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# Individualized medicine 2010

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## Abstract

Molecular and cell biology have revolutionized not only diagnosis, therapy and prevention of human diseases but also greatly contributed to the understanding of their pathogenesis. Based on modern molecular and biochemical methods it is possible to identify on the one hand point mutations and single nucleotide polymorphisms. On the other hand, using high throughput array technologies, it is possible to analyse thousands of genes or gene products simultaneously, resulting in an individual gene or gene expression profile (signature). These data increasingly allow to define the individual risk for a given disease and to predict the individual prognosis of a disease as well as the efficacy of therapeutic strategies (individualized medicine). In the following sections some of the recent advances of predictive medicine and their clinical relevance will be addressed.

**Keywords:** array analyses • genomics • transcriptomics • proteomics • metabolomics • microRNA • genome-wide association studies • disease risk • disease prognosis • prediction of therapeutic efficacy • individualized medicine

## Molecular analyses

The basic aspects of molecular and cell biology are not only integral part of biomedical research but are increasingly becoming also relevant for patient care. The genetic material of all living organisms is made up of DNA that in its entirety makes up the individual's genome. Genomics aim at the unravelling of the complete genetic information of an individual's genome [1]. In the context of the human genome organization project the complete sequence of the human genome was established almost 10 years ago [2, 3].

In order to utilize the sequence information from the human genome organization project for research as well as for clinical applications and to define the function(s) of newly identified genes, strategies were developed to globally analyse genomic DNA sequences as well as their cell-, tissue- or organ-specific expression profile that are collectively termed 'functional genomics'.

An important instrument of functional genomics are array analyses which are based on the complementary base-pairing of single-stranded DNA or RNA (genes) to form a double-stranded hybrid (hybridization). This principle has been successfully utilized for several decades for the analysis and characterization of DNA (Southern Blot) und RNA (Northern Blot). Different from these primarily research tools which allow the simultaneous analysis of a limited number of genes only, the more recently developed chip/array technologies now make the simultaneous analysis of large numbers of genes possible.

Using chips, also termed 'microarrays' with a surface of in general about 0.5 $cm^2$, thousands or ten thousands of single-stranded DNA species, reverse transcribed RNA or oligonucleotides of known sequence can be placed in a highly ordered pattern. These array analyses provide a global gene or gene

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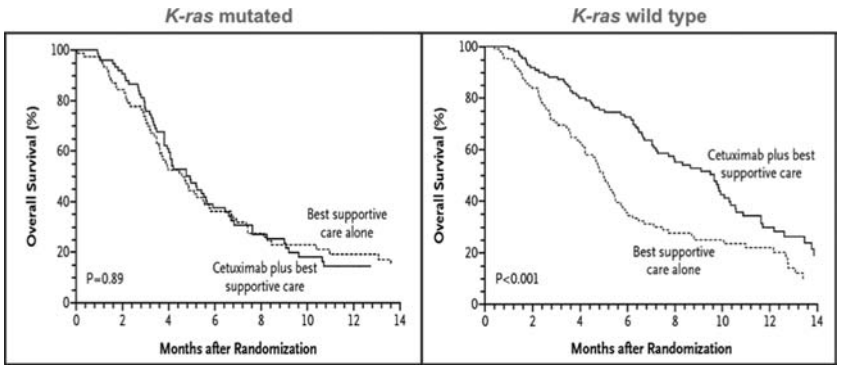


**Fig. 1** Prognostic (patient survival) and predictive relevance (therapeutic efficacy of cetuximab) of the KRAS mutation in colorectal carcinoma [14].

expression profile (signature) and allow identifying and characterizing complex genomic polymorphisms.

# Mutations – polymorphisms – HapMap

## Mutations

Mutations are alterations of DNA or RNA sequences, *e.g.* of human genes. Apart from deletions, insertions, loss of heterozygosity and other genetic alterations point mutations are of major biological and clinical importance. Apart from the start codon (ATG) and three stop codons (TAA/TAG/TGA) a defined base triplet codes for an amino acid (genetic code), *e.g.* AAA codes for lysine. At the same time, a given amino acid can be encoded by two or more base triplets, *e.g.* lysine is encoded by AAA and AAG (degeneracy of the genetic code). It follows from this that a mutation does not necessarily result in an amino acid substitution (silent mutations). Mutations resulting in an amino acid substitution can have different consequences: conservative mutations result in the replacement of an amino acid by a similar amino acid, *e.g.* the mutation GCC to GGC results in the replacement of alanine by glycine. Missense mutations, by comparison, result in a biologically significant amino acid substitution, *e.g.* the mutation GCC to cholangiocellular carcinoma (CCC) results in the replacement of alanine by proline that is frequently associated with a change of the conformation of the protein, resulting in functional consequences. The most severe mutations are non-sense mutations, *i.e.* the replacement of an amino acid by a stop codon. This results in the termination of transcription and a C-terminal truncation of the protein that is generally associated with a loss of function. Mutations are frequent but are usually recognized and corrected by the cellular DNA repair system. If the repair system is not functional, mutations may be fixed and then cause severe diseases, including malignancies.

## Mutations and diseases

Mutational analyses have in recent years increasingly become part of the clinical patient management, especially, for patients with malignant diseases. In colorectal cancer (CRC) tissues, for example, genetic markers have been identified that allow to predict the prognosis of the individual patient as well as the response to a 5-fluorouracil based therapy [4–6] Also for irinotecan, another chemotherapeutic agent used to treat patients with advanced CRC, a molecular marker was identified that predicts the response to therapy: only patients with a microsatellite instability in the tumour that results in a DNA repair defect benefit from this treatment [7]. Alternatively, the expression level of topoisomerase in the individual patient's CRC allows selecting patients who may benefit from irinotecan [8].

In recent years, the 'targeted therapy' of malignant diseases with monoclonal antibodies and low molecular weight tyrosine kinase inhibitors (TKI, nibs) alone or in combination with conventional chemotherapeutic strategies resulted for several tumour entities in a significantly improved overall or recurrence-free patient survival. For patients with metastasized CRC the monoclonal antibodies bevacizumab, cetuximab and panitumumab have already become part of the standard treatment in clinical practice [9, 10]. In this context it was recently shown that only patients with wild-type KRAS or wild-type BRAF tumours benefit from cetuximab or panitumumab [11–15]. Therefore, by determining the KRAS status before therapy allows predicting whether the patient will benefit from cetuximab (Fig. 1). For patients with a mutated KRAS or BRAF gene this mab therapy will, therefore, not be recommended, saving costs and not unnecessarily exposing patients to potential adverse events.

Another molecular marker was identified in patients with non-small lung cancer: a mutation of the epidermal growth factor receptor gene predicts a response to treatment with the TKI gefitinib [16]. Further, patients with a human epidermal growth factor receptor-2 $(HER2)^+$ metastasized gastric cancer, found in about 20% of the patients with this malignancy, benefit from treatment with the mab trastuzumab [17, 18], similar to patients with $HER2^+$ breast cancer. These examples make it likely that through the molecular characterization of tumours, it will increasingly be possible to identify subgroups of patients for whom the efficacy of a given drug can be predicted (individualized medicine). Studies addressing these predictive aspects are presently under way for many human tumours [19, 20].

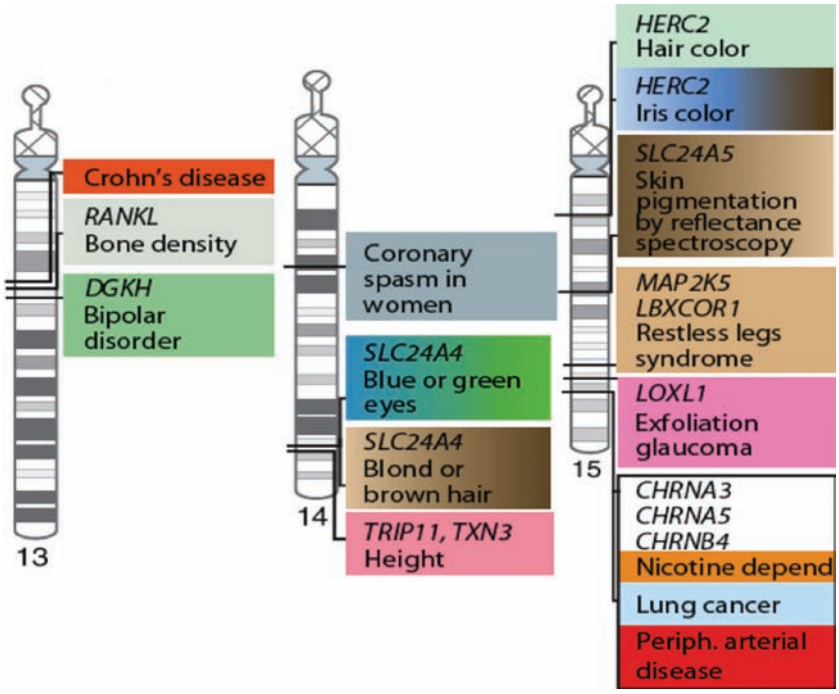


**Fig. 2** HapMap for chromosomes 13, 14 and 15 (modified from [28]).

## Polymorphisms

A single nucleotide polymorphism (SNP) is a mutation/ base substitution in a defined locus of the human genome that occurs in the population at a frequency of $>$1%. A practical example of significant epidemiological and clinical interest is the interleukin (IL)-28B gene that codes for interferon λ. For this gene 3 variants/ genotypes have been identified (haplotypes for alleles 1 and 2): T/T, C/C and T/C. The frequency of these genotypes in the general population depends among others from the individual's ethnic background: T/T is most frequent in Africans (60–80%) while in Asians genotype C/C predominates (about 90%).

## Polymorphisms and diseases or disease predispositions

Staying with the example of the polymorphisms of the IL-28B gene it was found that it has a major impact in patients with a hepatitis C virus (HCV) infection. Patients with the IL-28B genotype C/C eliminate the HCV infection spontaneously or during antiviral therapy much more frequently than patients with genotype T/T. Patients with genotype T/C have an intermediary frequency of spontaneous elimination or response to therapy [21–24]. In another study it was shown that a specific polymorphism resulting in a inosine triphosphatase deficiency protects from ribavirin induced haemolysis that presents a frequent problem in patients with chronic hepatitis C undergoing antiviral treatment [25].

## HapMap project

In the context of the international HapMap consortium there is a genome-wide search for polymorphisms of four ethnically different populations and their association with human diseases [26–29]. Through these genome-wide association [3] studies more than 150 gene loci have been identified that are associated with more than 60 frequent human traits, such as hair colour, eye colour and height or with the individual's disease-specific risk (Fig. 2) [28, 29]. GWA studies revealed gene loci that, for example, predict the individual's risk to develop coronary heart disease [30, 31], restless legs syndrome [32], sporadic amyotrophic lateral sclerosis [33] or multiple sclerosis [34]. Further, a polymorphism in the apolipoprotein C3 gene has recently been identified that is associated with non-alcoholic steatohepatitis and insulin resistance [35]. For hepatocellular carcinoma (HCC) a polymorphism in the epidermal growth factor gene was identified (genotype G/G) that is associated with a 4-fold increased risk in the development of an HCC [36]. Also with respect to the individual's breast cancer risk, several gene loci have been identified by GWA studies [28].

GWA studies, therefore, allow an increasingly better understanding of the pathogenesis of human diseases that potentially translates into improved diagnosis, therapy and prevention. In addition, the identification of defined polymorphisms may contribute to assess the individual disease-specific risk. However, the contribution of genetic polymorphisms to the risk assessment for a given disease must, for each disease entity, be carefully weighed against established clinical parameters. In this context, a recently

published study found an only marginal contribution of genetic data to the assessment of the individual's risk to develop breast cancer as compared to the clinically established Gail model [37]. Thus, there are also reservations regarding the clinical relevance of polymorphisms to predict different human traits or disease risks [38, 39].

## Signature analyses – omics

With the determination of the complete sequence of the human genome [2, 3], the identification and characterization of human genes and the regulation of gene expression became of paramount interest. For these projects methods were developed that allow the high-throughput analysis of the human genome (genomics), messenger RNAs (transcriptomics), proteins (proteomics) or metabolites (metabolomics) in cells, tissues or organs (omics). Since the start of the human metabolom project in 2005 more than 8000 metabolites have been identified/ characterized and deposited in a data bank that is freely accessible. Since the function of a given protein largely depends on its secondary and tertiary structure (folding) structural genomics aim at the definition of the complete folding inventory of a newly identified protein in order to define its potential functions. These methodologies increasingly allow an insight into cellular pathways and biological networks that are collectively termed 'systems biology' [1]. This approach should allow to further optimize the diagnosis, therapy and prevention of human diseases and to contribute to the prediction of the individual's prognosis and response to therapy [40].

The array technologies allow to simultaneously analyse tens of thousands of genes and their expression at the mRNA and/or protein level. It is thus possible to identify an individual gene or gene expression signature in malignant tumours or tumour-associated cells or tissues (microenvironment) that permits to predict the individual patient's prognosis and/or the response to a specific drug. In addition, pharmacogenetic analyses based on the individual's genomic DNA (pharmacogenetics) increasingly allow to predict the efficacy as well as potential side effects of drugs (individualized pharmacotherapy) [41].

In the following text, the contribution of signature analyses to the understanding of the pathogenesis, the prediction of the natural course and the clinical management of patients will be illustrated for selected clinical examples.

### Lymphoma

One of the very first clinical applications of the DNA chip technology was in patients with a diffuse large cell B cell lymphoma (DLBCL). The gene expression signatures allowed to defining two molecular subgroups with different prognosis and clinical risk score [42]: patients with a 'germinal' centre B- signature who have a good prognosis and patients with an 'activated B-like' DLBCL signature with a poor prognosis. More recently, it was shown that patient survival after chemotherapy is not only dependent on tumour cell characteristics but also on the microenvironment of the tumour [43]: patients with a good prognosis have a so-called 'stromal 1' signature characterized by the presence of histiocytes and connective tissue. By comparison, patients with an unfavourable prognosis have a 'stromal-2' signature characterized by a high density of blood vessels (angiogenesis).

Also for the classic Hodgkin's lymphoma, a microenvironment signature of tumour-associated $CD68^+$ macrophages was identified that predicts a poor prognosis [44]. By comparison, 100% of patients with Hodgkin's lymphoma survive if the microenvironment signature of tumour-associated $CD68^+$ macrophages was negative.

### Breast cancer

Based on a signature of RAS and other dysregulated genes in tumour tissues, a new classification of breast cancer was recently proposed [45]. Further, DNA microarray analyses of 70 genes defined two different gene expression profiles [46]. These allow predicting the prognosis of the disease ('good prognosis signature' *versus* 'poor-prognosis signature'), also with respect to the risk of lymph node metastases and tumour recurrence [47], as well as the response to chemotherapy [48]. In another study it could be shown that the amplification of HER2 in the breast cancer predicts the therapeutic efficacy of anthracyclines [49]. Further, Liu *et al.* [50] defined an 'invasiveness gene signature' (IGS) based on 186 different genes: 100% of patients with a favourable IGS had a 10 year overall survival and 81% had a 10 year metastasis-free survival, respectively, as compared to 60% and 57% of patients, respectively, with an unfavourable IGS. Importantly, the prognostic contribution of the IGS was independent from established clinical and histopathological parameters.

The identification of IGS, for example, and of pathogenesis networks may become relevant also for therapeutic decisions. Signatures predicting a poor prognosis may be an indication for a more aggressive chemotherapy. Conversely, signatures predicting a favourable prognosis may justify not recommending an adjuvant chemotherapy [51]. Array signatures thus reflect a major advance of medicine from a more empirical clinical management to an individually 'tailored' medicine based on the individual patient's genetic profile [52].

### Lung cancer

Based on proteome analyses it became possible to more precisely categorize the histology of lung tumours and to clearly distinguish between primary lung tumours and lung metastases [53]. Additional studies recently revealed that the individual prognosis of patients with non-small cell lung cancer (NSCLC) can be predicted by a 5-gene signature [54, 55]. Further, in a recent case report metabolomics could predict the response to treatment with the TKI erlotinib in a patient with a subtype of NSCLC [56].

### Prostate cancer

Metabolomic profiles were able to distinguish between benign prostate, localized prostate cancer and metastatic disease and identified sarcosine as an important metabolic intermediary involved in prostate cancer cell invasion and aggressiveness [57]. Further, metabolomics allowed predicting the risk of recurrence [58].

### Hepatocellular carcinoma

The HCC is world-wide frequent malignancy with an epidemiologically and genetically very heterogeneous background [59–61]. The gene and gene expression profiles of HCCs and their relevance for the patients' prognosis were analysed in numerous studies [62–64]. Depending on the etiological background of the underlying chronic liver disease a 3-gene and a 120-gene signature, respectively, were identified that allow distinguishing between dysplasia and neoplasia [61]. In addition, transcriptome analyses of HCCs identified several genes which suggest a novel HCC classification with therapeutic implications [65]. Further, microarray analyses of 6000 genes in completely resected HCCs showed that 12 genes predict a high risk for an early intrahepatic recurrence [66]. If validated by additional studies, it thus may become possible to preoperatively distinguish patients with a high risk of recurrence from those with a low risk of recurrence. This HCC sub-categorization may contribute to further optimize the therapeutic strategy for the individual patient.

Apart from clinical parameters and the gene or gene expression profiles of tumour-associated genes, such as glycipan-3, heat shock protein 70, survivin, LYVE1 and microRNA (miR) species (see below) in the tumour (tumour profiling), there is increasing evidence that the gene or gene expression signature in cells or tissues adjacent to the (later developing) HCC contributes to the risk of HCC development and the risk of HCC metastases, respectively (adjacent tumour profiling).

In cell biology and disease pathogenesis, miR species are increasingly attracting attention. The miR species are non-coding RNA molecules of 20–25 nucleotides length which suppress gene expression in the cell nucleus by complementary base-pairing with the 3′-non-translated region of mRNAs, thereby blocking translation. Based on this principle action, miR can function, among others, as tumour suppressors or oncogenes. Analyses addressing the role of miR in HCC development revealed that females have a higher expression of miR-26a and miR-26b in the non-tumourous liver tissue adjacent to the HCC than males. Further, the expression of these miR species is lower in the HCC as compared to the adjacent non-tumourous liver and patients with a low miR expression in the HCC have a poorer prognosis but respond better to interferon treatment than patients with a high miR expression in the tumour [67]. Other miR species have recently been identified that are involved in HCC development (miR221; [68]), HCC invasion and metastasis (miR-30d; [69]) as well as the prognosis of patients with this malignancy (miR-29; [70]).

### Cholangiocellular carcinoma

The early detection of CCCs is a major clinical challenge and is currently based on imaging analyses, brush cytology, histology and tumour markers in serum. Conceptually based on the fact that bile contains numerous metabolites, bile from healthy individuals as well as from CCC patients was analysed by nuclear magnetic resonance spectra, followed by orthogonal partial least square discriminating analyses, in order to detect and characterize the full complement of metabolites in bile (metabolomics). Metabolomics analyses indeed identified CCC patients with a sensitivity of 88% and a specificity of 81% [71]. If validated by additional studies, 'metabolomics' may indeed complement the (limited) diagnostic tools presently available in clinical practice.

### Non-malignant diseases

Recently it was discovered that specific miR species affect the biology of HCV, *i.e.* miR199a effectively suppresses HCV replication and thus may represent a novel antiviral strategy for patients with chronic HCV infection [72, 73]. Further, in patients with chronic hepatitis C a specific gene signature in liver tissue seems to predict the progression of chronic hepatitis C to liver fibrosis and cirrhosis [74, 75]. Metabolomics was also shown to have an impact on the diagnosis of celiac disease using serum or urine [76].

## Conclusions and perspectives

Recent advances in cell and molecular biology allow an increasingly detailed understanding of the pathogenesis of human diseases. With the rapid development of novel molecular and biochemical analyses it is now on the one hand possible to identify disease-related point mutations and SNPs and on the other hand, based on array technologies tens of thousands of genes or proteins can be analysed simultaneously.

In addition to malignant diseases, there are more and more examples of the predictive power of molecular and biochemical analyses for other disease entities. In this context, a recent study in patients after heart transplantation defined a gene expression profile in peripheral blood that closely correlates with the histological findings in endomyocardial biopsies [77]. If validated by further studies, such profiles may contribute to the non-invasive management of patients after heart transplantation. Further, genetic variants of the cytokine-inducible SRC homology 2 domain protein gene have been shown to be associated with an increased susceptibility to bacteremia, malaria and tuberculosis [78]. Another recent advance in the field of molecular analyses is the sequencing of the complete genome of an individual with a family history of vascular disease and sudden death. The interpretation of the sequence data obtained identified a genetic risk for myocardial infarction, diabetes mellitus type 2 and several malignancies [79, 80].

Based on recent advances in most fields of medicine we can expect an increasing number of clinically relevant applications of molecular analyses, including specific point mutations or SNPs as well as individual gene or gene expression profiles. These analyses should allow predicting the individual risk to develop a disease (HapMap project) and to assess the individual prognosis as well as the efficacy of therapeutic strategies in patients suffering from malignant or non-malignant diseases (individualized medicine).

## Conflict of interest

The authors confirm that there are no conflicts of interest.

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