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Whole-exome sequencing for monozygotic twins discordant for hemifacial microsomia

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ABSTRACT

Hemifacial microsomia (HFM) is the second most common congenital craniofacial malformation. Although many sporadic and familial cases have been studied to explore the etiology and pathogenesis of HFM, no common understanding has been reached. We aimed to further probe into the etiology of HFM through studying monozygotic twins. Here, we report two cases of pairs of monozygotic twins discordant for HFM, and performed whole-exome sequencing (WES) and bioinformatics analysis to help determine the underlying molecular mechanisms. We identified 93 and 83, and 101 and 104 genes containing rare germline mutations in the twins of the two pairs, respectively. No positive gene candidates were found among the samples, and none of the analyses results revealed a clear intersection with previously reported gene candidates. The pathogenesis of HFM twin pairs does not appear to be related to single nucleotide variants or small insertions/deletions. Thus, HFM may be caused by structure variations, epigenetic alterations, and/or instability of short repeat sequences, which requires further investigation in a larger cohort with sequencing technology for verification.

1. Introduction

Hemifacial microsomia (HFM) is the second most common congenital craniofacial malformation, which is mainly characterized by a hypoplastic mandible and microtia, and may be further complicated by other congenital signs such as facial paralysis, and ocular and vertebral deformities. Since the standard of diagnosis remains controversial, the morbidity as well as related research and clinical results show substantial variation. We have proposed using the severity of HFM and external ear malformations as the minimal diagnostic criteria (Tasse et al., 2005). HFM pathogenesis is still considered to be complex and heterogeneous despite extensive research effort on its etiology. To help better understand the etiology and molecular mechanisms of HFM, we here present the results of the first whole-exome sequencing (WES) analysis for two pairs of monozygotic twins discordant for HFM.

2. Material and Methods

2.1. Subjects

Since establishing the craniomaxillofacial group in the Department of Plastic and Reconstructive Surgery of the Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, we have treated over 500 patients with HFM, including two pairs of monozygotic twins discordant for HFM. This research project was approved by Independent Ethics Committee of Shanghai Ninth People's Hospital affiliated to Shanghai JiaoTong University School of Medicine, and corresponding informed consent forms were signed by all of the subjects' parents.

There was no consanguinity between the parents of these two pairs of twins, and all four parents reported being young and healthy

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at the time of the twins' conception, with no indication of any of the known risk factors for HFM, such as taking retinoid acid (Lammer et al., 1985), thalidomide (Smithells, 1963), primidone (Gustavson and Chen, 1985), or vasoactive drugs (Werler et al., 2004), diabetes (Wang et al., 2002), or smoking (Luquetti et al., 2012). The parents also claimed that all babies were born at full term and had no history of apnea or salvage. The two healthy twins were both normal in terms of appearance and physical examination at birth.

The first set of monozygotic twins (Case 1) involved 10-year-old boys (Fig. 1) born on May 25, 2000, one of whom was born with congenital left-side HFM and microtia, classified as O1M2aE3N0S1, and first sought our medical advice on March 27, 2010. The facial asymmetry became more and more obvious with age and the external ear structures were absent with ankylotia, leaving a sausage-like vestigial structure. Moreover, when the patient opened his mouth, the angle of the mouth and nasal alar on his left side became oblique, tilting upward and outward. The computed tomography (CT) scan revealed obvious hypoplasia of the mandible, especially the ramus part, and the temporomandibular joint was deformed but still functional (Fig. 1C). We hypothesized that the great difference between the bodily forms of the monozygotic twins may be ascribed to the abnormal occluding relationship caused by HFM, thus impeding regular feeding and nutrition intake. Results of other physical examinations and laboratory reports showed normal values.

The second set of monozygotic twins (Case 2) involved 13-year-old girls (Fig. 2) who were born on September 14, 2002, one of whom came to our department for improvement in her appearance on January 6, 2015. She had no normal structural configuration on her left ear and showed mild facial asymmetry, which was classified as O0M1E3N0S0 according to the OMENS classification. The CT scan showed only minimal mandibular hypoplasia with no apparent deviation. The electrocardiogram showed sinus arrhythmia and the hearing test indicated hearing loss on her left side. Other physical examinations such as abdominal and ophthalmological assessments did not show other positive signs, and the results of the biochemical tests were mostly within the normal ranges: red blood cell count, 5.06×10^{12}/L (normal range: $3.8–5.1 \times 10^{12}$/L); white blood cell count, 9.9×10^{9}/L ($3.5–9.5 \times 10^{9}$/L); hemoglobin, 143 g/L (115–150 g/L); platelet count, 333×10^{9}/L ($125–350 \times 10^{9}$/L); alkaline phosphatase, 160 U/L (45–129 U/L); serum albumin, 50 g/L (32–48 g/L).

2.2. DNA extraction

Genomic DNA of the four samples (two pairs of twins) was extracted using DNeasy Blood & Tissue Kit (QIAGEN, GmBH, Germany) following the manufacturer's instructions. Potential RNA contamination was eliminated with RNaseA (QIAGEN). We used a NanoDrop spectrophotometer (Thermo Fisher, Waltham, MA, USA)

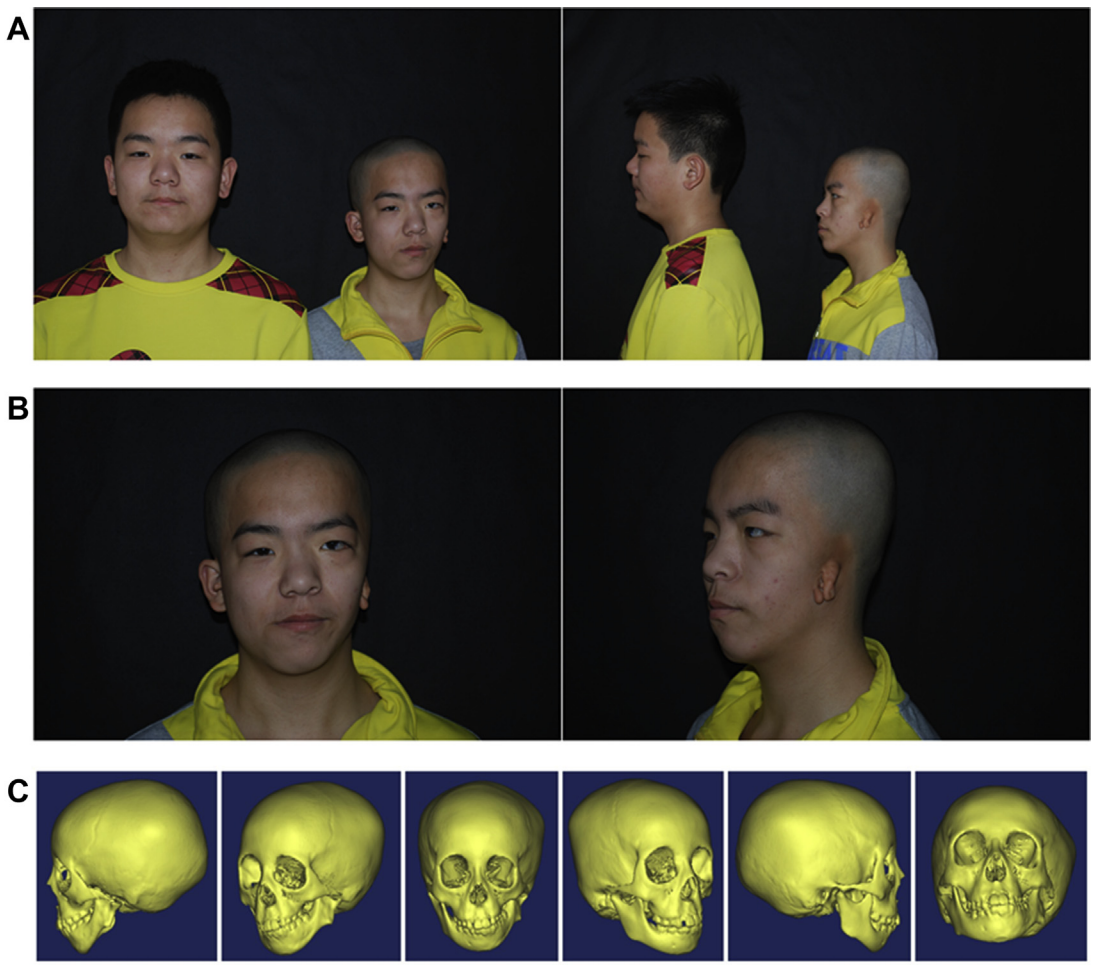

Fig. 1. (A) Monozygotic twins discordant for hemifacial microsomia (HFM) described as Case 1. Left: the healthy twin. Right: the HFM patient. (B) Front view and left-side view of the HFM patient. (C) Three-dimensional computed tomography scan of the HFM patient. Left to right represents the left, left anterior oblique, front, right anterior oblique, right, and upward view, respectively.

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