Nonspecific PCR Amplification of *CRYBB2*-Pseudogene Leads to Misconception of Natural Variation as Mutation

We recently attempted to amplify the *CRYBB2* gene in genomic DNA samples of congenital cataract patients using the primer sets specific for exon 5 of the *CRYBB2* gene (sense primer: 5′-CCCCTCACCCATACTCACTT-3′ and antisense primer: 5′-CCCCAGAGTCTCAGTTCCTG-3′) as adopted from the article by Weisschuh et al.[1] We observed an interesting result indicating three variations (rs2330991; rs2330992; rs4049504) in our sample. Upon searching for similar reports in other ethnicities, we came across an article by Hansen et al.,[2] wherein the authors have reported that these variations in cis position are pathogenic, possibly by transforming the secondary structure of the beta crystallin protein, in rare circumstances, due to gene conversion. Further screening of cataract probands from three other families (SEC12, VEC2, DKEC1) using the same primer set also depicted all three variations in them. Such a possibility could either mean a sequential artifact or nonspecificity of the primers.

To clarify further, an in silico PCR using the USC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgPcr; provided by the University of California at Santa Cruz) was done with the above primers,[1] which amplified two regions of the *CRYBB2* and *CRYBB2-P1* (NR_033,734.1) genes. *CRYBB2-P1* is a pseudogene, positioned below the normal *CRYBB2*. Sequence comparison of *CRYBB2* versus its pseudogene (*CRYBB2-P1*) detected all three variations referred to above in its pseudogene. To confirm, the same samples were amplified using primers cited in one of our earlier reports[3] (sense primer: 5-AGTGGTCATAGACACGTAGTGGGTGCAC-3 and antisense primer: 5-CTGTTCCCAAACTTAGGGACACACGC-3). Interestingly, the results did not show any of the three variations. This probably confirms that the said variations are due to mispriming and amplification of the pseudogene. When the primers used by Hansen et al.[2] were cross checked with the National Center for Biotechnology Information (NCBI) database, it was apparent that it matches both *CRYBB2* and *CRYBB2-P1*. The same concordant result was obtained by in silico PCR analysis using the UCSC Genome browser. Theoretically, this primer set fails to exclusively amplify the specific *CRYBB2* sequence and, hence, their data depicting the three variations in the family (*CC00133*) studied are rather unconvincing in accepting them as a pathogenic mutation underlying the phenotype. It is presumed that primers referred to by Hansen et al.[2] to amplify exon 5 actually denote exon 6. Further, we tested all the primers used by the authors through in silico PCR analysis using the UCSC Genome browser. It was observed that some of the primers were not specific to the genes that were screened (primers for *CRYAB* Exon 1 actually amplifies *CRYBB1*, primers for the exons *CRYBB1* amplified *CRYBA1* with the exception of exon 6 that amplified *CRYBB1*, and the reverse primer of exon 2 of *MAF* doesn't match with its gene sequence and, hence, showed no amplification in in silico PCR analysis).

Therefore, this situation exemplifies the importance that nonspecific amplification of a pseudogene needs to be addressed and scrutinized. With due appreciation of the interesting data presented by the authors, we wish to drive this point to the kind attention of the authors to enable rectification of the same as erratum. The authors discuss that their results may be due to gene conversion, which could have been validated by other means. The experiments and predictions by Hansen et al.,[2] therefore, need revision with cautious interpretation.

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