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Relationship between *H1* and *H2* haplotypes of the 17q21 inversion and pregnancy loss in Bosnian population: A case - control study

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Abstract

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The 17q21.31 band is one of the most structurally complex and evolutionary dynamic regions of the genome. Frequencies of two single nucleotide polymorphisms (SNPs): rs9468 and rs1800547 determine worldwide distribution of *H1* and *H2* haplotypes. Recent studies have demonstrated that *H2* haplotype is ancestral in hominoids and under positive selection in European population. The role of non-inverted orientation (*H1* haplotype) and inverted orientation (*H2*) remains unclear, where it is suggested that mothers who are *H1H2* heterozygotes on average tend to have more children than *H2H2* homozygotes. We investigated the prevalence of the inverted 17q21 haplotype in 154 women with pregnancy loss and 154 mothers with at least one live-born child, mean age: 33.0 (±5.4) y/o and 31.4 (±6.7) y/o. All 308 women were of Bosnian origin from Sarajevo. Following DNA extraction from buccal swabs, the genotyping was performed. For statistical analysis R CRAN software was used. Haplotypes distribution was compared between groups. In women with and without pregnancy loss we identified: 74.7% and 79.2% *H1H1*, 24.0% and 17.5% *H1H2* and 1.3% and 3.3% *H2H2* haplotypes. There were no significant differences between the distributions of haplotypes in women with and without pregnancy loss. Statistically significant difference between the average number of children in women with *H1H2* haplotype ($n_{avg.} = 1.54$) in comparison to women with *H2H2* haplotype ($n_{avg.} = 1.29$), was not found. Haplotype *H2* of the 17q21.31 inversion was not linked to pregnancy loss and number of children in Bosnian women.

Introduction

Recurrent pregnancy loss is a pregnancy complication characterized by two or more consecutive spontaneous abortions which affects 1 to 3% of fertile couples (Li et al., 2016). According to the previous reports, pregnancy loss (PL) may have multifactorial background: environmental, anatomical, immune, as well as endocrine or genetic. However, despite the awareness of the impact of the above-mentioned factors, approximately 50% of pregnancy loss cases still remain unexplained (Musters et al., 2011). Development of molecular techniques over the past decades resulted in elucidating the role of genetic risk factors in susceptibility to different conditions including pregnancy loss.

Inversions – one of genetic factors – could be associated with phenotypic effects of clinical importance and have important consequences in humans (Puig et al., 2015; Giner-Delgado et al., 2019). One of them is the approximately 900 kb chromosome inversion polymorphism at the 17q21.31 arising about 3 million years ago, including among others the *MAPT* (microtubule-associated protein tau) gene (GenBank: NC_000017.11, Gene ID: 4137), which defines two distinct lineages, *H1* and *H2* (Stefansson et al., 2005). Worldwide distribution of *H1* and *H2* haplotypes is linked to frequencies of two single nucleotide polymorphisms: rs9468 and rs1800547. The rs1800547A and rs9468T alleles are on *H1* haplotype background, and the G and C alleles are on *H2* background.

The role of non-inverted orientation (*H1* haplotype) and inverted orientation (*H2*) has not yet been fully elucidated. Some authors reported that the 17q21.31 inversion can be considered as a potential biomarker of Alzheimer's and Parkinson's diseases as well as mental disorders (Rao et al., 2010; Spencer et al., 2011; Okbay et al., 2016; Babic Leko et al., 2018). The inversion status has also shown to affect the expression of several genes in the 17q21.31 region (de Jong S et al., 2012). Analysis of the 17q21.31 inversion region demonstrated that *H2* haplotype is ancestral in hominoids (Stefansson et al., 2005). In Europeans, the inversion seems to have been favoured by natural selection in the past $1 \cdot 10^3$ years

and the distribution of *H2* haplotype varies in different populations. It ranges from 4.3% in Finns, 15.0% in Danes through 17.7% in the Irishs, 18.9% in French, 23.9% in Hungarians to 27.7% in Greeks and 37.5% in Sardinians (Donnelly et al., 2010). Decreasing gradient from south to north Europe is observed. In Africans this haplotype is rare (probably as a result of European admixture) while in Asians is almost absent (Donnelly et al., 2010).

A 900-kb inversion polymorphism at 17q21.31 with two haplotypes (*H1* and *H2*) was first described over a decade ago (Stefansson et al., 2005). Authors have suggested that *H1H2* heterozygotes women from Iceland, on average, have more children than *H2H2* homozygotes. Additionally, heterozygotes have higher recombination rates (Stefansson et al., 2005). The aim of our study was to establish the distribution of allele and genotype frequencies of the 17q21.31 inversion in women with PL and mothers with at least one live-born child from the general population of Sarajevo, Bosnia and Herzegovina (B&H). We also compared number of children in women that are *H1H2* heterozygotes and women that are *H2H2* homozygotes.

Material and methods

The present study was conducted in accordance with the standards of the Declaration of Helsinki (1975, revised 2000), and the protocol of the study was approved by the local Ethics Committees (decision ref. numbers: B&H: 10-1285-03-14; Poland: KB-0012/38/13 and KB-0012/119/18). Informed consent was obtained from all individual participants included in the study. According to the definition of ASRM (American Society of Reproductive Medicine) PL is spontaneous abortion of an embryo or fetus before the 20th week of pregnancy or when the fetus weighs less than 500 grams or measures less than 25 cm while RPL (recurrent pregnancy loss) refers to two or more failed pregnancies (Practice Committee of the American Society for Reproductive Medicine, 2008).

Subjects

All women were recruited from Institution of Health Protection of Women and Motherhood in Sarajevo, B&H. Twins and women with any serious illness

Table 1. Characteristics of group of women with pregnancy loss

	1 st trim. n= 118	2 nd trim. n=21	1 st +2 nd trim.* n=15
Age (years±SD)	32.8 (±5.4)	33.5 (±4.7)	33.3 (±3.7)
Weight (kg)	74.3 (±9.5)	72.2 (±9.8)	73.1 (±6.7)
No. of pregnancy loss, (average/range)	159 (1.3/1-4)	30 (1.4/1-3)	31 (2.1/1-3)
The average week of pregnancy loss, average (±SD)	9.1 (±1.6)	18.1 (±3.7)	13.5 (5.6)
Min. week of miscarriage	6	13	13
Max. week of miscarriage	12	24	28
No. of successful pregnancies, (average/ range)	172 (1.5/0-5)	24 (1.1/0-4)	18 (1.2/0-5)

*women with both first and second trimester losses

(including hepatic, pulmonary, renal disorders and cancer) were excluded from the study. For the purpose of the study, we chose 154 women with pregnancy loss (PL) and 154 mothers with at last one live-born child as a control group, mean age: 33.0 (±5.4) y/o and 31.4 (±6.7) y/o. The min. week of miscarriage in women with PL was 6., while max. was 28. In women with and without PL number of pregnancies was 1.4 (±1.1) and 1.2 (±0.8), respectively. All included pregnancies were anembryonic. Characteristics of the group of women with PL are shown in Table 1, as we previously reported in Adler et al. (2018).

DNA extraction and genotyping

Genomic DNA from buccal swabs was extracted using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Two SNPs: rs9468 and rs1800547, were selected from the NCBI SNP database (<http://www.ncbi.nlm.nih.gov/SNP>). Genotypes were determined by real-time PCR using StepOne™ Real-Time PCR System, Applied Biosystems and TaqMan SNP Genotyping Assays (Life technologies, Assay ID: C_7563752_10 and C_7563692_10, respectively) (Applied Biosystems, Foster City, CA, USA). The data were analyzed with Taq Man Genotyper Software v. 1.0.1. For quality control purposes, approximately 10% of the samples were re-genotyped in a blinded fashion and the same results were obtained.

Statistical analysis

All the tests were performed using the R CRAN statistical software (version 3.4.2) (R Core Team, 2017). Statistical analysis was performed using t-test

for two independent means. Haplotypes distribution was compared between groups. $P < 0.05$ was considered statistically significant. The distribution of genotypes was determined in women with and without PL, and comparison was made by the Kruskal-Wallis Test and Fisher's Exact Test. The function `HWE.chisq` for two alleles from package `genetics` was performed (Warnes et al., 2013).

Results and Discussion

Two haplotypes of the 17q21.31 inversion in women from B&H with PL and control group (mothers with at last one live-born child) were identified. In women with and without pregnancy loss we identified 115 and 122 *H1H1*, 37 and 27 *H1H2* and 2 and 5 *H2H2* haplotypes (see table 2.). There were no significant differences between the distributions of haplotypes in women with and without PL (P -value = 0.25). In women with and without pregnancy loss the distribution of *H1* haplotype was 267 (86.7%) and 271 (88.0%), while the distribution of *H2* was 41 (13.3%) and 37 (12.0%). Due to the small sample size we assessed the expected value of genotype prevalence in both groups, P values were 0.611 and 0.032.

The results of our study showed that women with *H1H2* haplotype in comparison to women with *H2H2* haplotype have more children ($n_{avg.} = 1.54$ vs. $n_{avg.} = 1.29$). Nevertheless, statistically significant differences in our study group ($n=308$) were not found. Similar results were obtained in the Stefansson et al. (2005) study ($n=29137$). It is important to mention that in Stefansson et al. (2005) study both women ($n=16959$) and men ($n=12178$)

Table 2. Distribution of allele and genotype frequencies of the 17q21.31 inversion in women with and without PL

Inversion 17q21.31	Observed frequency, n (%)		HW- expected		<i>P</i> value
	Women with PL ¹ , n=154	Women without PL ² , n=154	Women with PL ¹ , n=154	Women without PL ² , n=154	
Genotypes, n (%)					
<i>H1H1</i>	115 (74.7)	122 (79.2)	119.2 (75.2)	119.2 (77.4)	0.61 ¹ ; 0.03 ² , respectively
<i>H1H2</i>	37 (24.0)	27 (17.5)	36.1 (23.1)	32.6 (21.1)	
<i>H2H2</i>	2 (1.3)	5 (3.3)	2.7 (1.8)	2.2 (1.4)	

were subjects. Average number of offspring in subjects with *H1H2* haplotype in their study was 0.0796. To the best of our knowledge we were the first to analyze the distribution of *H1* and *H2* haplotypes in B&H population and its impact on increased number of offspring. We also tested possible relationship between *H1* and *H2* haplotypes of the 17q21.31 inversion and PL. Association of *H1* and *H2* haplotypes and pregnancy loss was not previously examined. In our study relationship between pregnancy loss and haplotypes of the 17q21.31 was not found. Inversions are structural variants in genome linked to phenotypic differences and adaptation of organisms through ages. They often have almost identical inverted repeats at their breakpoints which makes their detection very challenging. (Alkan et al., 2011; Puig et al., 2015). Therefore, there is still very little information on inversions in the human genome. It is unknown how many polymorphic inversions exist in humans, what are their global frequencies and distributions and what features are they related to (Martínez-Fundichely et al., 2014).

In the last decade a great effort has been devoted to characterizing the human genome (Auton et al., 2015). Unfortunately, a significant part of the genetic risk for common and complex diseases still remains unclear (Eichler et al. 2010). It should also be noted that even if the studies were conducted, not all variants have been studied at the same level of detail. Based on the above mentioned the role of inversion still remains to be solved.

Major limitation of the presented study is small sample size. It is important to point out that the distribution of *H2* haplotype observed in our study is congruent to those reported in previously mentioned studies from European populations.

Conclusion

In Bosnian population, haplotype *H2* of the 17q21.31 was not associated with number of children nor pregnancy loss as there were no significant differences in the distributions of haplotypes in women with and without PL.

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Conflict of Interest

The authors state there is no conflict of interest.

References

- Adler G, Mahmutbegovic E, Uzar I, Adler MA, Mahmutbegovic N, Valjevac A (2018) First Bosnian Study of the Relationship between APOE rs7412 and rs429358 Variants and Pregnancy Loss. *Folia Biol (Praha)*, 64:65-69.
- Alkan C, Coe BP, Eichler EE (2011) Genome structural variation discovery and genotyping. *Nat Rev Genet*, 12:363-376.
- Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, et al. (2015) The 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature*, 526:68-74.
- Babic Leko M, Willumsen N, Nikolac Perkovic M, Klepac N, Borovecki F, Hof PR, Sonicki Z, Pivac N, de Silva R, Šimić G (2018) Association of MAPT haplotype-tagging polymorphisms with cerebrospinal fluid biomarkers of Alzheimer's disease: A preliminary study in a Croatian cohort. *Brain Behav*, 8:e01128.
- de Jong S, Chepelev I, Janson E, Strengman E, van den Berg LH, Veldink JH, Ophoff RA (2012)

- Common inversion polymorphism at 17q21.31 affects expression of multiple genes in tissue-specific manner. *BMC Genom*, 13:458.
- Donnelly MP, Paschou P, Grigorenko E, Gurwitz D, Mehdi SQ, Kajuna SL, Barta C, Kungulilo S, Karoma NJ, Lu RB, Zhukova OV, Kim JJ, Comas D, Siniscalco M, New M, Li P, Li H, Manolopoulos VG, Speed WC, Rajeevan H, Pakstis AJ, Kidd JR, Kidd KK (2010) The distribution and most recent common ancestor of the 17q21 inversion in humans. *Am J Hum Genet*, 86:161-171.
- Eichler EE, Flint J, Gibson G, Kong A, Leal SM, Moore JH, Nadeau JH (2010) Missing heritability and strategies for finding the underlying causes of complex disease. *Nat Rev Genet*, 11:446-450.
- Giner-Delgado C, Villatoro S, Lerga-Jaso J, Gayà-Vidal M, Meritxell Oliva M, David Castellano D, Pantano L, Bitarello BD, Izquierdo D, Noguera I, Olalde I, Delprat A, Blancher A, Lalueza-Fox C, Esko T, O'Reilly, PF, Andres AM, Ferretti L, Puig M, Cáceres M (2019) Evolutionary and functional impact of common polymorphic inversions in the human genome. *Nat Commun*, 10:4222.
- Li L, Dou L, Leung PC, Chung TK, Wang CC (2016) Chinese herbal medicines for unexplained recurrent miscarriage. *Cochrane Database Syst Rev*, CD010568.
- Martínez-Fundichely A, Casillas S, Egea R, Ràmia M, Barbadilla A, Pantano L, Puig M, Cáceres M (2014) InvFEST, a database integrating information of polymorphic inversions in the human genome. *Nucleic Acids Res*, 42:D1027-D1032.
- Musters AM, Taminiau-Bloem EF, van den Boogaard E, van der Veen F, Goddijn M (2011) Supportive care for women with unexplained recurrent miscarriage: patients' perspectives. *Hum Reprod*, 26:873-877.
- NCBI SNP database Available from: <http://www.ncbi.nlm.nih.gov/SNP> (accessed February 10, 2018).
- Okbay A, Baselmans BM, de Neve JE, Turley P, Nivard MG, Fontana MA, et al. (2016) Genetic variants associated with subjective well-being, depressive symptoms and neuroticism identified through genome-wide analyses. *Nat Genet*, 48:624-633.
- Practice Committee of the American Society for Reproductive Medicine (2008) Definitions of infertility and recurrent pregnancy loss: a committee opinion. *Fertil Steril*, 89:1603.
- Puig M, Casillas S, Villatoro S, Cáceres M (2015) Human inversions and their functional consequences. *Brief Funct Genom*, 14:369-379.
- Rao PN, Li W, Vissers LE, Veltman JA, Ophoff RA (2010) Recurrent inversion events at 17q21.31 microdeletion locus are linked to the MAPT H2 haplotype. *Cytogenet Genome Res*, 129:275-279.
- R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: <https://www.R-project.org/> (accessed February 10, 2018).
- Stefansson H, Helgason A, Thorleifsson G, Steinthorsdottir V, Masson G, Barnard J, Baker A, Jonasdottir A, Ingason A, Gudnadottir VG, Desnica N, Hicks A, Gylfason A, Gudbjartsson DF, Jonsdottir GM, Sainz J, Agnarsson K, Birgisdottir B, Ghosh S, Olafsdottir A, Cazier JB, Kristjansson K, Frigge ML, Thorgeirsson TE, Gulcher JR, Kong A, Stefansson K. (2005) A common inversion under selection in Europeans. *Nat Genet*, 37:129-137.
- Spencer CC, Plagnol V, Strange A, Gardner M, Paisan-Ruiz C, Band G, et al. (2011) UK Parkinson's Disease Consortium; Wellcome Trust Case Control Consortium. Dissection of the genetics of Parkinson's disease identifies an additional association 5' of SNCA and multiple associated haplotypes at 17q21. *Hum Mol Genet*, 20:345-353.
- Warnes G, with contributions from Gorjanc G, Leisch F, Michael Man M (2013) Genetics: Population Genetics. R package version 1.3.8.1. <https://CRAN.R-project.org/package=genetics>
- WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. Available from: <https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/> (accessed February 10, 2018).