Placenta 82 (2019) 42–45

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Technical note

Altered levels of placental miR-338-3p and miR-518b are associated with acute chorioamnionitis and *IL6* genotype

Chaini Konwar[a,b], Irina Manokhina[a,b], Jefferson Terry[a,c], Amy M. Inkster[a,b], Wendy P. Robinson[a,b,*]

[a] BC Children's Hospital Research Institute, Vancouver, BC V5Z 4H4, Canada
[b] Department of Medical Genetics, University of British Columbia, Vancouver, BC V6H 3N1, Canada
[c] Department of Pathology, BC Children's Hospital, BC V6H 3N1, Canada

A R T I C L E I N F O

Keywords:
Placenta
Preterm birth
Acute chorioamnionitis
miRNAs
Biomarkers

A B S T R A C T

Placental-derived miRNAs are attractive candidates as biomarkers of placental health, but their associations with specific pathologies, such as acute chorioamnionitis (aCA), are not well explored. Samples of chorionic villi from 57 placentas (33 aCA and 24 non-aCA) were analyzed. Expression was quantified for six candidate miRNAs (miR-146a, miR-210, miR-223, miR-338-3p, miR-411, and miR-518b), using quantitative real-time PCR. miR-518b and miR-338-3p were differentially expressed between aCA cases and non-aCA cases (Bonferroni-corrected $p < 0.05$). Further, we observed that placental miR-518b expression was associated with an *IL6* SNP (rs1800796), a polymorphism we previously reported as a risk-conferring variant for aCA.

1. Introduction

Preterm birth (PTB) is a major health concern worldwide. Histologic evidence of inflammation in the placenta and fetal membranes is commonly observed in PTBs [1]. This often presents as acute chorioamnionitis (aCA), defined by an infiltration of maternal neutrophils into the fetal membranes, and/or chorionic plate [2,3]. Placental-derived miRNAs are promising biomarkers of pregnancy outcomes including aCA; as they are more stable than mRNAs and quantifiable in maternal circulation [4]. There is increasing interest in placental-specific miRNAs in preeclampsia, but few studies have investigated PTB-associated placental miRNA changes [5,6]. Additionally, studies often combine PTB-associated conditions rather than investigating single etiologies such as aCA. In the present study, we investigated the association of aCA with six inflammation-related miRNAs in a population of 57 placentas (33 aCA, 24 non-aCA).

2. Methods

Placentas for this study were selected from a larger ongoing study of genomic changes and pregnancy complication [7,8] with ethics approval granted by the University of British Columbia Children's & Women's Research Ethics Board (H04-70488). Of the 57 placentas included, 33 aCA cases were diagnosed using histologic criteria [2] and 24 non-aCA cases included 17 PTBs of mixed etiologies and 7 term controls (Table 1). Gestational age (GA) at delivery significantly differed between groups as aCA is associated with PTB. Ancestry was inferred with a panel of 50 ancestry informative SNP markers [7,9]. Ancestry coordinate 1, largely separating Europeans and East Asians, significantly differed between groups (Kolmogorov-Smirnov $p < 0.05$). GA and ancestry were accounted for in statistical analyses.

p-values are calculated by Wilcoxon-Mann-Whitney rank sum test for continuous variables, Fisher's exact test for fetal sex. ns = non-significant. Birth weight defined by its standard deviation relative to normative values by GA and fetal sex is presented in the form of a Z-score in the table.

Six miRNAs were investigated, chosen based on published reports of an association with either aCA (miR-223 and miR-338) [10,11], PTB (mir-210, mir-411) [12,13], or innate immune system responses (mir-146a) [14]. miR-518b is a member of the C19MC cluster and chosen as it has been associated with various reproductive pathologies [5,12,15]. miRNA from chorionic villi was extracted using the *mirVana*™ Isolation Kit and miRNA levels quantified by Nanodrop 1000 spectrophotometer. Next, reverse transcription was performed using specific *TaqMan*™ miRNA Assays, followed by quantitative real-time PCR (qPCR) reactions on the ABI ViiA7 system. Additional methodological details are

---

[*] Corresponding author. Department of Medical Genetics, University of British Columbia, Vancouver, BC V6H 3N1, Canada.
E-mail addresses: chaini@alumni.ubc.ca (C. Konwar), imanokhina@uvic.ca (I. Manokhina), jefferson.terry@cw.bc.ca (J. Terry), ainkster@bcchr.ca (A.M. Inkster), wrobinson@bcchr.ca (W.P. Robinson).

https://doi.org/10.1016/j.placenta.2019.05.009
Received 19 February 2019; Received in revised form 16 April 2019; Accepted 17 May 2019
0143-4004/

Table 1
Clinical characteristics of the discovery cohort.

Variables	aCA (n = 33)	Non-aCA (n = 24)	*p* value
Maternal age, years (range)	19.6–43.9	20.4–43.5	ns
GA at delivery, weeks (range)	18–36	22.6–40.7	0.0009
Birth weight (Z-score) (range)	−0.89–3.49	−1.36–3.1	ns
Fetal sex (M/total)	16/33	13/24	ns

provided in the supplementary data.

CpGs from the Illumina 850 K microarray [16] mapping to candidate miRNAs using Illumina's annotation, were tested for differential methylation by aCA diagnosis in a subset of the chorionic villus samples (n = 32/57; 19 aCA, 13 non-aCA). The subset of samples were selected based on available published DNA methylation data (GSE115508). Additionally, *IL6* genotype (rs1800796) data available for another subset of samples (n = 48/57) was used to characterize genetic regulation of miRNA expression.

Statistical analyses were performed using R version 3.4.1. Shapiro-Wilk normality test showed our miRNA expression data did not follow a normal distribution, and thus miRNA levels were compared using the non-parametric Kruskal-Wallis test.

3. Results

3.1. Expression of miR-338-3p and miR-518b is associated with aCA status

Expression of miR-518b was significantly decreased ($p = 0.004$), and miR-338-3p significantly increased in placentas with aCA ($p = 0.002$) (Fig. 1), even after adjustment for GA, fetal sex and ancestry (Supplementary methods). Although expression of miR-146a, miR-210, miR-223, and miR-411 was not significantly associated with aCA, most miRNAs trended in the expected direction, as reported in infection-mediated conditions [17,18].

To investigate sex-specific miRNA expression in aCA, we stratified aCA association tests by sex. For miR-338-3p, increased expression in aCA cases was observed in both males ($p = 0.05$) and females ($p = 0.02$); miR-518b expression was significantly associated with aCA status in females only (p = 0.009), suggesting possible sex-specific expression of this species in aCA (Supplementary Fig. S1).

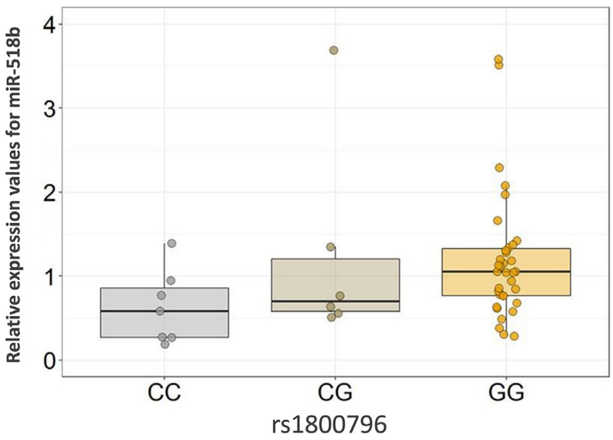

Fig. 2. Expression of aCA-associated miR-518b in chorionic villi is influenced by *IL6* rs1800786 genotype. Adjusted (for GA, sex and ancestry) miR-518b expression values are plotted on the y-axis w.r.t *IL6* genotype groups (CC = 7, GG = 35) on the x-axis. Individuals with the CC genotype showed increased expression levels in the placenta compared to individuals of GG genotype ($p = 0.02$). The heterozygous CG (n = 6) individuals showed intermediate levels of miR-518b expression compared to CC and GG, though not significant at $p < 0.05$. Additionally, although, not significant, expression patterns of miR-338-3p also displayed a trend related to the *IL6* genotype distribution (Supplementary Fig. S3).

3.2. Expression of miR-518b and miR-338-3p is associated with IL6 genotype

We identified 3 CpG sites (*cg06445981, cg11251554*, and *cg15993786*) and 11 CpG sites (*cg06807993*, *cg06869212*, *cg11600078*, *cg18637486*, *cg21473782*, *cg23176214*, *cg23295826*, *cg24085713*, *cg26068527*, *cg26766064*, and *cg06332842*) from the 850 kK array mapping to miR-518b and miR-338-3p, respectively. None of these CpGs showed differential methylation by aCA status (Supplementary Fig. S2).

As we previously reported altered *IL6* genotype in association with aCA [19], we wanted to explore the relationship with miRNA expression. Expression of miR-518b, but not mir-338-3p, was significantly associated with the *IL6* rs1800796 genotype: CC individuals (n = 7; 6 aCA, 1 non-aCA) showed significantly decreased miR-518b expression

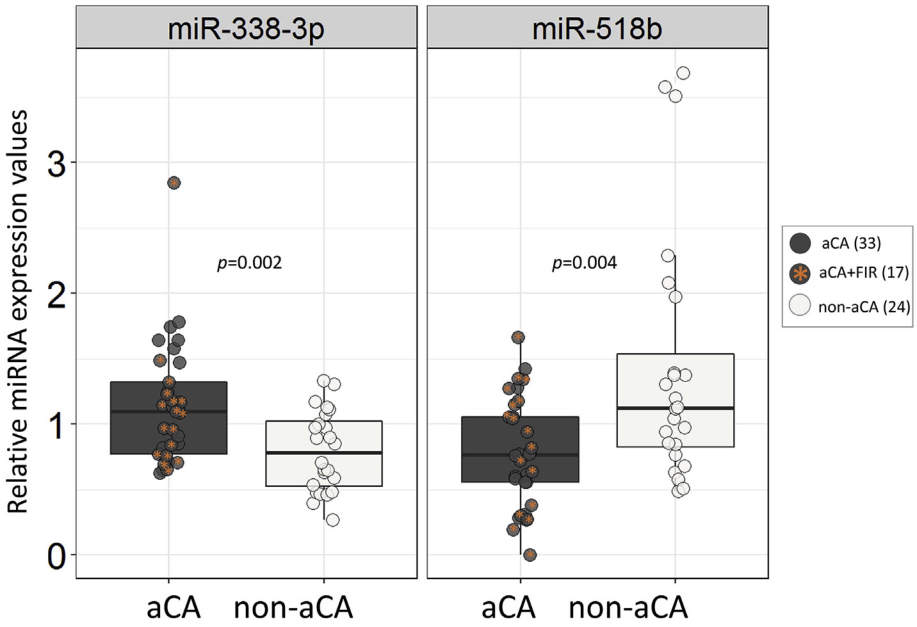

Fig. 1. Differential expression of miR-338-3p and miR-518b in placentas is associated with aCA status. Adjusted (for GA, sex and ancestry) expression values are plotted on the y-axis w.r.t study groups on the x-axis. Even after adjustment for GA, fetal sex, and ancestry, altered expression was observed between aCA cases and non-aCA cases. Based on our clinical records, it is unclear why the three females in the non-aCA group showed higher miR-518b expression compared to the remaining individuals within the non-aCA cases, however, we used the non-parametric Kruskal-Wallis test, which is relatively robust to outliers, and thus the magnitude of the difference in expression is less likely to drive the significance between the groups.

compared to GG individuals (n = 35; 16 aCA, 19 non-aCA) (Kruskal-Wallis $p = 0.02$) (Fig. 2).

4. Discussion

We identified altered chorionic villi expression of miR-338-3p and miR-518b in aCA-affected placentas, suggesting plausible involvement of these miRNAs in aCA. An independent study also demonstrated altered expression of miR-338-3p in chorioamniotic membranes affected with aCA [10]. Though the same study identified miR-223 as differentially expressed in aCA, we did not observe this association. Differences may be attributed to small sample size, accounting for confounding factors and/or reflect tissue-specific expression patterns.

Decreased placental expression of miR-518b has been reported in PPROM [5], intrauterine growth restriction [[20] and complete hydatidiform moles [21]. Although the exact role of miR-518b in placenta is not established, miR-518b has been shown to repress *RAP1B* [22], a predicted gene target for miR-518b. *RAP1B* deficient mice show increased neutrophil recruitment and migration into sites of inflammation [23]. Further, our study demonstrated that placental expression of miR-518b is associated with *IL6* (rs1800796) genotype, a placental polymorphism that may increase aCA risk: individuals carrying the C allele exhibited decreased placental expression of miR-518b compared to carriers of the G allele, and we observed an association between reduced miR-518b expression and aCA status. Of note, *IL6* is associated with many inflammatory disorders and is shown to alter immune responses against microbial infection [24], a well-characterized cause of aCA.

Overall, we observed placental-specific miRNA changes in aCA-affected placentas and attempted to characterize the processes that regulate expression of aCA-associated miRNAs. As miR-518b is detectable in maternal circulation throughout gestation [25,26], and shows possible diagnostic potential [25], further studies into these miRNAs are warranted. Clinical application would require integrating data from many such biomarkers, as well as an improved understanding of how genetic background influences miRNA expression.

Funding

This work was supported by an operating grant to WPR awarded from the Canadian Institutes of Health Research [#49520 to WPR]. WPR receives salary support through an investigatorship award from the BC Children's Hospital Research Institute.

Declarations of interest

The authors declare that they have no competing interests.

Acknowledgements

We would like to thank all the participants of this study who generously donated their placentas. We are thankful to K Louie and Dr. J Schuetz for patient recruitment and R Jiang for placental dissections. In addition we are grateful to the Robinson lab members for their thoughtful feedback and advice.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2019.05.009.

References

[1] W.W. Andrews, R.L. Goldenberg, O. Faye-Petersen, S. Cliver, A.R. Goepfert, J.C. Hauth, The Alabama Preterm Birth study: polymorphonuclear and mononuclear cell placental infiltrations, other markers of inflammation, and outcomes in 23- to 32-week preterm newborn infants, Am. J. Obstet. Gynecol. 195 (2006) 803–808 https://doi.org/10.1016/j.ajog.2006.06.083.

[2] R.W. Redline, O. Faye-Petersen, D. Heller, F. Qureshi, V. Savell, C. Vogler, Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns, Pediatr. Dev. Pathol. 6 (2003) 435–448 https://doi.org/10.1007/s10024-003-7070-y.

[3] R.W. Redline, Classification of placental lesions, Am. J. Obstet. Gynecol. 213 (2015) 21–28 https://doi.org/10.1016/j.ajog.2015.05.056.

[4] Y. Ouyang, J.F. Mouillet, C.B. Coyne, Y. Sadovsky, Placenta-specific microRNAs in exosomes – good things come in nano-packages, Placenta 35 (2013) 69–73 https://doi.org/10.1016/j.placenta.2013.11.002.

[5] I. Hromadnikova, K. Kotlabova, K. Ivankova, L. Krofta, Expression profile of C19MC microRNAs in placental tissue of patients with preterm prelabor rupture of membranes and spontaneous preterm birth, Mol. Med. Rep. 16 (2017) 3849–3862 https://doi.org/10.3892/mmr.2017.7067.

[6] G. do Imperio, E. Bloise, M. Javam, P. Lye, A. Constantinof, C. Dunk, F.M. dos Reis, S.J. Lye, W. Gib, T.M. Ortiga-Carvalho, S.G. Matthews, Chorioamnionitis induces a specific signature of placental ABC transporters associated with an increase of mir-331-5p in the human preterm placenta, Cell. Physiol. Biochem. 45 (2018) 591–604 https://doi.org/10.1159/000487100.

[7] C. Konwar, E.M. Price, L.Q. Wang, S.L. Wilson, J. Terry, W.P. Robinson, DNA methylation profiling of acute chorioamnionitis-associated placentas and fetal membranes: insights into epigenetic variation in spontaneous preterm births, Epigenet. Chromatin 11 (2018) 63–77 https://doi.org/10.1186/s13072-018-0234-9.

[8] S.L. Wilson, K. Leavey, B.J. Cox, W.P. Robinson, Mining DNA methylation alterations towards a classification of placental pathologies, Hum. Mol. Genet. 27 (2018) 135–146 https://doi.org/10.1093/hmg/ddx391.

[9] G.F. Del Gobbo, E.M. Price, C.W. Hanna, W.P. Robinson, No evidence for association of MTHFR 677C > T and 1298A > C variants with placental DNA methylation, Clin. Epigenet. 10 (2018) 34–50 https://doi.org/10.1186/s13148-018-0468-1.

[10] D. Montenegro, R. Romero, B.L. Pineles, A.L. Tarca, Y.M. Kim, S. Draghici, J.P. Kusanovic, J.S. Kim, O. Erez, S. Mazaki-Tovi, S. Hassan, Differential expression of microRNAs with progression of gestation and inflammation in the human chorioamniotic membranes, Am. J. Obstet. Gynecol. 197 (2007) 289e1-e6 https://doi.org/10.1016/j.ajog.2007.06.027.

[11] J. Lee, C.J. Kim, J. Kim, D. Lee, S. Ahn, B.H. Yoon, Increased miR-223 expression in foetal organs is a signature of acute chorioamnionitis with systemic consequences, J. Cell Mol. Med. 22 (2018) 1179–1189 https://doi.org/10.1111/jcmm.13377.

[12] X.M. Zhu, T. Han, I.L. Sargent, G.W. Yin, Y.Q. Yao, Differential expression profile of microRNAs in human placentas from preeclamptic pregnancies vs normal pregnancies, Am. J. Obstet. Gynecol. 200 (2009) 661.e1-e7 https://doi.org/10.1016/j.ajog.2008.12.045.

[13] D.A. Enquobahrie, M. Hensley, C. Qiu, D.F. Abetew, K. Hevner, M.G. Tadesse, M.A. Williams, Candidate gene and microRNA expression in fetal membranes and preterm delivery risk, Reprod. Sci. 23 (2016) 731–737 https://doi.org/10.1177/1933719115612925.

[14] K.D. Taganov, M.P. Boldin, K.J. Chang, Baltimore, D. NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses, P. Natl. Acad. Sci. 103 (2006) 12481–12486 https://doi.org/10.1073/pnas.0605298103.

[15] I. Hromadnikova, K. Kotlabova, M. Ondrackova, P. Pirkova, A.V. Kestlerova, L. Hympanova, L. Krofta, Expression profile of C19MC microRNAs in placental tissue in pregnancy-related complications, DNA Cell Biol. 34 (2015) 437–457 https://doi.org/10.1089/dna.2014.2687.

[16] S. Moran, C. Arribas, M. Esteller, Validation of a DNA methylation microarray for 850,000 CpG sites of the human genome enriched in enhancer sequences, Epigenomics 8 (2016) 389–399.

[17] J. Lemaire, G. Mkannez, F.Z. Guerfali, C. Gustin, H. Attia, R.M. Sghaier, K. Dellagi, D. Laouini, P. Renard, Sysco- Consortium, MicroRNA expression profile in human macrophages in response to Leishmania major infection, PLoS Neglected Trop. Dis. 7 (2013) e2478 https://doi.org/10.1371/journal.pntd.0002478.

[18] K. Kotaro, M. Hitomi, S. Masato, S.U. Aya Shinozaki, K. Taira, S. Takahito, M. Kim, M. Ogawa, Y.W. Iwasaki, H. Kayo, Y. Fukuda-Yuzawa, Epigenetic silencing of miR-210 increases the proliferation of gastric epithelium during chronic Helicobacter pylori infection, Nat. Commun. 5 (2014) 4495–4497 https://doi.org/10.1038/ncomms5497.

[19] C. Konwar, G.F. Del Gobbo, J. Terry, W.P. Robinson, BMC Med Genet 20 (1) (2019 Feb 22) 36, https://doi.org/10.1186/s12881-019-0768-0 PMID: 30795743.

[20] D. Wang, Q. Na, W. Song, G. Song, Altered expression of miR-518b and miR-519a in the placenta is associated with low fetal birth weight, Am. J. Perinatol. 31 (2014) 729–734 https://doi.org/10.1055/s-0033-1361832.

[21] Q. Na, D. Wang, W. Song, Underexpression of 4 placenta-associated MicroRNAs in complete hydatidiform moles, Int. J. Gynecol. Cancer 22 (2012) 1075–1080 https://doi.org/10.1097/IGC.0b013e3182574439.

[22] M. Liu, Y. Wang, H. Lu, H. Wang, X. Shi, X. Shao, Y.X. Li, Y. Zhao, Y.L. Wang, miR-518b enhances human trophoblast cell proliferation through targeting Rap1b and activating Ras-MAPK signal, Front. Endocrinol. 9 (2018) 100 https://doi.org/10.3389/fendo.2018.00100.

[23] S. Kumar, J. Xu, R.S. Kumar, S. Lakshmikanthan, R. Kapur, M. Kofron, M. Chrzanowska-Wodnicka, M.D. Filippi, The small GTPase Rap1b negatively regulates neutrophil chemotaxis and transcellular diapedesis by inhibiting Akt activation, J. Exp. Med. 211 (2014) 1741–1758 https://doi.org/10.1084/jem.20131706.

[24] J. Hoge, I. Yan, N. Jaenner, V. Schumacher, A. Chalaris, O.M. Steinmetz, D.R. Engel, J. Scheller, S. Rose-John, H.W. Mittrücker, IL-6 controls the innate immune response against Listeria monocytogenes via classical IL-6 signaling, J. Immunol. 190

(2013) 703–711 https://doi.org/10.4049/jimmunol.1201044.

[25] K. Kotlabova, J. Doucha, I. Hromadnikova, Placental-specific microRNA in maternal circulation – identification of appropriate pregnancy-associated microRNAs with diagnostic potential, J. Reprod. Immunol. 89 (2011) 185–191 https://doi.org/10.1016/j.jri.2011.02.006.

[26] K. Miura, S. Miura, K. Yamasaki, A. Higashijima, A. Kinoshita, K.I. Yoshiura, H. Masuzaki, Identification of pregnancy-associated microRNAs in maternal plasmA, Clin. Chem. 56 (2010) 1767–1771 https://doi.org/10.1373/clinchem.2010.147660.