McCune Albright Syndrome: new insights into the molecular diagnostic

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The McCune-Albright syndrome (MAS) is a rare paediatric disorder, characterized by the classical triad of polyostotic fibrous dysplasia, skin hyperpigmentation and endocrine disorders, including peripheral precocious puberty (PPP), hyperthyroidism, acromegaly and hyperprolactinemia.[1,2]

The MAS is due to a postzygotic activating mutation of the *GNAS* gene, that encodes for the alpha subunit of Gs protein (Gsα, Figure 1), occurring in the early embryonic development, leading to a mosaic distribution of the lesions. Because the mutation is often mild or absent in the blood, the mosaic distribution of the *GNAS* mutations in MAS is a major impediment to the molecular diagnostic, that often needs a bone biopsy, despite the development of sensitive methods with enrichment as Nested-PCR and PNA clamping.[3]

Consequently the diagnosis is essentially clinical, but the time lag between first feature (often PPP) and next leads to an important delay in diagnosis.[4]

The development of a sensitive and non-invasive test represents a significant challenge. The emergence of the liquid biopsy concept and the detection of somatic mutation in cell-free circulating DNA (cfDNA) from patients presented with cancer give new tracks for molecular diagnosis of MAS.[5]

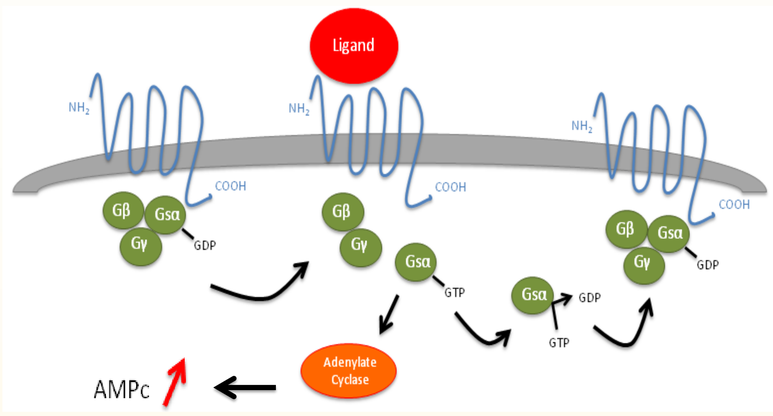

Figure 1: Signal transduction via the protein Gs.
The activating mutations of *GNAS*, are substitutions at arginine 201 codon or rarely glutamine 227 codon that constitutively activate Gsα by deleting the GTPase activity.

To avoid the pitfall of molecular diagnosis we develop the targeted screening of the two most common mutations of *GNAS*: R201C and R201H, in whole blood DNA by digital droplet PCR (ddPCR), a breakthrough technology of ultrasensitive quantitative PCR.
Second we made the assumption that cfDNA from mutated tissues should be detected in DNA extracted from plasma of MAS patients and test this hypothesis using ddPCRs.

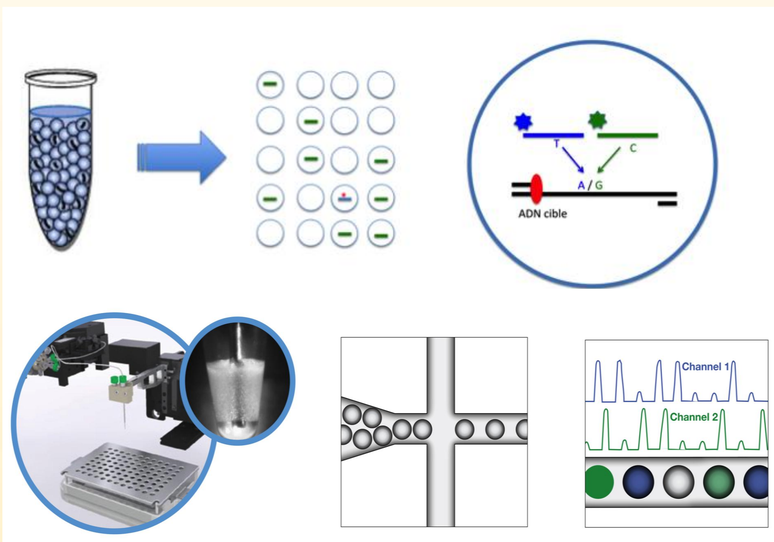

Figure 2: principle of ddPCR (BioRad, commercial documentation)
The DNA inputs are emulsified into 20,000 oil droplets, containing probes, primers and polymerase, transferred in a PCR plate and amplified by PCR following the manufacturer' instructions. Fluorescence is analysed by flow cytometry on the X100™ Droplet Digital™ PCR System (Biorad).

Material and Methods:

A retrospective study was first performed together with the laboratory of hormonology of the Hospital Lapeyronie (Montpellier, France) on multiple DNA specimens from normal and SMA patients previously analysed by Nested-PCR to validate the method. All specimens from patients suspected of having a MAS addressed in the laboratory were included. All patients or their parents have signed consent for genetic testing. DdPCR is performed using ddPCR™ Supermix for Probes (No dUTP) (Biorad) and PrimerPCR™ ddPCR™ Mutation Assays (Biorad) were used for both mutations analysis. Each assay comprises a wild-type (WT) probe, HEX-labelled, and a mutated probe 6-FAM-labelled (Figure 2 and 3).

Results:

Analytic tests and comparative studies show a specificity of 100% without false positive and sensitivity superior to the Nested-PCR method (data not shown).
Using ddPCR we simultaneously analysed 10 000 pieces of *GNAS* and detected very low rate of mutations (until 1 mutated copies/4000, patient 7).
We detected a *GNAS* mutation in 42,9% of patients (6/14) (Table 1), showing the superiority of ddPCR to PNA clamping and Nested-PCR (Table 2)
We detected a R201C mutation in whole blood DNA of a young girl (7) with PPP and GH excess. This results was confirmed on a second sample (2 years away), demonstrating a good stability of this marker.

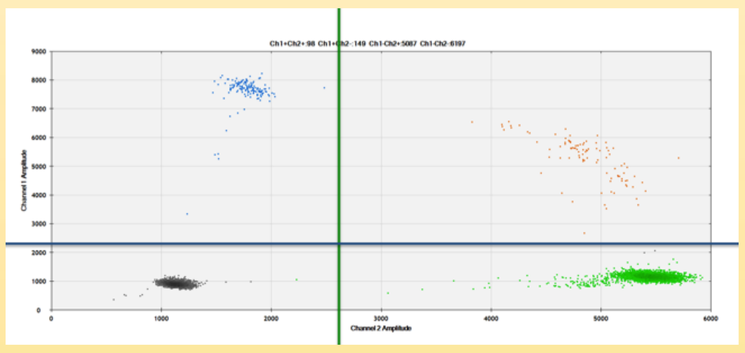

Figure 3:Raw data of ddPCR for a R201C mutated patient analyzed on QuantaSoft software(Biorad).
Right lines represent thershold. Each dot represent a droplet. Black dot: droplet not containing DNA inside. Green dot: droplet containing WT DNA, Blue dot: droplet containing mutated DNA, brown dot: droplet containing both mutated and WT DNA. The DNA distribution, following a Poisson distribution, allow to calculate the number of WT and mutated copies of *GNAS*.

Patient	nbr of SMA triad signs	R201C mutation		R201H mutation	
		WT copies	mutated copies	WT copies	mutated copies
1	3	10220	0	16080	0
2	3	5600	0	2660	0
3	2	131400	0	14480	4
4	3	34960	1680		
5	2	7460	0	8480	0
6	3	6020	0		
7	1	16380	4		
8	3	4240	252	4600	0
9	3	14285	0	7885	7
10	3	5283	1684	12794	0
11	3	15504	0	12821	0
7 bis	1	17060	16	4584	0
12	1	14969	0	7332	0
13	1	13906	0	10754	0
14	3	20163	0	14646	0

Table 1: Research of R201C and R201H mutations of GNAS in whole blood DNA of 14 patients.

blood DNA		
PNA*	Nested-PCR*	ddPCR
35,2% (25/71)	36,6% (26/71)	42,8% (6/14)

Table 2: Comparison between ddPCR, Nest-PCR and PNA clamping for molecular diagnostic of MAS. *Kalfa et al., EJE, 2006

Second we were able to detect R201C mutation in cfDNA extracted from plasma of two patients whereas the detection was negative in DNA extracted from blood (Table 3), increasing the sensibility of the method (Table 4).

Specimens	nbr of SMA triad signs	Mutation R201C	
		WT copies	Mutated copies
6 - blood	3	6020	0
6 - skin	3	32560	2
6 - cfDNA	3	84	1
12 - cfDNA	1	136	0
14 - cfDNA	3	164	2

Table 3: Results of ddPCR R201C test for cfDNA and tissue DNA of patients 6, 12 and 14.

Patients with 3 signs		
blood DNA	cfDNA	blood + cfDNA
44% (4/9)	100% (2/2)	67% (6/9)

Table 4: Percentage of patients presented with 3 signs of MAS having identified R201C mutation by ddPCR

Conclusion:

Here we demonstrate the relevance of targeted screening of GNAS activating mutations by ddPCR for molecular diagnostic of MAS.
Second we show that the somatic mutated cells of MAS patients release DNA in blood flow.
This mutated cfDNA is detectable by ddPCR, increasing the sensibility of the method.
DdPCR presents considerable benefits in terms of sensibility, acceptability and cost-effectiveness.

References: [1] Albright et al., NEJM, 1937 ; [2] McCune, Am J Dis Child, 1936 ; [3] Kalfa et al, EJE, 2006 ; [5] Agopiantz et al., Ann Endoc, 2016, [6] Re et al, Oncotarget 2016.