Allelic drop-out is a common phenomenon reducing the diagnostic yield of PCR-based target sequencing

Shestak A.G. [1], *Bukaeva A.A.* [1], *Saber S.* [2], *Zaklyazminskaya E.V.* [1]

[1] *Petrovsky National Research Center of Surgery, Moscow, Russia*

[2] *Cardiac Electrophysiology Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran*

anna.shestak87@gmail.com

Authors declare no conflict of interest

Allelic drop-out (ADO) is a known phenomenon of selective allele amplification representing the potential problem of correct DNA diagnostics. Both NGS and Sanger sequencing are PCR-based methods, Sanger sequencinq is used to verify NGS results.

The aim is to demonstrate the incidence of ADO reducing the diagnostic yield in primary cardiomyopathy genetic testing via semiconductor NGS and Sanger sequencing of target gene panels

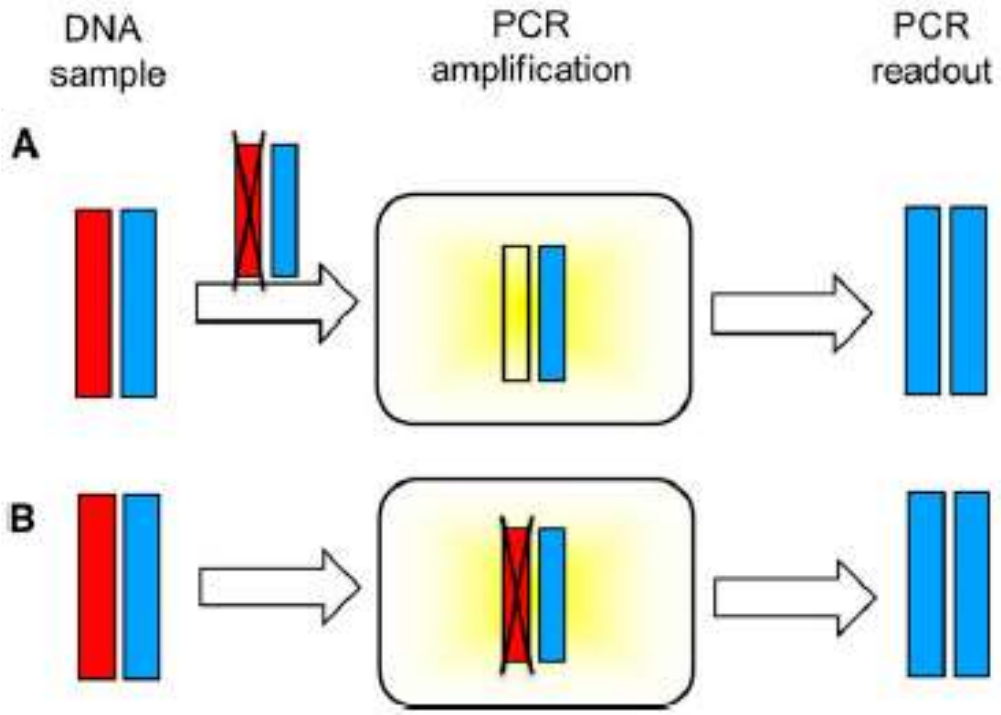


Figure 1. Two stages of allelic dropout
The red and blue bars are two allelic copies of a locus in a DNA sample.
A: Owing to sample-specific factors (low DNA concentration or poor DNA quality), one of the two alleles drops out when preparing DNA for PCR amplification.
B: Owing to locus-specific factors (low binding affinity between primers or polymerase and the target DNA sequences) or samplespecific factors (poor DNA quality), one of the two alleles fails to amplify with PCR
(Wang C, Schroeder KB, Rosenberg NA. Genetics. 2012;192(2):651-669).

METHODS

We formed 3 custom gene panels based on oligos automatically designed by AmpliSeq Designer® ("K^+/Na^+ ion channels", "Desmosomal proteins", and "Sarcomeric proteins") containing 1049 primer pairs for 37 genes, the total size is 152 kb. DNA samples of 140 probands were screened with at least one of these target gene panels. Data from Ion PGM™ System were processed with licensed Torrent Suite Software 5.6.0. List of variants found by NGS were visually compared with Sanger sequencing chromatograms.

RESULTS

We have detected 12 ADO cases both in PGM (7 cases) and Sanger (5 cases) sequencing data. We detected ADO causing variants in all cases. All ADO events had happened due to frequent or rare SNVs in the oligoprimer annealing sites and were detected because of the presence of «marker» SNV in the target DNA fragment. Three SNVs would be missed if were not revealed by re-sequencing with alternative method and alternative oligos.

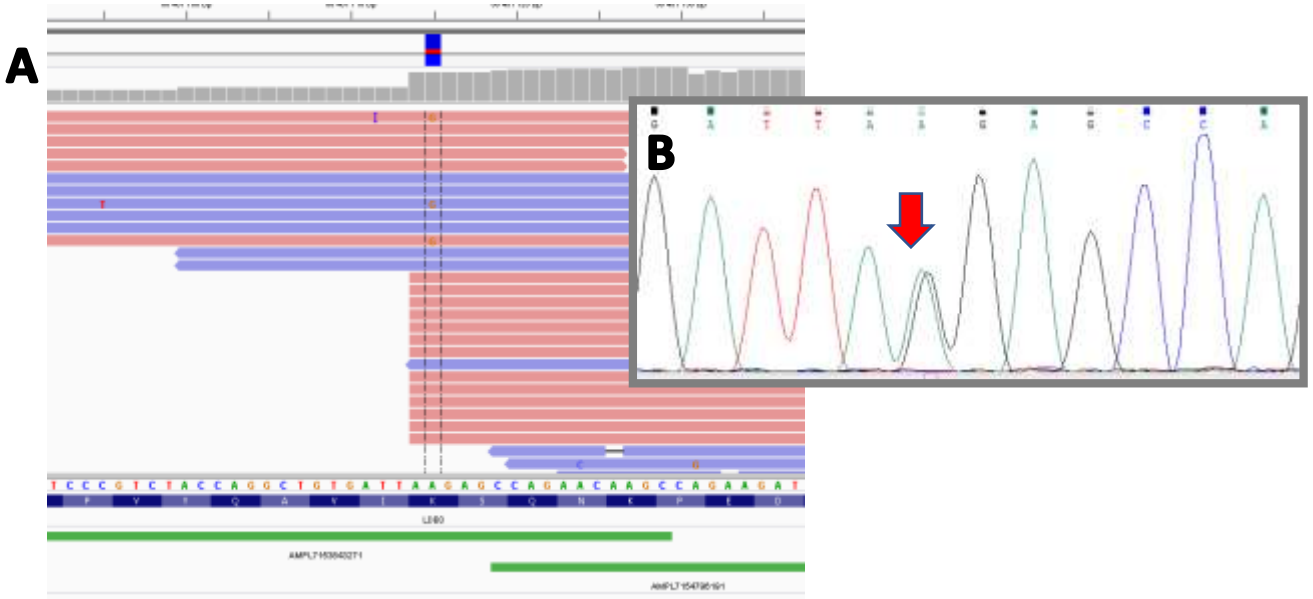
Table 1. Revealed cases of allelic drop-out in Ion Torrent sequencing data

Panel	Gene	Exon	ADO causing variant	Allele frequency in Europe (gnomAD)	Noted ADO cases
"K^+/Na^+ ion channels"	*SCN1B*	3	p.R214Q (c.641G>A)	0,42%	2
"K^+/Na^+ ion channels"	*SCN5A*	17	p.E1061E (c.3183A>G)	89%	many
"Desmosomal proteins"	*LDB3*	7	p.K251R (c.752A>G)	0.14%	1
"Sarcomeric proteins"	*LDB3*	10	p.T351A (c.1051A>G)	0,06%	1
"Desmosomal proteins"	*LDB3*	10	p.A358A (c.1074C>T)	4,4%	2
"Desmosomal proteins"	*FLNC*	10	p.Y538Y (c.1614C>T)	3,9%	1
"Desmosomal proteins"	*FLNC*	25	p.D1468D (c.4404T>C)	99,9%	many

Table 2. Revealed cases of allelic drop-out in Sanger sequencing data

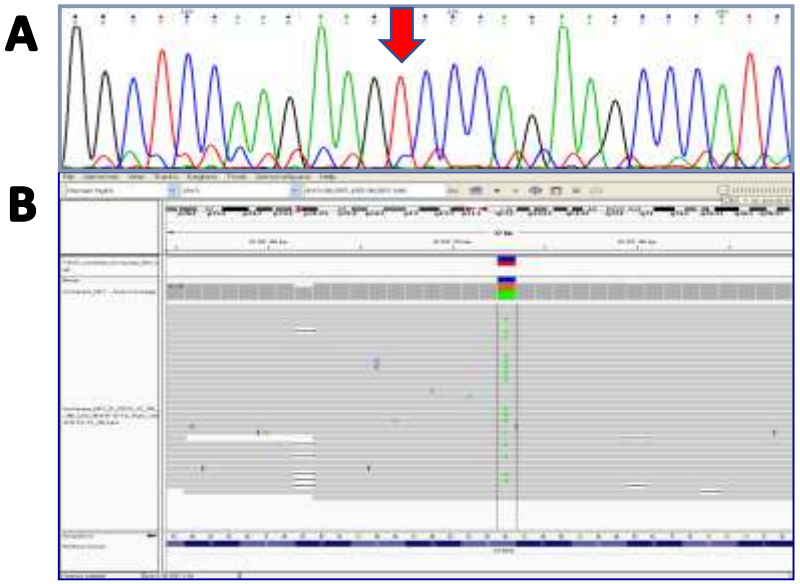
Gene	Intron	Oligo	SNV causing ADO	Allele frequency in Europe (gnomAD)
SCN5A	26	R	c.4542+89C>T	8,7%
PKP2	11	F	c.2300-195A>G	13,9%
DSP	14	F	c.1904-49T>A	41%
DSP	16	F	c.2298-85C>T	60,5%
DSP	22	F	c.3085-115C>T	67,6%

Fig. 2. Allelic drop-out in exon 7 of the LDB3 gene in proband NRF76 with arrhythmogenic right ventricular cardiomyopathy.



A: Ion Torrent sequencing results; genetic variant p.K251R (c.752A>G) is detected in only one of two overlapping amplicons;
B: Control Sanger sequencing chromatogram of exon 7 of the LDB3 gene in proband NRF76 with alternative pair of oligoprimers. Genetic variant p.K251R is heterozygous.

Fig.3. Allelic drop-out in exon 26 of the SCN5A gene occurred in patient sample.



A: Sanger sequencing chromatogram showing the presence of homozygous mutation p.P1506S in asymptomatic relative of the proband with Brugada syndrome; B: The same patient's Ion Torrent sequencing results proving p.P1506S to be heterozygous.

CONCLUSION

All PCR-based methods have a risk of ADO decreasing the diagnostic yield of genetic testing. Theoretically ADO might affect about 1% of amplicons. It seems that the real rate of ADO might be even higher and depends on numbers of oligoprimer's pairs. Specific software taking into account updating in SNVs distribution to avoid ADO in automatic oligo-primer's design would increase the accuracy of the molecular research.

Financial support of the study: Russian Science Foundation grant №16-15-10421