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Short Communication

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Epigenetic Status of Lebanese Dizygotic Twins

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Lebanon is an eastern Mediterranean country with a small population of only 4 million (Abbas et al. 2020). It is characterized for its distinct human genetic, cultural, ethnic, and religious diversity (Haber et al. 2011). The average live twinning births rate in Lebanon is 18.9/1000 (Romanos & Borjac, 2018). Twins can be either monozygotic (MZ) or dizygotic (DZ). The difference between them is how they are formed during embryogenesis (Hoekstra et al. 2008). When a single sperm fertilizes a single egg, it splits then in two genetically identical twins (Xu et al. 2015). However, in fraternal twins case, separate eggs are fertilized by separate sperm to yield genetically different twins (Martino et al. 2013).

Genomic methylation regions of MZs and DZs were the focus of many studies worldwide (Kaminsky et al. 2009, Martino et al. 2013). Epigenetics is defined as heritable changes in gene activity and expression that occurs without alteration in DNA sequence (Vidaki & Kayser, 2018). DNA methylation, a major epigenetic modification, is described as the addition of a methyl group at the 5' position of the Cytosine within CpG dinucleotides to create 5-mC (Park et al. 2016). Being rich in CpG residues, Alu elements known as short conserved repeated DNA sequences are the target of DNA methylation (Häsler & Strub, 2006). Studies on African, European and Caucasian populations showed that patterns of DNA methylation are population-specific (Adkins et al. 2011, Fraser et al. 2012). To determine the methylation patterns, bisulfite treatment is commonly used where unmethylated Cytosine are converted by sodium bisulfite to Uracil, while 5mC remains unconverted (Fraga et al. 2005). Being dynamic, DNA methylation varies in response to environmental stimuli. Difference in methylation patterns detected by bisulfite treatment leads to difference in G+C content and therefore difference in melting curve temperatures (Tm) (Vidaki et al. 2017). The high-resolution melting curve (HRMC) analysis is a novel, sensitive, and relatively cheap post-PCR technique that permits to measure Tm (Stewart et al. 2015).

In Lebanon, for forensic discrimination purpose, we recruited twenty-eight unrelated pairs of Lebanese MZs. Our first study showed that the use of rapidly mutating (RM) Y-STRs recently introduced in the forensic casework did not improve distinction among the male Lebanese MZs pairs (Romanos & Borjac, 2018). However, our second study showed that 71.42% of both male and female Lebanese MZs pairs were epigenetically discriminated despite the fact that individuals within the same MZ pair has the same genetic composition thus they share the same DNA profile (Romanos & Borjac, 2020). The use of epigenetics biomarkers, Alu-Sp & Alu-E2F3 located

respectively at chromosome 19p13 and at chromosome 6p22, tends to offer a new alternative forensic approach over the classical forensic genetics. Epigenetic discriminations through Alu-Sp & Alu-E2F3 for fourteen unrelated pairs of Lebanese DZs pairs (age 2-32 years old) were detected in a third study for a comparative analysis. All DZs volunteers were chosen randomly to participate in the research, they signed a written informed consent, and they answered a well designed questionnaire concerning their educational levels, lifestyles, health conditions...Participants, belonging to Christianity or Islam, were geographically distributed all over the country. Buccal swabs were collected from their clean cheeks using sterile cotton swabs; then the genomic DNA was extracted using the Pure Link Genomic DNA Mini kit (Thermo Fisher Scientific, USA), and quantified using Nanodrop 2000 (Thermo Fisher Scientific, USA). Genomic DNA samples were genotyped with the Identifiler Plus kit (Thermo Fisher Scientific, -UK) to confirm the zygotic status. All amplified samples were run in duplicate on ABI 3500 Genetic Analyzer. Bisulfite treatment of extracted DNA samples was accomplished using the Qiagen Epitect Bisulfite kit (Qiagen, Germany). Amplification of bisulfite converted DNA was performed using the Qiagen Epitect HRM PCR kit (Qiagen, USA) using the 5-Plex HRM Rotor-Gene Q instrument. Seventeen CpG residues in Alu-Sp and two CpG residues in Alu-E2F3 were targeted in the study. HRMC analysis steps as well as primers sequences in this test were based on previously published data (Stewart et al. 2015). All reactions were carried out in triplicate and results were analyzed using the Rotor-Gene Q 2.3.1 software. Data analysis was performed using the paired sample t-test. The average Tm was compared within each pair. Results with p value equal or less than 0.05 ($p < 0.05$) were considered significant and p value equal or less than 0.01 ($p < 0.01$) were considered highly significant.

Our interest resides in showing that two individuals within the same Lebanese dizygotic pair, although having different genetic composition, could have the same Tm. The aim of the study is pinpointing that, although having two different DNA profiles, sharing the same epigenetic signature could be obtained.

After the zygosity status of all participants was confirmed, DZs epigenetic results are obtained as shown in Table 1.

Table 1: Epigenetic discrimination status of Lebanese DZs for *Alu*-Sp and *Alu*-E2F3 biomarkers.

Epigenetic Biomarkers	Discrimination Status of the 14 DZs Pairs		
	No Discrimination	Significant Discrimination	Highly Significant Discrimination
Alu-Sp	2	5	7
Alu-E2F3	3	3	8

Their results showed no discrimination for two out of fourteen pairs for Alu-Sp including one male and one female DZs pairs; and three out of fourteen pairs for Alu-E2F3 including one male and two female DZs pairs. Significant discrimination was obtained for five out of fourteen pairs for Alu-Sp including one male/female, three males and one female DZs pairs; and three out of fourteen pairs for Alu-E2F3 including one male and two female DZs pairs. Their results also showed a highly significant discrimination for seven out of fourteen pairs for Alu-Sp including one male/female, two male and four female DZs pairs; and eight out of fourteen pairs for Alu-E2F3 including two male/female, four male and two female DZs pairs. Once compared, the highest discrimination was observed among a 26 years old DZ female twin pair for Alu-E2F3 with a 0.2 ^{O}C increase on the largest Tm difference obtained among MZs. Interestingly, no discrimination among two DZs pairs targeting Alu-Sp as well as three DZs pairs targeting Alu-E2F3 was obtained.

In our study, the differentiation based on Alu repeats was comparable for both Lebanese MZs and DZs pairs. The fact that living under similar environmental factors could not lead to any epigenetic discrimination is applicable not only for MZs who already share the same genetic composition, but also for DZs characterized by a complete different genetic composition.

In MZs and DZs cases, the methylation patterns power of discrimination is assumed to be only affected by the environmental factors exposure. By consequence, we can deduce that the two different DNA profiles of individuals within the same Lebanese DZ pair do not indicate necessarily two different Tm.

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