1st INTERNATIONAL SYMPOSIUM of DOHaD and Pandemic: LESSONS FROM COVID-19
10, 11, 12 of May 2023
Maringá - PR / Brazil
State University of Maringá

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HOW THE PAST SHAPES CURRENT STORIES: (EPI)GENETIC EPIDEMIOLOGICAL PROFILES OF METABOLIC SYNDROME IN BRAZILIAN MENNONITES

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Abstract

Mennonites present cc. 500 years genetic isolation and three bottleneck events that reduced their genetic diversity. In order to identify (epi)genetic markers for metabolic syndrome (MS), we used a modified version of the Brazilian National Health Survey to interview 762 Mennonites from three settlements, between 2016-2023. We compared 63 vs. 127 exomes (Illumina HiSeq) from Mennonites with/without metabolic syndrome (MS) and genotyped candidate variants in regulatory regions with mass spectrometry (iPLEX) and sequence-specific amplification (PCR-SSP). We also evaluated DNA methylation of the *NR3C1* and *FKBP5* genes in peripheral blood mononuclear cells (PBMCs) of up to 66 and 141 individuals with/without MS. MS prevalence was 35.8%, similar to Neobrazilians (34.8%), paradoxically followed by a three times lower prevalence of acute myocardial infarction (AMI). Among independent MS risk factors, we found lower maternal warmth in infancy (OR=1.59, P=0.019) and a higher susceptibility to AMI with the harshest migratory route to Brazil (OR=1.57, P=0.001). Thirty-nine variants of 34 genes were associated with MS ($p<0.02$), 41% create/disrupt CpG sites and 12 were associated with visceral adipose and/or cardiovascular tissue expression. There were no methylation differences between individuals with and without MS in PBMCs with the *NR3C1* and *FKBP5* genes, pointing to other epigenetic causal effects. In conclusion, Mennonites have a peculiar epidemiological profile marked by (epi)genetic founder effects that also affect their metabolism, calling for urgent action for prevention and to alleviate the burden of comorbidities due to late diagnosis.

Keywords: Mennonites; DNA methylation; metabolic syndrome.

1. Introduction

Mennonites are a Christian Anabaptist group of Frisian/Flemish origin with cc. 500 years genetic isolation, three bottleneck events and common migration waves that reduced their genetic diversity and changed their susceptibility to chronic diseases. Indeed, South Brazilian Mennonites have the highest genetically-determined lactase persistence and natural resistance to HIV infection reported in Latin America[1]. Metabolic Syndrome (MS) is a cluster of cardiovascular risk factors, associated with the activity of the hypothalamic–pituitary–adrenal (HPA) axis, which depends on epigenetic alterations[2].

2. Material and methods

This project was approved at several instances by the Ethics Committee in Health Sciences of the UFPR (CAAE 55528222.9.0000.0102). Aiming to integrate an extensive epidemiological screening with genomic and (epi)genetic results to identify markers that reflect the Mennonite demographic history and past traumatic events as well as current environmental challenges, we used a modified version of the Brazilian National Health Survey and self-applied, validated psychological inventories to interview 762 Mennonites, 286 from Colônia Nova (CON-RS), 226 from Colonia Witmarsum (CWI) and 259 from Curitiba (CWB-PR), between 2016-2023. Participants also had biometric measurements and cholesterol levels, measured. In addition, 338 exomes were sequenced for >30x coverage (Illumina HiSeq) and 63 exomes from Mennonites with suspected MS were compared with 127 unaffected. Candidate variants in regulatory regions were also genotyped with mass spectrometry (iPLEX, in collaboration with Prof Jennifer E Hundt from the Lübeck University, Germany) and sequence-specific amplification (PCR-SSP). We also evaluated DNA methylation in peripheral blood mononuclear cells (PBMCs) in up to 141 controls and 66 individuals with MS, investigating 11

CpG sites of a CpG island in the 1F exon of the glucocorticoid receptor encoded by the *NR3C1* gene, as well as 4 and 3 CpG sites in introns 2 and 7 of its regulator encoded by the *FKBP5* gene. To this end, genomic DNA was converted with bisulfite using the EZ DNA Methylation Kit (Zymo Research). Amplicons were generated using primers designed using the PyroMark Assay Design Software v. 2.0.1.15 (Qiagen). Pyrosequencing was performed using Pyro-Mark ID 96 and PyroMark Gold Q96 (Qiagen). Association analyzes between the variables obtained in the questionnaire were conducted on the STATA 9.1 platform. The exomes of 338 Mennonite individuals residing in Colônia Nova - RS (n= 200), Witmarsum/Palmeira (n= 1) and Curitiba - PR (n= 137) were sequenced on the Illumina HiSeq platform, in partnership with Dr Fabiana L. Lopes and Prof Francis McMahon from the National Institute of Mental Health and Alan Shuldiner from Regeneron (USA). Raw data were converted to the VCF (Variant Call Format) format and aligned to the reference genome GRCh38/hg38, verifying the quality of the sequencing using the ForestQC software. Data were analyzed using multivariate logistic regression with correction for family environment in childhood, age, sex, 1st degree relatives with type 2 Diabetes Mellitus and practice of moderate or vigorous physical activity. For case-control genetic association analyses, the PLINK 1.9 program was used with dominant, recessive and additive models for each associated variant. The p-value was corrected using the Monte Carlo permutation method. The allele frequencies of the Mennonite population were compared to those of different populations by Fisher's exact test. The allele frequencies of the Brazilian population were extracted http://abraom.ib.usp.br. The tests were performed in the R language (version 4.2.2).

3. Results and discussion

The prevalence of MS was 35.8%, similar to the admixed Brazilian population (34.8%). Paradoxically, the prevalence of acute myocardial infarction (AMI) was six times lower than in the German population (who contributed to the Mennonite population), and three times lower

than in non-Mennonite Brazilians. The development of MS was independently associated ($p<0.05$) with increasing age (OR=1.06, P=0.000), presence of relatives with type 2 diabetes (OR=1.74, P=0.037), being male (OR=2.47, P=0.034). Furthermore, we found an independent association of metabolic syndrome with lower maternal warmth in infancy (OR=1.59, 95%CI=1.08-2.34, P=0.019) and a higher susceptibility to AMI in descendants of Mennonites that took the harshest migratory route to Brazil (2nd and 3rd generation, OR=1.57, 95%IC=1.21-2.03, P=0.001), indicating possible early or even inter/transgenerational epigenetic susceptibility effects to these diseases.

On the other hand, a higher frequency of moderate to vigorous physical activity (OR=0.41, P=0.001) was independently associated with MS protection. Thirty-nine variants of 34 genes were associated with MS, 20 with a dominant effect, 21 with an additive effect and 18 with a recessive effect ($p<0.005$), regardless of the mentioned risk factors. Of these, all were associated with MS susceptibility ($p<0.02$), 4 are missense, and 12 of them are associated with difference in expression in visceral adipose and/or cardiovascular tissues. However, *NR3C1* polymorphisms were associated with MS susceptibility, regardless of other risk factors: rs10482605*G (p=0.026; OR=4.74), rs258763*A_rs6877893*A_rs7701443*A_rs72802813*G (p=0.019; OR=4.82), rs258763*A_rs6877893 *A_rs41423247*G (p=0.016; OR=6.15), and rs258763*A_rs6877893*G_rs41423247*G (p=0.008; OR=6.06), the forelast two as haplotypes with an additive, the last with a recessive susceptibility effect. The frequencies of 17 associated alleles differ from those of non-Finnish and Brazilian Europeans, five of them being more frequent among Mennonites ($p<0.05$), indicating a possible founder effect. Several of the implicated polymorphisms are eQTLs that create or disrupt CpG sites. There were, however, no methylation differences between PBMCs of individuals with and without MS in the *NR3C1* and *FKBP5* genes, even though the expression of these genes is similar in blood and pituitary/ adrenal glands.

4. Conclusion

In conclusion, Mennonites have a peculiar epidemiological profile marked by (epi)genetic effects that also affect their metabolism, calling for urgent action for prevention and to alleviate the burden of comorbidities due to late diagnosis.

Funding

This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Empresa Brasileira de Serviços Hospitalares (Ebserh) grant numbers 423317/2021-0 and 313741/2021-2 (8520137521584230), Research for the United Health SUS System (PPSUS-MS), CNPq, Fundação Araucária and SESA-PR, Protocol Nº: SUS2020131000106.

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