Brief Report

Whole-Blood miRNA Sequencing Profiling for Vasospasm in Patients With Aneurysmal Subarachnoid Hemorrhage

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Background and Purpose—Arterial vasospasm is a well-known delayed complication of aneurysmal subarachnoid hemorrhage (aSAH). However, no validated biomarker exists to help clinicians discriminating patients with aSAH who will develop vasospasm (VSP^+) and identifying those who then deserve aggressive preventive therapy. We hypothesized that whole-blood miRNAs could be a source of candidate biomarkers for vasospasm.

Methods—Using a next-generation sequencing approach, we performed whole-blood miRNA profiling between VSP^+ patients with aSAH and patients who did not develop vasospasm (VSP^-) in a prospective cohort of 32 patients. Profiling was performed on the admission day and 3 days before vasospasm.

Results—Four hundred forty-two miRNAs were highly expressed in whole blood of patients with aSAH. Among them, hsa-miR-3177-3p demonstrated significant ($P=5.9\times10^{-5}$; $P_{\text{Bonferroni corrected}}=0.03$) lower levels in VSP^- compared with VSP^+ patients. Looking for whole-blood mRNA correlates of hsa-miR-3177-3p, we observed some evidence that the decrease in hsa-miR-3177-3p levels after aSAH was associated with an increase in *LDHA* mRNA levels in VSP^- ($P<10^{-3}$) but not in VSP^+ ($P=0.66$) patients.

Conclusions—Whole-blood miRNA levels of hsa-miR-3177-3p could serve as a biomarker for vasospasm.

Clinical Trial Registration—URL: https://www.clinicaltrials.gov. Unique identifier: NCT01779713.

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Key Words: biomarkers ■ humans ■ microRNAs ■ prospective studies ■ vasospasm, intracranial

Intracranial aneurysm rupture is most frequently responsible for aneurysmal subarachnoid hemorrhage (aSAH), leading to a true cerebral aggression responsible for neurological insults but also impacting on many other organism's functions.[1] One of the more dreadful aSAH complications is the occurrence of cerebral vasospasm. Cerebral vasospasm consists in a thickening and temporary contraction of an artery vessel occurring in 30% of patients with aSAH, on average between 4 and 12 days after the bleeding. This contraction may lead to hypoxia, which may in turn lead to severe neurological sequela.

Although diagnostic markers have been proposed,[1,2] there are to date no validated biomarkers that can help discriminating patients with aSAH who will develop vasospasm (VSP^+) from those who will not (VSP^-). Any patient admitted in neurointensive care units for an aSAH usually undergoes an aggressive preventive treatment, consisting in an invasive monitoring and administration of a vasodilator drug, the nimodipine,[3] that is associated with severe side effects, such as cerebral and pulmonary edema.[4]

Hypothesizing that whole-blood miRNAs could be a suitable source of candidate biomarkers for vasospasm, we report here the result of the first whole-blood next-generation sequencing miRNA profiling in a cohort of 32 patients with aSAH prospectively followed for cerebral vasospasm.

Materials and Methods

VASOGENE study was registered on ClinicalTrials with the unique identifier NCT01779713. miRNA data described in this work are available in the European Genome-Phenome Archive platform under the acronym access code VASOGENE.

VASOGENE Study

The VASOGENE study was approved by its local ethics committees (Commission Nationale de l'informatique et des Libertés [CNIL] and Comité Consultatif sur le Traitement de l'Information en Matière

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de Recherche dans le Domaine de la Santé [CCTIRS]), and all VASOGENE participants provided informed written consent.

The VASOGENE cohort is composed of 89 patients with aSAH recruited from January 2013 to December 2016 at the neurointensive care unit of Pitié-Salpêtrière Hospital (Paris, France). Participants were patients with aSAH hospitalized in the 48 hours after the aneurysm rupture and treated in the first 96 hours by embolization or surgery. All patients were French individuals, excluding blacks, Hispanics, and Asians, aged ≥18 years. Patients were followed in the neurointensive care unit for at least 12 days. Each day, a transcranial Doppler sonography was performed to diagnose vasospasm. When transcranial Doppler was equivocal or for patients with poor temporal window, a digital subtraction angiography was performed to confirm the suspicion of vasospasm. For all patients with aSAH, a blood sample was collected daily from the entry in the neurointensive care unit till day 12.

mRNA/miRNA Substudy

The present study deals with a subsample of the whole VASOGENE cohort composed of 16 VSP$^+$ patients retrospectively matched to 16 patients with aSAH who did not develop vasospasm after 12 days (VSP$^-$), matching being performed as much as possible for age, sex, and hemorrhage severity. For these 16 VSP$^+$/VSP$^-$ pairs, we analyzed miRNA/mRNA levels on whole-blood samples collected at the admission day (D_0) and 3 days (D_{v3}) before the day VSP$^+$ patients developed vasospasm (or the corresponding day for their matched VSP$^-$ patients). Detailed description of the genome-wide gene and miRNA expression profiling is given in the online-only Data Supplement. The design of this study is summarized in Figure I in the online-only Data Supplement.

Statistical Association Analyses

Association between miRNA abundance and vasospasm was tested using a linear mixed model adjusted for age and sex (Methods in the online-only Data Supplement). A Bonferroni correction was applied to identify significant associations. miRNAs found significantly associated with the risk of vasospasm in the miRNA sequencing analysis were requantified by reverse transcription-quantitative polymerase chain reaction for technical validation of the results (online-only Data Supplement). Similar linear models were used to identify candidate mRNA correlates of significant miRNAs (Methods in the online-only Data Supplement).

Results

Clinical characteristics of the VASOGENE and of the miRNA substudy populations are shown in the Table.

In total, 1512 known mature miRNAs were detected among which only 442 were considered as expressed and tested for association with vasospasm. Full association results are summarized in the Q-Q plot shown in Figure II in the online-only Data Supplement and listed in Table I in the online-only Data Supplement. One miRNA, hsa-miR-3177-3p, was significantly (P=5.9×10^{-5}; $P_{\text{Bonferroni corrected}}$=0.03) associated with the risk of vasospasm, with higher level in VSP$^+$ than in VSP$^-$ patients (6.20±0.47 versus 5.62±0.61; Figure 1). Using reverse transcription-quantitative polymerase chain reaction measurements, the significant association of hsa-miR-3177-3p with vasospasm was confirmed (P=0.03; Figure III in the online-only Data Supplement). Looking deeply to these results revealed that hsa-miR-3177-3p levels slightly decreased between D_0 and D_{v3} in VSP$^-$ (5.89 versus 5.41; P=0.037), whereas no change was observed in VSP$^+$ patients (6.20 versus 6.18; P=0.63; Figure 1).

We then scanned for mRNA expressions that could associate with hsa-miR-3177-3p levels. No single association

Table. VASOGENE Cohort

	Whole Study			Ancillary miRNA Study		
	VSP$^+$	VSP$^-$		VSP$^+$	VSP$^-$	
	n=32	n=57	*P* Value*	n=16	n=16	*P* Value*
Age, y	49.53 (10.06)	55.33 (12.00)	0.01	49.19 (10.98)	51.62 (12.70)	0.57
Female sex (%)	21 (65.63%)	39 (68.42%)	0.70	11 (68.75%)	11 (68.75%)	1.0
Smoker (%)	22 (68.75%)	28 (49.12%)	0.14	11 (68.75%)	9 (56.25%)	0.72
Fisher grade			0.16			0.11
1	2	9		2	1	
2	6	11		0	5	
3	7	6		4	3	
4	16	27		10	7	
5	1	4		0	0	
WFNS score			0.02			0.13
1	13	23		6	10	
2	14	10		8	3	
3	0	4		0	2	
4	5	13		2	1	
5	0	7		0	0	
GCS>13	26	33	0.08	13	12	0.06

Shown data: mean (SD) for quantitative variable and count (%) for qualitative variable. GCS indicates Glasgow coma scale; VSP+, vasospasm positive; VSP–, vasospasm negative; and WFNS, World Federation of Neurological Surgeons.

*Association test *P* value derived from ANOVA and χ^2 test statistics for quantitative and qualitative data, respectively.

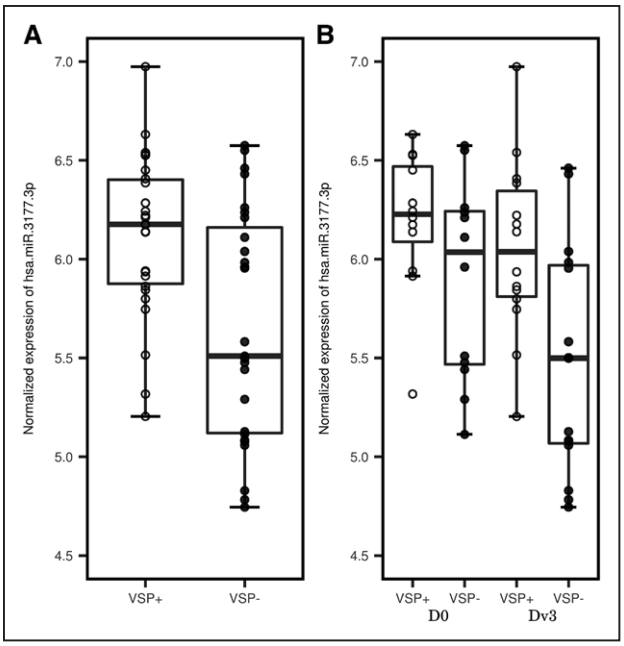

Figure 1. Whole-blood expression of hsa-miR-3177-3p in patients with aneurysmal subarachnoid hemorrhage with vasospasm (VSP$^+$) and without vasospasm (VSP$^-$) in the whole VASOGENE cohort (**A**) and separately at D_0 and D_{v3} (**B**).

reached the Bonferroni threshold of 2.3×10^{-6} (Table II in the online-only Data Supplement). However, among the 3 loci that exhibited suggestive statistical ($P<10^{-4}$) correlation with hsa-miR-3177-3p levels (Methods in the online-only Data Supplement), *LOC100506532* (ρ=0.45; $P=4.15\times10^{-5}$), *Mucin 1* (ρ=0.34; $P=4.76\times10^{-5}$), and *LDHA* (ρ=–0.38; $P=8.7\times10^{-5}$), we observed that the correlation between the mean difference of hsa-miR-3177-3p and the mean difference of *LDHA* mRNA was much stronger in VSP$^-$ (ρ=–0.81; P=0.001) than in VSP$^+$ (ρ=–0.14; P=0.657; Figure 2). Following an opposite pattern to that observed for hsa-miR-3177-3p, *LDHA* mRNA levels were rather constant between D_0 and D_{v3} (7.35±0.02 versus 7.36±0.06; P=0.69) in VSP$^+$ but slightly increased over time in VSP$^-$ (7.33±0.04 versus 7.36±0.05; P=0.12; Figure IV in the online-only Data Supplement).

We also sought for miRNAs whose mean expression difference between D_0 and D_{v3} could differ according to the vasospasm status but did not observe any miRNA that achieved statistical significance (Table III in the online-only Data Supplement).

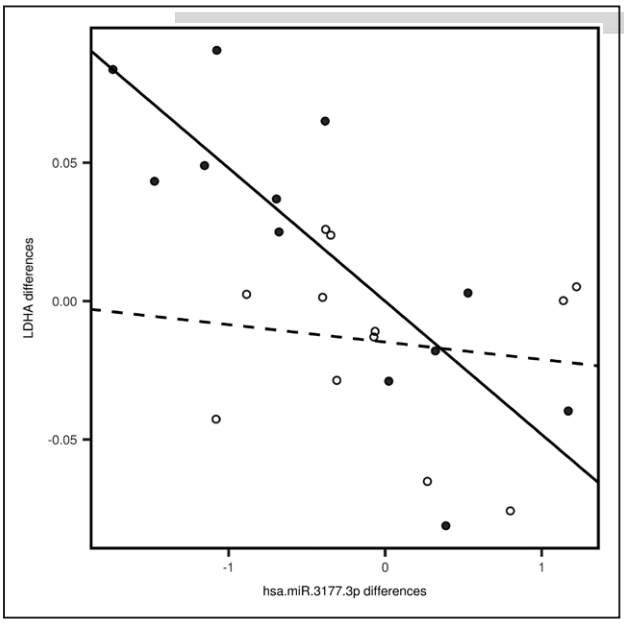

Figure 2. Correlation between changes in hsa-miR-3177-3p and in *LDHA* mRNA over time in whole-blood samples of patients with aneurysmal subarachnoid hemorrhage with vasospasm (VSP$^+$) and without vasospasm (VSP$^-$).

Discussion

We here deployed a next-generation sequencing approach to identify candidate miRNAs associated with vasospasm in whole-blood samples of patients with aSAH followed prospectively for vasospasm. To our knowledge, this is the first study using such integrative approach in the context of cerebral vasospasm and the largest cohort of patients with aSAH prospectively followed for vasospasm and studied for miRNAs and mRNAs.

This study revealed that increased hsa-miR-3177-3p levels were associated with vasospasm risk in patients with aSAH. Little is known about hsa-miR-3177-3p except it is highly expressed in the brain and cerebellum.[5] We also observed that this increase in hsa-miR-3177-3p levels was accompanied with a decrease in *LDHA* gene expression. Several works support the role of *LDHA*, which is also highly expressed in the brain,[6] as a good candidate for vasospasm. *LDHA* mRNA and protein levels have been shown to be modulated after cerebral artery occlusion in rats.[7] *LDHA* expression in brain microvascular endothelial cells was demonstrated to be influenced by hypoxia[8]—a key regulatory mechanism involved in vasospasm.[9] Finally, genetic variations at the *LDHA* locus have been reported to associate[10] with plasma concentrations of acute-phase serum amyloid A—an inflammatory marker known to be associated with cerebral disorders.[11,12]

Despite being supported by strong statistical and biological evidences, our results suffer from some limitations. The size of our cohort, despite the largest involved to date in a miRNA/mRNA study for vasospasm, is still relatively modest and our cohort limited to patients of European ancestry. Even if an association between miRNA and vasospasm reached statistical significance after multiple testing correction, we cannot exclude that we missed additional miRNA associations because of small sample size and power issues. Second, we do not provide replication of our main statistical findings in an independent cohort—a mandatory step to propose elevated hsa-miR-3177-3p levels in whole blood as a biomarker for vasospasm and to validate the association between hsa-miR-3177-3p and *LDHA*. Besides, further experimental works would be needed to investigate whether the observed association between hsa-miR-3177-3p levels and *LDHA* mRNA levels reflects a direct physical interaction between hsa-miR-3177-3p and one of its target genes or whether it involves an additional intermediate partner that remains to be identified. But this is out of the scope of the present epidemiological work.

Summary

We identified elevated hsa-miR-3177-3p levels in whole blood as candidate marker for the risk of vasospasm in patients with aSAH.

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Disclosures

Dr Clarençon is a consultant at or reports an advisory relationship with Balt, Medtronic, and Penumbra. The other authors report no conflicts.

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