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Beyond carrier frequency: a preliminary multicenter study of simultaneous couple-based comprehensive carrier screening for common and rare genetic disorders

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Abstract

Background Currently, the American College of Medical Genetics and Genomics (ACMG) carrier screening (CS) guidelines do not recommend routinely screening for conditions with carrier frequencies ≤ 1:200. Advances in DNA sequencing and declining costs, alongside growing awareness, make it increasingly feasible to expand CS to cover a broader spectrum of conditions. We aimed to design a CS panel regardless of carrier frequency to evaluate its utility in less common disease.

Methods We developed a large CS panel through gene curation, phenotypic severity evaluation, independent of carrier frequency. A cohort of 2010 couples across 24 Chinese cities underwent couple-based simultaneous screening. Gene carrier rate (GCR) was calculated, with subsequent assessment of at-risk couple rate (ACR) across varying GCR thresholds.

Results The results obtained from testing 1736 genes were presented. Among 2010 couples undergoing CS, 106 were identified as having an increased risk of offspring with at least one genetic condition. The initial ACR is 5.3% (106/2010). After excluding couples not carrying high-risk *CFTR* variants based on the ACMG-recommended 100-variant panel for *CFTR*, the adjusted ACR was 5.2% (105/2010). Furthermore, after excluding *GJB2*: c.109 G > A and *HFE*, the adjusted ACR decreased to 2.2% (45/2,010). Fifty genes with a GCR ≥ 1/200 were identified through individual carrier analysis that were absent from the ACMG Tier 3 list. Setting a GCR ≥ 1/200 threshold would fail to

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detect 14 high-risk couples associated with 16 rare conditions: two couples carried pathogenic variants linked to two rare conditions each. Notably, 53.3% of identified high-risk couples would remain undetected under ACMG Tier 3-recommended conditions, with the majority of missed conditions representing rare yet severe disorders. The simultaneous couple-screening model maintained manageable ACR while resolving all reproductive concerns in a single phase, thereby reducing genetic counseling demands compared to sequential approaches. This methodology also minimized false-positive interpretations through integrated variant analysis.

Conclusions This study obtained preliminary Chinese population-specific GCR for both common and rare recessive conditions, providing a foundation for future CS panel optimization. Couples at risk of having a child with rare conditions are often undetectable using conventional CS paradigms that set GCR levels at ≥ 1/200 thresholds, emphasizing the need to expand beyond ACMG Tier 3 standards to address rare severe genetic disorders. For large CS panels, simultaneous couple-based screening is an effective strategy.

Keywords Comprehensive carrier screening, Rare disease, Chinese population

Background

Carrier screening (CS) aims to assist couples in making informed reproductive decisions by identifying individuals at risk of having a child with a serious genetic condition. Diagnostic laboratories offer diverse genetic testing panels, which vary significantly in their composition. Decisions regarding panel design are guided by several clinical and technological criteria. These include the severity of the target diseases, population-specific carrier frequencies, and the clinical sensitivity of the assays in detecting pathogenic variants [1–3]. Carrier frequency is regarded as an important factor in the selection criteria for CS panels.

The American College of Medical Genetics and Genomics (ACMG) guidelines recommended screening for conditions with a carrier frequency ≥ 1:200 [4]. However, this threshold lacks strong empirical foundation and relies on databases (ClinVar/gnomAD) that underrepresent non-European ancestries [5], risking underestimation of carrier rates in underrepresented ethnic groups and for understudied diseases. There are inconsistencies in carrier test performance for underrepresented genetic ancestry groups in ACMG gene lists [6]. Consequently, they may not accurately reflect the carrier rates in the general population or among individuals seeking CS. Furthermore, there are many serious conditions at a carrier frequency below 1/200 [2], yet their true population-level carrier rates often remain unknown. This underscores the critical need for population-specific carrier rate data across all recessive conditions to inform evidence-based panel design.

Advances in next-generation sequencing (NGS) and the rapid decrease in costs of DNA sequencing over the last 15 years enable cost-effective, simultaneous detection of numerous genetic variants, making comprehensive CS increasingly accessible without significant additional reagent costs [7–12]. Consequently, the CS paradigm has transitioned from targeting populations with higher carrier frequencies to encompassing screening irrespective of ancestry, or combined strategies [5]. Notably, a recent study revealed that limiting screening to ACMG Tier 3 panels would fail to identify approximately 42% of high-risk couples [13]. Therefore, expanding CS beyond high carrier-rate genes is increasingly advocated, carrier frequency may not be the primary consideration for gene/condition selection. Instead, integrating screening for rarer disease into routine practice is essential to optimize reproductive outcomes and maximize public health benefits.

To address this need, we developed a large CS panel covering 1,736 genes irrespective of carrier frequency. We enrolled over 2,010 reproductive-age couples (including those planning pregnancy or in early pregnancy [< 12 weeks gestation]) and offered comprehensive CS in Chinese population, providing insights into their risk of conceiving a child with any of 1736 genes associated 1083 autosomal recessive and X-linked conditions.

Methods

Study design

This study is part of the National Key Research and Development Program of "Reproductive Health and Health Protection for Women and Children" (2022YFC2703400). It is a multi-center design involving 30 hospitals from 24 cities across China (Additional file 1:Fig.S1). It has been approved by the Ethics Committee of Xinhua Hospital affiliated with Shanghai Jiao Tong University School of Medicine (XHEC-C-2022-123-1). This study was conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

Identification of genes for assessment

Genes included in the assessment were identified through searching the OMIM database (Online Mendelian Inheritance in Man, https://omim.org/, accessed on May 26, 2023) [14]. Totally, 3150 OMIM genes with AR or X-linked recessive inheritance were found, among

which, genes with recessive inheritance (n = 2952) or X-linked recessive (n = 198).

Gene-disease pair selection criteria

a. Gene-disease pair are strong or definitive according to Clinical Genome Resource (ClinGen) Gene Curation Standard Operation Procedure, Version 5 (ClinGen SOP);
b. The condition is one for which an" average "couple would take steps to avoid the birth of a child with that condition [12] or the disease severity was classified as moderate or severe according to a systematic classification algorithm reported by Lazarin et al. [15].

Gene curation and phenotype classification protocol

Gene curation and phenotype classification were conducted by the 25 member units of the Genetic Counselling Branch of the Chinese Medical Association according to the established standard procedures.

Gene curation

Gene curators followed the ClinGen Gene Curation SOP, Version 5 (The ClinGen Gene Curation Working Group, 2017) [16].

Each gene-disease pair was independently curated by two specialists. For concordant results, laboratory directors reviewed all curation work to ensure correct framework application and scoring. When results conflicted, a third curator independently re-curated the gene. Laboratory directors then evaluated the combined findings, resolving discrepancies, implementing necessary revisions, and enforcing strict adherence to the ClinGen SOP. Throughout this process, evidence was systematically re-examined and integrated where applicable. The final classification and scoring were determined by consensus among three laboratory directors.

Phenotype evaluation

The classification of disease severity was performed according to the predefined selection criteria. For each gene-disease pair, two independent curators conducted parallel evaluations. When their assessments were concordant, the findings underwent subsequent review by a senior physician to verify proper application of the classification framework. In cases where there was inconsistency between the two curators, a senior physician from a third center would re-evaluate the results, propose necessary adjustments, and facilitate an agreement on the appropriate phenotypic severity classification by all three physicians.

Genes and conditions selection protocol

In the first round, the Australian Reproductive Genetic Carrier Screening Project, ("Mackenzie's Mission" [MM]), included ~ 1,300 genes associated with childhood onset, life-limiting or disabling conditions and for which early knowledge of carrier status may shape reproductive decision-making in their CS panel [12]. These conditions have been curated and assigned clinical validity classifications. These genes and conditions were directly included, after reviewed by curators and laboratory directors, 28 genes and conditions (Additional file 1:Table S1) were excluded for the correspondence between genes and diseases has been updated in OMIM. Genes and conditions in ACMG Tier 3 panel were also directly included. A total of 1286 genes were included in this protocol (Additional file 2: Table S2).

Then we included genes and conditions that had been previously curated and assigned strong or definitive classification: ClinGen gene curation expert panels had previously curated and assigned clinical validity classifications [17] or to more than three curator arrive at strong or definitive classification for a gene-disease pair in Gene Curation Coalition (GenCC) database [18]; or Genes and conditions had been curated according to ClinGen gene curation framework in a published study [19]; After reviewed by laboratory directors, these genes and conditions were included in the first round selection, and then these genes would follow phenotype evaluation. Finally, a total of 294 genes fulfilled selection criteria were included in this protocol (Additional file 2: Table S2).

To reduce the number of genes for further assessment, we excluded genes in the Human Gene Mutation Database (HGMD) [20] with fewer than 5 null variants (n = 782) as they were statistically insufficient to reach the 12-point threshold required for strong disease association under the ClinGen quantitative evidence framework [16]. The remaining genes and conditions firstly underwent phenotypic evaluation, and those that met the criteria proceeded to further gene-disease pair curation. Based on the gene curation process outlined above, the genes that met the standards were selected (n = 156) (Additional file 2: Table S2).

Additional file 2: Table S2 showed the details of selected genes. Finally, a total of 1736 genes and 1083 conditions were included.

Recruitment

To represent and cover the diversity of the population in different regions of China, we collaborated with 30 hospitals distributed across 24 different cities. All recruitment was conducted by reproductive centers, obstetricians, or genetic counselling centers of tertiary comprehensive hospitals, provincial/municipal maternal and child health hospitals, and selected district-level maternal and child

health hospitals in this pilot study. This approach ensured that participating couples had convenient access to certified genetic counselors, reproductive geneticists, prenatal diagnosis services (e.g., amniocentesis, chorionic villus sampling), and preimplantation genetic testing. Couples in which each partner was at least 20 years of age were eligible to participate if they were planning to conceive or if the female partner was pregnant at a gestation of less than 12 weeks.

Enrollment began with physicians providing prospective participants a research invitation, application form, and informed consent documents. During initial consultations, healthcare providers thoroughly explained the study objectives, screened disease spectrum, and test limitations. Couples who consented after reviewing the materials and having their questions addressed completed the application form and provided written informed consent for the CS. Following consent, each participant provided a 2–4 mL EDTA-anticoagulated peripheral blood sample. These samples were then securely transported from participating centers to a designated central laboratory for standardized genetic analysis.

Finally, a total of 2010 reproductive couples were enrolled. Information on geographic distribution, age, family history of genetic conditions, history of adverse pregnancy outcomes, and consanguinity was collected at the time of enrollment.

Next-generation sequencing (NGS) and data analysis

A comprehensive carrier screening panel was developed in this study, targeting the coding regions of 1,736 genes along with their flanking 5-bp intronic sequences. Detected variants include single-nucleotide variants (SNVs), small insertions/deletions (InDels, < 50 bp), and some selected large deletions/duplications, including deletions/duplications of three or more consecutive exons in the *DMD* gene, exon 7 deletions in the *SMN1* gene, and common large deletions in the *HBA* gene (--SEA, -α3.7, -α4.2, and --THAI).

Peripheral blood was collected from all individuals, and genomic DNA was extracted using the MagMax™ DNA Multi-Sample Ultra 2.0 kit (ThermoFisher, USA) according to the manufacturer's protocol. The extracted genomic DNA was fragmented, ligated with sequencing adapters, purification, probe hybridization capture, and library quantification to generate a final genomic library suitable for sequencing. Custom hybridization probes were designed and synthesized by Twist Bioscience (South San Francisco, CA, USA). Libraries were sequenced on a NovaSeq™ 6000DX-CN-BG platform (Berry Genomics, China) with 150 bp paired-end reads, achieving a quality threshold of Q30 ≥ 85%. The mean sequencing depth was approximately 200×, with over 97% of the target region covered by at least 20 reads. Fastq files were preprocessed using FASTP software for automated quality control, filtering, trimming, and other preprocessing [21]. Preprocessed sequencing data were aligned to the human reference genome (GRCh38/hg38) using BWA-MEM software (v0.7.15) [22]. Variant calling for SNVs and InDels was performed using Verita Trekker® (Berry Genomics, China), while copy number variations (CNVs) were detected using Sprinkle software (Berry Genomics, China). Identified SNVs/InDels variants were annotated using the Enliven® variant annotation system (Berry Genomics, China), which includes basic variant information (gene, variant location and type), pathogenic variant databases (OMIM, HGMD, and ClinVar) [14, 20, 23], population frequency databases (1000 Genomes, ExAC, and gnomAD) [24, 25], over 50 functional prediction tools (e.g., REVEL, SpliceAI) [26, 27], automatic ACMG-based classification, gene-related disease and clinical manifestations, inheritance mode, etc. Similarly, CNVs were annotated and classified using CNVisi® (Berry Genomics, China), including basic variant segment information (gene, chromosome location, etc.), pathogenic variant databases (GlinGen, OMIM, HGMD, ClinVar, and DECIPHER) [14, 20, 23, 28], population databases (gnomAD, Database of Genomic Variants) [25, 29], automatic ACMG-based classification, gene-related disease and clinical manifestations, etc. Variant pathogenicity was interpreted following the standards and guidelines of the ACMG and the Association for Molecular Pathology (AMP) [30], the ClinGen sequence variant interpretation working group guidelines and detailed rules (https://clinicalgenome.org/), and the Association for Clinical Genomic Sciences (ACGS) variant pathogenicity interpretation guidelines (2019 version, https://www.acgs.uk.com/).

It should be noted that *SMN1* and *SMN2* share a high degree of sequence homology. Therefore, copy number analysis of the *SMN1* gene primarily relies on the differential nucleotide positions that distinguish *SMN1* from *SMN2* [31]. The analysis process involves three steps: (1) calculating the *SMN1* to *SMN2* copy number ratio based on their differential sites; (2) estimating the total *SMN* copy number by comparing the coverage depth of *SMN1* and *SMN2* with that of a negative background dataset; and (3) determining the *SMN1* copy number by integrating the *SMN1*:*SMN2* ratio with the total *SMN* copy number.

Bioinformatic analysis for autosomal recessive conditions was performed with combined data from both reproductive partners; individual carrier status was not reported. Results were reported as "increased risk" for the following condition: both reproductive partners had a pathogenic or likely pathogenic variant/CNV in the same autosomal gene, the female partner had a pathogenic or

likely pathogenic variant/CNV in an X-linked gene, or both scenarios were present; Variants of uncertain significance were not reported. "Incidental risk" was reported for one of the reproductive partners had two pathogenic or likely pathogenic variants/CNV in the same autosomal gene, or the male partner had a pathogenic or likely pathogenic variant/CNV in an X-linked gene. The turnaround time was 30 days. A separate analysis of individual carrier status for all participants was also performed to calculate the individual gene carrier rate.

Clinical management of results

For couples planning pregnancy, the study protocol implemented a stratified communication framework guided by genetic risk stratification. Those identified with a reduced risk of transmitting inheritable disorders received phone notification. In contrast, couples who were found to have an increased chance were directly engaged by certified genetic counsellors affiliated with the research project. These specialists provided comprehensive counselling services at no cost, with intervention strategies tailored to pregnancy status: preconception couples received guided consultations about assisted reproductive technologies and family planning alternatives, while currently pregnant pairs obtained detailed information about available invasive diagnostic procedures.

Results

Study population

As summarized in Fig. 1, a total of 2010 reproductive couples enrolled in the study. 1904 couples (94.7%) were planning to conceive, and 106 couples (5.3%) had a female partner who was pregnant at a gestation of less than 12 weeks. Participants demonstrated a broad geographic distribution. However, given the relatively small sample size in this preliminary analysis, achieving uniform coverage across each province was difficult. Therefore, enrolled participants were grouped according to China's seven major geographic regions (Additional file 1:Fig.S1 and Table S3). As summarized in Table S3, 1.1% (23/2010) of participants reported a relevant family history of a genetic condition. Among females, 14.4% (290/2010) reported a history of adverse pregnancy outcomes. The majority of individuals (70.6%) were aged between 25 and 34 years. The 0.4% (9/2010) couples with consanguinity was reported.

Screening outcomes

Gene carrier rate (GCR)

A separate analysis of individual carrier status for all participants was also performed to calculate the individual GCR. For illustrative purposes, Table 1 shows the genes with a GCR > 0.5%. As can be seen, the highest GCR for a

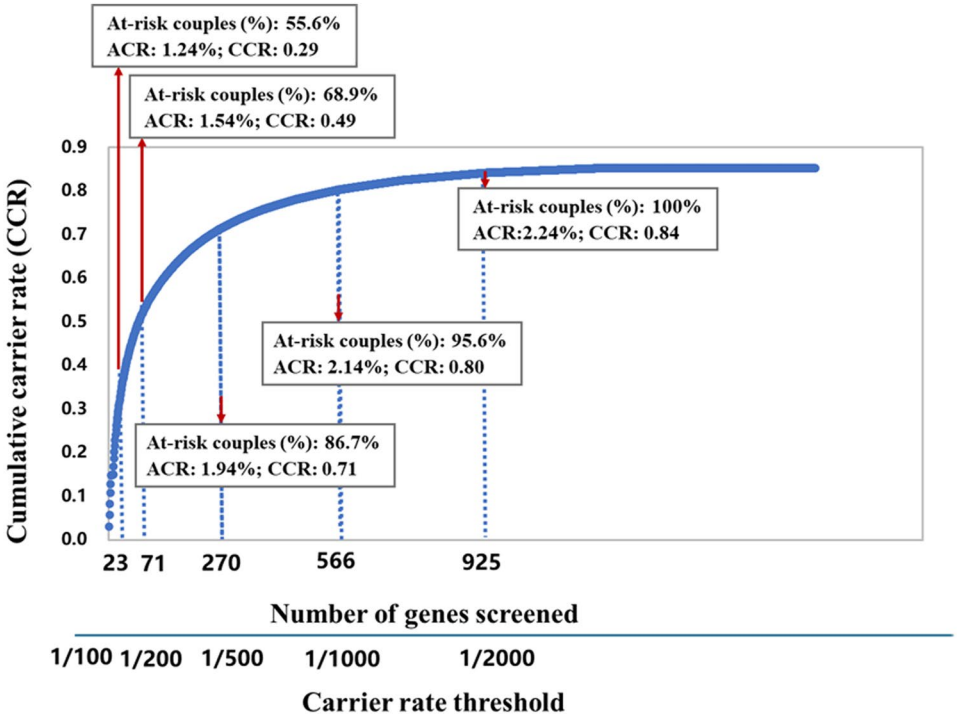

Fig. 1 Cumulative carrier rate (CCR), at-risk couple rate (ACR), and at-risk couple detection percentage at varying carrier frequency thresholds (when screening all genes, only genes with GCR ≥ 1/100, GCR ≥ 1/200, or GCR ≥ 1/500, or ≥ 1/1000 or ≥ 1/2000), following exclusion of *HFE*, *GJB2*:c.109G > A, and non 100 ACMG-recommended variants for *CFTR*

Table 1 Genes with carrier rate > 0.5% (1/200)

Gene	Carrier frequency (%)	Inheritance	Gene	Carrier frequency (%)	Inheritance
GJB2	14.28%/2.76%[#]	AR/DD	*CAPN3*	0.72%	AR
HFE	6.99%/0.32%[*]	AR	UGT1A1	0.72%	AR
GALC	3.03%	AR	*TYR*	0.70%	AR
ATP7B	2.76%	AR	*ADGRV1*	0.70%	AR/DD
HBA1/HBA2	2.69%	AR	*LDLR*	0.67%	AD/AR
SLC26A4	2.29%	AR	*ABCG5*	0.67%	AR
TTN	2.29%	AR	*DYSF*	0.67%	AR
SMN1	2.19%	AR	*GNE*	0.67%	AR
PAH	2.14%	AR	*C6*	0.65%	AR
USH2A	1.94%	AR	*CYP24A1*	0.65%	AR
CYP4V2	1.74%	AR	*MYO15A*	0.65%	AR
ABCA4	1.57%	AR	*PI4KA*	0.65%	AR
SLC22A5	1.47%	AR	*CUL7*	0.63%	AR
GAA	1.24%	AR	*EYS*	0.63%	AR
YY1AP1	1.26%	AR	*APOB*	0.60%	AR
IVD	1.19%	AR	*CEP135*	0.60%	AR
CFTR	1.14%/0.32%[&]	AR	*RP1*	0.57%	AD/AR
SGCA	1.07%	AR	*LAMA2*	0.57%	AR
HBB	1.00%	AR	*PMM2*	0.57%	AR
MMACHC	0.97%	AR	*PTPRQ*	0.57%	AR
F5	0.95%	AR	*TH*	0.57%	AR
OBSL1	0.95%	AR	*ACADSB*	0.55%	AR
ACY1	0.95%	AR	*ALPL*	0.55%	AR
OTOGL	0.92%	AR	*CEP152*	0.55%	AR
CYP27A1	0.90%	AR	*LPL*	0.55%	AR
FMO3	0.90%	AR	*NEB*	0.55%	AR
ROM1	0.90%	AD/AR/DD	*OCA2*	0.55%	AR
ABCC6	0.85%	AR	*POLG*	0.55%	AR
MYO7A	0.85%	AR	*SLC3A1*	0.53%	AD/AR
TRIOBP	0.80%	AR	*GCDH*	0.53%	AR
DNAH9	0.77%	AR	*OTOG*	0.53%	AR
PKLR	0.75%	AR	*PKD1L1*	0.53%	AR

[#]after excluding *GJB2*: c.109 G>A (p.V37I), [*]after excluding *HFE*: c.187C>G (H63D), [&]after excluding non-ACMG-recommended variants for *CFTR, AD: Autosomal dominant, AR: Autosomal recessive, DD: Digenic dominant*

single gene is 14.3% for *GJB2*, followed by *HFE* (7.0%) and *GALC* (3.0%).

Initially, *GJB2* showed the highest GCR (14.3%). For the *GJB2* gene, the most frequently detected variant was c.109 G > A (p.V37I), which resulted in 35 couples at risk for having a child who is homozygous for c.109 G > A (p.V37I) and 12 couples at risk for having a child who is compound heterozygous for c.109 G > A (p.V37I). Notably, individuals homozygous or compound heterozygous for c.109G > A typically manifest mild to moderate hearing loss with variable expressivity and low penetrance [32]. Following the exclusion of the c.109G > A variant, the GCR for *GJB2* decreased to 2.8%. Similarly, for the *HFE* gene, the most frequently detected variant was c.187 C > G (H63D), leading to 14 couples at risk for having a child who is homozygous for c.187 C > G. Individuals who are homozygous or heterozygous for c.187 C > G (H63D) are not at increased risk for hereditary hemochromatosis (HH), although they may display elevated serum iron and transferrin levels [33]. Following the exclusion of the *HFE*: c.187 C > G (H63D) variant, the GCR for *HFE* decreased from 7.0% to 0.3%. To provide a more accurate representation of the disease burden within the study population, we therefore used the recalculated GCRs of 2.8% for *GJB2* and 0.3% for *HFE*.

CFTR is related to cystic fibrosis, and the GCR rate was 1.1% (46/4020) when considering all P/LP variants. To better illustrate the disease burden in the study population, we reported only the 100 variants for *CFTR* recommended by ACMG [34]. After the adjustment, only seven P variants were reported among 4020 individuals and made the GCR of *CFTR* to be 0.3% (13/4020).

The carrier rates of each gene were shown in Additional file 2: Table S5 (excluding *HFE*, GJB2:*c.109G > A*, and non-ACMG-recommended 100-variant for *CFTR*).

At-risk couples

Among 2010 couples undergoing reproductive genetic CS, 106 (5.3%) were identified as having an increased risk of offspring with at least one genetic condition. Three couples carried variants associated with two conditions (*GJB2/DNAH5*, *GJB2/HBA1/2*, *CSPP1/HSD17B4*); One couple carried variants associated with three conditions (*DST/KLHL40/HBA1/2*). Of the at-risk couples, 101 (95.3%) had elevated risk for autosomal recessive conditions, while 5 couples (4.7%) had risk for X-linked conditions. Increased risk involved variants in 36 genes (32 autosomal, 4 X-linked) and one gross deletion in Xp21.1p11.3. Variants found in 106 couples were listed in Additional file 1: Table S4.

To better characterize disease burden in the study population, we excluded couples not carrying high-risk *CFTR* variants based on the ACMG-recommended 100-variant panel for *CFTR* [34]. This resulted in an adjusted ACR of 5.2% (105/2,010). Furthermore, as previously noted, classifying couples where both partners carry homozygous or compound heterozygous genotypes *GJB2*: c.109G > A, p.V37I or *HFE*: c.187 C > G, p.H63D variants as "high-risk" is clinically inappropriate. When excluding couples with either homozygous or compound heterozygous genotypes for these two *GJB2* and *HFE* variants, the ACR declined further to 2.2% (45/2,010). Finally, excluding all couples carrying variants in these two genes yielded a detection rate of 2.1% (43/2010). Within the cohort excluding these *GJB2* and *HFE* variants: One couple carried pathogenic variants associated with two conditions (*CSPP1* and *HSD17B4*); One couple carried variants associated with three conditions (*DST*, *KLHL40*, and *HBA1/2*). Notably, among the identified at-risk couples, 40 (88.9%) had an elevated risk for autosomal recessive conditions involving pathogenic variants in 30 distinct genes.

Cumulative carrier risk (CCR) and ACR at different GCR thresholds

To estimate the yields of the panel, we calculated the cumulative carrier risk (CCR). We ranked each gene (excluding *HFE*, *GJB2*: c.109G > A and non-ACMG-recommended 100-variant for *CFTR*) in descending order by its at-risk ratio and plotted the CCR as increasing numbers of genes are screened (Additional file 2: Table S5). As can be appreciated in Fig. 1, there is initially a rapid increase in the CCR, reflecting a small number of genes with high GCRs. This is then followed by a long tail of genes that contribute asymptotically to the CCRs. ACMG Tier-3/ACOG recommended that genes with GCR ≥ 1/200 or 1/100 be prioritized for CS. We investigated how screening only genes with GCR ≥ 1/200 or 1/100 in the current population affected the CCR and ACR. We discovered that raising the GCR ≥ 1/200 or 1/100 threshold significantly reduced the CCR by 35% or 55% (Fig. 1). As seen in Table 2, the involved gene/conditions listed according to different GCR thresholds. With the GCR threshold set at ≥ 1/200, CS would fail to detect 14 at-risk couples harboring variants associated with 16 genetic conditions (Table 2). Within this cohort: one couple carried pathogenic variants linked to two rare conditions (*CSPP1* and *HSD17B4*); Another couple carried variants associated with two additional rare conditions (*DST* and *KLHL40*).

Incidental findings

In this study, we identified incidental genetic findings in twelve individuals (Table 3). Specifically, seven individuals harbored homozygous *GJB2* c.109G > A variants. Follow-up revealed no hearing abnormalities in four of these individuals, while three exhibited mild hearing impairment without impact on daily life or work activities. Two individuals carried homozygous *STRC* variants lost to follow-up. One individual with a homozygous *ALPL* variant was found to have dental hypoplasia presented since childhood. One male carried a hemizygous *RS1* variant and reported progressive vision decline starting in high school; notably, his maternal uncle has a similar presentation. Finally, one individual with a homozygous *TNXB* variant showed no related clinical manifestations during follow-up.

Reproductive outcomes follow-up

In a follow-up study of the initial 106 high-risk couples without excluding *GJB2* and *HFE*, 16 were lost to follow-up. Among the remaining 90 couples, 22 pregnancies were achieved (either ongoing or resulting in live birth). Of these pregnancies, four couples conceived using in vitro fertilization with preimplantation genetic testing for monogenic disorders (IVF-PGT-M), while eighteen conceived naturally. Among the natural conceptions: five couples opted for prenatal genetic testing of the fetus; four received normal results, while one had an affected pregnancy and chose termination. The remaining thirteen couples declined prenatal diagnosis (Fig. 2). Within this group: one couple carried compound heterozygous *GJB2*: c.109 G > A variants, eight couples carried homozygous *GJB2*: c.109 G > A variants; Two couples carried homozygous *HFE* c.187 C > G variants. At follow-up, these thirteen couples had delivered seven unaffected babies, with six pregnancies still ongoing.

Among the 68 couples with non-pregnant female partners at follow-up, 24 opted for reproductive risk mitigation strategies (specifically: prenatal testing with the

Table 2 Involved genes, associated hereditary disorders in high-risk couples

Gene	At-Risk couples	Inheritance/Disease	ACMG Tier3 list?	Gene	At-Risk couples	Inheritance/Disease	ACMG Tier3 list?
GCR≥1/100				1/500≤GCR＜1/200			
*HBA1/2**	5	AR, Thalassemia, alpha	Y	*PDZD7*	1	AR, Deafness, autosomal recessive 57	N
ATP7B	4	AR, Wilson disease	Y	*CSPP1&*	1	AR, Joubert syndrome 21	N
SLC26A4	3	AR, Deafness, autosomal recessive 4, with enlarged vestibular aqueduct	Y	*RPGRIP1*	1	AR, Cone-rod dystrophy 13	N
SMN1	3	AR, Spinal muscular atrophy	Y	*ITGA2B*	1	AR, Glanzmann thrombasthenia 1	N
GJB2	2	AR/DD, Deafness, autosomal recessive 1A	Y	*DNAH5*	1	AR, Ciliary dyskinesia, primary, 3, with or without situs inversus	N
ABCA4	2	AR,Cone-rod dystrophy 3	N	*ERCC8*	1	AR, Cockayne syndrome, type A	N
GALC	1	AR, Krabbe disease	N	*DST**	1	AR, Neuropathy, hereditary sensory and autonomic, type VI	N
AR	1	XLR, Androgen insensitivity	N	TGM1	1	AR, Ichthyosis, congenital, autosomal recessive 1	N
AP1S2	1	XLR, Pettigrew syndrome	N	*HEXA*	1	AR, Tay-Sachs disease	Y
FMR1	1	XL, Fragile X syndrome	Y	*PROC*	1	AR, Thrombophilia 3 due to protein C deficiency	N
FGD1	1	XLR, Aarskog-Scott syndrome	N	1/1000≤GCR＜1/500			
Xp21.1p11.3	1	XL	N	*CERKL*	1	AR, Retinitis pigmentosa 26	N
USH2A	1	AR, Usher syndrome, type 2A	Y	*KLHL40**	1	AR, Nemaline myopathy 8	N
1/200≤GCR＜1/100				*PNPLA2*	1	AR, Neutral lipid storage disease with myopathy	N
CAPN3	1	AR, Muscular dystrophy, limb-girdle, autosomal recessive 1	N	*HSD17B4&*	1	AR, D-bifunctional protein deficiency	N
TYR	1	AR, Albinism, oculocutaneous, type III	Y	1/2000≤GCR＜1/1000			
C6	1	AR, C6 deficiency	N	*LCA5*	1	AR, Leber congenital amaurosis 5	N
MYO15A	1	AR, Deafness, autosomal recessive 3	N	*COL27A1*	1	AR, Steel syndrome	N
GCDH	1	AR, Glutaricaciduria, type I	N				
PKD1L1	1	AR, Heterotaxy, visceral, 8, autosomal	N				

Couples carried more than one condition: *DST/KLHL40/HBA1/2, &CSPP1/HSD17B4, Y: included in ACMG Tier3 list, N: not included in ACMG Tier3 list, AD: Autosomal dominant, AR: Autosomal recessive, DD: Digenic dominant, XL: X-linked, XLR:X-linked recessive

intention of termination if affected, or IVF-PGT-M); 35 were undecided regarding future reproductive plans (Fig. 2); and of the residual nine couples choosing neither intervention (instead using genetic information to plan for the potential birth of an affected child), seven out of nine had a variant combination predicted to cause mild or asymptomatic phenotype (four couples with homozygous *GJB2*: c.109G > A genotype, one with c.109G > A compound heterozygous genotype, and two with homozygous *HFE*: c.187 C > G genotype).

Discussion

This study, without considering carrier frequencies, selected a CS panel for over 1,700 genes based on gene curation, phenotypic evaluation, and factors influencing reproductive decisions. We then conducted CS for over 1,700 genes in 4,020 individuals from the Chinese population. Consequently, we determined an initial ACR of 5.3%. However, after excluding *GJB2* and *HFE*, the adjusted ACR decreased to 2.1%. This result is comparable to an Australian CS study that reported an ACR of 1.9% after testing approximately 1300 genes and also excluding these two genes [13]. And we obtained data on the carrier status of both common and rare diseases, shedding light on carrier frequencies for previously underexplored, less common conditions. This data lays a foundation for larger-scale population screenings in the future. Additionally, the findings provide essential reproductive counselling for high-risk couples, helping them make informed family planning decisions.

Our research investigating the carrier rates for common and uncommon recessive genetic disorders (characterized by GCR and CCR) within the general Chinese population provides valuable guidance for the development of effective CS panels. Carrier frequency stands as a key consideration in the selection of genes for such panels. Critically, GCR exhibits significant population specificity. In this study, we identified 50 genes meeting the criterion of GCR ≥ 1/200 within the Chinese cohort that are excluded from the ACMG Tier 3 list. Notably, several genes—including *GALC*, *ABCA4*, *SLC22A5*, and *SLC25A13*—demonstrate carrier frequencies exceeding

Table 3 Incidental genetic findings and their follow-up results

Number	Sex	Age (y)	Gene	Variant	ACMG classification	Condition	Phenotype at follow-up
1	F	29	*GJB2*	NM_004004.6:c.109G > A:p.V37I hom	P	AR/DD: Deafness, autosomal recessive 1 A	Mild hearing impairment
2	M	32	*ALPL*	NM_000478.6:c.407G > A:p.R136H hom	P	AR: Hypophosphatasia, infantile	Dental developmental abnormalities since early childhood
3	M	28	*STRC*	Exon 16–18 deletion, hom	LP	AR: Deafness, autosomal recessive 16	Loss follow-up
4	F	27	*GJB2*	NM_004004.6:c.109G > A:p.V37I hom	P	AR/DD: Deafness, autosomal recessive 1 A	Hearing normal
5	F	24	*GJB2*	NM_004004.6:c.109G > A:p.V37I hom	P	AR/DD: Deafness, autosomal recessive 1 A	Hearing normal
6	F	31	*GJB2*	NM_004004.6:c.109G > A:p.V37I hom	P	AR/DD: Deafness, autosomal recessive 1 A	Mild hearing impairment
7	F	29	*GJB2*	NM_004004.6:c.109G > A:p.V37I hom	P	AR/DD: Deafness, autosomal recessive 1 A	Mild hearing impairment
8	F	24	*GJB2*	NM_004004.6:c.109G > A:p.V37I hom	P	AR/DD: Deafness, autosomal recessive 1 A	Hearing normal
9	M	29	*RS1*	NM_000330.4:c.52G > A:p.A18T hom	P	XLR: Retinoschisis	Decreased visual acuity
10	M	/	*STRC*	Exon 16–18 deletion, hom	LP	AR: Deafness, autosomal recessive 16	Loss follow-up
11	M	27	*TNXB*	Exon 39–41 deletion, hom	LP	AR: Ehlers-Danlos syndrome, classic-like, 1	No relevant phenotype
12	F	36	*GJB2*	NM_004004.6:c.109G > A:p.V37I hom	P	AR/DD: Deafness, autosomal recessive 1 A	Hearing normal

AR: Autosomal recessive, DD: Digenic dominant, P: pathogenic, LP: likely pathogenic

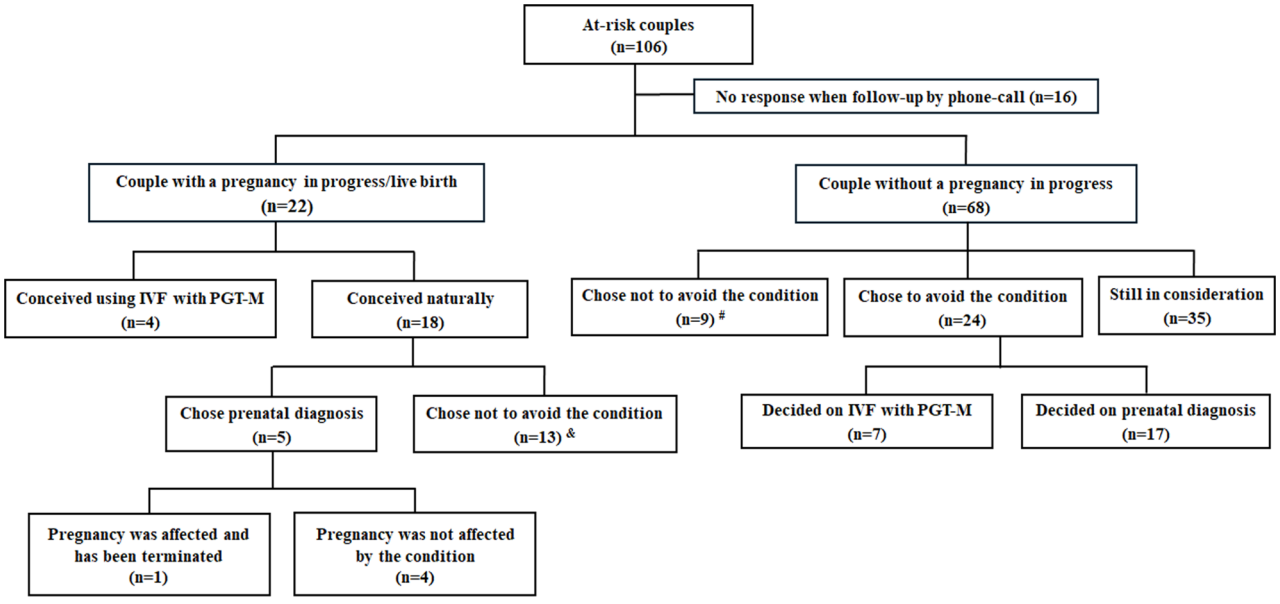

Fig. 2 Reproductive choices made by 106 at-risk couples. #:*GJB2*:c.109G >A/c.109G>A (n=4); c.109G>A/ c.235del (n=1); *HFE*: c.187C>G/ c.187C>G (n=2); other genes (n=2), &:*GJB2*:c.109G >A/c.109G>A (n=7); c.109G>A c.235del (n=2); *HFE*: c.187C>G/ c.187C>G (n=2); other genes (n=2)

1/100 specifically in the Chinese population [35–37]. Therefore, developing carrier screening panels optimally suited for this population necessitates utilizing genetic carrier data derived directly from the target local population to inform the selection of appropriate genes.

Our findings and the results of another study [2] have challenged the criterion of 1/200 cut-off, suggesting it warrants reconsideration. Although implementing more stringent GCR thresholds (1/200) requires minimal expansion of current gene panels. However, this approach carries significant clinical limitations:

restricting screening to ACMG Tier 3 panels would fail to detect approximately 53% of high-risk couples- specifically those with increased risk of conceiving offspring in our study. This finding aligns with another nationwide CS study testing 1281 genes which showed ACMG Tier 3 panels miss 42% of actionable carrier pairs in their cohort [13]. Furthermore, if the carrier rate decreases from 1/200 to 1/500 or 1/1000, this would facilitate the identification of an additional 8 or 12 high-risk couples through expanded CS in this study. Notably, more than half of the genetic disorders identified in these couples are classified as severe or profound conditions. For example, *HSD17B4*-associated D-bifunctional protein deficiency is an autosomal recessive inborn error of peroxisomal fatty acid oxidation [38], affected individuals exhibit profound clinical severity characterized by infantile-onset hypotonia, seizures, and distinctive facial features. This condition demonstrates rapid progression with a high mortality rate, as most patients died within the first 2 years of life without achieving any developmental milestones. The primary cause of death is infection-induced cardiopulmonary failure, with a mean age of death at 2 years and 11 months. Mutations in the *KLHL40* gene are a common cause of severe or even lethal nemaline myopathy conditions [39]. This disorder similarly presents with severe phenotypic manifestations and catastrophic clinical outcomes, 60% of the patients died within the first 4 years of life. These results underscore a critical implication on current frequency-based screening paradigms exclude couples at risk for rare yet devastating genetic conditions, which lays a detection gap.

Rare diseases have emerged as a critical global public health priority due to their multifaceted impact on patients and families. Affected individuals face a constellation of challenges including prolonged diagnostic odysseys, lifelong physical disabilities, inadequate compensatory support systems, and limited therapeutic options with exorbitant costs [40, 41]. These cumulative burdens exert profound detrimental effects across health, psychosocial, and socioeconomic domains for rare disease families [42, 43]. The economic implications are particularly striking. In 2019, the national cost of 379 rare diseases in the United States totaled US$966 billion includes $418 billion in direct medical costs and $548 billion in indirect/non-medical costs, with a per-person excess cost of $26,887 compared to non-affected individuals [41]. Notably, these figures represent only 5% of the approximately 7,000 recognized rare diseases worldwide, suggesting substantial underestimation of the true global burden. In this context, expanded CS programs present a cost-effective prevention strategy.

The evolution of CS strategies for rare diseases necessitates lower GCR thresholds. Our results revealed unrestricted multi-gene panels achieve exceptionally high CCRs, with about 84% of individuals being carriers for at least one condition. If implementing a sequential screening strategy (testing partners only after initial positive findings) would substantially increase retesting rates, thereby elevating data analysis burdens, reporting workloads, and genetic counselling burden, as well as unnecessary concern for screened couples. Median total time spent per participant in results disclosure following CS with genome sequencing is 64 min, and preparation work was the most time-consuming activity [44]. In contrast, couple-based simultaneous screening model maintains manageable ACR levels; like in this study, the adjusted ACR (after excluding *GJB2*:c.109G > A, p.V37I or *HFE*: c.187 C > G, p.H63D) is 2.2%, while resolving all potential reproductive risks in a single testing phase, ultimately reducing genetic counselling demands and service pressures compared to sequential models and minimizes false-alarm scenarios through concurrent variant interpretation. Therefore, couple-based screening model is suitable for large screening panel (> 1000 genes).

In this study of couples identified as having an increased genetic risk, we observed that among those currently pregnant, having achieved a live birth, or not yet having initiated a pregnancy at follow-up, one-third of the cohort either utilized prenatal or preconception testing (including IVF-PGT-M) or expressed intention to utilize IVF-PGT-M or prenatal testing to pursue pregnancy. We acknowledge that stated intentions may not reflect future actions, and one-third of the cohort remained undecided regarding whether and how to avoid the genetic condition. Consequently, we will continue longitudinal follow-up of this cohort to report longer-term reproductive outcomes. Approximately one-third of the study cohort chose not to avoid the condition associated with their carrier status. The majority of these individuals carried combined variants in *GJB2* (c.109G > A, p.V37I) or *HFE* (c.187C > G, p.H63D), which are associated with milder clinical manifestations. Supporting this observation, another CS study found that 75 out of 219 ARCs were *GJB2* carriers. However, despite the high carrier frequency of *GJB2* variants, only 8 of these 75 ARCs opted for PGT-M targeting the specific *GJB2* variant identified [35]. A significant factor influencing this decision is the nature of the *GJB2* c.109G > A (p.V37I) variant. Although the p.Val37Ile variant is pathogenic for autosomal recessive nonsyndromic hearing loss, it primarily causes mild to moderate hearing loss with late onset and low penetrance. Studies estimate its penetrance in Chinese children homozygous for this variant is only 17% [32, 45]. Similarly, in our incidental findings, the majority of homozygous carriers remained asymptomatic or exhibited only mild hearing impairment at their current follow-up age, which did not affect their daily life or work. In the same way, individuals

homozygous or heterozygous for the *HFE* variant c.187C > G (p. H63D) are not generally considered at significant risk for developing clinically significant HH [33]. Genetic screening for *HFE*-associated HH remains controversial, and population screening is not recommended due to its low penetrance and the unpredictable natural history of untreated individuals. Consequently, some experts argue that current evidence is insufficient to support population screening recommendations for *HFE*-associated HH. Furthermore, the primary goal of CS is to identify ARCs during the preconception period, enabling couples to make informed alternative reproductive decisions. Therefore, understanding what types of genetic risk information participants consider 'meaningful' and actually contribute to their reproductive decision-making is paramount. Based on the characteristics outlined above (low penetrance, mild/moderate manifestations, controversy/practical limitations regarding clinical actionability), we decided to exclude the *GJB2* c.109G > A variant and the *HFE* gene from our subsequent screening panels.

Our study has several important limitations that warrant consideration. First, while the inclusion of rare disease screening improved detection rates for Mendelian disorders, the relatively limited cohort size resulted in undetected carriers for certain low-frequency pathogenic variants (GCR < 0.025%). Future implementations would benefit from expanded population sampling. Second, our attempt to account for geographical heterogeneity in carrier frequencies through multi-regional sampling across China remains constrained by uneven regional representation, which introduces potential ascertainment bias that may affect generalizability. This underscores the need for stratified sampling frameworks in subsequent nationwide studies. Third, while our gene selection prioritized conditions with moderate-to-severe phenotypes, the clinical interpretation framework faced inherent challenges: some identified variants resided in genes with variable penetrance and expressivity, making definitive assessment of phenotypic severity problematic.

Conclusions

This population-based study determined carrier frequencies for both common and rare autosomal recessive disorders. These findings establish an evidence-based framework for optimizing CS panels in future large-scale screening. For comprehensive CS, implementing concurrent couple-based screening protocols significantly enhanced the efficiency of comprehensive risk assessment. This approach reduced burdens associated with data analysis, reporting workload, and genetic counseling compared to sequential individual testing. Notably, 53.3% of identified high-risk couples would have remained undetected using ACMG Tier 3-recommended screening criteria, half of which involved rare conditions. This underscores the necessity to expand beyond ACMG Tier 3 standards to address rare severe genetic disorders.

Abbreviations

CS	Carrier screening
GCR	Gene carrier rate
ACR	At-risk couple rate
ACMG	American College of Medical Genetics and Genomics
HGMD	Human Gene Mutation Database
NGS	Next-generation sequencing
SNVs	Single-nucleotide variants
InDels, <50 bp	Small insertions/deletions
ClinGen	Clinical Genome Resource
GenCC	Gene Curation Coalition
SOP	Standard Operation Procedure
CNV	Copy number variation
AMP	ACMG and the Association for Molecular Pathology
CCR	Association for Clinical Genomic Sciences (ACGS); Cumulative carrier rate
HH	At-risk couple rate (ACR); Hereditary hemochromatosis
IVF-PGT-M	In vitro fertilization with preimplantation genetic testing for monogenic disorders
P/LP	Pathogenic and likely pathogenic

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13073-026-01628-8.

Additional file 1. Fig. S1: Geographical distribution of participants. Table S1: The 28 genes included in Mackenzie's Mission were removed due to correspondence between genes and diseases has been updated in OMIM. Table S3: Population demographics of enrolled couples. Table S4: Increased chance results found in this study.

Additional file 2. Table S2: The details of selected genes in this study. Table S5: Gene carrier rate, cumulative carrier rate, at-risk ratio, cumulative at-risk ratio, and identified at-risk couples of selected genes following exclusion of *HFE*, GJB2:c.109G>A, and non 100 ACMG-recommended variants for *CFTR*.

Additional file 3. Data sharing statement.

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Authors' contributions

The study was designed by YongguoYu, Bing Xiao, and Hongqian Liu. Gene curation and phenotype evaluation were performed by Chinese Medical Doctor Association of Genetics Counselling Board. Samples were enrolled and collected by Xiaotian Su, Xiaoxiao Xie, Zhengfeng Xu, Feng Ren, Ying Peng, Ling Hui, Liangpu Xu, Chunhua Zhang, Jie Duan, Juan Zhang, Juan Li, Shuyuan Xue, Shixiu Liao, Ling Liu, Zhuoshu Zhao, Dejian Yuan, Juan Qiu, Fuqing Zhang, Yang Huang, Yingchun Ha, Lei Yu, Kai Tang, Xiaozhou Li, Wei Pan, Jing Shu, Jiansheng Zhu, Ling Meng, and Chenming Xu. Next generation sequencing data was analyzed by Yu Sun, Aiping Mao, and Nannan Yan. The first draft of the manuscript was written by Bing Xiao and Hongqian Liu, and it was reviewed by Yongguo Yu. All authors read and approve the final manuscript.

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Data availability

The datasets generated and/or analyzed during the current study (including the complete de-identified patient data set, genomic data, and associated clinical information) are not publicly available due to strict patient privacy, confidentiality agreements, and institutional ethical restrictions. However, they are available from the corresponding author upon reasonable request. A data

sharing statement provided by the authors is available with the full text of this article in Additional file 3.

Declarations

Ethics approval and consent to participate
This study was approved by the ethic board of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (XHCC-C-2022-077-1). This study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent for genetic analysis was obtained from the participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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