Genetic spectrum and clinical characteristics of 3β-hydroxy-Δ⁵-C₄₇-steroid oxidoreductase (HSD3B7) deficiency in China

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Abstract

Background: Biallelic variants in HSD3B7 cause 3β-hydroxy-Δ⁵-C₂₇-steroid oxidoreductase (HSD3B7) deficiency, a life-threatening but treatable liver disease. The goal of this study was to obtain detailed information on the correlation between the genotype and phenotype of HSD3B7 deficiency and to report on responses to primary bile acid therapy.

Methods: The medical records of a cohort of 39 unrelated patients with genetically and biochemically confirmed HSD3B7 deficiency were examined to determine whether there exist genotype-phenotype relationships in this bile acid synthesis disorder.

Results: In all, 34 of the 44 variants identified in HSD3B7 were novel. A total of 32 patients presented early with neonatal cholestasis, and 7 presented after 1-year of age with liver failure (n = 1), liver cirrhosis (n = 3), cholestasis (n = 1), renal cysts and abnormal liver biochemistries (n = 1), and coagulopathy from vitamin K1 deficiency and abnormal liver biochemistries (n = 1). Renal lesions, including renal cysts, renal stones, calcium deposition and renal enlargement were observed in 10 of 35 patients. Thirty-three patients were treated with oral chenodeoxycholic acid (CDCA) resulting in normalization of liver biochemistries in 24, while 2 showed a significant clinical improvement, and 7 underwent liver transplantation or died. Remarkably, renal lesions in 6 patients resolved after CDCA treatment, or liver transplantation. There were no significant correlations between genotype and clinical outcomes.

Conclusions: In what is the largest cohort of patients with HSD3B7 deficiency thus far studied, renal lesions were a notable clinical feature of HSD3B7 deficiency and these were resolved with suppression of atypical bile acids by oral CDCA administration.

Keywords: Bile acid synthesis, Chenodeoxycholic acid, Genetic spectrum, HSD3B7, Renal lesions, 3β-hydroxy-Δ⁵-C₂₇-steroid oxidoreductase deficiency
Chenodeoxycholic acid (CDCA) has been shown to be effective and life-saving [8–10]. If untreated, HSD3B7 deficiency-associated liver disease may lead to liver failure requiring liver transplantation [9]. Comprehensive information on the clinical and genetic features of HSD3B7 deficiency is limited by the fact that worldwide there have been < 100 cases reported of this rare disorder and consequently there is a paucity of data on genotype-phenotype associations. [1, 2, 4, 7, 10–21]. Due to the lack of urinary analysis by mass spectrometry to establish the biochemical diagnosis in some regions of the world, the more frequent use of panel or whole exome sequencing has led to molecular analysis playing an increasing role in establishing an early diagnosis. However, interpreting clinical significance of genetic variants remains a critical roadblock [22, 23]. Underlying pathogenic variants are often classified as variants of uncertain significance (VUS) for lack of data, which could lead to under-recognition of this treatable disorder.

The aim of this study was to present the genetic spectrum, clinical features and treatment outcome of a large cohort of Chinese patients with a confirmed HSD3B7 deficiency, and discuss the possible impacts of HSD3B7 variants on the clinical phenotype.

Methods

Patients

We retrospectively reviewed the findings from 39 patients who were diagnosed with HSD3B7 deficiency at Children's Hospital of Fudan University between the years 2009–2020. This included five patients (P5, P9, P11, P13, and P14) that were reported previously [17–19, 24]. In 33 patients, the diagnosis was established by clinical features, serum liver biochemistries, urinary bile acid analysis by fast atom bombardment ionization mass spectrometry (FAB-MS), and molecular analysis. In 6 cases (P3, P6, P36–P39) where urine was not available for analysis, the diagnosis was suspected based on clinical characteristics and serum liver biochemistries, and then confirmed by genetic studies with parental verification. The following information was collated from patient records: gender, geographical origin, age at disease onset, age at first visit to our hospital, clinical features, laboratory findings, radiological studies, genetic data, type and duration of therapies, and responses to treatment.

This study was approved by the Ethics Committees on Human Research of the Children's Hospital of Fudan University.

Genetic study

Before December 2015, all exons and adjacent introns of HSD3B7 (RefSeq NM_025193.4) were Sanger sequenced as described previously [17]. After January 2016, panel sequencing and Sanger confirmation were performed [25]. Large fragment deletions were confirmed by quantitative polymerase chain reaction (qPCR). Variants were annotated for frequency in public databases (Genome Aggregation Database and Exome Aggregation Consortium) and predicted pathogenicity in PROVEAN (http://provean.jcvi.org), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (http://mutantintaster.org), SIFT (http://sift.jcvi.org) and FATHMM (http://fathmm.biocompute.org.uk). Variants with minor allele frequency < 0.005 and predicted to be pathogenic by at least one of the five programs were considered as predicted pathogenic variants. The American College of Medical Genetics and Genomics (ACMG) guidelines was used for clinical sequence interpretation [26].

To explore the possible genotype-phenotype association, all variants were categorized into two classes. Frameshift, nonsense, classical splicing variants and large fragment deletions predicted to result in nonsense mRNA decay or protein truncation were defined as null variants. Other variants, including missense, non-classical splicing and non-frameshift small indel types, were defined as non-null variants.

Urinary bile acid analysis

Urine samples were collected before any treatment with the primary bile acid, chenodeoxycholic acid (CDCA), and analyzed at the Cincinnati Children's Hospital Medical Center using FAB-MS mass spectrometry [9]. In patients with a suspected bile acid synthesis disorder, treatment with UDCA was terminated 5–7 days prior to collection of urine samples. Diagnosis of a HSD3B7 deficiency was based on the finding of a lack of the normal primary bile acid conjugates and the presence of the pairs of ions at m/z 469/485 (sulfate conjugates) and m/z 526/542 (glyco-sulfate conjugates) representing the atypical 3β-hydroxy-Δ5-bile acids that are the signature metabolites for this bile acid synthesis disorder. FAB-MS analysis of urine was also used to monitor the therapeutic response to primary bile acid therapy [1, 27, 28].

Management

After the confirmation of the diagnosis, CDCA (initially 4–10 mg/kg/d) was prescribed. Serum biochemistries were measured every week until the jaundice resolved and thereafter monthly until the normalization of liver function tests was achieved. Urinary bile acid analysis and renal ultrasound were repeated every 6 months. Dose adjustments of CDCA were based on the findings of reductions in the levels of atypical 3β-hydroxy-Δ5-bile acids from the urinary bile acid analyses combined with
changes in the serum biochemistries, including serum transaminases and GGT.

Statistical analysis
Statistical analysis was performed using SPSS 17. Mann-Whitney test, Fisher’s exact test and Spearman correlation were performed. Values for \( p < 0.05 \) was considered statistically significant.

Results
The genetic spectrum of HSD3B7 deficiency
There were 44 pathogenic/predicted pathogenic variants identified (Table 1, Additional file 2: table S1 and S2). Of these, 23 were nonsense variants (42.3%), 5 were splice site variations (16.7%), 3 were small (< 15 bp) deletions or insertions (34.6%) and one was a 1.2-kb deletion (1.3%). Information regarding paternal and maternal revealed homozygotes in 14 patients (35.9%) and compound heterozygotes in 17 patients (43.6%). In eight patients (20.5%), parental verification was not performed (Table 1).

Among the 44 variants, 10 were reported previously in the literature and 34 were novel [16–19, 24, 29]. All 34 novel variants were absent or with very low frequency (less than 1/10,000) in Genome Aggregation Database and Exome Aggregation Consortium. All were predicted to cause deleterious disruptions to the protein by at least one of the five programs: PROVEAN, MutationTaster, PolyPhen-2, SIFT and FATHMM software (Additional file 2: Table S1). According to ACMG standards and guidelines, 1 out of 34 novel variants were assigned as a “pathogenic variant,” 14 as “likely pathogenic,” and the remaining 19 as “VUS” (Additional file 2: Table S1).

The variants identified were spread throughout the HSD3B7 gene. Over 75% of patients carried an HSD3B7 variant on exon 4, 5 or 6 (Fig. 1). The four most common variants were c.45_46delAG (n = 6, 7.7%) in exon 1, c.503G>A (n = 9, 11.5%) in exon 4, c.543dupG (n = 6, 7.7%) and c.683G>A (n = 5, 6.4%) in exon 5.

Clinical data and laboratory evaluation
Among the 39 patients enrolled, 24 were male and 15 were female. Four patients (P2, P6, P24, P26) had one sibling respectively with neonatal cholestasis that died before 3 years of age. Table 2 summarizes the clinical features, liver biochemistries, urinary bile acid analysis, medical treatment, and outcome.

The median age of onset of symptoms was 10 days (range 2 days–16.8 years old). The median age at diagnosis was 4.8 months (range 1.7 months–17.2 years old). Depending on the onset age, we classified our patients into two groups. The first group included 32 patients presenting with neonatal cholestasis. The second group included 7 patients presented with a broad spectrum of symptoms after one year of age, including adolescence-onset cholestasis and liver failure (P6), liver cirrhosis with (P22) or without (P31, P35) a history of transient neonatal cholestasis, recurrent cholestasis (P15), renal cysts and abnormal liver biochemistries with transient neonatal cholestasis (P2), and coagulopathy of vitamin K1 deficiency and abnormal liver biochemistries (P8).

Neonatal cholestasis with low serum GGT and serum total bile acids (sTBA), the latter measured by immunoassay, is a common feature of HSD3B7 deficiency. The serum GGT levels in the patients who were referred before one year of age ranged 8–70U/L and the range of the sTBA concentration was 0.2–85.4µmol/L. The concentration of sTBA was between 10 and 30 µmol/L in eight patients, five who had stopped UDCA treatment for five days, and >30µmol/L in three patients of whom two (P4 and P38) were on UDCA therapy and one (P21) was in liver failure. These high sTBA would be expected in these three patients.

Renal images were collected from 35 patients before treatment with CDCA, of whom 10 (28.6%) had renal lesions, including renal cysts (n = 6), renal stones (n = 2), calcium deposition (n = 2), renal enlargement (n = 1) and multiple abnormal echoes in the calyx (n = 1) (Fig. 2; Additional file 1: figure S1 and Additional file 1: S2). In these patients, the serum creatinine levels and urinalysis were all within the normal range. The patients with renal lesions (median age 3.1 years, range 3.7 months to 17.2 years) were referred significantly later in age than patients that did not have identifiable renal lesions (median age 4.5 months, range 1.7 months to 5.2 years, \( P < 0.001 \)).

Urinary bile acid analysis
Urine samples from 33 patients were collected and analyzed using FAB-MS. The profiles of 32 patients showed an absence or a lack of the normal primary bile acid conjugates and marked elevations in sulfate and glyco-sulfate conjugates of dihydroxy- and trihydroxy-cholenoic acids (ions at m/z 469, 485, sulfate conjugates; m/z 526, 542, glyco-sulfate conjugates) that are the biomarkers for the HSD3B7 deficiency. Compared with typical bile acid metabolites, the profile of Patient 21, who was in liver failure, showed only traces of these ion features, presumably because of significant loss of quantitative synthetic function (Fig. 3).

Clinical follow-up and outcome
Apart from 2 patients (P4 and P6) that died before a diagnosis of HSD3B7 deficiency was established, 1 patient (P23) that refused oral CDCA therapy and 3 patients (P3, P7 and P11) that were lost to follow-up, 33 patients...
| Patients | Zygosity | Location | Nucleotide change (NM_025193.4) | Predicted amino acid change (NP_079469.2) | ACMG classification<sup>‡</sup> | Parental derivation | Geographical origin |
|----------|----------|----------|-------------------------------|-----------------------------------|---------------------------------|-----------------|-------------------|
| P1       | Hom      | Ex6      | c.1031 A > G                  | p.Tyr344Cys                        | LP                              | PS3 + PM2_S + PP4 | Paternal/maternal  | Zhejiang          |
| P2       | Het      | Ex1      | c.45_46delAG                  | p.Gly17Leuφ*26                    | P                               | PS1 + PS4 + PM2_S | Maternal          | Jiangxi           |
| P3       | Hom      | Ex6      | c.988_990delACC               | p.Thr329del                        | LP                              | PM2_S + PM3 + PM4 + PP3 | Paternal          | Jiangxi           |
|          | hom      | Ex6      | c.968 C > T                   | p.Thr323Met                        | VUS                             | PM2_S + PP3      | Paternal/maternal  | Jiangsu           |
| P4       | Het      | Ex5      | c.683G > A                    | p.Arg228Gln                        | LP                              | PS4 + PM2_S + PM3 + PP3 | Paternal          | Shandong          |
|          | Het      | Ex6      | c.1040delT                    | p.Gly88Arg                         | VUS                             | PM2_S + PM3 + PM3 | Paternal          | Yunnan            |
| P5       | Het      | Ex1      | c.45_46delAG                  | p.Gly17Leuφ*26                    | P                               | PS1 + PS4 + PM2_S | Maternal          | Shandong          |
|          | Het      | Ex2      | c.262G > C                    | p.Gly347Argφ*70                    | P                               | PS1 + PS4 + PM2_S | Maternal          | Yunnan            |
|          | Het      | Ex6      | c.988_990delACC               | p.Thr329del                        | VUS                             | PM2_S + PM3 + PM4 | Paternal          | Jiangsu           |
| P6       | Het      | Ex4      | c.484_485delinsCC             | p.Thr329del                        | VUS                             | PM2_S + PM3 + PM4 | Paternal          | Guizhou           |
| P7       | Het      | Ex5      | c.544delC                     | p.Leu182Cysφ*4                    | LP                              | PS1 + PM2_S      | Paternal/maternal  | Shandong          |
| P8       | Hom      | Ex4      | c.474delC                     | p.Tyr159Leuφ*27                   | LP                              | PS1 + PM2_S      | Paternal/maternal  | Hebei             |
| P9       | Het      | Ex5      | c.543dupG                     | p.Leu182Cysφ*16                    | P                               | PS1 + PS4 + PM2_S + PM3 | Maternal          | Hebei             |
| P10      | Het      | Ex6      | c.781G > A                    | p.Asp261Asn                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | NA                | Jiangxi           |
| P11      | Het      | Ex3      | c.401G > A                    | p.Gly347Argφ*70                    | P                               | PS1 + PS4 + PM2_S | NA                | Jiangxi           |
| P12      | Het      | Ex5      | c.682 C > T                   | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | NA                | Anhui             |
|          | Het      | Ex6      | c.1016G > C                   | p.Asp261Asn                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | NA                | Anhui             |
| P13      | Het      | Ex4      | c.503G > A                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | NA                | Hebei             |
|          | Het      | Ex5      | c.683G > A                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | NA                | Hebei             |
| P14      | Het      | Ex4      | c.1474G > A                   | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | NA                | Hubei             |
| P15      | Het      | Ex5      | c.503G > A                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | NA                | Xingjiang         |
|          | Het      | Ex5      | c.569G > A                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | NA                | Xingjiang         |
| P16      | Het      | Ex5      | c.682 C > T                   | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | Paternal          | Jehol/Hebei       |
| P17      | Hom      | Ex6      | c.988_990delACC               | p.Thr329del                        | VUS                             | PM2_S + PM3 + PM4 + PP3 | Paternal          | Henan             |
| P18      | Het      | Ex5      | c.543dupG                     | p.Leu182Cysφ*16                    | P                               | PS1 + PS4 + PM2_S + PM3 | Maternal          | Gansu             |
| P19      | Het      | Ex6      | c.683G > A                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP3 | Maternal          | Gansu             |
|          | Het      | Ex1      | c.45_46delAG                  | p.Gly17Leuφ*26                    | P                               | PS1 + PS4 + PM2_S | NA                | Sandong           |
| P20      | Het      | Ex6      | c.770 A > G                   | p.Tyr25Cysφ*70                     | P                               | PS1 + PS4 + PM2_S | NA                | Sandong           |
|          | Het      | Ex5      | c.683G > A                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP3 | NA                | Guangxi           |
|          | Het      | Ex5      | c.683G > T                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP3 | NA                | Guangxi           |
| P21      | Het      | Ex5      | c.561T > G                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP3 | NA                | Hunan             |
|          | Het      | Ex5      | c.586G > A                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP3 | NA                | Hunan             |
| P22      | Het      | Ex3      | c.346T > C                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | Maternal          | Hainan            |
|          | Het      | Ex6      | c.683_685dup                  | p.Leu249Aalφ*16                    | LP                              | PS1 + PS4 + PM2_S + PM4 | Maternal          | Hainan            |
| P23      | Hom      | Ex4      | c.503G > A                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP3 | NA                | Hainan            |
|          | Het      | Ex5      | c.676 C > T                   | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | Maternal          | Shandong          |
| P24      | Het      | Ex5      | c.683G > A                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | Maternal          | Shandong          |
| P25      | Het      | Ex4      | c.503G > A                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | Maternal          | Hebei             |
|          | Het      | Ex6      | c.743G > C                    | p.Arg228Gln                        | VUS                             | PM2_S + PM3 + PM4 + PP4 | Maternal          | Hebei             |

<sup>‡</sup> Assumed de novo
were treated with CDCA (initial dose ranging 3-10 mg/kg/d) and regularly monitored. The median follow-up period was 26 mo (range 10 days to 10 + years). Of these, 24 (73%) achieved a complete normalization of serum liver biochemistries, 2 (6%) showed significant clinical improvement, 5 (15%) underwent liver transplantation, and 2 (6%) died. There was no significant difference in the age at diagnosis between the patient group consisting of the one that had a liver transplant and the deceased cases combined (median 4.9 mo, n = 7, range 1.8 mo–11.5 mo) the group comprising the native liver survivors (median 4.8 mo, n = 26, range 1.4 mo–6.6 y, \( P = 0.874 \)).

Of the 10 patients with renal lesions, one (P6) died before a definite diagnosis of HSD3B7 deficiency was made, two other patients (P15, P35) have yet to undergo repeat renal imaging. Renal ultrasonography was repeated in the other seven patients: Six patients were on continuous CDCA therapy, and one underwent a liver transplant (P21) 10 days after initiating bile acid therapy. Renal lesions eventually disappeared in all of these patients after a median duration of 13 mo (range 4 mo to 36 mo) and concomitant with a decrease or disappearance of atypical bile acids in urine and normalization of serum liver biochemistries (Fig. 2), save patient P37. In patient P37, renal ultrasound revealed bilateral renal enlargement improved after 11 months of CDCA treatment (left 87.9 mm*27.6 mm*24.3 mm, right 83.1 mm*31.6 mm*37.6 mm, compared 105 mm*25.1 mm*29.7 mm and 89.8 mm*29.1 mm*32.5 mm, respectively).

Genotype-phenotype relationship

Genotypically, 12 patients were classified as harboring biallelic null variants, 15 patients as one null and one non-null variants, and 11 patients as biallelic non-null variants. Phenotypically, 32 patients were classified

Table 1 (continued)

| Patients | Zygosity | Location | Nucleotide change (NM_025193.4) | Predicted amino acid change (NP_079469.2) | ACMG classification \(^{a}\) | Parental derivation | Geographical origin |
|----------|----------|----------|---------------------------------|------------------------------------------|----------------|---------------------|-------------------|
| P27      | Hom      | Ex4      | c.485_487delGCA                 | p.Ser162del                              | VUS           | PM2-S + PM4 + PP4   | Paternal/ maternal | Zhejiang          |
| P28      | Het      | Ex5      | c.683G>T                       | p.Arg228Gln                              | LP            | PS4 + PM2-S + PP3   | Paternal           | Hunan             |
| P29      | Het      | In5      | c.694_427delG                  | p.Val586GluF*14                           | LP            | PS/1 + PM2-S + PM3  | Maternal           | Hunan             |
| P30      | Het      | Ex2      | c.173_174del                  | p.Leu144Pro                              | VUS           | PM2-S + PM3 + PP3   | Paternal           | Shandong          |
| P31      | Het      | Ex5      | c.557 G>T                     | p.Leu186Met                              | VUS           | PM2-S + PP3         | Paternal           | Shandong          |
| P32      | Het      | Ex6      | c.968 C>G                     | p.Leu182Alafs*16                          | VUS           | PM2-S + PP3         | Paternal           | Shandong          |
| P33      | Het      | Ex6      | c.698 A>G                     | p.Asn233Ser                              | VUS           | PM2-S + PM3 + PP3   | Paternal           | Shandong          |
| P34      | Het      | Ex6      | c.920_931delGGCTGC            | p.Trp307_310delinsSer                    | LP            | PS/1 + PM2-S        | Paternal           | Shandong          |
| P35      | Het      | Ex2      | c.45_6delAG                   | p.Gly171Leufs*26                          | P              | PS/1 + PS4 + PM3    | Paternal           | Shandong          |
| P36      | Het      | Ex2      | c.319 C>T                     | p.Glu107Ter                              | LP            | PS/1 + PM2-S        | Maternal           | Shandong          |
| P37      | Het      | Ex2      | c.45_6delAG                   | p.Gly170Leufs*26                          | P              | PS/1 + PS4 + PM2    | Maternal           | Shandong          |
| P38      | Het      | Ex6      | c.206_210del                  | p.Pro115Alafs*2                           | LP            | PS/1 + PM2-S + PM3  | Maternal           | Anhui             |
| P39      | Het      | Ex6      | c.206_210del                  | p.Trp318Alafs*16                          | P              | PS/1 + PM2-S + PM3  | Paternal and maternal | Anhui          |
| P40      | Het      | Ex6      | c.206_210del                  | p.Trp318Alafs*16                          | P              | PS/1 + PM2-S + PM3  | Paternal and maternal | Yunnan           |

Het heterozygous, Hom homozagous, Ex exon, In Intron, P pathogenic, LP likely pathogenic, VUS variant of uncertain significance; PVS, pathogenic very strong, PS pathogenic strong, PM pathogenic moderate, PP pathogenic supporting

\(^{a}\) According to the American College of Medical Genetics and Genomics interpretation guidelines

\(^{b}\) Without confirmation of paternity and maternity
as neonatal cholestasis onset, 7 with childhood onset. The clinical outcome were classified as excellent for 27 patients (native liver survivors), and poor outcome for 12 (either died or were transplanted). No significant differences were observed in terms of age of disease onset or clinical outcome among the patients with different genotypes (Table 4). Similarly, there was no significant differences among patients with novel variants and other known variants (Additional file 2: table S4).

**Discussion**

This study, the first of its kind, details the genotypic and phenotypic features of the largest collection of patients with HSD3B7 deficiency reported to date. Genetic analysis revealed 34 novel pathogenic or predicted pathogenic variants in the HSD3B7 gene. Furthermore, our observation that 10 patients had renal lesions, and remarkably, treatment with oral CDCA or liver transplantation resolved these lesions concomitant with a suppression of the atypical 3β-hydroxy-Δ5-bile acids biomarkers, highlights renal lesions as an important clinical feature of this bile acid synthesis disorder.

We have described 34 novel variants in our patients; 19 novel variants were assigned as VUS, including 17 missense variants, 1 non-classical splice site variant and 1 non-frameshift (3 bp) deletion, which were absent or with very low frequency in public databases and were predicted pathogenicity by at least one of the five programs used. The diagnosis of these subjects was based on not only genetic analysis, but also on definitive features of the urinary bile acid profile, combined with the clinical features and liver biochemistries. The bile acid profiles of 14 patients with 17 variants assigned as VUS were consistent with HSD3B7 deficiency which is important information for the pathogenicity assessment of these variants if they are detected in future patients. In two patients with the remaining two variants of uncertain significance (c.968 C>T and c.484_485delinsCC), serum TBA concentrations (measured by enzyme immunoassay) were low (<10µmol/L) and consistent with expectations for a bile acid synthesis disorder [20]. Elevated atypical urinary bile acids and low serum TBA (measured when off UDCA therapy) enabled us to make the final diagnosis and to prove that these 19 novel variants of uncertain significance are likely pathogenic.

During the study period, 5086 patients with neonatal cholestasis were referred to our center. In our HSD3B7 deficiency patients, 32 presented as neonatal cholestasis. It is likely that HSD3B7 deficiency accounts for 0.6% of neonatal cholestasis in our single liver center. This would
| Patients | Gender | Age at onset | Age at first referral | Presenting symptoms | Liver biochemistries | Urinary bile acids profiling§ | Treatment after diagnosis | Status/age at last follow-up | Liver biochemistries |
|----------|--------|--------------|----------------------|---------------------|---------------------|-----------------------------|---------------------------|-------------------------|----------------------|
|          |        |              |                      |                     | TB/DB (µmol/L) ALT/AST (U/L) | TB/DB (µmol/L) ALT/AST (U/L) |                          |                         | TB/DB (µmol/L) ALT/AST (U/L) |
| P1       | M      | 1.5mo        | 5.7mo                | Neonatal cholestasis, hepatomegaly | 85.6/366           | 159/154                     | UDCA × 2y, CDCA × 10y1mo | Normal/12y              | 5.6/2.4, 32/22         |
| P2       | M      | 10d          | 16.5mo               | Renal cysts, abnormal liver biochemistries, hepatomegaly with a history of transient neonatal cholestasis | 24.7/20.1          | 128/72                      | UDCA × 25 y, CDCA × 7y6mo | Normal/11.2y            | 11.4/4, 13.7/23        |
| P3       | M      | 5d           | 4.5mo                | Neonatal cholestasis, hepatomegaly | 133.9/65.5         | 36/85                       | NA                         | NA                     | 488.4/343.1, 268/356   |
| P4       | F      | 7d           | 4.5mo                | Neonatal cholestasis, hepatosplenomegaly | 137.3/102         | 51/164                      | NA                         | Died/10mo              | NA                    |
| P5       | M      | 5d           | 3.7mo                | Neonatal cholestasis, hepatosplenomegaly | 157.7/122.3       | 521/356                     | CDCA × 7y                  | Normal/7.3y            | 11.3/47, 5/15          |
| P6       | M      | 16.8y        | 17.2y                | Cholestasis, hepatosplenomegaly and then liver failure | 96/68              | 62/46                       | NA                         | Died/17.2y             | 720/593, 179/104       |
| P7       | M      | 1mo          | 2.2mo                | Neonatal cholestasis, coagulopathy, abdominal hematoma | 123.9/75.7        | 157/132                     | NA                         | Lost follow-up/22mo     | 260.7/195.5, 244/625  |
| P8       | F      | 3.5y         | 4.3y                 | Coagulopathy of vitamin K1 deficiency, abnormal liver biochemistries, hepatosplenomegaly | 32/24             | 51/70                       | CDCA × 6y2mo              | Normal/10.4y           | 13/2.6, 25/9           |
| P9       | M      | 1mo          | 6.6mo                | Neonatal cholestasis, hepatomegaly | 151.3/108.75       | 812/819                     | CDCA × 5y8mo               | Normal/6.2y            | 6.1/2.1, 16.3/25.3     |
| P10      | M      | 2.3d         | 3.4mo                | Neonatal cholestasis, hepatosplenomegaly | 77.4/55.1         | 71/76                       | CDCA × 6y                  | Normal/6.3y            | 6/2.6, 10.6/27.3       |
| P11      | M      | 2d           | 5.2mo                | Neonatal cholestasis, hepatomegaly | 164.1/109.9        | 376/297                     | CDCA × 12d                 | Liver biochemistries worsen/6mo | 163.4/134.5, 340/370   |
Table 2 (continued)

| Patients | Gender | Age at onset | Age at first referral† | Presenting symptoms | Liver biochemistries | Urinary bile acids profiling§ | Treatment after diagnosis | Status/age at last follow-up | Liver biochemistries |
|----------|--------|--------------|------------------------|---------------------|---------------------|------------------------------|--------------------------|-------------------------|----------------------|
|          |        |              |                        |                     | TB/DB (µmol/L)      | ALT/AST (U/L)                |                          |                         | TB/DB (µmol/L)        |
| P12      | F      | 1.5mo        | 2.6mo                  | Neonatal cholestasis| 191.4/123.1         | 152/210                     | +                        | CDCA × 2y4mo            | 10.6/2.4              |
| P13      | F      | 10d          | 2mo                    | Neonatal cholestasis, hepato-megaly | 103.3/85.9      | 284/216                     | +                        | CDCA × 3y10mo           | 12.4/3.7              |
| P14      | M      | 2mo          | 6.3mo                  | Neonatal cholestasis | 335.9/236.8        | 768/608                     | +                        | CDCA × 4y               | 16.6/2.14             |
| P15      | F      | 3d           | 6.6y                   | Recurrent cholestasis, splenomegaly | 46.2/143       | 26/34                       | +                        | CDCA × 2y11mo           | 11.5/2.4              |
| P16      | F      | 3d           | 5.8mo                  | Neonatal cholestasis | 98/59.3           | 181/276                     | +                        | CDCA × 3y4mo            | 5.1/1.9               |
| P17      | F      | 2mo          | 4.8mo                  | Neonatal cholestasis, hepato-megaly | 81.9/37.7      | 75/197                      | +                        | CDCA × 2y5mo            | 9.7/3.2               |
| P18      | F      | 1mo          | 4.6mo                  | Neonatal cholestasis | 82.5/51.1         | 83/97                       | +                        | CDCA × 2y9mo            | 7.6/2.8               |
| P19      | M      | 3d           | 1.7mo                  | Neonatal cholestasis | 214.7/151        | 212/282                     | +                        | CDCA × 1y9mo            | 3.1/1.7               |
| P20      | M      | 2d           | 5.5mo                  | Neonatal cholestasis, hepato-splenomegaly | 138.1/68.8  | 327/485                     | +                        | CDCA × 2y5mo            | 7/2                   |
| P21      | M      | 10d          | 11.5mo                 | Neonatal cholestasis, liver failure, hepato-splenomegaly, pneumonia | 309/213.6   | 72/154                      | +                        | CDCA × 10d, then liver transplanted | 14.2/48              |
| P22      | M      | 3‑4d         | 4.9y                   | Liver cirrhosis, hepatosplenomegaly with a history of transient neonatal cholestasis | 20.2/13.8     | 47/61                       | +                        | UDCA × 9mo              | 5/1.9                 |
| P23      | M      | 1mo          | 8.7mo                  | Neonatal cholestasis | 41.4/23.3        | 291/204                     | +                        | Hyperbilirubinemia resolved and transaminase slightly elevated | 19.7/10.1            |

Note: TB/DB = Total Bilirubin/Direct Bilirubin; ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase; CDCA = Chenodeoxycholic Acid; UDCA = Ursodeoxycholic Acid; § = urinary bile acids profiling includes conjugated and unconjugated bile acids; † = age at first referral refers to the age at which the patient was referred for the first time for any reason related to their liver disease.
| Patients | Gender | Age at onset | Age at first referral† | Presenting symptoms | Liver biochemistries | Urinary bile acids profiling§ | Treatment after diagnosis | Status/age at last follow-up | Liver biochemistries |
|----------|--------|--------------|------------------------|---------------------|---------------------|-------------------------------|---------------------------|-----------------------------|---------------------|
|          |        |              |                        |                     | TB/DB (µmol/L) ALT/AST (U/L) |                              |                           |                             | TB/DB (µmol/L) ALT/AST (U/L) |
| P24 M    | 11d    | 2.4mo        |                        | Neonatal cholestasis, hepatosplenomegaly | 141.2/70.1 134/131 | +                           | CDCA × 2y1mo Normal/2.3y | 7.9/3                       | 31.4/43.2 |
| P25 M    | 3d     | 3mo          |                        | Neonatal cholestasis, hepatosplenomegaly | 204.9/101.3 279/393 | +                           | CDCA × 3mo, then liver transplanted | 327.2/150.2                   | 116/289 |
| P26 M    | 1mo    | 2.2mo        |                        | Neonatal cholestasis | 88.9/495 107/137 | +                           | CDCA × 3y2mo Normal/3.4y | 5/08                        | 31/54   |
| P27 M    | 7d     | 2.2mo        |                        | Neonatal cholestasis | 125/85 40/132 | +                           | CDCA × 1y1mo Normal/1.3y | 15.2/5.2                    | 41.2/44.1 |
| P28 M    | 18d    | 8mo          |                        | Neonatal cholestasis | 165/59 46/294 | +                           | CDCA × 3mo, then liver transplanted | Died/11mo                   | 201.3/62                  | 182/662 |
| P29 M    | 3d     | 4.6mo        |                        | Neonatal cholestasis | 96/37 111/167 | +                           | CDCA × 2y2mo Normal/2.5y | 6.3/1.5                     | 16/28   |
| P30 M    | 7d     | 7.8mo        |                        | Neonatal cholestasis | 128.6/69.3 84/406 | +                           | CDCA × 3mo, then liver transplanted | Alive/2.7y                   | 126.9/68.6                | 377/518 |
| P31 F    | 4y     | 5.2y         |                        | Liver cirrhosis, splenomegaly | 15.3/36 40/NA | +                           | CDCA × 2y6mo Normal/7.7y | 22.8/8.3                    | 25/33   |
| P32 F    | 3d     | 3.3mo        |                        | Neonatal cholestasis | 170.4/93.9 290/153 | +                           | CDCA × 1y5mo Normal/1.8y | 8.4/5                       | 31/45   |
| P33 F    | 3d     | 5mo          |                        | Neonatal cholestasis | 74.6/42.8 100/200 | +                           | CDCA × 12mo Normal/1.4y | 5.5/1.1                     | 31/49   |
| P34 M    | 3d     | 1.8mo        |                        | Neonatal cholestasis | 141.2/92.1 1198/136.7 | +                           | CDCA × 3mo, then liver transplanted | Alive/1.3y                   | 333.5/273                  | 585.1/668.1 |
| P35 F    | 45y    | 4.7y         |                        | Liver cirrhosis, splenomegaly | 29.4/17.9 37.6/50.2 | +                           | CDCA × 11mo Hypersplenism improved/5.7y | 11.8/48                     | 16/24.9 |
| P36 M    | 1mo    | 4.4mo        |                        | Neonatal cholestasis | 436/327.1 9384/1526.8 | NA                           | CDCA × 1mo Died/6mo | 863.1/508.8                | 2845/321.7 |
| P37 M    | 1mo    | 1.8y         |                        | Neonatal cholestasis, liver failure | 45/35.2 2172/385 | NA                           | CDCA × 12mo Normal/2.4y | 9.1/4.1                     | 23.78/38.21 |
| P38 F    | 3d     | 4mo          |                        | Neonatal cholestasis | 88/66 1893/170.5 | NA                           | CDCA × 4mo Hyperbilirubinemia resolved and transaminase slightly elevated | 8.2/3.1                     | 67.06/62.44 |
Table 2 (continued)

| Patients | Gender | Age at onset | Age at first referral† | Presenting symptoms | Liver biochemistries | Urinary bile acids profiling§ | Treatment after diagnosis | Status/age at last follow-up | Liver biochemistries |
|----------|--------|--------------|------------------------|---------------------|---------------------|-----------------------------|-------------------------|------------------------|---------------------|
| P39      | F      | 2d           | 4.7mo                  | Neonatal cholestasis| 186.8/155.6         | 265/428.8                    | CDCA × 2.5mo            | Died/7mo               | 494.5/291.5          |
| Reference range | 3.4–17.1/0–6 | 9–50/15–40 |                        |                     | TB/DB (µmol/L)          | ALT/AST (U/L)               |                         |                        | TB/DB (µmol/L)          |

† age at first visit to our center; ‡If renal imagine indicate renal lesions, the result is positive; §If FAB-MS profile show an absence or a lack of the normal primary bile acid conjugates and marked elevations of atypical 3β-hydroxy-Δ5-bile acids, the result is positive and supports a diagnosis of 3β-HSD deficiency; TB, total bilirubin; DB, direct bilirubin; ALT, alanine transaminase; AST, aspartate transaminase;
be consistent with the previously reported incidence of all bile acid synthesis disorders accounting for about 2% of unexplained cholestasis cases, with the HSD3B7 deficiency being the most common of the disorders [9]. A consistent finding was that liver biochemistries, revealed elevated serum conjugated hyperbilirubinemia, and transaminases, but normal GGT, consistent with previously reported cases [16]. Care is required when interpreting a routine serum TBA level obtained when the patient is receiving UDCA therapy because an elevated or slightly elevated serum TBA may not necessarily exclude a diagnosis of HSD3B7 deficiency in neonates. Although most patients with HSD3B7 deficiency showed good compliance to CDCA therapy, there were seven patients that did not respond to therapy, presumed to be due to the intrinsic hepatotoxicity of CDCA. For the patient P39, liver function indices worsened after contracting pneumonia and the patient later died at 7 months of age. Thus, infection might be another reason for the poor prognosis of some patients.

Our findings show that renal lesions in the face of normal renal chemistries have a prevalence of 28.6% in HSD3B7 deficiency and the most common renal involvement was renal cysts (5/10). Renal cysts have been described in a few patients but a causal association has not been previously confirmed [30]. In patients with HSD3B7 deficiency, primary bile acids are not synthesized and instead there is an accumulation of hepatotoxic 3β-hydroxy-Δ5-bile acids that leads to cholestasis that often progresses to subsequent liver failure. Urinary excretion consequently becomes the major route of elimination of these atypical bile acids. The cause of renal lesions is unclear but animal studies suggest that high concentrations of bile acids can be toxic to renal tubules and may generate or initiate renal lesions [31]. Whether chronic exposure of the kidney to high concentrations of the atypical 3β-hydroxy-Δ5-bile acids associated with HSD3B7 deficiency can explain the renal disease is conjecture. Significant was our finding that renal lesions appeared mainly in the older children and that these

### Table 3: Manifestations of renal lesion and its evolution in patients with HSD3B7 deficiency

| Patient | Age at first imaging | Renal lesion before chenodeoxycholic acid (CDCA) administration | Renal Tests | Management | Status of renal lesions/age at last follow-up |
|---------|----------------------|---------------------------------------------------------------|-------------|------------|---------------------------------------------|
| P2      | 16.5mo               | Medullary sponge kidney with calcification                    | Multiple small cystic high signal in bilateral renal medulla | 33.8 Normal CDCA × 2.5 y, CDCA 10 mg/kg/day × 7y6mo | Normalized/11.2y |
| P5      | 3.7mo                | Multiple abnormal echoes in the calyx                          | NA          | 13 Normal CDCA 10 mg/kg/day × 7y            | Normalized/7.3y |
| P6      | 17.2mo               | Renal stones                                                  | Renal cysts | 29 Normal CDCA 10 mg/kg/day × 6y2mo        | NA               |
| P8      | 4.3y                 | Renal stones                                                  | NA          | 18 Normal CDCA 8 mg/kg/day × 2y8mo         | Normalized/10.4y |
| P15     | 6.6y                 | Renal cysts with calcification                                | NA          | 36 Normal CDCA 8 mg/kg/day × 3mo, 10 mg/kg/day × 2y8mo | NA               |
| P16     | 5.8mo                | Renal cysts                                                   | Progressively abnormal signals | 14 Normal CDCA 10 mg/kg/day × 3y4mo | Normalized/3.8y |
| P21     | 11.5mo               | Calcium deposition                                            | NA          | 8 Normal CDCA 8 mg/kg/day × 7d, 6 mg/kg/day × 4d, then liver transplanted × 18mo | Normalized/4.8y |
| P22     | 4.9y                 | Renal cysts                                                   | NA          | 25 Normal CDCA 8 mg/kg/day × 21d, 5 mg/kg/day × 4mo, 6 mg/kg/day × 31mo | Normalized/7.9y |
| P35     | 4.7y                 | Bilateral renal enlargement                                   | NA          | 29 Normal CDCA 3 mg/kg/day × 11mo          | Improved/5.7y   |
| P37     | 1.8y                 | Renal cysts                                                   | Renal cysts | 17 Normal CDCA 4.5 kg/kg/day × 4mo         | NA               |

NA not available
resolved upon suppression of bile acid synthesis, or after liver transplantation, both of which eliminate the production of 3β-hydroxy-Δ5-bile acids. No common variant was associated with renal lesions of HSD3B7 deficiency. These findings suggest that it may be the accumulation over time of 3β-hydroxy-Δ5-bile acids that appear to underlie the renal pathology.

In conclusion, this study presents a comprehensive description of the the HSD3B7 genetic spectrum and clinical characteristics of HSD3B7 deficiency in a large cohort of infants and children from China. It concludes that the genotype is not a good predictor of the phenotype, or the clinical outcome. Furthermore, our data highlight the significant prevalence of renal lesions in HSD3B7 deficiency and that these lesions can be resolved by primary bile acid therapy. Thus, targeted renal evaluation, including serum biochemistries, renal ultrasound, and urinalysis, should be included in the standard work-up of children with HSD3B7 deficiency.
Fig. 3 The negative ion FAB-MS spectrum of the urine for A a patient with HSD3B7 deficiency revealing marked elevations in sulfate and glyco-sulfate conjugates of dihydroxy- and trihydroxy-cholenoic acids (i.e. unsaturated C24 bile acids) evidenced by the pairs of ions at m/z 469, 485 (sulfate conjugates) and m/z 526, 542 (glyco-sulfate conjugates) and B the mass spectrum of the urine from patient 21 which shows low intensity ions for these atypical 3β-hydroxy-Δ5 bile acid that are the biomarkers for HSD3B7 deficiency due to the more advanced liver disease and loss of synthetic function

Table 4 Correlation of genotype and phenotype in patients with HSD3B7 deficiency

|                         | Biallelic null variants (n = 12) | Single null variant (n = 15) | Biallelic non-null variants (n = 12) | Total (39) | Analysis (Spearman correlation) |
|-------------------------|----------------------------------|-------------------------------|--------------------------------------|-------------|---------------------------------|
| Group by onset age       |                                  |                               |                                      |             |                                 |
| Neonatal cholestasis     | 9 (75%)                          | 12 (80%)                      | 11 (92%)                             | 32 (82%)    | rs = 0.170, p = 0.300           |
| Childhood onset          | 3 (25%)                          | 3 (20%)                       | 1 (8%)                               | 7 (18%)     |                                 |
| Clinical outcome         |                                  |                               |                                      |             |                                 |
| Native liver survivors   | 8 (67%)                          | 12 (80%)                      | 7 (58%)                              | 27 (69%)    | rs = -0.071, p = 0.668          |
| Liver transplanted or death | 4 (33%)                          | 3 (20%)                       | 5 (42%)                              | 12 (30%)    |                                 |
Abbreviations
HSD3B7: 3β-hydroxy-delta5-C27-steroid oxidoreductase; CA: Cholic acid; CDCA: Chenodeoxycholic acid; VUS: Variants of uncertain significance; FAB-MS: Fast atom bombardment ionization mass spectrometry; qPCR: Quantitative polymerase chain reaction; ACMG: The American College of Medical Genetics and Genomics; UDCA: Ursodeoxycholic acid; GGT: Gamma-glutamyl transpeptidase; TCH: Total cholesterol; TBA: Total bile acids.

Supplementary Information
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Additional file 1. Renal images in additional patients.
Additional file 2. Table S1. Pathogenicity prediction of novel variants in HSD3B7. Table S2. Previously reported variants in HSD3B7. Table S3. Serum liver biochemistries at first referral and at last follow-up. Table S4. Correlation of genotype and phenotype in patients with HSD3B7 deficiency.

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Author’s contribution
JZ performed the genetic studies, statistical analysis and manuscript preparation. KDRS and JEH contributed to the analysis and interpretation of urinary bile acids, and manuscript preparation; YG and YHS were involved in renal imaging studies; J-SW contributed to obtaining funding, study concept, design, supervision and manuscript preparation. All authors involved in acquisition, analysis and interpretation of data, and manuscript revision and final approval of its publication.

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Availability of data and materials
The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. All data generated and analyzed during this study are included in this article and its supplementary tables.

Declarations
Ethics approval and consent to participate
This study was approved by the Ethics Committees on Human Research of the Children’s Hospital of Fudan University.

Consent for publication
Not applicable.

Competing interests
KDRS and JEH have minor equity in Asklepion Pharmaceuticals and are consultants to Retrophin. JSW consulted for Ethyptharm. The other authors disclose no conflicts.

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