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LETTER TO THE EDITOR

A bias in genotyping the miR-27a rs895819 and rs11671784 variants

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To the Editor,

Due to the oncogenetic function of miR-27a, SNPs affecting this gene are frequently investigated for an influence on cancer risk. Yang et al. [1] has reported the association between the minor [G] allele of rs895819 on miR-27a and a reduced familial breast cancer risk in German population by direct sequencing. One study in Chinese Han population also observed a tumor suppressive effect of the rs895819 [G] allele in gastric cancer using MALDI-TOF MassARRAY [2].

One very recent publication has analysed the association of rs895819 with familial breast cancer risk in Italian population using TaqMan allelic discrimination assay [3]. However, this genotyping method appears to be inappropriate for rs895819 (chr19:13808292) due to the adjacent variant rs11671784 (chr19:13808296, which is located only 4 nucleotides distance and represents a rare variant, with the rare [T] allele frequency of 2.4 % and 1.9 % in German familial breast cancer cases and healthy controls, respectively [1]). Unfortunately, commercially available pre-designed assays of Applied Biosystems do not consider SNPs (especially SNPs with a low allele frequency) in their probe design nor provide access to the probe sequences [4]. Any TaqMan probe overlapping with the respective other SNP will give false calls in case of certain SNP constellations. To be sure about the primer and probe design, we ordered a self-designed TaqMan allelic discrimination probes for rs895819 and rs11671784 genotyping using the Applied Biosystems Assay-by-Design service. The two TaqMan probes comprise only the target SNP sites and avoid any overlap with the adjacent SNP (please see the primers and probes in Table 1). The observed genotyping bias introduced by the TaqMan allelic discrimination assay was as follows:

First, we genotyped rs895819 in 1,217 German familial breast cancer patients and 1,422 unrelated healthy German controls as the samples described by Yang et al. [1] by both sequencing and TaqMan allelic discrimination method. All the uncertain or inconsistent genotypes determined by both assays were investigated for the second time for confirmation. As a result, the rs895819 genotyping using and TaqMan allelic discrimination probes were not concordant with our sequencing results (Table 2). The genotype constellation rs895819-AA/rs11671784-TT all failed when genotyping; the genotype constellation rs895819-AG/rs11671784-CT resulted in false rs895819-GG instead of rs895819-AG calls (Table 2). Remarkably, researchers will not notify the bias via genotyping duplicates as the bias will be the same in both duplicates. As the genotype constellation frequencies of rs895819-AA/rs11671784-TT and rs895819-AG/rs11671784-CT are rather rare in Caucasian population (about 1.9 % in familial breast cancer cases and 1.4 % healthy controls [1]), it is also possible that the genotyping results are in Hardy–Weinberg equilibrium (HWE) although using biased genotyping assay. Nevertheless, this bias might have important implications on the final result.

We further genotyped rs11671784 in a subset of 413 samples containing all the observed genotypes by TaqMan

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Table 1 TaqMan primers and probes for allelic discrimination assays used for genotyping rs895819 and rs11671784

db SNP rs #	Taqman primers 5′–3′	Taqman probes 5′–3′
rs895819	F: GGGCGGAACTTAGCCACTG	VIC-TGAACACGACTTGGCGTG
	R: CAGGGCTTAGCTGCTTGTGA	FAM-TGAACACGACTTGGTGTG
rs11671784	F: GCCACTGTGAACACGACTTG	VIC-TGAGCAGGGTTCAC
	R: TGAGGAGCAGGGCTTAGCT	FAM-TGAGCAGGGTCCAC

Table 2 DNA samples genotyped for rs895819 by sequencing and TaqMan allelic discrimination methods

Sequencing			TaqMan assay of rs895829	
Genotype combination		Samples	Genotypes	Samples
rs895819	rs11671784			
AA	CC	1,112	AA	1,085
			AG	14
			Undetermined	13
AA	CT	66	AA	65
			Undetermined	1
AA	TT	3	Undetermined	3
AG	CC	1107	AA	7
			AG	1,084
			GG	6
			Undetermined	10
AG	CT	39	GG	38
			Undetermined	1
GG	CC	278	AA	1
			AG	5
			GG	268
			Undetermined	4

Table 3 DNA samples genotyped for rs11671784 by sequencing and TaqMan allelic discrimination methods

Sequencing			TaqMan assay of rs11671784	
Genotype combination		Samples	Genotypes	Samples
rs895819	rs11671784			
AA	CC	181	CC	180
			Undetermined	1
AA	CT	12	CT	12
AA	TT	3	TT	2
			Undetermined	1
AG	CC	167	CC	163
			Undetermined	4
AG	CT	8	TT	4
			Undetermined	4
GG	CC	42	CC	1
			Undetermined	41

allelic discrimination method and compared the results with sequencing data (Table 3). All the uncertain or inconsistent genotypes determined by both assays were investigated for the second time for confirmation. Although rs895819 and rs11671784 are closely located, no linkage between the two SNPs was observed in the German population (unpublished data by Yang. et al.). The TaqMan allelic discrimination assay for rs11671784 also introduced a bias and provided false calls. The genotype constellation rs895819-AG/rs11671784-CT half failed in the genotyping and half resulted in rs11671784-TT instead of rs11671784-CT; almost all of the genotype constellation rs895819-GG/rs11671784-CC failed in genotyping (Table 3). In together, about 12 % of the samples were failed in the genotype determination for rs11671784 by the TaqMan allelic discrimination assay.

Given these facts, we would like to point out that the use of direct sequencing, restriction or MALDI-TOF MassARRAY using primer extension from one direction (not overlapping with any SNP), is essential when investigating rs895819 and rs11671784. Generally, sequence polymorphisms in the vicinity of the SNP to be analysed should be carefully considered. In certain situation, it is of great importance to verify a portion of genotyping results of TaqMan allelic discrimination assay using an independent method like sequencing.

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Conflict of interest None.

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