

Carrier Screening of 400 Variations Related 11 Recessive Diseases in the Daur Ethnicity in China

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Abstract

Background: Single gene disorders are common diseases that cause birth defect. Carrier screening is an effective method to reduce the affected children with single gene disorders. However, incidence rates and carrier positive rates vary among ethnic groups.

Results: In the present study, four hundred alleles associated with 11 recessive disease were detected in the Daur ethnicity of China. Among the 246 individuals, 25 individuals were identified as heterozygous carriers of at least one for 11 recessive disorder, carrier rate was 10.16%. A total of 19 females were carrier positive among 143 individuals with a 13.29% positive rate, however, only 6 out of 103 males were carrier positive with a 5.83% positive rate. The most common in the Daur was HLD (2.85%) and congenital hearing loss (2.85%), followed by CAH (2.44%), PKU (1.22%), SMA (0.41%), MMA (0.41%), and X-linked ichthyosis (0.41%).

Conclusions: These results estimated the distribution of carrier frequencies in the Daur, and showed that several of these diseases may be considered for inclusion in carrier screening in the Daur population. Further large-scale study should be performed to identified the results.

Introduction

In China, the number of birth defects each year accounts for about 20% of the world. According to the "China birth defects prevention and control report (2012)" released by the Ministry of Health, the total incidence of birth defects in China is about 5.6%, and the number of new birth defects is as high as 900,000 annually. As an important part of birth defects, child with single gene disorders still lack an effective treatment despite significant scientific and medical advances in the last few decades.

Carrier screening for genetic disease is the practice of diagnosing individuals and predicting birth defects, beginning in 1970 by using a biochemical enzymatic assay to screen carrier status for (AJ) individuals [1–5]. In the absence of family history, carrier screening is an effective method for measure the risk of fetus affected by recessive disease. However, scientific advances over the past decades have discovered thousands of single gene disorder, which disorders should include to screen remains a huge challenge [6]. In addition, carrier frequency is varied in different populations. Considering the population ethnic admixture, it was reasonable to screen [7–9]. Nowadays, carrier screening for many genetic diseases ally has been common in many countries due to the recommendation of ACOG and ACMG, such as cystic fibrosis (CF), spinal muscular atrophy (SMA)[10, 11]. In 2011, Bell et al first reported carrier screening for severe childhood recessive diseases by NGS. In the study, 104 unrelated individuals were screened for 448 kinds of severe recessive genetic diseases, and it was found that each person carried 2.8 pathogenic mutations (rang from 0 to 7) [12]. And among Ashkenazi Jewish individuals, the carrier frequency is as high as 43.6%, and one out of 2.3 people on average is a carrier of recessive disorders.

China is a multi-ethnic country with 56 ethnic groups living together on a vast territory. There are differences in genetic background including genetic phenotypes, genetic diseases, incidence rate and

susceptibility to many diseases. The Daur is one of the ethnic minorities with relatively small populations in China, and they are mainly distributed in the Heilongjiang Province and the Inner Mongolia Autonomous Region. In recent years, many studies have been reported the carrier frequencies for one or several diseases in Han population, but there was no any report on the Daur nationality [7, 13–15].

Carrier screening to the general population is an economical way to predict and reduce the incidence of recessive diseases. Here, we test about four hundred alleles associated with 11 recessive diseases in the Daur population and summarize clinical data from different population demographics. The purpose of the present study is to understand the distribution of carrier frequencies of the Daur and inform future genetic counseling efforts.

Results

Population characteristic

In total, 257 Daur individuals were included the present study, of which 11 out of 257 Daur individuals are parent-child and 11 child samples are excluded in the primary results due to their relationship. Among 246 individuals, 143 were females, and 103 were males. The number of single detection and couple detection was 168 and 78. All participants provided written informed consent.

Carrier Status

About 400 alleles associated with 11 recessive disease were identified in the Daur population. In the present research, as shown in Table 1, among the 246 individuals, 25 individuals were identified as heterozygous carriers of at least one for 11 recessive disorder, carrier rate was 10.16%, one individual was found to be carrier of two disorders (X-linked ichthyosis and SMA). A total of 19 females were carrier positive among 143 individuals with a 13.29% positive rate, however, only 6 out 103 males were carrier positive with a 5.83% positive rate. There was a gender difference in the positive rate of the males (5.83%) and the females (13.29%) in Daur, but the difference was not statistical due to sample size ($\chi^2 = 3.651, P = 0.056$). In addition, among 11 children, three were detected as carriers from their parents.

Table 1
Characteristic of included individuals and carrier rate.

Types	N	Carrier positive	Male		Female	
			N	Carrier positive	N	Carrier positive
Single detection	168	15	64	2	104	13
Couple detection	78	10	39	4	39	6
Total	246	25 (10.16%)	103	6 (5.83%)	143	19 (13.29%)

Disorders Identified Among Carriers

As shown in Table 2, the most common in the Daur was HLD (2.85%) and congenital hearing loss (2.85%), followed by CAH (2.44%), PKU (1.22%), SMA (0.41%), MMA (0.41%), and X-linked ichthyosis (0.41%).

Table 2
Disorders' type of individuals with carrier positive.

Disorders	Male (N = 103)	Female (N = 143)	Total
Thalassaemia	0 (0)	0 (0)	0 (0)
HLD	1 (0.97%)	6 (4.20%)	7 (2.85%)
Congenital hearing loss	2 (1.94%)	5 (3.50%)	7 (2.85%)
CAH	1 (0.97%)	5 (3.50%)	6 (2.44%)
SMA	0 (0)	1 (0.70%)	1 (0.41%)
PKU	1 (0.97%)	2 (1.40%)	3 (1.22%)
MMA	1 (0.97%)	0 (0)	1 (0.41%)
Fragile X syndrom	0 (0)	0 (0)	0 (0)
X-linked ichthyosis	0 (0)	1 (0.70%)	1 (0.41%)
DMD	0 (0)	0 (0)	0 (0)
Hemophilia	0 (0)	0 (0)	0 (0)
Total	6 (5.83)	20 (13.99%)	26 (10.57%)

Identified Variants

Two variant types were detected in seven HLD carriers, and the most prevalence variant in HLD was ATP7B c.3316 G > A, another was ATP7B c.2621 C > T. In six CAH carriers, half of them were CYP21A2 c.923 dupT, two were CYP21A2 c.1069 C > T, and one was CYP21A2 gene fusion. In seven congenital hearing loss carriers, GJB2 c.235 delC was detected in five individuals, and GJB2 c.299_300 delAT was detected in two individuals. Three PKU carriers were detected positive with PAH c.611 A > G, c.721 C > T, and c.1238 G > C. The result of identified variants was shown in Table 3.

Table 3
Disorders and variants identified in the Daur population.

Disorder	Gene	Identified variant[†]	Detected times
HLD	ATP7B	c.3316 G > A	5
		c.2621 C > T	2
Congenital hearing loss	GJB2	c.235 delC	5
		c.299_300 delAT	2
CAH	CYP21A2	c.923 dupT	3
		c.1069 C > T	2
		Gene fusion	1
PKU	PAH	c.611 A > G	1
		c.721 C > T	1
		c.1238 G > C	1
SMA	SMN1	SMN1 deletions	1
MMA	MMACHC	c.609 G > A	1
X-linked ichthyosis	STS	STS deletion	1
† All variants are heterozygous.			

Table 4
Disorders and variants identified in 500 Han population in China.

Disorder	Gene	Identified variant	Detected times	Ethnicity [†]
Thalassaemia	HBA	- $\alpha^{3.7}$	6	Han
		- α^{SEA}	5	Han
		- $\alpha^{4.2}$	3	Han
		c.377 T > C	1	Han
		c.-100 G > A	1	Han
		c.126_129delCTTT	3	Han
		c.316-197C > T	1	Han
		c.85dupC	1	Han
		HLD	ATP7B	c.3316 G > A
c.2333 G > T	3			Han
c.2975 C > T	2			Han
c.1846 C > T	1			Han
c.2621 C > T	1/2			Han/Daur
c.2755 C > G	1			Han
c.3426 G > C	1			Han
c.3443 T > C	1			Han
c.3809 A > G	1			Han
c.994 G > T	1			Han
CAH	CYP21A2			c.955 C > T
		c.293 - 13 A/C > G	3	Han
		c.923 dupT	3	Daur
		c.518 T > A	1	Han
		c.332_339 del	1	Han
		c.1069 C > T	1/2	Han/Daur
		Gene fusion	2/1	Han/Daur

[†] The data in Han population was provided by Genesky Biotech, Co., Ltd., Shanghai, China .

Disorder	Gene	Identified variant	Detected times	Ethnicity [†]
Congenital hearing loss	GJB2	c.235 delC	5/5	Han/Daur
		c.299_300 delAT	2/2	Han/Daur
		g.20398370–20523823 del	1	Han
	SLC26A4	c.919-2 A > G	3	Han
		c.1174 A > T	1	Han
		c.1229 C > T	1	Han
PKU	PAH	c.1975 G > C	1	Han
		c.728 G > A	3	Han
		c.1256 A > G	1	Han
		c.611 A > G	1/1	Han/Daur
		c.320 A > G	1	Han
		c.482 T > C	1	Han
		c.716 G > A	1	Han
		c.721 C > T	1/1	Han/Daur
		c.1238 G > C	1/1	Han/Daur
			PTS	c.155 A > G
SMA	SMN1	SMN1 deletions	12/1	Han/Daur
MMA	MMACHC	c.658_660 delAAG	2	Han
		c.482 G > A	1	Han
		c.567 dupT	1	Han
		c.609 G > A	1/1	Han/Daur
		c.626 dupT	1	Han
			MUT	c.729_730 insTT
Fragile X syndrome	FMR1	CGG 30/74	1	Han
		CGG 30/87	1	Han
DMD	DMD	EX01-05DUP	1	Han
X-linked ichthyosis	STS	STS deletion	1/1	Han/Daur

[†] The data in Han population was provided by Genesky Biotech, Co., Ltd., Shanghai, China .

Discussion

Birth defects are a heavy blow to the family and a heavy economic burden to the country. Because one's health is not affected by carrier status, most parents are unaware their "harm" gene until the birth of newborn with defect, which leads to the absence of genetic counseling and preconception carrier testing.

Carrier screening in healthy individuals with no a positive family history can help to testing carrier status for genetic diseases. Carrier screening early screening for genetic diseases with high incidence of ethnic populations in some geographical regions [16, 17]. Since autosomal recessive disorders are more common in certain populations, early screening work was carried out on the basis of race. The international carrier screening program has been carried out for many years, expanding from a single race to a multi-ethnic group, and the types of diseases are gradually increasing, from high-risk groups to low-risk groups.

China is a country with a high incidence of birth defects, with an average of one birth defect every 30 seconds. China is still in the early stage of research and development, and population-based carrier screening has not yet been carried out on a large scale due to limitation of associated harms and costs, especially in ethnic minority. As a minority located in north China for generations with a population of over 120,000, the Daur has their own genetic background so that the genetic characteristics of the Daur nationality are not allow to be neglected. By screening the hot spot mutation of genetic diseases, it can take the initiative to prevent some ethnic genetic diseases and block the chance of genetic diseases in the offspring.

At present, there is no reporting the prevalence of rare genetic diseases of the Daur and describing the attitudes of people towards carrier screening. In the present study, about 400 alleles associated with 11 relative common recessive disease in the Daur population were detected. Carrier frequency in the Daur population was 10.16%. The carrier positive rate of congenital hearing loss, CAH, and PKU were detecting more than 1% in the Daur population. Comparing with the Han population in China, it's found that the distributions of carrier variation is relatively simple in the Daur (Table 4). Thalassaemia is one of the most common genetic disorders in the world, mainly distributed in coastal areas, and 4.54% and 11.07% of the population in Hunan [18] and Guangdong [19] represented heterozygous carriers of α -thalassaemia and β -thalassaemia. Different from the other populations located in the south of China, no individuals were detected with thalassaemia carrier in the Daur population. In addition, genetic deafness is highly heterogeneous, but mutations in the GJB2, SLC26A4, and MT-RNR1 are the majority in diverse populations. However, only mutation c.235 delC and c. 299_300 delAT of the GJB2 gene in 85 related variations were screened positive in the Daur population.

HLD, also known as Wilson's disease, is another common detected disease with heterozygous carrier in our present study. However, only two variation types were found, ATP7B c.3316 G > A mutation was the most common variants in the Daur population, and another variation was ATP7B c.2621 C > T.

SMA is the most common neuromuscular AR disorder caused by SMN1 mutation, and SMA carrier screening is beneficial to genetic counseling and reduce the number of affected children. ACMG has recommended offering carrier screening for SMA to couples regardless of ethnicity [20]. SAM carrier frequency has been reported as 1 in 72 to 1 in 47, which was different in ethnic. It has been reported that the carrier frequency in China is about 2.2% in Taiwan, 1.6% in HongKong, 1.9% in Shanghai, and 1.2 in Liuzhou population [21]. In 2020, a largest-scale carrier screening for SMA in mainland China showed that 1.77% women were identified as carrier in 13069 women (approximately 1:56 in the population) [15]. However, the frequency of Daur is lower than that of these populations, only one out of 246 individuals was detected to be a positive carrier.

Fragile X is a kind of X-linked disorders, which are due to mutation in genes on the X chromosome. Female carries of an X-linked disorder are at 50% risk for having an affected male newborn and a carrier female newborn. In the present study, we found there was one carrier of Fragile X in the Daur population. According to ACMG and ACOG, Fragile X should be screened based on family history. DMD, a common disease in rare diseases, also is a X-linked disorder, comparing with the treatment, screening test is an effective method to prevention it [22]. In our study, we failed to find one carrier in the Daur population.

Our study has some limitations. First, the carrier frequency is low for many diseases, so it is necessary to expend number of included samples. Secondly, the failure to perform a more in-depth analysis due to the lack of detailed information

Conclusions

In conclusion, our study showed the distribution of carrier frequencies of the Daur. Results of the present study identified the importance of expending carrier screening in the future in a healthy population of the Daur nationality. Meanwhile, it is helpful for us to guide pregnancy planning based on their ethnicity and contribute to the professional discussion on the clinical application of carrier screening and genetic counseling.

Methods

Study population

A total of 257 individuals with healthy phenotype from the Daur ethnicity were collected for the present study. DNA was extract from 200 ul whole blood using the QIAamp blood kit (Qiagen, Hilden, Germany). 5% random duplication samples were detected to guarantee the quality of test.

Carrier Screening

About Four hundred alleles associated with 11 recessive diseases were tested using iMLDR, SNaPshot, long-PCR, CNVplex, AQ-PLP, Fast-target technology by Genesky Biotech, Co., Ltd., Shanghai, China. The

information of disease and allele was summarized in Additional file 1:Table S1.

Statistical analysis

The SPSS version 11.0 (SPSS, Inc., Chicago, IL, USA) was used to perform all statistical analyses. Carrier positive rates were calculated as the number of individuals heterozygous divided by the total number of individuals in certain population. Carrier positive rate was compared between different populations and sex. Chi-square was used to assess the differences of carrier frequencies and P value < 0.05 was considered statistically significant.

Abbreviations

DMD: Duchenne muscular dystrophy; SMA: spinal muscular atrophy; PKU: phenylketonuria; MMA: methylmalonic academia; CAH: congenital adrenal cortical hyperplasia; HLD: hepatolenticular degeneration

Declarations

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Authors' contributions

Q.L, J.Y and S.F conceived and designed the study; Q.L and T.Z, B.D, M.J collected blood sample and extracted DNA. Q.L and T.Z, B.D, X.J, J.B analyzed the data. Q.L, T.Z, B.D, X.J, J.B, J.Y and S.F drafted the manuscript. All authors revised and approved the final draft.

Ethics approval and consent to participate

The study was approved by Ethics Committee of Harbin Medical University. All participates provided written informed consent.

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Data availability statement

The data that support the findings of this study are available on reasonable request from the corresponding author.

Consent for publication

Not applicable

Competing interests

All authors declare no competing interests.

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