Droplet Digital PCR Techniques to detect R201 mutations in Mccune-Albright Syndrome

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Background

MAS is a rare disorder, this syndrome is classically characterized by a triad of physical signs: cafe-au-lait skin pigmentation(SP) , fibrous bone dysplasia（FD）, peripheral precocious puberty(PPP). In children, the most frequent initial presentation of MAS is PPP.MAS is caused by postzygotic activating mutations at the R201 codon of the GNAS gene, leading to a state of somatic mosaicium. In MAS patients, the frequency of mutations is expected to be generally low in clinically unaffected tissues such as peripheral blood leukocytes (PBL). Our aim to improve the mutation detection rate and quantify the presence of R201 GNAS mutations in PBL of MAS patients.

Methods

Patients: 73 girls with MAS and 30 PT as controls. 14 girls with triad signs,46 girls with 2 signs,13 girls with vaginal bleeding with ovarian cysts.
PAP and ddPCR techniques were used to search for R201 mutations in the DNA of blood from 73 MAS patients and 30 PT girls. The ability of ddPCR to provide quantitative data was tested in the serial dilution of wild type,R201H, R201C cloned peripheral blood leukocytes.00

Result

Compared with the PAP results, the results of ddPCR were compared（table1）. The results showed that the analysis results of GNAS gene were obviously superior to the PAP method, especially for the non classic MAS, which was very difficult to be diagnosed clinically (77% and 7.69%). Patients' clinical characteristics are summarized in table 2.

Table1 Comparison of two detection methods

	ddPCR	PAP
（n）	73/30	73/30
positive（n）	56/0	21/0
negative（n）	17/30	52/30
percent	76.71%	28.76%
Triad MAS	14(100%)	7(50.00%)
2 signs	32(69.5%)	13(28.2%)
1 sign	10(76.9%)	1(7.69%)

Table2 Clinical characteristics of 73 girls with MAS and 30 controls

groups	N	Newly diagnosis age(y)	LH (mIU/ml)		FSH (mIU/ml)		E2 （pg/ml）	Uterine volume (ml)	Ovarian volume (ml)	Ovarian cyst volume(ml)	BA-CA(y)
			baseline	peak	baseline	peak					
Triad MAS	14	2.76±2.02	0.08±0.03	0.31±0.38	0.96±0.97	2.61±2.58	48.25±12.4	4.4±4.1	1.8±2.5	9.0±6.7	1.63±2.88
2signsMAS	46	4.21±2.69	0.23±0.98	1.16±3.65	1.05±9.45	3.15±8.04	45.88±20.8	4.6±5.0	2.6±2.1	7.6±7.5	1.29±2.58
1sign MAS	13	4.55±2.96	0.15±2.17	0.59±2.54	1.14±11.87	2.95±7.18	50.5±20.6	4.6±3.6	2.2±2.0	7.0±8.5	0.97±1.64
PT	30	6.60±1.06	0.10±0.08	2.44±0.27	2.08±1.17	11.95±2.76	9.90±0.10	0.89±0.22	0.85±0.39	-	0.21±0.75

Conclusion

ddPCR techniques to the clinical screening of MAS molecular defects for the first time, and its efficiency is far higher than that of ordinary PCR and PAP. ddPCR is close to positive rate of 76.7%, so it is currently in MAS.