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MOLECULAR BIOLOGICAL INVESTIGATIONS OF ENDEMIC NEPHROPATHY

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Abstract

Today, the investigation of different human diseases cannot be successful without involving of this disease molecular aspect. It means modern medicine and molecular biology is fortunately irreversible connected disciplines that together provide disease investigation on the level of informatics macromolecules (DNA and RNA). It is now possible to detect causative agent of some disease4 very fast with sensitivity and specificity almost 100%. Molecular biology provide important inform ations about many human disease such as Diabetes mellitus-1, Familial adenomatous polyposis, Cystic fibrosis and in the same time is crucial in prenatal diagnostics and transplantation medicine.

Molecular aspect of endemic nephropathy investigation, of course, provides also confirmation of wide accepted hypothesis about genetic predispositions of humans to this disease. BEN (Balkan endemic nephropathy) is disease of not yet known ethiology. By using of several molecular methods it is determined that 3q21.3-3q27.3 region of human 3 chromosome is connected with BEN. The investigation of polymorphic microsatelite DNA sequences of this region will be useful in solving of BEN genetic background. But priority surely will be finding of main aethiological factors included in making of BEN specific genetic disorders. Only multidisciplinary approach in solving of this disease will be successful, in which molecular biology will play very important role.

Introduction

Many epidemiological aspects of Balkan endemic nephropathy (BEN) are very similar to those determined in countries in which it occurs (Romania, Bulgaria, Yugoslavia, Croatia and Bosnia). Despite of

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numerous investigations, the aetiology of this disease remains not yet known. The main clinical feature is tubulo-interstitial nephropathy. All things considered many of scientist included in research of BEN agreed that secondary metabolite of Aspergillums and Penicillium species, possible viral infection, environmental conditions as well as genetic predispositions are important crucial factors in BEN development.

Recently, modern molecular-biological investigations of BEN provide new important informations about phenotype-genotype correlations associated with this disease. But, unfortunately, molecular mechanisms which cause specific genetic changes of BEN responsible genes and main aehiological factors which cause that, remain a big misters and will be the object of molecular investigations in the near future.

Molecular methods of BEN investigation

Fluorescent in City Hybridisation (FISH) – is method that is used of DNA target sequences in chromosome cell or tissue. By FISH is possible direct identification of candidate genes, part gene, specific chromosome regions or whole chromosome. It is base on hybridisation of two complementary sequences in metaphase or interphase nuclei: FISH analysis provides also information's about caryotype changes that are invisible by classical cytogenetics method. For BEN investigations all FISH probes were applied in combination with alternatively labelled chromosome 11 centromeric probes, because of FGF3 and FGF4 genes (Fibroblast growth factor protein genes) location at human 11 chromosomes. (*Toncheva etal., 2002.)*

Polymerase Chain Reaction (PCR) – By PCR is investigating polymorphism of microsatelite DNA sequences (associated with BEN) at human 3 chromosome (3q 21.3.3q 27.3 region).

Isolation of human DNA is performed usually by QIAGEN extraction kit.

Amplification in thermal cycle according specific primers and detection of PCR products by gel electrophoresis with specific DNA markers.

Phlyacrylamid – gel electrophoresis (PAGE) is important in separation of nucleic acid, by using of silver staining procedure and specific markers.

By DNA sequencing it is possible to detect mutations of genes and their analysis as well.

But it is necessary before DNA sequencing perform PCR amplification of target nucleotide sequences.

Discussion of obtained results of BEN molecular investigations

Genetic predispositions and different environmental conditions are surely factors very important for BEN appearance including possible viral aetiology. By using of molecular methods (FISH, PCR, DNA-sequencing, PAGE-polyacrylamid gel electrophoresis) there is provided new information's crucial for solving and better understanding of BEN genetic background. Now, it is widely accepted hypothesis about multifactorial aetiology of this disease.

Molecular investigation of BEN patients provide the following information's:

- the polymorphisms of Nat 2 and MDR genes (3 chromosome) can mean increased possibility for BEN development.
- CYP2DG allele distribution, other human 3 chromosome anomalies in the region 3q24 – 3q27.3 may be markers for BEN susceptibility.
- Frequent changes of chromosome X could be associated with the female BEN predominance. *(Atanasova et al., 2002)*
- Investigation of FGF3 and FGF4 genes (Fibroblast growth factor genes located at 11Q13 region may be useful for better understanding of BEN tumors.

Modern investigation of BEN cannot be imagined without great possibilities at molecular biology. In fact recent medicine and molecular biology go together into 21 century. Molecular biological methods provide presimtomatic, early fast and high specific diagnostics and realisation of preventive therapy.

Investigation of many diseases such as Diabetes mellitus-tipl, familiar adenomatous polyposis, Cystic fibrosis, Hemophilia is successful by using of molecular methods that are very important also in prenatal diagnostics and transplantation medicine as well.

The realisation of BEN investigation project must include molecular aspect and multidisciplinary approach in solving this great medical problem and institutions that already have infrastructure for specific aspect of researches. *(Zahaaireva et al., 2002; Atanasova et al., 2002;)*

Apstrakt

Savremena istraživanja različitih humanih oboljeja nebi bila tako uspješna bez uključivanja molekularnog aspekta. To podrazumjeva da su moderna medicina i molekularna biologija neodvojive discipline koje zajednički omogućavaju istraživanje bolesti na nivou informacionih makromolekula (DNK i RNK). Moguće je detekovati uzročnika bolesti veoma brzo sa senzitivnošću i specifičnošću od skoro 100%.

Molekularna biologija obezbjeduje važne informacije o mnogim humanim oboljenjima kao što su *Diabetes melitus* tip I, Familijarna adenomatozna polipoza, Cistična fibroza, a istovremeno je od krucijalnog značaja u prenatalnoj dijagnostici i transplantacionoj medicini.

Rezultati molekularnog aspekta istraživanja endemske nefropatije omogućavaju potvrdu opšteprihvaćene hipoteze o genetičkim predispozicijama ljudi na ovu bolest. BEN (Balkanska endemska nefropatija) je oboljenje još uvijek nepoznate etiologije.

Upotrebom nekoliko molekularnih metoda determiniran je 3q21.3-3q27.3 region na 3 humanom hromosomu koji je povezan sa BEN. Istraživanje polimorfizma mikrosatelitskih DNA sekvenci koje se nalaze u ovom regionu, biće korisna u rješavanju genetičke osnove BEN. Međutim, svakako da će prioritet biti pronalaženje genetičke promjene specifične za Balkansku endemsku nefropatiju. Jedino će multidisciplinaran pristup istraživanja ove bolesti biti uspješan u kome će molekularna biologija imati značajnu ulogu.

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