypercholanemia, the clinical condition of this case raised the question whether NTCP deficiency is linked to premature loss of fetus.

To unravel the clinical phenotypes and to investigate the medical consequences of the p.Ser267Phe mutation in SLC10A1, we have identified eight individuals with homozygous p.Ser267Phe mutation in SLC10A1. We performed in-depth medical investigations and exome sequencing in the homozygous individuals, and compared their BA levels with 170 wild-type and 107 heterozygous healthy individuals. Here we report our findings and their clinical implications.

Materials and Methods
Identification of individuals with homozygous p.Ser267Phe mutation in SLC10A1 and recruitment of the cohort. During routine employer-sponsored health assessments, in which testing for total serum BAs (tsBAs) was included, two individuals (individuals 1 and 2) had higher levels of tsBAs than the hospital normal range in 2009 and 2014 respectively (Fig. 1a). The two individuals were asymptomatic. Our detailed medical investigations excluded all the causes of hypercholanemia that were known at the time, including viral hepatitis, obstruction of biliary tract, toxic factor, medication factor, autoimmune liver disease, alcoholic liver disease, fatty liver, liver cirrhosis, liver fibrosis and gastrointestinal diseases. As SLC10A1 was a gene for which we did not know whether mutations cause any phenotype, we sequenced the gene and found that both individuals were homozygous p.Ser267Phe mutations. To further investigate the medical significance of this mutation, we started to recruit individuals who are homozygous p.Ser267Phe in SLC10A1 from the Third Affiliate Hospital, Sun Yat-sen University, Southern China, in 2014. Our criteria were that individuals must be homozygous p.Ser267Phe in SLC10A1 and have excluded all the known conditions that may cause increased blood tsBAs; must not have any other predicted deleterious mutations by SIFT (http://sift.jcvi.org/) among variations reported in the 1000 Genome Project (http://browser.1000genomes.org/Homo_sapiens/Search/Results?site=ensembl&q=SLC10A1) and literature (Supplementary Table S1) on SLC10A1; must have normal renal and liver function; must be unrelated to any other individuals in the cohort; and must not be on NTCP-targeting or interacting drugs (consecutive samples, June 2014–April 2016). We also recruited healthy...
and unrelated individuals who were either heterozygous or wild-type for p.Ser267Phe and did not bear any other deleterious mutations in SLC10A1 as mentioned for the homozygous individuals from the Physical Checks Department (consecutive, February 2015–April 2016 for heterozygous and January 2016–April 2016 for wild-type individuals). In total we recruited 285 individuals, including 8 individuals who were homozygous for the p.Ser67Phe mutation (Table 1), 107 healthy heterozygous individuals, and 170 healthy wild-type individuals (Supplementary Table S2). tsBAs were measured in all the individuals. We performed in-depth medical examinations of the homozygous individuals, traced all their available medical records, and followed them up for 8–90 months (Fig. 1a, Table 1).

Written informed consent was obtained from all participants. The study was approved by the Ethical Committee for Human Study, Sun Yat-sen University, and the Principles in the Declaration of Helsinki were followed.

Clinical laboratory investigations. Serum from the homozygous, heterozygous, and wild-type individuals was collected in the morning after at least 8 hours of fasting. The initial and follow-up tsBAs measurements were conducted in the hospital laboratory by the enzyme circle method using a Total Bile Acids Assay kit (Maccure, Chengdu, China) on a Hitachi 7180 automatic biochemical analyzer (Hitachi, Ibaraki, Japan). The tsBAs and six BA species were re-tested by the independent KingMed Diagnostics Lab (http://www.kingmed.com.cn) on a separate blood sample (individual 6 had three separate blood collections and tests) using the enzyme circle method and a Total Bile Acid Reagent Kit (Dongou, Wenzhou, China) on a Roche P800 automatic biochemical analyzer (Roche, Mannheim, Germany). Liquid chromatography-tandem mass spectrometry analysis was used to measure six species of BAs—taurodeoxycholic acid (TDCA), glycodeoxycholic acid (GDCA), cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), and ursodeoxycholic acid (UDCA) on a Triple Quad 5500 mass spectrometry (AB Sciex, California, USA) using the respective reagents (Sigma-Aldrich, Missouri, USA and C/D/N Isotopes Inc, Pointe-Claire, Canada) as previously reported.27 Blood lipids, sex and adrenocortical hormones, and vitamin A and D levels of the homozygous individuals were measured by the hospital laboratory or the KingMed Diagnostics Lab (Supplementary Tables S3 and 4). Bone density was measured by dual energy X-ray absorptiometry (DXA, Discovery DXA System, Hologic Inc, Bedford, USA).  

Sequencing and genotyping. Sanger sequencing was performed to genotype p.Ser267Phe in DNA extracted from blood. We also used Sanger sequencing to exclude all other mutations in SLC10A1 that are predicted to be deleterious by SIFT among the Chinese in the 1000 Genome Project and in two recent reports15, 16 in the homozygous, heterozygous, and wild-type individuals in DNA extracted from blood (Supplementary Table S1). We also used Sanger sequencing to genotype the available parents of individuals 4 and 8. To exclude mosaicism, p.Ser267Phe was also sequenced from buccal samples of the homozygous individuals by Sanger sequencing.

To exclude possible contribution by other BA metabolism-associated genes to the hypercholanemia, exome sequencing was performed on the Illumina and Proton platforms in the eight homozygous individuals (NCBI accession codes SRA438551; Supplementary Tables S5–7, text page 7–10, Supplementary Materials). We selected all of the genes known to cause hypercholanemia and genes coding for BA transporters in enterohepatic circulation in OMIM and in the literature7 (18 genes, Supplementary Table S8), and 48 other genes involved in BA metabolism in OMIM and in the literature7, 17 (Supplementary Table S8). We retrieved and compared all the
variations in the 66 genes with minor allele frequencies lower than 30% among Eastern Asian/Chinese (http://browser.1000genomes.org/Homo_sapiens) in the eight homozygous individuals (Supplementary Table S8).

To examine whether there is population stratification in the cohort (170 wild-type individuals, 107 p.Ser267Phe heterozygous individuals, and 8 p.Ser267Phe homozygous individuals), we genotyped 21 ancestry-informative markers (AIMs) in all samples in the three groups as previously reported (Supplementary Table S9)\(^1\) and designed a statistical test based on random sampling and using the strategy as we previously reported (text page 10–11, Supplementary Materials)\(^2\).

### Statistical analyses

We performed separate linear regressions for each of the six BA species and tBAs as dependent variables and on the p.Ser267Phe genotypes as an independent variable using the R software package (http://www.r-project.org) on values obtained from the KingMed Diagnostics Lab. For all statistical regression analyses we normalized each of the bile acids using a log-transform, since the raw data for each was left-skewed. For each of the BA phenotypes we explored regressions on p.Ser267Phe under both recessive and additive models. All regressions were adjusted for age and gender. For the single individual (individual 6) with three measurements of each bile acid, we used their average values in the regressions. Statistical significance was ascribed according to a Bonferroni \(P\)-value threshold, which was adjusted for the total number of BA phenotypes that were tested and for the use of two genetic models (i.e., our threshold for declaring significance was \(P = 0.05/14 = 0.0036\)). The effect estimates from our linear regressions are interpreted as the mean change in log-bile acid associated with an extra copy of the p.Ser267Phe mutation under additive models, or with the homozygous genotype under recessive models. Under both models, a negative effect indicates the mutation is associated with a reduction in the bile acid, and a positive effect is associated with an increase in tBAs or BA species.

### Data availability statement

The raw data of the Illumina and Proton sequence reads of the whole exome sequence in the 8 individuals who are homozygous p.Ser267Phe in SLC10A1 have been deposited in the NCBI Sequence Read Archive, accession codes SRA438551.

### Results

#### The homozygous p.Ser267Phe mutation in SLC10A1 is associated with hypercholanemia.

All tBAs tests that were performed in the hospital laboratory showed consistent hypercholanemia in all homozygous individuals (Fig. 1a). Moreover, all heterozygous and wild-type individuals were normocholanemic. The hospital measurement was confirmed by the analyses of the KingMed Diagnostics Lab, where tBAs and 6 species of BAs were also measured in another blood collection (Table 2, Supplementary Table S2). The median of tBAs was 3.63 times the maximal reference of the KingMed Diagnostics Lab (Fig. 1b). Under both models, the recessive and additive genetic models, tBAs, and two species of conjugated BAs—TDCA and GDC—were significantly elevated (Under recessive genetic model \(P = 5.8 \times 10^{-29}\), \(2.4 \times 10^{-14}\), \(2.9 \times 10^{-8}\), respectively; Under additive genetic model \(P = 1.0 \times 10^{-25}\), \(2.6 \times 10^{-13}\), \(1.1 \times 10^{-8}\), respectively; Table 2). One species of unconjugated BAs—CA—was also moderately increased (Under recessive genetic model \(P = 4.2 \times 10^{-4}\); Under additive genetic model \(P = 1.4 \times 10^{-5}\); Table 2). Analysis using the additive model also showed a moderate increase in the unconjugated DCA (\(P = 0.0013\), Table 2).

Our Sequenom assay revealed no population stratification among the homozygous, heterozygous, and wild-type individuals, and the 21 ancestral informative markers were within the Hardy-Weinberg equilibrium (Fig. S1, Supplementary Table S9 and text page 10–11, Supplementary Materials).

These results demonstrated that homozygosity for p.Ser267Phe was associated with hypercholanemia made up of conjugated BAs, including TDCA, GDCA, and to a less extent, unconjugated CA.

### Exome sequencing excluded the involvement of other BA metabolism-associated genes

Our exome sequencing detected a total of 23 variations in the 66 genes involved in BA metabolism in the homozygous individuals, besides p.Ser267Phe in SLC10A1 (Supplementary Table S8). Among the 23 variations, 5 were predicted to be deleterious by SIFT. Individual 1 carried the heterozygous mutation p.Val174Ala in SLC01B1 (solute carrier organic anion transporter family member 1B1); individual 4, 5, and 8 carried a heterozygous mutation p.Arg918His in MYO5B (myosin VB) (Supplementary Table S8); individual 1 and 3 carried a heterozygous mutation p.Val186Phe in AMACR (alpha-methylacyl-CoA racemase); individual 7 had a heterozygous mutation p.Arg35Trp in ACOX2 (acyl-CoA oxidase 2); and individual 8 had a heterozygous

---

| Individual 1 | Individual 2 | Individual 3 | Individual 4 | Individual 5 | Individual 6 | Individual 7 | Individual 8 |
|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Sex          | Male         | Male         | Male         | Female       | Male         | Female       | Female       |
| Age (years)  | 48           | 41           | 64           | 7            | 48           | 39           | 29           | 22           |
| Menopausal age | N/A*        | N/A          | N/A          | N/A          | N/A          | N/A          | N/A          |
| Occupation   | Medical Specialist | Medical Specialist | Farmer | Student | Cleaner | Businessman | Nurse | Medical Student |
| Body mass index | 26.2        | 20.0         | 23.4         | 14.5        | 20.1         | 25.9         | 17.4         | 19.7         |
| Follow up (months) | 90           | 30.5         | 21           | 25           | 13           | 10           | 8            | 8            |
| Number of children | 1            | 1            | 4            | N/A          | 2            | 1            | 1            | N/A          |

Table 1. Clinical and Demographic Data of the Individuals Who Are Homozygous for p.Ser267Phe in SLC10A1. N/A: not applicable. §The normal body mass index range according to Chinese standards for individual 4 (7 years and 9 months old) is 13.0–20.6\(^{13}\).
blood cell analysis were normal in all homozygous individuals (Supplementary Table S13). Our threshold for declaring significance is \( P = 0.05 \) for each log-bile acid (BA) per genotype and for the total serum BAs (tsBAs), adjusted for age and gender. The \( P \)-values were obtained from separate linear regressions. CA: cholic acid. DCA: deoxycholic acid. CDCA: chenodeoxycholic acid. UDCA: ursodeoxycholic acid. CI: confidence interval. The mean change in log-bile acid associated with having the homozygous mutation under the recessive models, or with each extra mutation under the additive models. §Mean change in log-bile acid associated with having the homozygous mutation under the recessive models, or with each extra mutation under the additive models. Under both the homozygous and heterozygous settings, a positive effect indicates a reduction in the bile acid, and a positive effect is an increase in tsBAs or BA species.

### Table 2. Statistical analyses of total serum bile acids (tsBAs) and six BA species for association with p.Ser267Phe genotype in SLC10A1.

| Trait                 | Gene       | Recessive genetic model | Additive genetic model |
|-----------------------|------------|-------------------------|------------------------|
| tsBAs                 |            | Effect 95% CI           | Effect 95% CI          |
|                       |            | \( 2.84 (2.39, 3.28) \) | \( 0.84 (0.70, 0.99) \) |
| TDCA                  |            | \( 2.56 (1.93, 3.18) \) | \( 0.71 (0.51, 0.91) \) |
| GDCa                  |            | \( 2.87 (1.88, 3.86) \) | \( 0.92 (0.61, 1.23) \) |
| CA                    |            | \( 1.60 (0.72, 2.48) \) | \( 0.61 (0.34, 0.89) \) |
| DCA                   |            | \( 1.10 (0.17, 2.04) \) | \( 0.48 (0.19, 0.77) \) |
| CDCA                  |            | \( 0.72 (-0.09, 1.53) \) | \( 0.20 (-0.06, 0.45) \) |
| UDCA                  |            | \( 0.55 (-0.33, 1.43) \) | \( 0.00 (-0.28, 0.27) \) |

### Table 3. Vitamin D and adrenocortical hormones (cortisol and aldosterone) in individuals who are homozygous for p.Ser267Phe in SLC10A1.

| Individual | 25-hydroxyvitamin D* (normal range: 75.00–150.00) (nmol/L) | Cortisol (normal range: 118.60–618.00) (at 8:00 am) (nmol/L) | 24-hour Urinary Free Cortisol* (normal range: 3–8 y, 1.40–20.00; ≥ 18 y, 3.50–45.00) (μg/24 h) | Aldosterone (normal range: 40.00–310.00) (orthostatic) (pg/mL) |
|------------|-----------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| Individual 1 | 54.00                                                    | 208.22                                                        | 46.40                                                         | 140.56                                                        |
| Individual 2 | 28.00/30.00/25.00                                        | 424.31                                                        | 22.30                                                         | 169.74                                                        |
| Individual 3 | 76.00/56.00/44.00/63.00/46.00                             | 495.20/384.25                                                | 19.70                                                         | 150.33                                                        |
| Individual 4 | 48.00                                                    | 264.37                                                        | 14.90                                                         | 187.08                                                        |
| Individual 5 | 42.00                                                    | 213.10                                                        | 20.10                                                         | 178.18                                                        |
| Individual 6 | 53.00                                                    | 392.63                                                        | 15.50                                                         | 192.49                                                        |
| Individual 7 | 40.00                                                    | 184.11                                                        | 77.00                                                         | 109.70                                                        |
| Individual 8 |                                                          | 259.67                                                        | 15.40                                                         | 163.21                                                        |

mutation p.Val61Phe in FAB (fumarylacetate hydrolase) (Supplementary Table S8). However, as the five genes are recessive, these heterozygous variations are unlikely to be the cause of their hypercholanemia. In contrast, each and all of the hypercholanemic individuals were homozygous for p.Ser267Phe in SLC10A1 (Supplementary Table S8). This indicates that homozygosity of p.Ser267Phe in SLC10A1 is the likely cause of their hypercholanemia.

Sanger sequencing of buccal swabs produced the same results in p.Ser267Phe in SLC10A1 as the blood samples in all homozygous individuals. The available parents of individuals 4 and 8 were heterozygous p.Ser267Phe carriers. These results imply that homozygous p.Ser267Phe mutation likely originated in the germline and not through mosaicism.

The homozygous p.Ser267Phe mutation in SLC10A1 is associated with low vitamin D levels and differences in steroid hormones and blood lipids. All of the homozygous individuals were asymptomatic. Our in-depth medical examinations showed that all of the individuals who were homozygous for the p.Ser267Phe mutation had normal liver and renal function and normal bilirubin levels. Their medical history showed that they did not have jaundice, pruritus, liver disease, or any of the conditions known to cause hypercholanemia. However vitamin D levels were reduced in all individuals relative to the preferred range (Table 3). Osteoporosis or osteopenia was diagnosed in 3 of the 6 adults who consented to testing. But none had rickets, osteomalacia, a fracture, or back or joint pain. Owing to difficulties in assessing bone density in children, we did not classify individual 4, the 7-year-old boy, into any category (Table 4). Various sex hormones in the homozygous individuals were deviated when compared with the Chinese normal range, but all homozygous adults had well-developed secondary sexual characteristics and were fertile (Table 1 and Supplementary Table S10). Various blood lipids also deviated from the normal range (Supplementary Table S11), and 24-hour urinary free cortisol levels were increased in individuals 1 and 7 (Table 3). Levels of vitamin A and aldosterone, and bleeding and coagulation times were normal in all individuals (Table 3, Supplementary Table S12). And other biochemistry and blood cell analysis were normal in all homozygous individuals (Supplementary Table S13).
Table 4. Bone mineral density of individuals who are homozygous for p.Ser267Phe in SLC10A1²⁵. Figures that deviate from the normal range are in bold italics and are underlined. *Dual energy X-ray absorptiometry (DXA) measurements were made on a Discovery DXA System (Hologic Inc, Bedford, USA). The WHO criteria for classification in adults: Male age ≥ 50 or post-menopausal phase women: Normal: T-score > –1.0; Osteopenia: –2.5 < T-score < –1.0; Osteoporosis: T-score ≤ –2.5. Male Age < 50 or non-menopause women: Normal: Z-score > –2.0, Osteoporosis: Z-score ≤ –2.0. †N/A: not applicable.

Discussion

We have identified a new type of hypercholanemia that is associated with homozygosity for the p.Ser267Phe mutation in SLC10A1. This type of hypercholanemia is different from the previously recognized hypercholanemia in that it is associated with a different gene, and the individuals were all asymptomatic. It is different from the single reported case of the homozygous p.Arg252His mutation in SLC10A1, in whom neurological and developmental delay were present⁶, besides conjugated hypercholanemia. The germline origin of the mutation and the age range of our homozygous group suggest that this type of hypercholanemia is likely to begin early in life. The allele frequency of this mutation varies in different populations, with the highest incidence occurring in Southern China (8% and 12% in Chinese Han and Dai respectively) and Vietnam (11%) (http://browser.1000genomes.org/Homo_sapiens/Variation/Population?db=vdb;vdb=variation;vf=1673765). Taking these observations together, we propose that this “hidden” hypercholanemia affects 0.64% of the Southern Han, 1.44% of the Dai Chinese population, and 1.21% of the Vietnamese population.

The most notable finding in the homozygous individuals was the increase in conjugated and unconjugated serum BA levels. We suggest that this finding is most likely due to reduced BA transport from the portal circulation into hepatocytes. This supports the hypothesis that the physiological function of the enterohepatic circulation is not only to recycle BAs but also to clear BAs from the circulation to achieve homeostasis⁶. Alternatively, the liver may synthesize increased levels of BAs to compensate for the reduced enterohepatic recirculation in the homozygous carriers. As NTCP also transports unconjugated BAs, the increase of the unconjugated BA species in the liver may synthesize increased levels of BAs to compensate for the reduced enterohepatic recirculation in the homozygous carriers. As NTCP-targeted therapies. All homozygous individuals were asymptomatic and had excluded gastrointestinal diseases, there was no evidence to support that low vitamin D level are the consequence of intestinal malabsorption. The p.Ser267Phe mutation loses most of its function of bile acid uptake in in vitro experiments. Hypercholanemia may lead to lower level of bile acids in the intestine in all homozygous individuals. As bile acids play roles in emulsification and absorption of fat and fat-soluble vitamins, insufficient bile acids in the intestine could lead to fat-soluble vitamin D deficiency in these homozygous individuals. And studies on the related mechanisms are underway.

The deviations of levels of sex hormones, blood lipids, and urinary free cortisol seemed to show no consistent patterns in the homozygous individuals. This may be due to an adaptive response to chronic hypercholanemia, wherein the expression levels of multiple genes involved in metabolism of BAs, blood lipids and steroid hormones are altered. Whether these deviations are specifically associated with this novel form of hypercholanemia described here, and whether they are clinically significant, will require further investigation and longer follow-up. Overall results from our study demonstrate a remarkable genomic resilience and seemed reassuring for the new NTCP-targeting drugs that show strong potential for the treatment of hepatitis B and D.

The limitation of this study is the sample size. As the homozygous individuals are asymptomatic, it has taken considerable effort to recruit eight individuals.

In this study we report the identification of a new type of hypercholanemia. And our research about homozygous p.Ser267Phe individuals has implications for personalized medicine. The results from our study suggest that SLC10A1 mutations should be screened for in individuals with asymptomatic hypercholanemia. We also
recommend that levels of vitamin D be monitored or supplemented, while bone density and sex hormones, cortisol, and blood lipids should be carefully monitored in carriers who are homozygous p.Ser267Phe of SLC10A1 and in patients who are undergoing long-term NTCP-targeted therapies, including the drugs that are currently in clinical trials, such as myrcludex B (anti-HBV/HDV infection)10.

References
1. Boyer, J. L. Bile formation and secretion. Compr Physiol 3, 1035–1078 (2013).
2. Yan, H. et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. Elife 1, e00049 (2012).
3. Anwer, M. S. & Steiger, B. Sodium-dependent bile salt transporters of the SLCA10A1 transporter family: more than solute transporters. Pfugers Arch 466, 77–89 (2014).
4. Dong, Z., Ekins, S. & Polli, J. E. Structure-activity relationship for FDA approved drugs as inhibitors of the human sodium taurocholate cotransporting polypeptide (NTCP). Mol Pharm 10, 1008–1019 (2013).
5. Lapham, K. et al. Inhibition of Hepatobiliary Transport Activity by the Antibacterial Agent Fusidic Acid: Insights into Factors Contributing to Conjugated Hyperbilirubinemia/Cholestasis. Chem Res Toxicol 29 (2016).
6. Bogomolov, P. et al. Treatment of chronic hepatitis D with the entry inhibitor myrcludex B: First results of a phase Ib/Ila study. J Hepatol 65, 490–498 (2016).
7. Qiu, Y. L. et al. Defects in MYO5B are associated with a spectrum of previously undiagnosed low gamma-glutamyltransferase cholestasis. Hepatology (2016).
8. Vaz, F. M. et al. Sodium taurocholate cotransporting polypeptide (SLC10A1) deficiency: conjugated hypercholelasmia without a clear clinical phenotype. Hepatology 61, 260–267 (2015).
9. Karpen, S. J. & Dawson, P. A. Not all (bile acids) who wander are lost: the first report of a patient with an isolated NTCP defect. Hepatology 61, 24–27 (2015).
10. Ho, R. H., Leake, B. F., Roberts, R. L., Lee, W. & Kim, R. B. Ethnicity-dependent polymorphism in Na+-taurocholate cotransporting polypeptide (SLC10A1) reveals a domain critical for bile acid substrate recognition. J Biol Chem 279, 7213–7222 (2004).
11. Pan, W. et al. Genetic polymorphisms in Na+-taurocholate co-transporting polypeptide (NTCP) and ideal apical sodium-dependent bile acid transporter (ASBT) and ethnic comparisons of functional variants of NTCP among Asian populations. Xenobiotica 41, 501–510 (2011).
12. Yan, H. et al. Viral entry of hepatitis B and D viruses and bile salt transport share common molecular determinants on sodium taurocholate cotransporting polypeptide. J Virol 88, 3273–3284 (2014).
13. Deng, M. et al. Clinical and molecular study of a pediatric patient with sodium taurocholate cotransporting polypeptide deficiency. Exp Ther Med 12, 3294–3300 (2016).
14. Van Herpe, F. et al. NTCP deficiency and persistently raised bile salts: an adult case. J Inherit Metab Dis (0032017).
15. Yang, J. et al. A genetic variant of the NTCP gene is associated with HBV infection status in a Chinese population. BMC Cancer 16, 211 (2016).
16. Hu, H. H. et al. The rs2296651 (S267F) variant on NTCP (SLC10A1) is inversely associated with chronic hepatitis B and progression to cirrhosis and hepatocellular carcinoma in patients with chronic hepatitis B. Gut 65, 1514–21 (2016).
17. Scherer, M., Gnewuch, C., Schmitz, G. & Liebisch, G. Rapid quantification of bile acids and their conjugates in serum by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 877, 3920–3925 (2009).
18. Alrefai, W. A. & Gill, R. K. Bile acid transporters: structure, function, regulation and pathophysiological implications. Pharmacol Rev 24, 1803–1823 (2007).
19. Deng, M. et al. The p.Ser267Phe variant in SLC10A1 is associated with resistance to chronic hepatitis B. Hepatology 61, 1251–1260 (2015).
20. Lou, H. et al. A 3.4-kb Copy-Number deletion near EPASI is significantly enriched in High-Altitude tibetans but absent from the Denisovan sequence. Am J Hum Genet 97, 54–66 (2015).
21. Qin, P. et al. A panel of ancestry informative markers to estimate and correct potential effects of population stratification in Han Chinese. Eur J Hum Genet 22, 248–253 (2014).
22. Holick, M. F. Vitamin D deficiency. N Engl J Med 357, 266–281 (2007).
23. Li, H., Ji, C. Y., Zong, X. N. & Zhang, Y. Q. [Body mass index growth curves for Chinese children and adolescents aged 0 to 18 years]. Zhonghua Er Ke Za Zhi 47, 493–498 (2009).

Acknowledgements
This project is supported by the National Natural Science Foundation of China (31471193, 81570539, 81370535, 91331204 and 31525014). S.X. acknowledges financial support from the Strategic Priority Research Program (XDB13040100) and Key Research Program of Frontier Sciences (QYZDJ-SSW-SYS009) of the Chinese Academy of Sciences (CAS). We thank all participants of the study. We also thank Prof. Jianping Wang, 6th affiliated Hospital, Sun Yat-sen University for his support for this study.

Author Contributions
Study concept and design: Y.W.(Yiming Wang), L.P., Z.G., R.L., C.C., X.X., P.H.M., S.X.; Acquisition of data: R.L., C.C., E.L., H.H., R.D., Y.W.(Ye Wang), B.Z., Q.P., D.J., H.Z.; Analysis and interpretation of data: R.L., C.C., F.L., Q.L., Q.W., P.J.N., S.X., M.C., Y.D., X.L., Z.L., M.D., W.D., P.S., Y.C., P.H.M.; Statistical analysis: P.J.N., S.X., P.S., Q.L.; Drafting of the manuscript: Y.W.(Yiming Wang), L.P., Z.G., R.L., C.C., Q.L., P.J.N., F.L., S.X., H.H., R.D., Y.W.(Ye Wang), B.Z., Q.P., D.J., H.Z.; Critical revision of the manuscript for important intellectual content: Y.W.(Yiming Wang), L.P., X.X., P.H.M., S.X., Q.W., M.C., Y.D., X.L., Z.L., M.D., W.D., P.S., Y.C.; Obtained funding: Y.W.(Yiming Wang), L.P., S.X.; Study supervision: Y.W.(Yiming Wang), L.P. All authors reviewed the manuscript.

Additional Information
Supplementary information accompanies this paper at doi:10.1038/s41598-017-07012-2
Competing Interests: The authors declare that they have no competing interests.
Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
