UDC 577:575:614.8.086 (470.54)
DOI: 10.21668/health.risk/2023.2.15.eng

Read online

Research article

POLYMORPHISM OF THE APOE GENE AS A RISK FACTOR OF OBESITY IN WORKERS EXPOSED TO OCCUPATIONAL HAZARDS AT FERROUS METALLURGY ENTERPRISES

D.D. Polyanina, I.A. Bereza, A.M. Amromina, D.R. Shaikhova, S.G. Astakhova, M.P. Sutunkova, V.B. Gurvich

Yekaterinburg Medical Research Center for Prophylaxis and Health Protection in Industrial Workers, 30 Popov Str., Yekaterinburg, 620014, Russian Federation

Obesity contributes to the development of severe concomitant diseases and substantially degrades the quality of life. This pathological condition is caused by multiple risk factors including hazardous workplace exposures and genetic predisposition. The ApoE gene participates in regulation of lipid metabolism. Its most significant polymorphisms are rs429358 and rs7412 with the resulting e2, e3 and e4 alleles.

This study did not consider effects of electromagnetic fields generated by office electrical equipment or the lifestyle of the subjects. The sample included people with a large age difference due to the rarity of the apolipoprotein e2 and e4 alleles. The sample was not standardized by age and years of work experience.

The aim of this study was to investigate associations between the ApoE gene polymorphisms and body mass index in workers employed at a metallurgic plant.

We examined 328 male office workers and workers of a converter workshop. The body mass index (BMI) was calculated based on the results of instrumental measurements of weight and height using the conventional formula. DNA was isolated from peripheral blood using the LumiPure DNA gel extraction kit, and polymorphisms were determined using amplification by Calero et al with modifications and horizontal agarose gel electrophoresis. The data were analyzed using the Kruskal – Wallis test.

Statistically significant differences were established in the blue-collar workers. The highest mean BMI value was established in the e2 allele carriers.

We found that people with the e2 allele in their genotype were more prone to obesity. We also assume a potential association between the unsafe work environment and a more pronounced manifestation of the phenotype. These findings can be used for identifying individuals at risk and taking timely preventive measures.

Keywords: *ApoE, obesity, risk factors, BMI, lipid metabolism, ferrous metallurgy, harmful working conditions, cholesterol.*

Obesity is one of the main risk factors for the development of serious cardiovascular diseases, such as stroke, coronary heart disease, pulmonary embolism, etc., and musculoskeletal disorders, with osteoarthritis being the principal concomitant pathology leading to disability [1–3]. According to the Federal State Statistics Service (Rosstat), in the year 2019, 17.8 % of men and 24.5 % of women in the Russian Federation were obese (obesity

Daria D. Polyanina – Laboratory Assistant at the Department of Molecular Biology and Electron Microscopy (e-mail: polyaninadd@ymrc.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0009-0007-5046-0207).

Ivan A. Bereza – Researcher at the Department of Molecular Biology and Electron Microscopy (e-mail: berezaia@ymrc.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0002-4109-9268).

Anna M. Amromina – Junior Researcher at the Department of Molecular Biology and Electron Microscopy (e-mail: amrominaam@ymrc.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0001-8794-7288).

Daria R. Shaikhova – Researcher at the Department of Molecular Biology and Electron Microscopy (e-mail: darya.boo@mail.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0002-7029-3406).

Svetlana G. Astakhova – Head of the Department for Organization of Clinical Activities and Medical Statistics (e-mail: astahova@ymrc.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0002-6027-704X).

Marina P. Sutunkova – Doctor of Medical Sciences, Director (e-mail: sutunkova@ymrc.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0002-1743-7642).

Vladimir B. Gurvich – Doctor of Medical Sciences, Scientific Director (e-mail: gurvich@ymrc.ru; tel.: +7 (343) 253-87-54; ORCID: https://orcid.org/0000-0002-6475-7753).

classes I to III) while 46.9 % of men and 34.7 % of women were overweight [4].

It has been previously shown that people aged over 40 and those having low physical activity are more likely to be overweight and obese. Little is known, however, about the prevalence of obesity in people employed in industries with unsafe working conditions. It is generally accepted that excess adipose tissue accumulates when energy intake exceeds energy expenditure; yet, both occupational risk factors and genetic predisposition can also contribute to higher prevalence of obesity among the working population [5–8].

Apolipoprotein E (APOE) is one of the regulators of fat metabolism in the body of mammals. It is involved in the transport of triglycerides and cholesterol to various tissues by interacting with lipoprotein receptors of target cells. It is a key regulator of cholesterol redistribution [9–11]. The most important polymorphisms for the APOE gene are rs429358, which is characterized by the substitution of the amino acid cysteine for arginine at position 112, and rs7412 with the substitution of arginine for cysteine at position 158 [12, 13].

The study of the relationship between genetic predisposition, occupational risk factors, and morbid obesity makes it possible to identify criteria for assessing health risks and contributes to elaboration of effective preventive measures for the population at risk.

Our **objective** was to investigate associations between the APOE gene polymorphisms and the body mass index in workers employed at a metallurgic plant.

Materials and methods. We examined 328 male employees of a ferrous metallurgy enterprise aged 23 to 66 years (mean age: 43.61 ± 9.04 years). The sample was divided into 142 cases, all working in a basic oxygen furnace shop and exposed to occupational hazards, and 186 controls selected from administrative/management personnel.

The body mass index (BMI) was calculated based on the results of instrumental body height and weight measurements using the conventional formula. DNA was isolated from peripheral blood using the LumiPure DNA gel extraction kit and polymorphisms were then determined using amplification by Calero et al with modifications and horizontal agarose gel electrophoresis [14].

PCR mixtures. We prepared two PCR mixtures: mixture A based on Arg primers (Arg112 and Arg158) and mixture B based on Cys primers (Cys112 and Cys158). Each mixture contained 10 µl of BioMaster HS-Taq PCR-Color (2x), 0.4 µL of each primer, 0.8 µL of a common primer, DMSO, water, and 2.5 µl of the DNA sample. Amplification was carried out using a T100 Thermal Cycler (BIO RAD, USA) under the following conditions: preliminary denaturation for 5 min at 95 °C followed by 35 three-step cycles: denaturation for 30 s at 95 °C, primer annealing for 30 s at 61 °C, elongation for 60 s at 72 °C, followed by the final elongation during 15 min at 72 °C. The results were visualized using horizontal agarose gel electrophoresis.

For statistical analysis, the data on each patient were recorded twice in order to compensate for the data loss when selecting only one allele, so each study participant with a heterozygous genotype was taken into account in both groups at once. The use of genotypes for the analysis was considered inappropriate due to resulting small sample sizes impeding application of appropriate statistical methods. The data were analyzed using the Kruskal – Wallis test in Statistica 12 (StatSoft Inc).

Results and discussion. In this study, we examined the relationship between APOE gene alleles and the body mass index in employees of a ferrous metallurgy enterprise presented in the table.

Each cohort was divided into three subcohorts depending on the allele present. When analyzing the whole sample, no statistical differences were found, but we observed a tendency towards manifestation of the phenotype in people carrying the e2 allele ($p = 0.074$).

The mean body mass index in workers with different APOE gene alleles

Cohort	e2	e3	e4	*p*
Administrative / management personnel	27.48 ± 4.45 (*n* = 7)	28.05 ± 4.54 (*n* = 316)	28.11 ± 4.32 (*n* = 49)	0.683
Workers of the basic oxygen furnace shop	29.24 ± 4.53 (*n* = 21)	28.21 ± 4.52 (*n* = 225)	28.36 ± 4.55 (*n* = 38)	**0.038**

N o t e s : e3 – normal genotype; e2 and e4 – mutant genotypes. The table shows mean values and the error of the mean; the number of alleles considered is in brackets; statistically significant differences ($p \leq 0.05$) are in bold. The comparison was made within cohorts between alleles.

No statistically significant differences were found within the control cohort ($p = 0.683$), which probably indicates the absence of an external factor affecting the manifestation of phenotypic traits in office workers.

In contrast to the controls, BMI values in workers of the basic oxygen furnace shop were allele dependent ($p = 0.038$), with the highest mean value established in the APOE e2 carriers. It is worth noting that the sample included people with a large age difference, so the data might be distorted by the absence of phenotype manifestations in younger subjects and the presence of concomitant diseases leading to weight gain in the older ones. We decided to include these groups of workers due to the rarity of the e2 and e4 alleles.

Hence, we can assume the relationship between the presence of the e2 allele of the APOE gene and weight gain. A statistically significant effect of this APOE variant on obesity has not yet been established, so it is impossible to ascertain that the correlation found in this study will be true for other populations, since anthropometric data are strongly influenced by such factors as ethnicity, sex, lifestyle, and concomitant diseases [15–17].

It is worth mentioning, however, that there exists a relationship between conformation of the APOE protein and its binding activity. Weisgraber states that APOE3 and APOE4 were equally effective at binding to low density lipoprotein receptors on cultured human fibroblasts, while APOE2 showed only 1 % binding capacity. This fact is associated with the development of type 3 hyperlipoproteinemia, which can lead to obesity if not managed. According to the author, the presence of the e2 allele is not an absolute prerequisite of hyperlipoproteinemia and, as mentioned earlier, external factors play the main role in the development of this metabolic syndrome [18–20].

Conclusion. We established that people with the e2 allele in the genotype are more likely to develop obesity; besides, the relationship between the exposure to occupational hazards and a more pronounced manifestation of the phenotype is possible. These findings can facilitate identification of individuals at risk and promote timely preventive measures.

Research limitations. The study did not take into account health effects of electromagnetic fields generated by office electrical equipment and lifestyle of the subjects. The sample consisted of people with a large age difference owing to the rarity of the apolipoprotein e2 and e4 alleles. The sample was not standardized by age and years of work experience.

Funding. This research received no external funding.

Conflict of interest. The authors have no conflicts of interest to declare.

References

1. Obesity and overweight. *World Health Organization*, 2021. Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (March 03, 2023).

2. Guh D.P., Zhang W., Bansback N., Amarsi Z., Birmingham C.L., Anis A.H. The incidence of co-morbidities related to obesity and overweight: A systematic review and meta-analysis. *BMC Public Health*, 2009, vol. 9, pp. 88. DOI: 10.1186/1471-2458-9-88

3. Malik V.S., Willet W.C., Hu F.B. Nearly a decade on – trends, risk factors and policy implications in global obesity. *Nat. Rev. Endocrinol.*, 2020, vol. 16, no. 11, pp. 615–616. DOI: 10.1038/s41574-020-00411-y

4. Rosstat izuchil ratsion rossiyan [Rosstat studied Russians' diets]. *Rosstat*, 2019. Available at: https://rosstat.gov.ru/folder/313/document/70761 (March 03, 2023) (in Russian).

5. Luckhaupt S.E., Cohen M.A., Li J., Calvert G.M. Prevalence of obesity among US workers and associations with occupational factors. *Am. J. Prev. Med.*, 2014, vol. 46, no. 3, pp. 237–248. DOI: 10.1016/j.amepre.2013.11.002

6. Salem V., AlHusseini N., Razack H.I.A., Naoum A., Sims O.T., Alqahtani S.A. Prevalence, risk factors, and interventions for obesity in Saudi Arabia: A systematic review. *Obes. Rev.*, 2022, vol. 23, no. 7, pp. e13448. DOI: 10.1111/obr.13448

7. Kontsevaya A., Shalnova S., Deev A., Breda J., Jewell J., Rakovac I., Conrady A., Rotar O. [et al.]. Overweight and Obesity in the Russian Population: Prevalence in Adults and Association with Socioeconomic Parameters and Cardiovascular Risk Factors. *Obes. Facts*, 2019, vol. 12, no. 1, pp. 103–114. DOI: 10.1159/000493885

8. Van der Valk E.S., van den Akker E.L.T., Savas M., Kleinendorst L., Visser J.A., Van Haelst M.M., Sharma A.M., van Rossum E.F.C. A comprehensive diagnostic approach to detect underlying causes of obesity in adults. *Obes. Rev.*, 2019, vol. 20, no. 6, pp. 795–804. DOI: 10.1111/obr.12836

9. Riedel B.C., Thompson P.M., Brinton R.D. Age, APOE and sex: Triad of risk of Alzheimer's disease. *J. Steroid Biochem. Mol. Biol.*, 2016, vol. 160, pp. 134–147. DOI: 10.1016/j.jsbmb.2016.03.012

10. Gylling H., Miettinen T.A. Cholesterol absorption and synthesis related to low density lipoprotein metabolism during varying cholesterol intake in men with different apoE phenotypes. *J. Lipid Res.*, 1992, vol. 33, no. 9, pp. 1361–1371.

11. Nunes V.S., Cazita P.M., Catanozi S., Nakandakare E.R., Quintão E.C.R. Decreased content, rate of synthesis and export of cholesterol in the brain of APOE knockout mice. *J. Bioenerg. Biomembr.*, 2018, vol. 50, no. 4, pp. 283–287. DOI: 10.1007/s10863-018-9757-9

12. Mahley R.W., Rall S.C. Jr. Apolipoprotein E: Far More Than a Lipid Transport Protein. *Annu. Rev. Genomics Hum. Genet.*, 2000, vol. 1, pp. 507–537. DOI: 10.1146/annurev.genom.1.1.507

13. Hatters D.M., Peters-Libeu C.A., Weisgraber K.H. Apolipoprotein E structure: insights into function. *Trends Biochem. Sci.*, 2006, vol. 31, no. 8, pp. 445–454. DOI: 10.1016/j.tibs.2006.06.008

14. Calero O., Hortigüela R., Bullido M.J., Calero M. Apolipoprotein E genotyping method by Real Time PCR, a fast and cost-effective alternative to the TaqMan and FRET assays. *J. Neurosci. Methods*, 2009, vol. 183, no. 2, pp. 238–240. DOI: 10.1016/j.jneumeth.2009.06.033

15. Tejedor M.T., Garcia-Sobreviela M.P., Ledesma M., Arbones-Mainar J.M. The Apolipoprotein E Polymorphism rs7412 Associates with Body Fatness Independently of Plasma Lipids in Middle Aged Men. *PLoS One*, 2014, vol. 9, no. 9, pp. e108605. DOI: 10.1371/journal.pone.0108605

16. Ozen E., Mihaylova R.G., Lord N.J., Lovegrove J.A., Jackson K.G. Association between APOE Genotype with Body Composition and Cardiovascular Disease Risk Markers Is Modulated by BMI in Healthy Adults: Findings from the BODYCON Study. *Int. J. Mol. Sci.*, 2022, vol. 23, no. 17, pp. 9766. DOI: 10.3390/ijms23179766

17. Jones N.S., Rebeck G.W. The Synergistic Effects of APOE Genotype and Obesity on Alzheimer's Disease Risk. *Int. J. Mol. Sci.*, 2018, vol. 20, no. 1, pp. 63. DOI: 10.3390/ijms20010063

18. Weisgraber K.H. Apolipoprotein E: Structure-Function Relationships. *Adv. Protein Chem.*, 1994, vol. 45, pp. 249–302. DOI: 10.1016/s0065-3233(08)60642-7

19. Ruiz J., Kouiavskaia D., Migliorini M., Robinson S., Saenko E.L., Gorlatova N., Li D., Lawrence D. [et al.]. The APOE isoform binding properties of the VLDL receptor reveal marked differences from LRP and the LDL receptor. *J. Lipid Res.*, 2005, vol. 46, no. 8, pp. 1721–173. DOI: 10.1194/jlr.M500114-JLR200

20. Matsunaga A., Saito T. Apolipoprotein E mutations: a comparison between lipoprotein glomerulopathy and type III hyperlipoproteinemia. *Clin. Exp. Nephrol.*, 2014, vol. 18, no. 2, pp. 220–224. DOI: 10.1007/s10157-013-0918-1

Polyanina D.D., Bereza I.A., Amromina A.M., Shaikhova D.R., Astakhova S.G., Sutunkova M.P., Gurvich V.B. Polymorphism of the ApoE gene as a risk factor of obesity in workers exposed to occupational hazards at ferrous metallurgy enterprises. Health Risk Analysis, 2023, no. 2, pp. 155–159. DOI: 10.21668/health.risk/2023.2.15.eng

Received: 14.04.2023
Approved: 19.06.2023
Accepted for publication: 23.06.2023