



CYP21A2 mutation spectrum in Bulgarian CAH patients



Medical University
Sofia

Albena Todorova^{1,2}, Andrey Kirov^{1,2}, Iva Stoeva³, Tihomir Todorov², Ganka Dineva³,
Antoaneta Kostova³, Ani Aroyo³, Vanio Mitev¹

¹Department of Medical Chemistry and Biochemistry, Medical University, Sofia, Bulgaria;

²Genetic Medico-Diagnostic Laboratory "Genica"; ³Univercity Pediatric Hospital, "Screening and Functional Endocrine Diagnostics" Medical University, Sofia, Bulgaria

INTRODUCTION

Congenital adrenal hyperplasia (CAH) is a group of inborn errors of steroidogenesis. It is mainly caused by steroid 21-hydroxylase coding gene (*CYP21A2*) mutation. More than 30% of the *CYP21A2* mutations are deletions, with ethnic specific differences. The Bulgarian mutational spectrum of *CYP21A2* gene is currently unknown.

MATERIALS AND METHODS

Nineteen patients, picked up by 17-OHP neonatal screening (nation-wide implementation 2010), were enrolled in the study. The MLPA method was chosen as a first step genetic testing and in many cases targeted sequencing may prove point mutations detected by the MLPA. Sequencing of the whole gene was chosen as a second step.

The mutation nomenclature is based on Human Genome Variation Society (HGVS) recommendations and NCBI Reference Sequence: NM_000500.7.

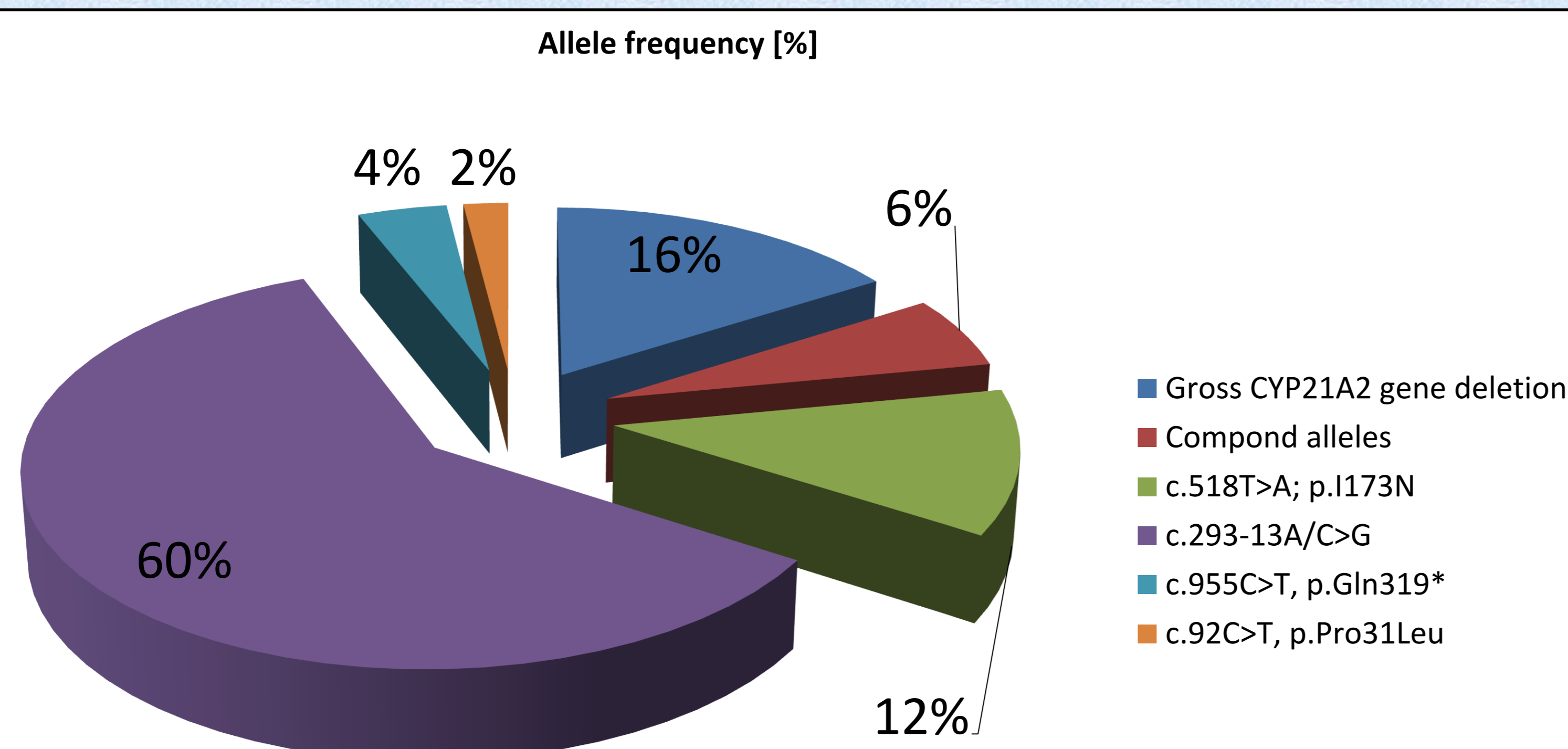


Fig 1. *CYP21A2* gene allele frequency in Bulgarian CAH patients

RESULTS

The molecular genetic testing detected a heterozygous deletion of *CYP21A2* gene (16% allele frequency). The whole gene sequencing revealed the splice site mutation c.293-13A/C>G (60%), missense mutations – c.518T>A, p.1173N (12%) and c.92C>T, p.Pro31Leu (2%); nonsense mutation c.955C>T, p.Gln319* (4%) – (Fig 1.) Three of our patients are carrier of compound allele with more than one mutation (Fig 2).

The patient reveals classic salt-wasting CAH phenotype caused by a homozygous c.923dupT mutation and a heterozygous mutation c.334G>A. The double mutated allele was inherited from the mother and it is most probably formed due to non-allelic homologous recombination (NAHR) between the *CYP21A2* gene and its pseudogene (Fig 3).

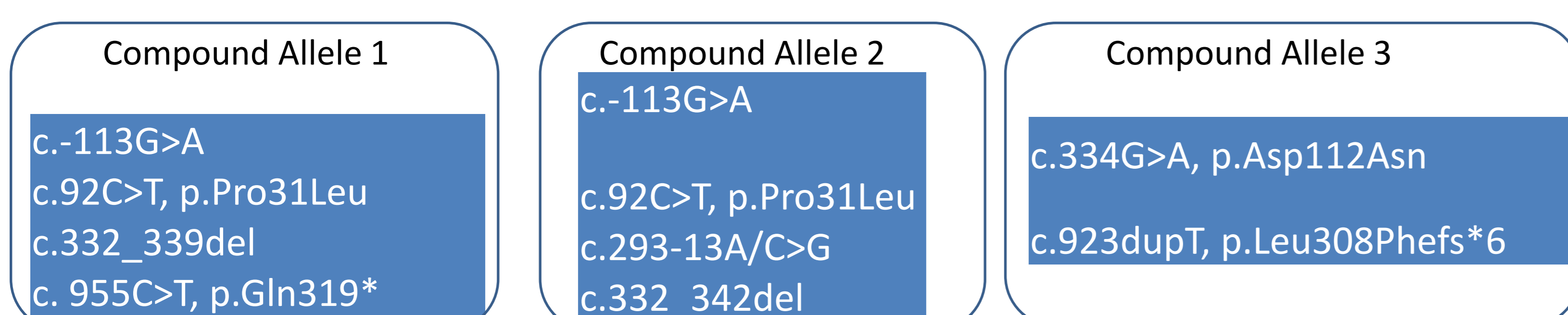


Fig 2. Generation of deletion rearrangement by NAHR

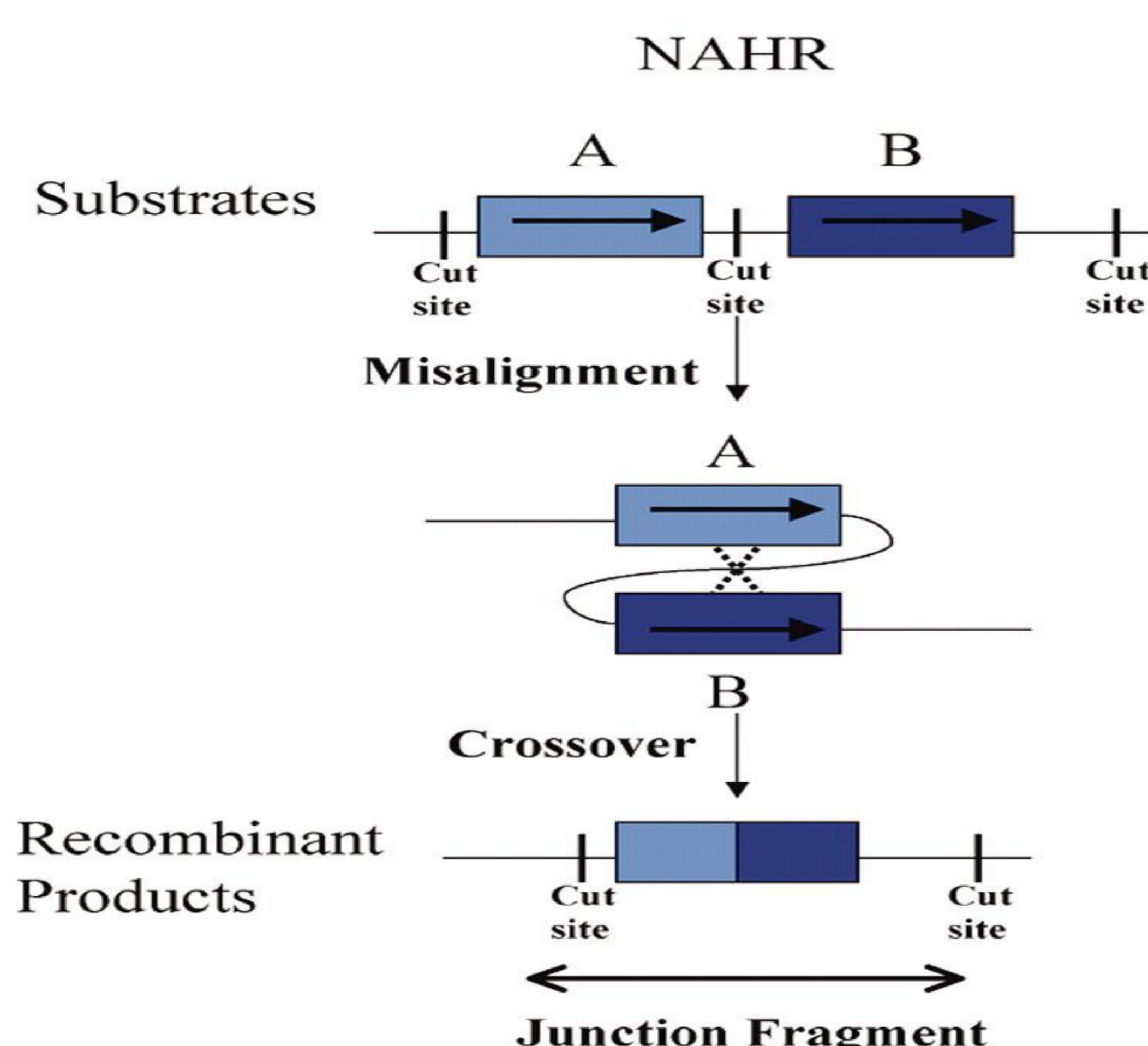


Fig 3. Generation of deletion rearrangement by NAHR

CONCLUSIONS

Our results showed that the *CYP21A2* genetic screening in Bulgarian patients should begin with the most common mutations c.293-13A/C>G and c.518T>A, followed by MLPA analysis. The patients without mutations or with one mutation are subjected for whole gene sequencing.

Although the proposed algorithm is effective for genetic confirmation of the clinical diagnosis, we should keep in mind that due to possible NAHR involving pseudogene some patients may carry more than 2 mutations in the *CYP21A2* gene and some of the genetic changes could be *de novo*. This is important for *CYP21A2* mutation carrier status determination in relative and in case of prenatal diagnostics.

Acknowledgements: The study was supported by the Grants No 30/2013 and 42D/2013, Sofia Medical University, Bulgaria

contacts <todorova_albena@abv.bg>, <stoeva_i@abv.bg>