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Table 1; Figure 2

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Abstract

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 aSAH patients that will develop vasospasm 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 aSAH patients developing vasospasm (VSP^+) and patients that did not (VSP^-) in a prospective cohort of 32 patients. Profiling was performed on the admission day and three days before vasospasm.

Results

442 miRNAs were highly expressed in whole blood of aSAH patients. Among them, hsa-miR-3177-3p demonstrated significant ($p = 5.9x10^{-5}$, $p_{Bonferroni\text{-}corrected} = 0.03$) lower levels in VSP^- compared to 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 *Lactate Dehydrogenase A* 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://clinicaltrials.gov. Unique Identifier: NCT01779713.

Intracranial aneurysm rupture is most frequently responsible for subarachnoid hemorrhage (aSAH), leading to a true cerebral aggression responsible for neurological insults but also impacting on many other organism's fucntions.[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 aSAH patients, on average between 4 and 12 days following the bleeding. This contraction may lead to hypoxia, which may in turn lead to severe neurological sequela.

While diagnostic markers have been proposed,[1,2] there is so far no validated biomarkers that can help discriminating aSAH patients that will develop vasospasm from those who will not. Any patient admitted in Neuro-Intensive 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 oedema.[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 aSAH patients 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 EGA platform under the acronym access code VASOGENE.

VASOGENE study

The VASOGENE study was approved by its local ethics committees (CNIL, CCTIRS) and all VASOGENE participants provided informed written consent.

The VASOGENE cohort is composed of 89 aSAH patients recruited from January 2013 to December 2016 at the Neuro-intensive care unit (nICU) of Pitié-Salpêtrière Hospital (Paris, France). Participants were aSAH patients hospitalized in the 48 hours following 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 or more. Patients were followed in the nICU for at least 12 days. Each day, a transcranial Doppler (TCD) sonography was performed to diagnose vasospasm. When TCD was equivocal or for patients with poor temporal window, a digital subtraction angiography (DSA) was performed to confirm the suspicion of vasospasm (VSP^+). For all aSAH patients, a blood sample was collected daily from the entry in the nICU 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 aSAH patients 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 three 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 Supplements. The design of this study is summarized in Supplemental Figure I.

Statistical association analyses

Association between miRNA abundance and vasospasm was tested using a linear mixed model adjusted for age and sex (Supplemental Methods). A Bonferroni correction was applied to identify significant associations. miRNAs found significantly associated with the risk of vasospasm in the miRNA sequencing analysis were re-quantified by RT-qPCR for technical validation of the results (Supplemental). Similar linear models were used to identify candidate mRNA correlates of significant miRNAs (Supplemental Methods).

Results

Clinical characteristics of the VASOGENE and of the miRNA substudy populations are shown in Table 1.

In total, 1,512 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 Quantile-Quantile plot shown in Supplementary Figure II and listed in Supplementary Table I. One miRNA, hsa-miR-3177-3p, was significantly ($p = 5.9x10^{-5}$, $p_{Bonferroni\text{-}corrected} = 0.03$) associated with the risk of vasospasm, with higher level in VSP^+ than in VSP^- patients (6.20 ± 0.47 *vs* 5.62 ± 0.61) (Figure 1). Using RT-qPCR measurements, the significant association of hsa-miR-3177-3p with vasospasm was confirmed ($p = 0.03$) (Supplementary Figure III). Looking deeply to these results revealed that hsa-miR-3177-3p levels slightly decreased between D_0 and D_{v3} in VSP^- (5.89 *vs* 5.41, $p = 0.037$) while no change was observed in VSP^+ patients (6.20 *vs* 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 reached the Bonferroni threshold of $2.3x10^{-6}$ (Supplementary Table II). However,

among the 3 loci that exhibited suggestive statistical ($p < 10^{-4}$) correlation with hsa-miR-3177-3p levels (Supplemental Methods), *LOC100506532* (ρ=0.45, $p = 4.15 \times 10^{-5}$), *Mucin 1*(ρ=0.34, $p = 4.76 \times 10^{-5}$), *Lactate dehydrogenase A* (*LDHA*) (ρ=-0.38, $p = 8.7 \times 10^{-5}$), 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 *vs* 7.36±0.06, p = 0.69) in VSP^+, but slightly increased over time in VSP^- (7.33±0.04 *vs* 7.36±0.05, p = 0.12) (Supplementary Figure IV).

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 achieve statistical significance (Supplementary Table III).

Discussion

We here deployed a next-generation-sequencing approach to identify candidate miRNAs associated with vasospasm in whole blood samples of aSAH patients 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 aSAH patients 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 aSAH patients. 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 vasospam.[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 so far 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 due to small sample size and power issues. Second, we do not provide replication of our main statistical findings in an independent cohort, a mandatory step in order 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 remain 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 aSAH patients.

Disclosures: none

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Table 1 Description of the VASOGENE cohort

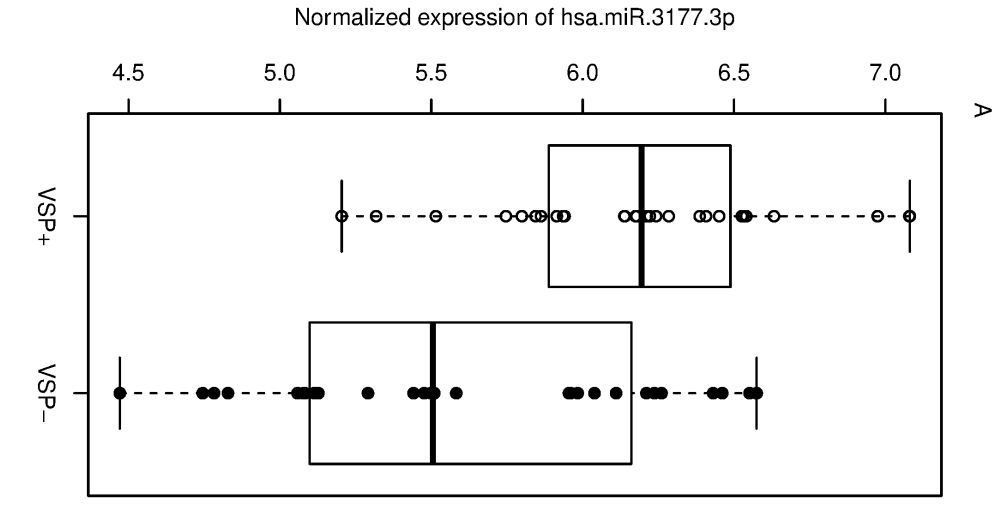
Figure 1 Whole blood expression of hsa-miR-3177-3p in aSAH patients with (VSP^+) and without (VSP^-) vasospasm. In the whole VASOGENE cohort (A) and separately at D_0 and D_{v3} (B)

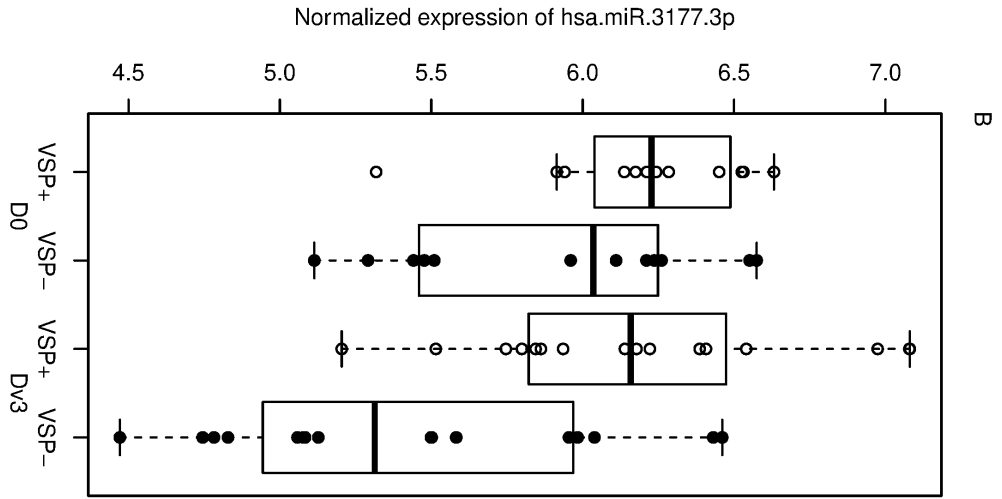
Figure 2 Correlation between changes in hsa-miR-3177-3p and in *LDHA* mRNA over time in whole blood samples of aSAH patients with (VSP^+) and without (VSP^-) vasospasm.

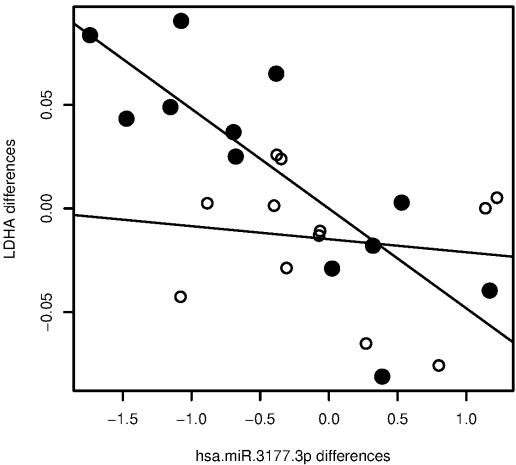
Table 1: VASOGENE Cohort

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

[1]Association test P-value derived from ANOVA and Chi-square test statistics for quantitative and qualitative data, respectively.
Shown data: mean (SD) for quantitative variable and count (%) for qualitative variable.
[2]World Federation of Neurological Surgeons score
[3]Glasgow Coma Scale


Normalized expression of hsa.miR.3177.3p
4.5
5.0
5.5
6.0
6.5
7.0
A
VSP+
VSP−


Normalized expression of hsa.miR.3177.3p
4.5
5.0
5.5
6.0
6.5
7.0
B
D0
VSP+
VSP−
Dv3
VSP+
VSP−


LDHA differences
0.05
0.00
−0.05
−1.5
−1.0
−0.5
0.0
0.5
1.0
hsa.miR.3177.3p differences