Screening for Pathogenic Genes of Stargardt Disease

Identification of 37 novel *ABCA4* mutation sites using DNBSEQ-G400 sequencing platform

Based on years of research, and using targeted exon sequencing technology and DNBSEQ-G400 sequencing platform, researchers at the Eye Institute, Eye and ENT Hospital, College of Medicine, Fudan University found 37 novel *ABCA4* gene mutations in Chinese patients with Stargardt disease. They also determined the mutation frequency and mutation sites in the gene.

The relevant results of this study were published in 2019 in the journal *Frontiers in Genetics*, under the title "*ABCA4* Gene Screening in a Chinese Cohort With Stargardt Disease: Identification of 37 Novel Variants"[1].

Recommended application: Disease omics (complex disease)

Recommended model: DNBSEQ-G400RS

- ### Rapid detection and identification of genetic diseases

  DNBSEQ-G400 platform supports the rapid detection of genetic diseases and identification of mutation sites, thereby mapping a more detailed mutation atlas.

- ### Automatic operation compatible

  The automated extraction and library preparation equipment of MGI significantly save labor costs in high-throughput sequencing and improve processing efficiency.

Background

Stargardt disease (STGD1, OMIM 248200) is a genetic macular dystrophy. Patients have bilateral or continuous central vision loss in early adolescence[2,3]. STGD1 is one of the leading causes of macular dystrophy in children, accounting for approximately 7% of patients with retinal dystrophy, and has an incidence of 1:10000.

Patients with STGD1 often develop maculopathy in the fundus, with lipofuscin deposition in the retinal pigment epithelium (RPE). This causes RPE atrophy and photoreceptor cell death, leading to blindness[1]. STGD1 is an autosomal recessive hereditary disease, where patients have mutations in the ATP binding cassette subfamily A member 4 (*ABCA4*) gene[4].

Located on chromosome 1p22.1, *ABCA4* (OMIM 601691) contains 50 exons and is specifically expressed in retinal photoreceptor cells. The gene plays a role in the transport of N-retinylidene-phosphatidylethanolamine (NRPE), an intermediate metabolite of vitamin A. It can transport NRPE from the outer segment of photoreceptors to the cytoplasm, thus contributing to the clearance of toxic retinal phospholipid compounds in photoreceptor cells. The inactivation of its function may lead to degeneration of photoreceptor cells[5,6]. *ABCA4* mutations can lead to diseases such as STGD1, retinitis pigmentosa (RP), cone rod dystrophy (CRD), and retinal dystrophy[7]. However, research findings suggest that *ABCA4* is the only disease-causing gene related to STGD1. At present, approximate 1,200 types of mutations have been found in *ABCA4*, of which nearly 900 are related to STGD1 and most are missense mutations. Among different patients with STGD1, the probability of detecting at least one *ABCA4* mutation is 70%~90%[8]. Studies have shown that *ABCA4* gene mutation is race-specific. For example, c.2588G>C occurs more in Western European, p.Y808* in Chinese, and c.5714+5G>A in Greek subjects[1].

Study description

To determine the *ABCA4* gene mutation spectrum and frequency in a Chinese cohort with STGD1, researchers at the Eye Institute, Eye and ENT Hospital, College of Medicine, Fudan University performed *ABCA4* gene targeted exon sequencing on the DNBSEQ sequencing platform. They found 37 novel *ABCA4* mutation sites associated with STGD1 disease in a Chinese cohort . These results expanded the mutation spectrum of the *ABCA4* gene. The researchers identified the common *ABCA4* gene mutation sites in patients with STGD1 in Eastern China, and found the *de novo* mutation c.4561delA.

Materials and Methods

Sample collection and DNA extraction

The team collected peripheral blood samples from 153 volunteers in the Eye Institute, Eye and ENT Hospital,Fudan University from 2016 to 2018. Samples included 25 family cases (25 patients with primary disease and their relatives) and 71 sporadic cases. Their genomic DNA was extracted using the DNA extraction kit. After assessing DNA integrity using 1% agarose gel electrophoresis (AGE), samples were stored at -20°C for subsequent analysis.

Targeted exon library preparation and sequencing

For this study, the Target_Eye_792_V2 chip covering 792 common genetic eye disease gene exons and noncoding regions (UTRs) was designed. The process to prepare the targeted exon library was as follows: after genomic DNA fragmentation, exon, flanking intron and promoter regions were captured using the Agilent SureSelect Target Enrichment reagent kit, and the enriched targeted exon library was converted into DNA nanospheres (DNBs) before loading onto the MGISEQ-2000 sequencer for sequencing. In the case of large sample sizes, the automated extraction and library preparation technology provided by MGI can cut labor costs significantly and improve efficiency.

Genetic analysis

Sequencing data were aligned to the human reference genome (hg38) using Burrows-Wheeler Aligner (BWA, http://bio-bwa.sourceforge.net/), and the identified mutants were annotated using these databases: 1,000 Genomes Project (http://browser.1000genomes.org./), dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), ESP6500 (http://evs.gs.washington.edu/EVS/), and ExAC (http://exac.bro adinstitute.org).

Mutants with a lessallele frequency (MAF) were selected to search for possibly detrimental mutations (MAF<0.1%). These potentially detrimental mutations were predicted using the following website tools: Sorting Intolerant from Tolerant (SIFT, http://sift.jcvi.org/), Polymorphism Phenotyping v2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster software (http://www.mutationtaster.org/). The remaining mutations were selected according to their potential detriment, genotype relationships, and mutation-related reports, using ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/),HGMD (http://www.hgmd.cf.ac.uk/ac/index.php), and Online Mendelian Inheritance in Man (OMIM, http://www.omim.org/). According to the American College of Medical Genetics (ACMG) and genome guide, these mutations are divided into detrimental mutations, possibly detrimental mutations, mutations of uncertain detriment, mutations that may occur, and mutations that have occurred. In addition, Sanger sequencing technology played a significant role in this study.

Sample collection	Library preparation and sequencing	Bioinformatics Analysis	Result analysis
Peripheral blood samples from 153 volunteers, including 25 family cases (25 patients with primary disease and their relatives) and 71 sporadic cases	 Agilent SureSelect Target Enrichment reagent kit  DNBSEQ-G400 genetic sequencer	BWA SIFT PolyPhen-2 MutationTaster ClinVar HGMD OMIM	Calculation of *ABCA4* mutation detection rate, Summary of *ABCA4* mutation types, Identification of new mutations of *ABCA4*

Results

Clinical research results

The team took samples from 153 volunteers (including 25 family cases and their relatives, and 71 sporadic cases), Volunteers were on average 33 years of age, 87% came from Eastern China (Fig. 1), and 101 were confirmed cases of STGD1.

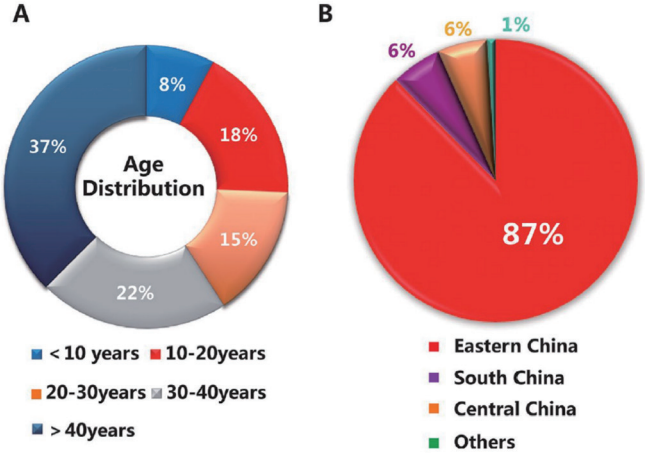

Fig. 1. Basic information of 153 patients in this study. (A) Age distribution of patients; (B) Geographical distribution of patients.

ABCA4 mutation detection rate

The team performed a genetic screening of all 153 volunteers using targeted exon sequencing technology. They found that mutations occurred in the *ABCA4* gene of 129 patients, with a total mutation detection rate of 84.3%, distributed as 56.2% with two or three allele mutations, 28.1% with one allele mutation, and 15.7% with no mutation. Furthermore, the mutation detection rates of family cases and sporadic cases were 87.8% and 80.3%, respectively (Table 1). In the 25 primary cases in 25 families, only one case had three allele mutations, 28 cases had two allele mutations, and one had no mutation. In the sporadic cases, 80.3% had possibly detrimental *ABCA4* mutations. The results were consistent with previous findings, and most patients with STGD1 had complex heterozygous mutations.

Genetic analysis

Analyses detected 96 *ABCA4* gene mutations, including 37 new mutations and 59 known muta-

tions. They were all distributed in 50 exons of *ABCA4*, of which 6 different mutations were distributed in exons 3 and 13 respectively, 4 different mutations in exons 8, 22, 23, 35, and 1-3 different mutations in the remaining 29 exons respectively. ABCA4 protein has 6 functional domains and the above mutations were distributed mainly in the transmembrane domain 1 (TMD1), nucleotide binding domain 1(NBD1), and nucleotide binding domain 2 (NBD2); (Fig. 2).

The 96 *ABCA4* gene mutations included missense mutations (64%), nonsense mutations (6%), splicing mutations (6%), frameshift mutations (12%), and small indel mutations (2%). Among these, 38 were detrimental mutations (39.5%), 26 were possibly detrimental mutations (27.1%), and 32 mutations were of uncertain detriment(33.4%). The latter were mostly new mutations. The 64 detrimental/possibly detrimental mutations also included missense mutations (53%), splicing mutations (19%), frameshift mutations (17%), nonsense mutations (9%), and small indel mutations (2%) (Fig. 3A). 10 common *ABCA4* gene mutation sites were found in the 153 volunteers (Table 2), all of which were detrimental and mainly missense mutations. Three of the most common mutations were c.101_106 delCTTTATp.Ser34_Leu35del (allele frequency of 10.5%), c.2894A>Gp.Asn965Ser (6.5%) and c.6563T>Cp.Phe2188Ser (4.6%).

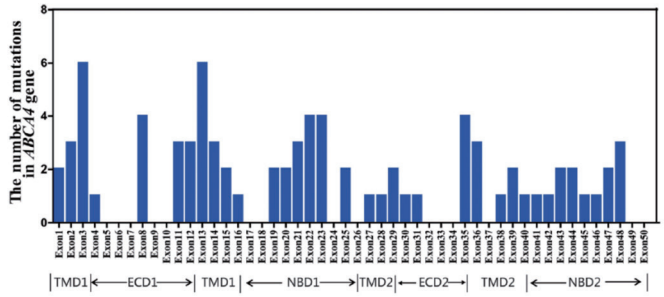

Fig. 2. Distribution and frequency of *ABCA4* gene mutation sites identified in this study.

Variance per cases	Families (no./percentage)	Sporadic cases (no./percentage)	Total cases (no./percentage)
3	1/1.2	2/2.8	3/2.0
2	28/34.2	55/77.5	83/54.2
1	43/52.4	0/0	43/28.1
0	10/12.2	14/19.7	24/15.7
	82/100	71/100	153/100

Table 1. Mutation detection rate of *ABCA4* in this study.

New mutations of *ABCA4* gene

In this study, a total of 37 novel *ABCA4* gene mutations were identified, including missense mutations (15/37, 40%), frameshift mutations (8/37, 22%), splicing mutations (7/37, 19%), nonsense mutations (6/37, 16%), and small indel mutations (1/37, 3%) (Fig. 3B).

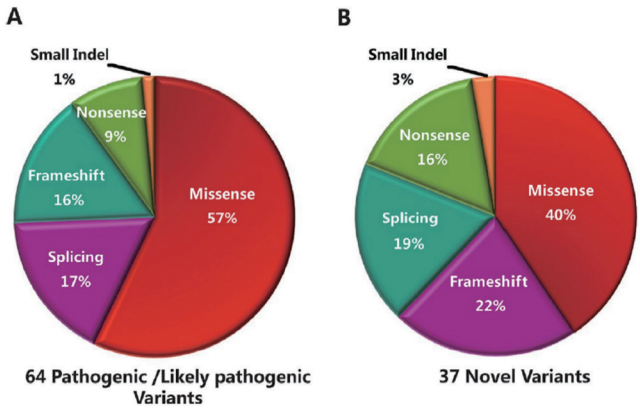

Fig. 3. Genetic analysis of *ABCA4* gene mutation sites.

Gene	Nucleotide change	Amino acid change	Clinical significance[1]	Allele frequency
ABCA4	c.101_106 delCTTTAT	p.(Ser34_Leu35del)	Pathogenic	10.5%
ABCA4	c.2894A>G	p.(Asn965Ser)	Pathogenic	6.5%
ABCA4	c.6563T>C	p.(Phe2188Ser)	Pathogenic	4.6%
ABCA4	c.1819G>A	p.(Gly607Arg)	Pathogenic	3.3%
ABCA4	c.1006delT[2]	p.(Ser336Profs*38)	Pathogenic	3.3%
ABCA4	c.5761G>A	p.(Val1921Met)	Pathogenic	2.6%
ABCA4	c.1804C>T	p.(Arg602Trp)	Pathogenic	2.6%
ABCA4	c.6282+1G>A	p.?	Pathogenic	2.6%
ABCA4	c.6389T>A	p.(Met2130Lys)	Pathogenic	2.6%
ABCA4	c.1561delG	p.(Val521Serfs*47)	Pathogenic	2.6%

Table 2. 10 types of common *ABCA4* gene mutation sites in 153 volunteers.

Summary

The described study characterized the *ABCA4* mutation spectrum of Chinese people and determined the total mutation detection rate to be 84.3%. A total of 37 novel STGD1-related mutations were also found, expanding the known *ABCA4* mutation spectrum. A novel heterozygous mutant, namely c.4561delA, was also found. Gene detection in patients with STGD1 could make clinical diagnosis more accurate. This study expanded the *ABCA4* mutation spectrum of Chinese people, which allows for improved genetic screening of patients with STGD1.

The research team sequenced the *ABCA4* gene using targeted exon capture technology on the DNBSEQ-G400 sequencing platform independently developed by MGI. The DNBSEQ-G400 sequencer is a comprehensive and flexible sequencer with a throughput scale that meets the sequencing application and data analysis needs of scientific research, clinical medicine, forensic medicine, agriculture, and other fields.

DNBSEQ-G400RS Genetic Sequencer

Reference

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Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	DNBSEQ-G400RS Genetic Sequencer	900-000170-00
	MGISP-100RS Automated Sample Preparation System	900-000206-00
	MGISP-960RS Automated Sample Preparation System	900-000146-00
Software	MegaBOLT Bioinformatics analysis accelerator	900-000555-00
Library Prep	MGIEasy Circularization Module V2.0（16 RXN）	1000005260
Sequencing Reagents	DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100)	1000016950

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2. For HotMPS sequencers: This sequencer is only available in selected countries, and its software has been specially configured to be used in conjunction with MGI's HotMPS sequencing reagents exclusively.

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