



Letter to the editor: “The association of CD40 polymorphism (rs1883832C/T) and soluble CD40 with the risk of systemic lupus erythematosus among Egyptian patients”

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To the editor:

We have read the article titled “The association of CD40 polymorphism (rs1883832C/T) and soluble CD40 with the risk of systemic lupus erythematosus among Egyptian patients” published by Mousa et al. [1], and we have great interest in clarifying the methodological information mentioned by the authors and that, consequently, have an impact on the interpretation of the results reported in the study.

The authors performed the genotyping of the rs1883832 (-1C/T) *CD40* SNP in the study population by the PCR-RFLP technique using the *NcoI* enzyme for the restriction assay. *NcoI* is an enzyme that recognizes the CCATGG sequence; it cuts between the two cytosines. In line with the above, the presence of the minor allele T of the rs1883832 *CD40* SNP changes the sequence to CTATGG, preventing the enzyme from performing the cut.

According to the information presented in the manuscript by Mousa et al., the criteria for genotyping the rs1883832 *CD40* SNP seem to be incorrect. In the

electrophoretic restriction patterns, the cut of the amplified DNA fragment was in the presence of the minor allele T. As a result of the above, it seems that the genotypic and allelic frequencies reported by the authors are incorrect since the presence of the minor allele is overrepresented. Furthermore, the interpretation regarding the soluble CD40 levels in association with the genotypes of the *CD40* SNP would also be affected.

We [2] and other authors [3–6] have performed the genotyping of the -1C/T *CD40* SNP following the PCR-RFLP method using the *NcoI* enzyme and consistently reported the C allele as the one recognized for the restriction assay. It is worth mentioning that some of the studies mentioned above have confirmed their results by sequencing. Besides, we have confirmed this using the sequence of the *CD40* gene (NG_007279.1) as the reference and a restriction analysis tool [7] (Fig. 1).

Taking the above into account, we consider it necessary for the authors to carry out a thorough review or a subsequent validation of their results.

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a)
Homo sapiens CD40 molecule (CD40), RefSeqGene (LRG_40) on chromosome 20

NCBI Reference Sequence: NG_007279.1

[FASTA](#) [Graphics](#)

Go to:

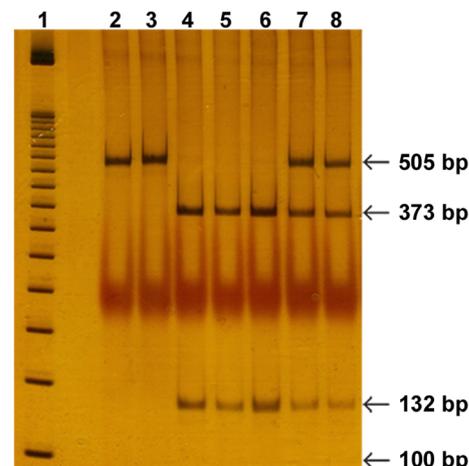
LOCUS NG_007279 11492 bp DNA linear PRI 16-SEP-2018
 DEFINITION Homo sapiens CD40 molecule (CD40), RefSeqGene (LRG_40) on chromosome 20.
 ACCESSION NG_007279 REGION: 4988..16479
 VERSION NG_007279.1
 KEYWORDS RefSeq; RefSeqGene.
 SOURCE Homo sapiens (human)

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4861 ggcgtccctaa actccccgggg gaattagact tgtgggaatg tctctggggaa actcctgcgc
4921 ggtgaattgc tgggggctcc gcccccccga taggtggacc gcgattgggc tttgaagacc
4981 ccgccccttt cctgggcggg gccaaaggctg gggcagggga gtcagcagag gcctcgctcg
5041 gggccccaagt ggtcctgccc cctggtctca cctcgctatggttctgctctg cctctgcagtg
5101 cgtcctctgg ggcctgcttc tgaccgctgt gaggttgttt tgcccggacc agacgggagt
5161 tgggagtggg gaatgagaag gaaagggaaag gaagacttcg gggaaagaggc cttcctggct
5221 gatttttttg ggggcaggag ggtgggtggg agctgggcaa ggtgcccccg ctctcggtctg
5281 aatgggggtg gctgcctctc tcttctcccg ggctggggtc ccgggagcgg cctacagggg
5341 ccgctcaggg aaggcaactg ctgcccaagc gtgcctagac ggcctggacg ggttttaggga
5401 gcctcagagg ctggccacac agagactggt agggggttca gagggcggga agtgaggcgg
5461 accaagggaa ggggcgggctc tggcccgttt cctgtccctt tcttattgtg gacagatgac
  
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Fig. 1 *CD40* gene reference sequence and *CD40* -1 C/T SNP restriction pattern with *NcoI* enzyme. **a** Segment of the *CD40* gene sequence showing the 505-bp fragment of DNA that is amplified for the -1C/T SNP identification delimited by the primers (highlighted in green); the recognition sequence of the *NcoI* enzyme (marked in bold black) and the -1C/T SNP position (marked in bold red) are also shown. **b** Representative

b) *NcoI*
 5' ... C[✓] C A T G G ... 3'
 3' ... G G T A C_▲ C ... 5'



polyacrylamide gel showing the restriction patterns obtained from the digestion of the 505-bp PCR product with the *NcoI* enzyme for the genotyping of the *CD40* -1C/T SNP. Lane 1: 50-bp molecular weight marker; lanes 2 and 3: T/T genotype; lanes 4–6: C/C genotype; lanes 7 and 8: C/T genotype. In the upper part, the recognition sequence of the *NcoI* enzyme and the cutting site are shown

Compliance with ethical standards

Disclosures None.

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