Targeted Sequencing of Maternal Plasma for Haplotype-based Noninvasive Prenatal Testing of Spinal Muscular Atrophy

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Objective

•To investigate the feasibility study of haplotype-based noninvasive prenatal testing (NIPT) of Spinal Muscular Atrophy (SMA).

Methods

• Five families with pregnancy• and a child affected by spinal muscular atrophy (SMA) were recruited between November 2014 and March 2015. Deletions of exons 7 and/or 8 in the SMN1 gene were identified by multiplex ligation dependent probe amplification (MLPA).Clinical information for the five couples and their proband child are shown in Table 1.For target capture sequencing of genomic and maternal plasma DNA, a custom-designed 221.43-kilobase (kb) NimbleGen EZ array was used, containing a 28-kb coding region of the SMN1 AQ10 gene and 2011 SNPs from 3 megabases (Mb) upstream to 3 Mb downstream of the SMN1 gene.

Results

• Parental haplotypes across the SMN1 gene and flanking region were constructed based on the genotyping information from the mother, father and proband child (Figure 1). The number of informative SNPs on the 221.43-kb target region used for fetal haplotype construction in each family ranged from 95 to 1186. According to parental haplotypes and maternal plasma DNA sequencing data, the PAHP-assisted method identified two normal fetuses (Cases 1 and 5), two carriers (Cases 2 and 3) and one affected fetus (Case 4) (Table 1 and Figure S1). The results were consistent with the standard prenatal diagnosis method using MLPA of AF or CV samples, with no false-positive or -negative result.

• Table S1 may be found in the online version of this article

Conclusions

• NIPT strategy for SMA through targeted sequencing of maternal plasma DNA is accurate and the haplotype-based method should be evaluated systematically in a larger population and may serve as a robust and accurate NIPT for SMA.

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Table 1. Clinical information and molecular diagnosis

Case	Sample ID	Clinical symptoms	MA	GW	MLPA Diagnosis	Pregnancy Outcome
F01	mother	No obvious abnormality	35Y		Hete. DEX7	Natural labor, Healthy female fetus
	father	No obvious abnormality	43Y		Hete. DEX7	
	proband	Type Ⅲ	2Y		Homo DEX7	
	maternal plasma	Singleton live pregnancy, ultrasonic normal		12W+6D	Normal	
	CV	Singleton live pregnancy, ultrasonic normal		12W+6D	Normal	
F02	mother	No obvious abnormality	23Y		Hete. DEX7-8	Natural labor, Healthy male fetus
	father	No obvious abnormality	27Y		Hete. DEX7-8	
	proband	Type Ⅱ	8M		Homo DEX7-8	
	maternal plasma	Singleton live pregnancy, ultrasonic normal		12W+5D	Hete. DEX7-8	
	AF	Singleton live pregnancy, ultrasonic normal		12W+5D	Hete. DEX7-8	
F03	mother	No obvious abnormality	32Y		Hete. DEX7-8	Cesarean section Healthy male fetus
	father	No obvious abnormality	-		Hete. DEX7-8	
	proband	Type Ⅱ	5Y		Homo DEX7-8	
	maternal plasma	Singleton live pregnancy, ultrasonic normal		13W+1D	Hete. DEX7-8	
	CV	Singleton live pregnancy, ultrasonic normal		13W+1D		
F04	mother	No obvious abnormality	32Y		Hete. DEX7-8	Termination of Pregnancy
	father	No obvious abnormality			Hete. DEX7-8	
	proband	Type Ⅲ	4Y		Homo DEX7-8	
	maternal plasma	Singleton live pregnancy, ultrasonic normal		13W+5D	Homo DEX7-8	
	AF	Singleton live pregnancy, ultrasonic normal		13W+6D	Homo DEX7-8	
F05	mother	No obvious abnormality	26Y		Hete. DEX7-8	Cesarean section Healthy male fetus
	father	No obvious abnormality			Hete. DEX7-8	
	proband	Type Ⅲ	1Y		Homo DEX7-8	
	maternal plasma	Singleton live pregnancy, ultrasonic normal		12W	Normal	
	AF	Singleton live pregnancy, ultrasonic normal		12W+2D	Normal