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Using next-generation sequencing as a genetic diagnostic tool in rare autosomal recessive neurologic Mendelian disorders

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ABSTRACT

Next-generation sequencing was used to investigate 9 rare Chinese pedigrees with rare autosomal recessive neurologic Mendelian disorders. Five probands with ataxia-telangectasia and 1 proband with chorea-acanthocytosis were analyzed by targeted gene sequencing. Whole-exome sequencing was used to investigate 3 affected individuals with Joubert syndrome, nemaline myopathy, or spastic ataxia Charlevoix-Saguenay type. A list of known and novel candidate variants was identified for each causative gene. All variants were genetically verified by Sanger sequencing or quantitative polymerase chain reaction with the strategy of disease segregation in related pedigrees and healthy controls. The advantages of using next-generation sequencing to diagnose rare autosomal recessive neurologic Mendelian disorders characterized by genetic and phenotypic heterogeneity are demonstrated. A genetic diagnostic strategy combining the use of targeted gene sequencing and whole-exome sequencing with the aid of next-generation sequencing platforms has shown great promise for improving the diagnosis of neurologic Mendelian disorders.

1. Introduction

The advent of next-generation sequencing (NGS), which is high throughput, functional, and cost effective, has provided a new approach to identify novel causal mutations and genes for several unresolved, rare, Mendelian disorders (Ku et al., 2011; Majewski et al., 2011; Ng et al., 2009, 2010a, 2010b). NGS has already been used successfully to discover many causative genes, including *TGM6* and *PRRT2*, which were identified in our previous study (Wang et al., 2010, 2011), thus strengthening its identity as a promising technology to pinpoint the genetic basis of monogenic diseases. In addition, NGS provides valuable information on known disease genes and related loci, especially for Mendelian disorders characterized by obvious genetic and phenotypic heterogeneity.

In some Mendelian disorders with genetic heterogeneity, a causal mutation may be present in several causative genes. In addition, some mutations, including point mutation, small indel, copy numbers variations (CNVs), and so forth, can be found in 1 causative gene. Consequently, the unique diagnostic properties of conventional Sanger sequencing make it unsuitable for diagnosing these disorders (Botstein and Risch, 2003). Recently, the use of high-throughput sequence-capture methods and NGS technologies has become well established, demonstrating their diagnostic usefulness in the investigation of heterogeneous diseases (Bonnefond et al., 2010; Choi et al., 2009; Montenegro et al., 2011; Pierson et al., 2012; Sailer et al., 2012; Wei et al., 2011). On the other hand, some Mendelian disorders caused by abnormal repetitive sequence stretches, such as trinucleotide repeat expansions responsible for some subtypes of hereditary spinocerebellar ataxias (SCAs), cannot be accurately determined by NGS for technical reasons (Sailer et al., 2012). Moreover, inadequate sequence coverage as well as the read depth of target regions resulting from technical hurdles may lead to loss of valuable information and artificial bias for assessment. Thus, optimization of NGS is helpful

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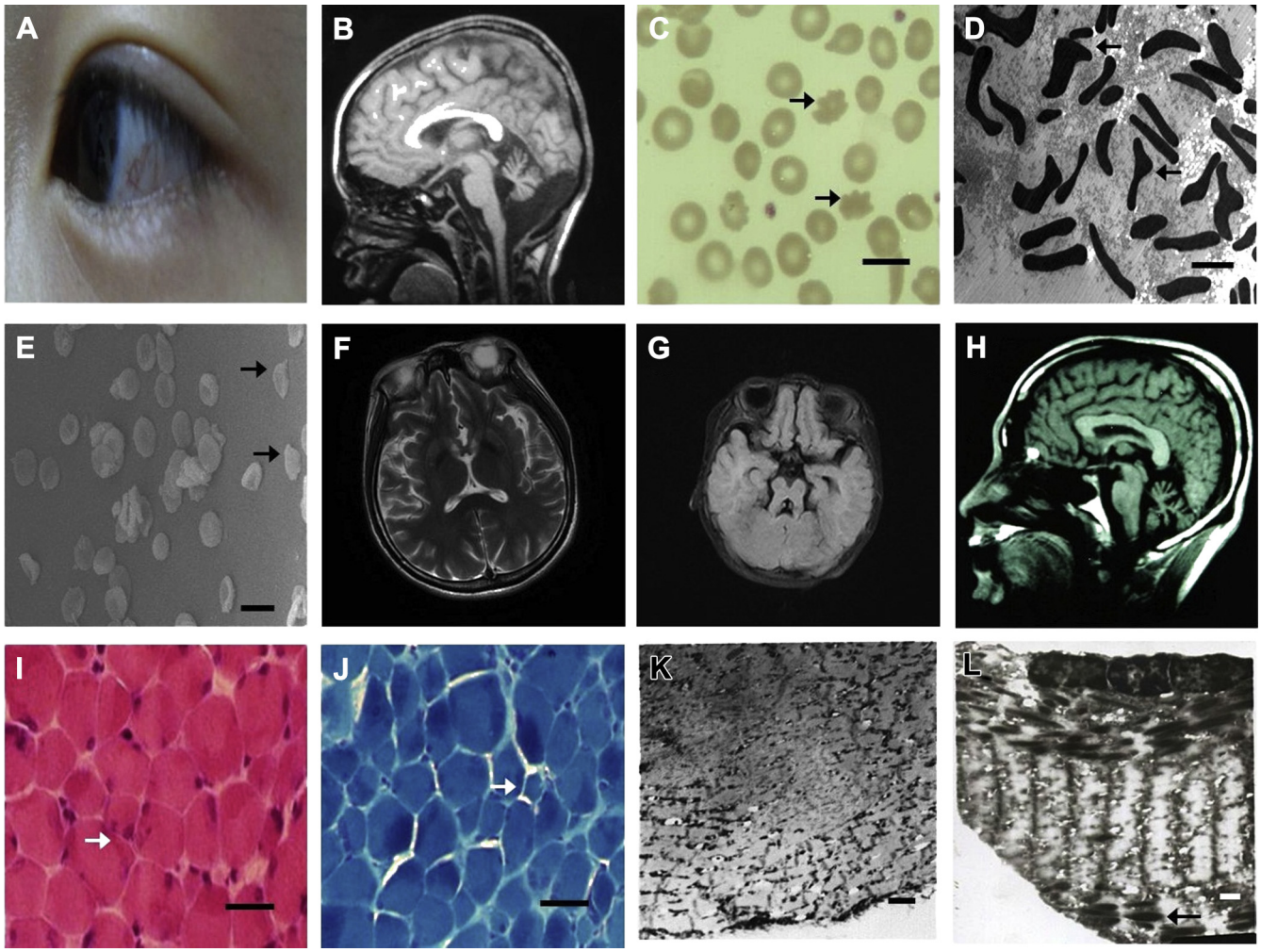

Fig. 1. Clinical supportive evidence. (A) Ocular telangiectasia was indicated in a proband with ataxia-telangectasia. (B) Brain magnetic resonance imaging (MRI) indicated cerebellar atrophy (sagittal T1) in a proband with ataxia-telangectasia. Acanthocytes with spiky projections are seen on a peripheral blood smear under a light microscope (May-Grünwald-Giemsa stain; ×1000; scale bar = 10 μm) (C), a transmission electron microscope (×5000; scale bar = 2 μm) (D), and a scanning electron microscope (×1500; scale bar = 10 μm) in a patient with chorea-acanthocytosis (arrows represent aberrant erythrocytes) (E). (F) No obvious change was found on brain MRI in the proband with chorea-acanthocytosis. (G) Brain MRI of the infant with Joubert syndrome revealed abnormal superior cerebellar peduncles and a deepened interpeduncular fossa (sagittal T1), comprising the molar tooth sign. (H) Brain MRI indicated cerebellar atrophy (sagittal T1) in a proband with spastic ataxia of Charlevoix-Saguenay (clinically diagnosed with hereditary spastic paraplegia or Machado-Joseph disease). Modified Gomori trichrome treatment (×200; scale bar = 10 μm) revealed nemaline bodies of dark-red staining in the myofibril cytoplasm (I), which was also observed as purple-red staining followed by hematoxylin-eosin treatment (×200; scale bar = 10 μm) (arrows represent nemaline bodies) (J). An electron microscopic observation showed focal myofilament disorganization and dissolution (×6000; scale bar = 1 μm) (K), as well as numerous subsarcolemmal and perinuclear rodlike structures (nemaline bodies) (×5000; scale bar = 1 μm) (arrow represents nemaline bodies) (L).

for clinical diagnostics, and this crucially depends on the development of analytical strategies as well as efficiency in targeted capture and sequencing.

In this study, we report the genetic analysis of 9 Chinese Han pedigrees with autosomal recessive neurologic disorders (ataxia-telangectasia [AT], chorea-acanthocytosis [ChAc], Joubert syndrome [JS], nemaline myopathy [NM], and spastic ataxia of Charlevoix-Saguenay [SACS]). Clinical assessments were determined based on medical history, physical examination, radiologic studies, biochemical tests, or pathologic biopsy. Our evaluations using targeted gene sequencing (TGS) and whole-exome sequencing (WES) on NGS platforms revealed several known mutations in causative genes. Also, the findings of novel potential pathogenic variations provided inherited clues for further functional research, indicating great promise for NGS in genetic diagnosis.

2. Methods

2.1. The patients

Thirteen patients from the 9 autosomal recessive pedigrees, diagnosed by 2 or more experienced neurologists, were enrolled in our study. Clinical diagnoses were made based on supportive evidence (Fig. 1, Table 1). Seven affected individuals from 5 families were diagnosed with AT, characterized by progressive cerebellar ataxia, ocular telangiectasia as well as cerebellar atrophy that was indicated by brain magnetic resonance imaging (MRI). An affected individual diagnosed with ChAc from 1 family presented with progressive dysarthria and dysphagia accompanied by aberrant erythrocytes and increased serum creatine levels in the blood but a normal brain MRI. An infant diagnosed with JS from 1 family exhibited hypertelorism, horizontal nystagmus, and ptosis of the