Gene expression profiles of recurrent acute pancreatitis risk in patients with sustained chylomicronemia

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Abstract. A fasting triglyceridemia >10 mmol/L is associated with chylomicronemia (CM) and an increased recurrent acute pancreatitis (RAP) risk. The number of pancreatitis episodes varies significantly between patients with CM. The objective of this study was to investigate gene expression profiles of RAP in patients with CM. A total of 47 CM subjects participated in this study. Prior to the analyses, all patients were divided into three groups covering a wide spectrum of RAP: 0 (n = 21), 1–3 (n = 10) or >4 (n = 16) pancreatitis episodes. Gene expression profiles were compared to those of 15 healthy normolipidemic controls. Differential expression moderated T-tests between studied groups were performed using a linear model of the Bioconductor package Limma. The False discovery rate was controlled using the Benjamini-Hochberg procedure. At a p-value <0.01, a false discovery rate of 5% and a >2-fold change expression significance levels, a set of 41 probes have been found differentially expressed in CM subjects with no pancreatitis, 103 in the CM group with 1 to 3 pancreatitis, and 94 in the group with ≥4 pancreatitis compared to healthy controls. Of the identified annotated probes, 14 are shared by all CM groups; 3 are specific to CM with no pancreatitis; 11 are specific to CM with 1 to 3 pancreatitis, and 17 are specific to CM with ≥4 pancreatitis. Most of the annotated biomarkers are involved in inflammatory, immune, lipoprotein kinetics or signalling biological pathways. These results reveal gene expression signatures of RAP in patients with CM.

Key words: Recurrent acute pancreatitis, Gene expression, Chylomicronemia

CHYLOMICRONS are large triglyceride (TG)-rich lipoproteins produced in the gut wall following a meal and usually rapidly cleared from the bloodstream through TG hydrolysis by the lipoprotein lipase. A defective or otherwise saturated lipoprotein lipase will lead to an abnormal accumulation of chylomicrons, even observed at the fasting state, a rare condition called chylomicronemia (CM) [1, 2]. CM is therefore associated with important increases in TG levels and fasting TG >10 mmol/L is a clinical indication of its presence [3]. For the most, CM is a multifactorial disorder, most often polygenic, in which severe hypertriglyceridemia (TG >5 mmol/L) persists and chylomicronemia (TG >10 mmol/L) recurrently reappears despite tight control of treatable secondary causes. In some rarer cases, termed familial chylomicronemia syndrome, CM will be of primary origin, most often monogenic, due to null loss-of-function mutations in lipoprotein lipase or in genes encoding interacting factors, such as apolipoprotein C2, apolipoprotein A5, lipase maturation factor 1 and glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 [1, 3, 4]. For these patients, clinical management is far more complex and fasting TG levels very rarely go under 10 mmol/L.

CM is associated with an increased risk of acute pancreatitis (AP), which is certainly one of its most serious clinical outcomes, regardless of its causes, although patients with familial chylomicronemia syndrome are at greater risk than those with multifactorial CM [5, 6]. The underlying pathophysiology of CM-associated AP, however, is not completely understood. Although TG levels are closely related to its severity [7], its expression does not seem to be explained only by this variable. There are important inter-individual variations in AP incidence and severity; while some patients will experience several recurrent AP (RAP), others with apparently a more severe disorder will either have no episode or only one or a few throughout their lifetime [5]. Some risk modulators have been identified, but we still do not have the complete picture [8, 9]. The objective of this study was to document gene expression profiles of RAP in patients.
with CM and to identify biomarkers potentially involved in its pathophysiology.

**Material and Methods**

**Subjects and clinical data**

This study comprised a sample of 62 French-Canadian subjects from the Saguenay–Lac-Saint-Jean founder population (Quebec, Canada), including 15 healthy normolipidemic control subjects and 47 patients with sustained CM. Occurrence of AP caused by chylomicronemia was documented using questionnaires and patient’s medical charts, applying the Atlanta classification criteria [10]. AP due to other causes than chylomicronemia were excluded. CM patients were divided according to the past occurrence of AP: 0 (n = 21), 1–3 (n = 10) and ≥4 (n = 16). We expect that the comparison of patients with no pancreatitis to those with several pancreatitis will help us to more easily identify potential risk/protection factors. Subjects gave their informed consent to participate in this study and were assigned a code that systematically de-identifies all clinical data [11]. This study, which was conducted as part of a research program on the natural history of severe hypertriglyceridemia (SMASH: Systems and Molecular Approaches of Severe Hyperlipidemias), was approved by IRB Services (now Advarra) and was conducted in accordance with the Declaration of Helsinki.

**Biochemical and gene expression analyses**

Blood samples were obtained after a 12-hour overnight fast from the antecubital vein into serum separation tubes and PAXgene RNA tubes (Qiagen, Valencia, CA, USA). Cholesterol and TG were measured by enzymatic assays on a CX7Analyser (Beckman, Fullerton, CA, USA). Total cholesterol was determined in plasma and high-density lipoprotein (HDL) after precipitation of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) in the infranatant. Plasma LDL-cholesterol level was estimated using the Friedewald formula [13] or direct measurement when TG level was >4.5 mmol/L. Gene expression analyses were conducted on RNA using Affymetrix Human Gene ST 2.0 microarrays (Santa Clara, CA, USA). Robust multi-array average was applied to raw intensities [14].

**Statistical analysis**

Categorical variables were compared using the Pearson χ² or Fisher’s exact tests, whereas continuous variables were compared with one-way ANOVA, using log₁₀-transformed data, followed by Bonferroni post hoc tests, or Kruskal-Wallis analyses followed by Mann-Whitney U-tests for variables staying non-normally distributed after transformation. Differential expression moderated T-tests between studied groups were performed using a linear model of the Bioconductor package Limma. The false discovery rate was controlled using the Benjamini-Hochberg procedure [15]. Data were analyzed using QIAGEN’s Ingenuity® Pathway Analysis (IPA®, Redwood City, CA USA) and SPSS package (IBM SPSS Statistics, Version 25.0. Armonk, NY, USA).

**Results**

According to Table 1, age, gender, smoking status and alcohol consumption are not differently distributed according to the past occurrence of AP. Prevalence of familial chylomicronemia syndrome and TG levels, both historical and current, are the variables that increase the most among subjects with several RAP, while the latter have the lowest median values of body mass index, HDL-cholesterol and LDL-cholesterol, which are all well-known to be lower in the familial form of chylomicronemia [1, 5] (p < 0.001).

Gene expression analyses revealed that, at a p-value <0.01, a false discovery rate of 5% and a ≥2-fold change expression significance level, a set of 41 probes were found differentially expressed in CM subjects without pancreatitis, 92 in the CM group with 1 to 3 pancreatitis, and 94 in the group with ≥4 pancreatitis compared to healthy controls (data not shown). Among the identified annotated probes (biomarkers), 14 are shared by the three CM groups, 3 are specific to CM with no pancreatitis, 11 are specific to CM with 1 to 3 pancreatitis, and 17 are specific to CM with ≥4 pancreatitis (Fig. 1). Most of the annotated biomarkers are involved in inflammatory, immune, lipoprotein kinetics or signalling biological pathways (Fig. 2).

**Discussion**

This study led to the identification of genetic biomarkers specifically expressed among patients with several RAP and others only expressed among CM patients with no or few AP suggesting some potential risk and protection factors (or markers), respectively. Most of these biomarkers are known to be involved in either inflammation, immune response, lipoprotein kinetic or cell signalling pathways. Among them, some genes previously associated with pancreatic cancer and chronic pancreatitis (IL1RAP, RPL27 and SERPING1) [16, 17] have been found differentially expressed among CM patients with ≥4 pancreatitis. Although none of these patients had pancreatic cancer or chronic pancreatitis, such observation leads to interesting hypotheses regarding potential similarities between these pancreatic disorders and stim-
ulates further researches. Some markers differentially expressed among patients with ≥4 pancreatitis have also been found differentially expressed among patients with familial CM as compared to patients with multifactorial CM [18]. The study of this apparent overlap would surely gives additional useful data for the study of AP expression. Besides, CLEC4E was found differentially expressed among CM patients with no history of pan-

Table 1  Subjects’ characteristics

|                  | Control subjects (n = 15) | Subjects with sustained chylomicronemia | p-value |
|------------------|---------------------------|----------------------------------------|---------|
|                  | Number of pancreatitis    |                                        |         |
|                  | 0 (n = 21)                | 1 to 3 (n = 10)                        | ≥4 (n = 16) |
| Age, years       | 51.0 (41.0–65.0)          | 58.0 (52.0–63.5)                      | 48.5 (35.0–63.8) | 53.0 (39.8–57.8) | NS |
| Men, n (%)       | 8 (53.3)                  | 14 (66.7)                              | 5 (50.0)  | 6 (37.5) | NS |
| Body mass index, kg/m² | 24.0 (22.0–28.0)         | 30.0 (27.0–33.0)                      | 27.5 (23.7–30.2) | 23.0 (21.0–24.8) | <0.001 |
| Type 2 diabetes, n (%) | 0                        | 9 (42.9)                              | 2 (20.0)  | 4 (25.0) | 0.03 |
| Alcohol abstinence, n (%) | 1 (6.7)                  | 4 (19.0)                              | 5 (50.0)  | 5 (31.3) | NS |
| Smoking, n (%)   | 9 (60.0)                  | 11 (57.9)                             | 6 (60.0)  | 11 (68.8) | NS |
| FCS, n (%)       | 0                         | 0                                     | 5 (50.0)  | 14 (87.5) | <0.001 |
| Historical total TG, mmol/L | 1.0 (0.9–1.5)*        | 16.7 (11.3–27.4)                      | 15.3 (11.7–22.9) | 26.9 (18.8–34.3) | <0.001 |
| Continuous variables are median (Interquartile range). * Only the current lipid profile was available for normal control subjects. FCS, Familial chylomicronemia syndrome; LDL, Low-density lipoprotein; HDL, High-density lipoprotein; TG, Triglyceride; Smoking, Past or current smokers Significantly different (p < 0.05) from 1Controls subjects, 2Subjects with no pancreatitis or 3subjects with 1 to 3 pancreatitis. NS: p > 0.05

Fig. 1 Venn diagram representing the distribution of differentially expressed biomarkers among chylomicronemia (CM) recurrent acute pancreatitis studied groups (|Fold change| ≥2). CM without pancreatitis group (A), CM with 1 to 3 pancreatitis group (B), and CM with ≥4 pancreatitis group (C).
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creatitis. Some studies have suggested that CLEC4E, a still little-known member of the C-type lectin family of immune receptor, could be involved in regulation of ectopic lipid accumulation and inflammation, making it an interesting candidate for AP risk modulation [19]. However, until now there is no observation that could allow to associate this receptor to the pathophysiological pathways of AP expression. Although some interesting hypotheses can be put forward, potential pathophysiological mechanisms that could link these genes to RAP remain to be explored.

AP is a highly prevalent and devastating clinical consequence of CM [3, 5, 6]. Not only it is a disabling condition that significantly interferes with patients’ quality of life [20], but it is also responsible for hospitalizations, including intensive care unit (ICU) cares, surgery, pregnancy termination and death, the overall mortality rate being between 3% and 15%, and even higher (up to 30%) in case of persistent organ failure [21]. At present, the expression of AP in CM is not fully understood, nor is it easy to predict or curable. It is therefore critical to advance the knowledge of AP physiopathological mechanisms by the documentation of new markers that can ultimately lead to the identification of potential therapeutic targets.

Study designs performed on a rare conditions [2], such as the one used here, will generally benefit from only small samples, in which covariates can have a much larger effect. In our sample, age, gender, smoking status and alcohol consumption, all known factors affecting pancreatitis risk, which are also well-known modulators of expression levels for several genes, were not significantly differently distributed according to the recurrence of AP. Significant differences between groups only include the higher prevalence of familial chylomicronemia syndrome among subjects with ≥4 RAP and median values of variables well-known to be higher (TG) or lower (body mass index, HDL-cholesterol and LDL-cholesterol) in the familial form of chylomicronemia. These differences therefore only reflects the higher prevalence of familial chylomicronemia in subjects with several RAP [1, 5]. This relative similarity between groups, in addition to the characteristic relative genetic homogeneity of the founder population from which the subjects came, are important strengths of our study [22].

In conclusion, our study suggest that gene expression profiling may contribute to identify key mechanisms of RAP in patients with CM. Next steps will be to identify genetic variants using exome sequencing and to perform functional studies with top candidate genes, including plasmatic RNA expression validation and protein quantification.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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