# Karika koja nedostaje između genotipa, fenotipa i kliničkih nalaza

# The missing link between genotype, phenotype and clinics

Giuseppe Lippi[1], Emmanuel J Favaloro[2]

[1]Odsjek za kliničku kemiju, Odjel za morfološke i biomedicinske znanosti, Sveučilište u Veroni, Verona, Italija

[1]Institute of Clinical Chemistry, Department of Morphological and Biomedical Science, University of Verona, Verona, Italy

[2]Odsjek za hematologiju, Institut za kliničku patologiju i medicinska istraživanja, Bolnica Westmead, Westmead, Australija

[2]Department of Hematology, Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, Westmead, Australia

**Sažetak**

Broj genetskih testova bilježi eksponencijalan rast u kliničkim laboratorijima, zahvaljujući izvanrednim znanstvenim i tehnološkim dostignućima, uz potporu relativno jeftinih, brzih, pouzdanih i visoko propulzivnih tehnika. Sasvim je neupitno da je genetsko testiranje pomoglo u identificiranju molekularne osnove monogenskih bolesti, kao i mnoštva gena upletenih u većinu poligenskih patologija. To je pak omogućilo individualizaciju liječenja i farmakološke terapije. Međutim, to je isto tako stvorilo jedan paradoks u upravljanom liječenju bolesnika prema kojemu se genetsko testiranje sad često vidi kao *panacea,* „lijek za sve", pretpostavljajući kako je svaki pojedini genetski polimorfizam udružen sa specifičnim, pojedinim fenotipom i/ili kliničkom slikom. Nažalost, više procesa koji reguliraju ekspresiju proteina ostaje i dalje nepoznato, dok njihova biološka osnova još nije konačno prepoznata, pa veza između genotipa, fenotipa i kliničkih nalaza nije uvijek očita te je često još teže ispitati u kojoj će mjeri veza između gena i okoline utjecati na liječenje bolesnika. Cilj ovoga članka je pružiti kritički osvrt na složen i višeslojan odnos koji povezuje gene, biokemiju i kliničke podatke, s naglaskom na prednosti i nedostatke genetskog testiranja kod monogenskih i poligenskih bolesti, te u predviđanju farmakološkog odgovora.

**Ključne riječi:** genotip; fenotip; klinički nalazi; genetsko testiranje

**Abstract**

Due to outstanding scientific and technological progresses, the number of genetic tests is growing exponentially in clinical laboratories, supported by relatively inexpensive, fast, reliable, high-throughput techniques. It is unquestionable that genetic testing has helped to identify the molecular bases of monogenic disorders as well as a variety of genes involved in most multifactorial pathologies. This in turn has allowed for personalized treatments and pharmacological therapies. However, it has also produced a paradox in the managed care of patients, in that genetic testing is now often perceived as a *panacea*, with assumptions that each single genetic polymorphism is associated with a specific, individual phenotype and/or clinical picture. Unfortunately, several processes regulating protein expression are still unknown, and their biological background has not been definitely recognized, so that the link between the genotype, the phenotype and the clinics is not always obvious, and it is often even more challenging to address how much the link between genes and environment will impact on the managed care of the patients. The present article aims to critically review the complex and multifaceted relationship linking genes, biochemistry and clinics, highlighting advantages and drawbacks of genetic testing in monogenic disorders, polygenic pathology and in the prediction of the pharmacological response.

**Key words:** genotype; phenotype; clinics; genetic testing

*Pristiglo: 2. ožujka 2009.* *Received: March 2, 2009*

*Prihvaćeno: 21. travnja 2009.* *Accepted: April 21, 2009*

## Uvod

Genetika je znanost o nasljeđivanju i raznovrsnosti živih organizama, koja istražuje funkciju gena, ustrojstvo genoma, organizaciju kromatina, stopu rekombiniranja, procese mutacije i povijest evolucije, kako bi pružila suvislo shvaćanje ljudskog genoma i njegovog složenog odnosa s biologijom, fiziologijom i bolešću. Genomika se pak u pravilu definira kao intenzivni napori da se odredi cjelokupna sekvenca DNA pojedinih organizama te da se provede podrobno genetsko mapiranje uključujući stu-

## Introduction

Genetics is the science of heredity and variation in living organisms, which investigates gene function, genome structure, chromatin organization, recombination rate, mutation processes, and evolutionary history, to provide a coherent understanding of the human genome and its complex relationship with human biology, physiology and disease. Genomics is instead typically defined as the intensive efforts to determine the entire DNA sequence of organisms and fine-scale genetic mapping efforts,

dije intragenomskih pojava kao što su heteroza, epistaza, pleiotropija i druge interakcije između lokusa i alela unutar genoma. Genetsko testiranje se prvotno razvilo prije gotovo dvadeset godina, a njegova izvorna primjena bila je ograničena na savjetovanje i prenatalnu dijagnostiku kod nekoliko nasljednih bolesti. Zahvaljujući završetku projekta humanog genoma (engl. *Human Genome Project*, HGP) (1) te projektu koji je potom uslijedio International HapMap Project, (od kojih su oba opisali genetske varijacije, uglavnom polimorfizme jednog nukleotida među različitim pojedincima te uzroke varijacija u genomu) (2), i uz izvanredna tehnološka dostignuća, genetsko testiranje je danas široko rasprostranjeno u kliničkim laboratorijima, uz pomoć relativno jeftinih, brzih i pouzdanih visoko propulzivnih tehnika. Genetska analiza doista je ljudima omogućila da shvate molekularnu osnovu nekih bolesti, izazvala je paradigmatski pomak u dijagnostici i kliničkom pristupu monogenskim bolestima (npr. hemofiliji, talasemiji, itd.), pomogla je identificirati razne gene upletene u većinu pretežito poligenskih bolesti (npr. šećerna bolest, tumori, venska tromboembolija, srčanožilne bolesti), omogućila je individualizaciju liječenja i osobito farmakoloških terapija. Međutim, ona je isto tako dovela do paradoksa u liječenju bolesnika, a to je da se genetsko testiranje većinom smatra *panaceom*, „lijekom za sve", uz pretpostavku da je svaki pojedini genetski polimorfizam udružen sa specifičnim, pojedinačnim fenotipom i kliničkom slikom. No, istina je drukčija, kako za monogenske, tako osobito za poligenske bolesti. O nekoliko primjera govori se niže u tekstu.

## Monogenske bolesti

Hemofilija A je teška nasljedna bolest krvarenja uzrokovana X-vezanim recesivnim mutacijama koje dovode do nedostatka funkcionalnog faktora VIII (FVIII). Učestalost bolesti u populaciji (otprilike 1 na 5000–10.000 muških živorođenih) uzrokovana je visokom stopom mutacije, poglavito u muškim zametnim stanicama, odnosno *de novo* somatskim mutacijama u ranoj embriogenezi (3). Tradicionalno se smatra da su najteži oblici nedostatka FVIII (npr. oni s aktivnošću FVIII < 1%) udruženi s izrazito povećanim rizikom od krvarenja zbog običnih ozljeda, koje zahvaća zglobove, mišiće, probavni sustav i mozak. Kod umjereno teških oblika bolesti (npr. aktivnosti FVIII između 1% i 5%) krvarenja mišića i zglobova također su česta, ali je moguć i nastup krvarenja u probavnom sustavu i mozgu (4,5). Glavni problem u upravljanom liječenju hemofiličara je personalizacija profilakse i terapije. Iako se većina znanstvenika i kliničara oslanja na tradicionalnu klasifikaciju bolesti (teška, umjerena, blaža), višestruki klinički dokazi govore kako se težina simptoma uvelike razlikuje među bolesnicima, kao i među onima koji nose iste (genetske) molekularne defekte. Složenosti ovoga pitanja

including studies of intragenomic phenomena such as heterosis, epistasis, pleiotropy and other interactions between loci and alleles within the genome. Genetic testing was originally developed nearly twenty years ago and its original applications were limited to counseling and prenatal diagnosis of a few hereditary diseases. Due to the completion of the human genome project (HGP) (1), the subsequent International HapMap Project (which both have described genetic variations, mostly single nucleotide polymorphisms, among individuals and the patterns of variation across the genome) (2), and thanks to outstanding technological improvements, genetic testing is now widespread in clinical laboratories, supported by relatively inexpensive, fast, reliable high-throughput techniques. Indeed, genetic analysis has allowed humans to understand the molecular basis of several disorders, has produced a paradigm shift in diagnosis and clinical management of single gene disorders (e.g., hemophilias, thalassemias, etc.), has helped identify a variety of genes involved in most multifactorial prevailing pathologies (e.g., diabetes, cancer, venous thromboembolism, cardiovascular disease), has allowed the personalization of treatments and especially pharmacological therapies. However, it has also produced a paradox in the managed care of patients, in that most would perceive genetic testing as a *panacea*, with the assumption that each single genetic polymorphism is associated with a specific, individual phenotype and clinical picture. But, the truth is different, both for single gene disorders and, especially, for multifactorial pathologies. Some examples will be discussed below.

## Monogenic disorders

Hemophilia A is a severe inherited bleeding disorder caused by X-linked recessive mutations resulting in deficiency of functional plasma factor VIII (FVIII). Its prevalence in the population (approximately 1 *per* 5000-10,000 males born) is due to a high mutation rate predominantly in male germ cells, namely, *de novo* somatic mutations in early embryogenesis (3). It is traditionally assumed that the most severe forms of FVIII deficiency (e.g., those with FVIII levels < 1%) are associated with a severely increased risk of bleeding from common injuries, which involves joints, muscles, gastrointestinal tract and brain. In the moderately severe forms of disease (e.g., FVIII levels between 1% and 5%), muscle and joint hemorrhages are also frequent, whereas digestive tract and cerebral hemorrhages might occur as well (4,5). The major problem in the managed care of hemophiliacs is personalization of prophylaxis and therapy. Although most scientists and clinicians rely on the traditional classification of disease (severe, moderate, mild), several lines of clinical evidence attest that the severity of symptoms varies widely among patients, also among those carrying the same (genetic) molecular defects. To further increase the complexity, no

doprinosi i nepostojanje čvrste korelacije između kliničke težine hemofilije i rezultata koagulacijskih pretraga (aktivnost nedostatnih faktora, vrijeme zgrušavanja pune krvi, tromboplastinski test probira), jer 10-15% bolesnika s teškom hemofilijom (< 1% aktivnosti faktora zgrušavanja) imaju relativno blagu kliničku bolest, rijetko krvare i ne trebaju profilaktičnu terapiju (4,5). Štoviše, nastup najteže komplikacije kod hemofiličara, tj. razvoj antitijela na FVIII (nazvana „inhibitorima") je vrlo heterogen (6). Iako je objavljeno kako bi razvoj inhibitora mogao biti češći u bolesnika s teškom hemofilijom A i mutacijama koje predviđaju nulti alel, nego u onih s *missense* mutacijama, sveukupna prediktivna vrijednost genetskog testiranja je upitna, jer bi se 33% do 78% hemofiličara moglo pogrešno klasificirati prema njihovom pojedinačnom genetskom riziku (7).
Usprkos ovim podacima, zaključili bismo da se pojedini genotipovi kod hemofiličara (kao i kod drugih monogenskih bolesti) ne odražavaju uvijek jednakim biokemijskim i kliničkim fenotipovima. Prvo i najvažnije, aktivnost FVIII u plazmi (FVIII:C) slična je, ali se ne preklapa u bolesnika s jednakim mutacijama, jer je poluživot FVIII u plazmi navodno kraći u bolesnika s krvnom grupom O i niskom aktivnošću von Willebrandova faktora (VWF) (5). Prema tome, današnji pristup kojim se nedostatak ispravlja nadomjesnom terapijom (rekombinantni FVIII ili rjeđe svježa smrznuta plazma) zasnovan je na kliničkim podacima i postojećoj aktivnosti FVIII:C u plazmi, a ne na pojedinačnom genotipu. Uz raznoliku udruženost genotipa s ostatnom aktivnosti faktora zgrušavanja, drugi dokazi ukazuju na to da farmakokinetika primijenjenih koncentrata faktora zgrušavanja, prisutnost protrombotskih biljega (8) i varijacije drugih koagulacijskih proteina procijenjene testovima sveopće hemostaze te fibrinolitički sustav mogu djelovati na trenutnu aktivnost FVIII:C u plazmi i kliničku težinu krvarenja (4,5,9). Sve u svemu, ovi dokazi navode na neizbježan zaključak kako je podrijetlo velike heterogenosti fenotipova kod teške hemofilije multifaktorsko, pa bi doprinos genetskog testiranja stoga bio ograničen; zato će se upravljana skrb o bolesnicima u bliskoj budućnosti uvelike voditi kliničkim značajkama i fenotipskim laboratorijskim pretragama kao što su testovi aktivnosti faktora i moguće testovi stvaranja trombina. Čak i kad predviđa kliničku težinu hemofilije, složeni sustav za procjenu težine hemofilije, *Hemophilia Severity Score* (HSS), previđa genotip, a umjesto toga uključuje samo godišnju incidenciju krvarenja zglobova, procjenu zglobova prema *World Federation of Hemophilia Orthopedic score* i godišnji čimbenik potrošnje (10).

## Poligenske bolesti

Srčanožilna bolest je prvi uzrok pobola i smrtnosti u zapadnim zemljama te predstavlja znatno ekonomsko opterećenje na razini pojedinca, ustanova i države. Točna

definite correlation exists between the clinical severity of hemophilia and the results of coagulation tests (activity of deficient factors, whole blood clotting time, thromboplastin screening test) either, since up to 10%-15% of patients with severe hemophilia (< 1% clotting factor activity) have relatively mild clinical disease, only rarely bleed and do not need prophylactic therapy (4,5). Moreover, it is also acknowledged that the onset of the development of antibodies to FVIII (called "inhibitors") as the most severe complication in hemophiliacs might be heterogeneous (6). Although it has been reported that patients with severe hemophilia A and mutations predicting a null allele might develop inhibitors more frequently than those with missense mutations, the overall predictive value of genetic testing is questionable because 33% to 78% of hemophiliacs might be misclassified according to their individual genetic risk (7).
In face of these data, we would conclude that particular genotypes in hemophiliacs (as for other single gene disorders) are not always mirrored by identical biochemical and clinical phenotypes. First and foremost, the activity of FVIII in plasma (FVIII:C) is similar but not overlapping in patients with identical mutations because the half-life of FVIII in plasma is reportedly shorter in patients with blood group O and low von Willebrand factor (VWF) antigen levels (5). Accordingly, the current approach to correct the deficit by replacement therapy (recombinant FVIII or less frequently fresh frozen plasma) is driven by the clinics and the presenting plasma FVIII:C level rather than by the individual genotype. Apart from variable associations of the genotype with the levels of residual clotting factor activity, there is evidence to suggest that pharmacokinetics of administered clotting factor concentrates, the presence of prothrombotic markers (8), and variations in other coagulation proteins as assessed in tests of global hemostasis as well as the fibrinolytic system can affect both the presenting plasma FVIII:C and clinical severity of bleeding (4,5,9). Taken together, this evidence leads to the inevitable conclusion that the origin of the large heterogeneity of phenotypes in severe hemophilia is multifactorial and the contribution of genetic testing would therefore be only limited, so that the managed care of patients will be driven largely by clinical features and phenotypic laboratory tests such as factor activity and potentially thrombin generation assays in the near future. Even when predicting the clinical severity of hemophilia, the composite Hemophilia Severity Score (HSS) overlooks the genotype, and only includes the annual incidence of joint bleeds, World Federation of Hemophilia Orthopedic joint score, and annual factor consumption instead (10).

## Polygenic disorders

Cardiovascular disease is the first cause of morbidity and mortality in Western countries and poses a substantial

procjena različitih razina rizika i utvrđivanje najprimjerenije trijaže bolesnika vodeći su ciljevi za optimalno kliničko i isplativo bavljenje ovom patologijom (11). Uza svu svojstvenu fenotipsku složenost, prisutnost višestrukih uzročnih čimbenika predstavlja dodatni izazov u istraživanju genetske osnove srčanožilne bolesti (12). Zapravo, iako postoji znatno preklapanje među raznim fenotipovima, osnovna patofiziologija obično se razlikuje, jer ishemijski ispadi (odnosno akutni infarkt miokarda) često, ali ne uvijek proizlaze iz prsnuća krhkog aterosklerotskog plaka, a čimbenici rizika i njihove interakcije koji dovode do te krhkosti razlikuju se od onih koji utječu na koronarno aterosklerotsko opterećenje (12). Tako su tisuće znanstvenih epidemioloških studija provedenih u posljednjim desetljećima dovele do otkrića snažne ali složene genetske osnove koja se ogleda u poremećenim koncentracijama lipida i lipoproteina, poremećajima u metabolizmu glukoze (osobito šećerna bolest), pretilosti, hipertenziji, upali, oksidacijskom stresu i hiperkoagulabilnosti (12).

Uz mogući doprinos dijagnostici monogenskih bolesti, genetsko testiranje bi se u budućnosti moglo pokazati vrijednim i kod poligenskih bolesti kao što je srčanožilna bolest, gdje bi ono omogućilo individualni pristup bolesniku, uključujući određivanje razine rizika za primarnu ili sekundarnu profilaksu, trajanje antikoagulantne terapije te u obiteljska ispitivanja. U takvoj situaciji učestalost specifičnih nasljednih čimbenika (npr. hipertenzija, hiperkolersterolemija) te značajan rizik što ga čini kombinacija dodatnih, rjeđih genetskih defekata dodatno ukazuje na potrebu provođenja genetskih analiza. Međutim, kako je gore napomenuto, kombinirani učinci genotipova i/ili haplotipova tako su složeni i mnogostruki da se kod nekih bolesnika mogu razviti ishemijske komplikacije bez ikakvih čimbenika rizika koje bi bilo moguće utvrditi, dok se kod drugih to neće dogoditi iako su nositelji „visoko rizičnih" genotipova. Uzimajući kao gotovu činjenicu da brojni rizični čimbenici potiču razvoj infarkta miokarda, od životnih navika koje se mogu promijeniti (npr. pušenje, tjelesna aktivnost) do genetske predodređenosti, analize gena kandidata provođene tijekom dva desetljeća nisu dosad uspjele objasniti molekularnu osnovu genetske predodređenosti za ovu patologiju (13). Štoviše, još preostaje dokazati jesu li sve alelne varijante koje se dovode u vezu sa srčanožilnim bolestima doista udružene s funkcionalnim učincima koji utječu na predviđanje rizika, te dostižu li objavljene veličine učinka te udruženosti dovoljan stupanj kliničke značajnosti (14).

Moguće je da će odgovarajuća i diskretna primjena genetskih testova, u razumnim uvjetima i prema kliničkoj situaciji (probir ili dijagnostika), otvoriti velike mogućnosti. Zbog sve većih ograničenja troškova i stoga upitne uporabe za klasificiranje rizika procijenjeni trošak ne opravdava preventivni probir u velikim populacijama zdravih ljudi (15). Međutim, osobe s visokim obiteljskim rizikom

economic burden at the individual, institutional, and national levels. The accurate stratification of the risk and identification of the most appropriate triage for the patients are the leading goals for optimal clinical and economical management of this pathology (11). Besides the inherent phenotypic complexity, the presence of multiple causal factors is an additional challenge in investigating the genetic basis of cardiovascular disease (12). If fact, although a considerable overlap exists among various phenotypes, the underlying pathophysiology is usually different, since ischemic events (namely, acute myocardial infarction) often but not always results from rupture of a vulnerable atherosclerotic plaque, and the risk factors and their interactions to generate plaque vulnerability differ from those that influence coronary atherosclerotic burden (12). Accordingly, thousands of epidemiological research studies over past decades have led to the discovery of a strong but complex genetic background, which is mirrored by abnormal levels of several lipids and lipoproteins, abnormalities in glucose metabolism (especially diabetes), obesity, hypertension, inflammation, oxidative stress, and hypercoagulability (12).

In addition to its potential contribution to the diagnosis of monogenic disorders, genetic testing might also be of future value in multifactorial, polygenic pathologies such as the cardiovascular disease, in that it would allow the construction of personalized frameworks including risk stratification for primary or secondary prophylaxis, duration of anticoagulant therapy, and family studies. In such a situation, the rationale to perform genetic analyses is further highlighted by the prevalence of specific inherited factors (e.g., hypertension, hypercholesterolemia) and for the significant risk conferred by a combination of additional less frequent genetic defects. As previously mentioned, however, the combined effects of genotypes and/or haplotypes are so complicated and multifaceted that some patients might develop ischemic complications with no identifiable risks factors, whereas others will not despite carrying "high risk" genotypes. Taking for granted that the development of myocardial infarction is promoted by numerous risk factors, ranging from rather modifiable lifestyle habits (e.g., smoking, physical activity) to genetic predisposition, two decades of candidate gene analyses have failed as yet to explain the molecular basis for the genetic predisposition to this pathology (13). Moreover, it has yet to be proven whether all the allelic variants which have been linked to cardiovascular disease are really associated with functional effects that affect risk prediction, and whether the reported effect sizes of the associations achieve a sufficient degree of clinical significance (14).

Conceivably, the appropriate and discretionary use of genetic tests, under reasonable conditions and according to the clinical setting (screening or diagnosis), will currently provide great opportunities. Due to increasing

mogle bi imati koristi od strategija ranog otkrivanja te od biokemijskih pretraga i testova na osnovi DNA, kojima bi se njihov rizik mogao podrobnije definirati. Osobe s najvišim obiteljskim rizikom možda su nositelji mendelijanskih poremećaja udruženih s velikim rizikom za preranu koronarnu arterijsku bolest, pa treba razmisliti o njihovu upućivanju na genetsku procjenu (16).

## Farmakogenetika

Kumarinski antikoagulansi čine glavnu skupinu lijekova u oralnoj antikoagulantnoj terapiji (OAT), a među njima je varfarin najčešće rabljeni antikoagulans širom svijeta. Upravljano liječenje bolesnika na OAT vrlo je zahtjevno zbog raznolikosti odgovora na određenu dozu lijeka, pa bi personalizirani odgovori pružili vrijednu mogućnost za ograničavanje opterećenja neželjenim zdravstvenim ishodima. Danas nekoliko vrsta dokaza potvrđuje kako je 50-60% pojedinačnih farmakoloških odgovora na varfarin i druge antikoagulantne lijekove genetski određeno pod utjecajem polimorfizama gena koji kodiraju reduktazu epoksida vitamina K (VKOR) i citokrom P450 CYP2C9 (17). Stoga se farmakogenetsko testiranje predlaže kao vrijedna pomoć u predviđanju odgovora na početnu dozu tijekom terapije te za procjenu razlika u dozi održavanja. Međutim, genetsko testiranje ni ovdje ne predstavlja *panaceu*, jer je podložno višestrukim ograničenjima koja uključuju optimalan sastav zbirke testova, ograničene podatke o razlikama među pojedincima, nedostatak specifikacija kvalitete i osobito nedostatak analiza konačnog ishoda koje bi nedvosmisleno potvrdile isplativost ovoga pristupa. Glavna prepreka proizlazi iz prevladavajućeg oslanjanja na pojedinačni farmakološki odgovor na varijable okoline i „slučajne" događaje kao što su različit unos vitamina K, probavna funkcija, metaboličke nenormalnosti koje utječu na sintezu čimbenika zgrušavanja ovisnih o vitaminu K, alkohol, te drugi lijekovi (18,19). Zato je prerano zaključiti da bi rutinsko genetsko testiranje dovelo do nedvojbeno povoljnog odnosa troškova i koristi, iako bi razvoj i kliničko vrednovanje jednostavnih ali sveobuhvatnih algoritama koji objedinjavaju najinformativnije genske polimorfizme s nekim demografskim i kliničkim varijablama mogao pružiti vrijedno sredstvo za pojedinačno upravljano liječenje bolesnika varfarinom.

## Zašto i gdje nedostaje karika

Ekspresija proteina se regulira na nekoliko razina uključujući: a) transkripcijske/posttranskripcijske/predtranslacijske procese koji utječu na kvantitativnu razinu ekspresije mRNA; b) translacijske procese koji reguliraju pretvorbu mRNA u proteine; i c) posttranslacijske/degradacijske procese koji utječu na stabilnost poluživota proteina. Postoji i nekoliko drugih mogućih procesa koji su još nepoznati ili

cost constraints and their questionable utility for classifying risk, the estimated expense does not justify preventive screening in large populations of healthy people (15). However, individuals with a high familial risk might benefit from early detection strategies and biochemical and DNA-based testing, which can further refine their risk. Individuals with the highest familial risk might carry mendelian disorders associated with a large magnitude of risk for premature coronary artery disease, so that referral for genetic evaluation should be considered (16).

## Pharmacogenetics

Coumarin derivatives are the mainstay of oral anticoagulant therapy (OAT) and comprise the most often used anticoagulant medication worldwide (warfarin). The managed care of patients on OAT is challenging, due to considerable variability in the dose-response, so that personalizing responses would represent a great opportunity in limiting the burden of adverse health outcomes. Several lines of evidence now attest that 50%-60% of the individual pharmacological response to warfarin and other anticoagulant drugs is genetically determined, influenced by polymorphisms in the genes encoding for vitamin K epoxide reductase (VKOR) and cytochrome P450 CYP2C9 (17). Pharmacogenetic testing has thereby been proposed as a valuable tool to aid prediction of dose response during initial anticoagulation and assess variability in dose maintenance. Once again, however, genetic testing does not provide a *panacea*, being influenced by several limitations that include the optimal composition of test panels, the limited information provided on the inter-individual variability, the lack of quality specifications, and, especially, the lack of definitive outcome analyses that unequivocally confirm the cost-effectiveness of this approach. The major hurdle comes from the predominant reliance of the individual pharmacological response on environmental variables and "random" events such as variation of vitamin K intake, gastrointestinal function, metabolic abnormalities that influence the synthesis of vitamin K-dependent coagulation, alcohol, and other drugs (18,19). It is therefore premature to conclude that routine genetic testing would carry an unequivocally favorable cost-to-benefit ratio, although the development and clinical validation of simple but comprehensive algorithms integrating the most informative gene polymorphisms with some demographic and clinical variables may provide a valuable tool in the individually managed care of patients on warfarin therapy.

## Why and where the link is missing

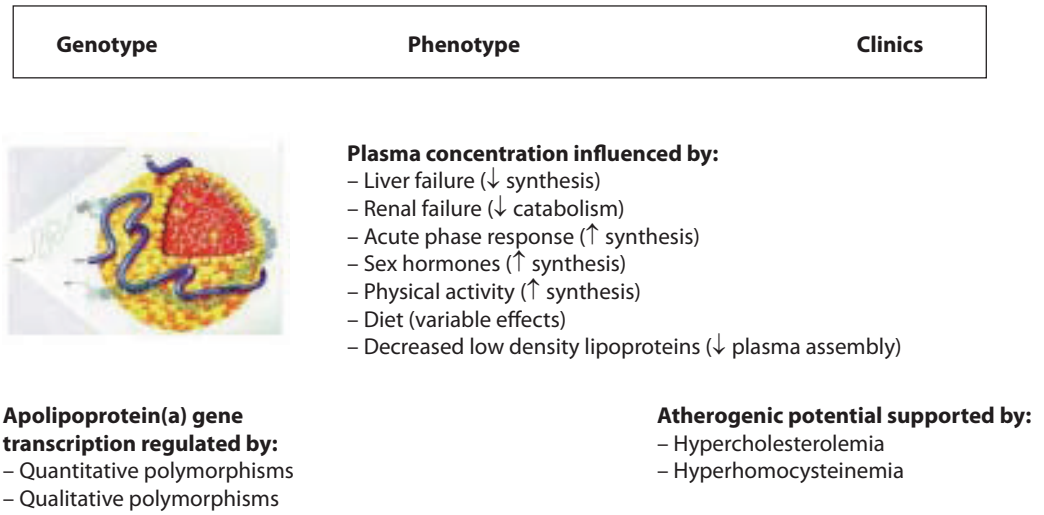
Protein expression is regulated at several levels, including (a) transcriptional/posttranscriptional/pretranslational processes that impact the quantitative level of expression

njihova biološka osnova nije prepoznata. Uz gensku transkripciju, ima dodatnih aspekata koji će utjecati na ekspresiju proteina, uključujući genske interakcije, istodobno prisutne bolesti, biološku varijabilnost i okolinu.

Lipoprotein(a) (Lp(a)) je paradigmatski primjer kako sve te varijable mogu djelovati na prevođenje genotipa u fenotip. Metabolizam ovoga neobičnog i visoko aterogenog lipoproteina navodno podliježe strogoj genetskoj regulaciji, jer kvantitativni i kvalitativni polimorfizmi na lokusu gena apolipoproteina(a) utječu na 90% njegove koncentracije u plazmi (20). S obzirom na to, očekivali bismo da će genetsko testiranje moći pouzdano predvidjeti biokemijski fenotip i kliničke nalaze koji u konačnici pokazuju koncentraciju u plazmi i aterogenost. To je tek djelomice točno, jer postoji očito razmimoilaženje između genotipa, fenotipa i kliničkih podataka (Slika 1.). Zapravo, prisutnost nekih elemenata koji reagiraju na interleukin 6 u sekvenci ovoga gena uzrokuje znatan porast koncentracije u plazmi kao odgovor na upalu (21). Slično tomu, spolni hormoni i tjelesna aktivnost također reguliraju gensku transkripciju prema višim vrijednostima, bubrežno zatajenje može dovesti do nakupljanja imunoreaktivnog Lp(a) u plazmi, jer se Lp(a) također izlučuje putem bubrega, dok su neki prehrambeni sastojci (npr. alkoholna pića) i zatajenje jetre udruženi sa znatnim sniženjem njegove koncentracije u plazmi, jer je ovaj organ glavno mjesto sinteze apolipoproteina(a) (21,22). Kako se konačna čestica Lp(a) sklapa u plazmi kovalentnim spajanjem dijelova apolipoproteina(a) i apolipoproteina B lipoproteina niske gustoće, koncentracija ovoga potonjeg u plazmi ima snažan utjecaj na razine Lp(a) (21). I konačno, glede aterogenosti Lp(a), u tijeku je rasprava o tome jesu li visoke razine Lp(a) sukladne s dugovječnosti, pa bi se ova pojava mogla pokazati

of mRNA, (b) translational processes that modulate the conversion of the mRNA blueprint into protein, and (c) posttranslational/degradative processes that impact the stability or half-life of the protein product. Several other potential processes exist but remain unknown, or their biological background has not been recognized. Other than gene transcription, additional aspects will impact on protein expression, including gene to gene interactions, comorbidities, biological variability, the environment, etc.

Lipoprotein(a) (Lp(a)) is a paradigmatic example of how all the variables can impact on the genotype to phenotype translation. The metabolism of this peculiar and highly atherogenic lipoprotein is reportedly under strict genetic regulation, in that more than 90% of its plasma concentration is influenced by quantitative and qualitative polymorphisms at the apolipoprotein(a) gene locus (20). One would expect, accordingly, that genetic testing can reliably predict the biochemical phenotype and the clinics that are ultimately plasma concentration and atherogenicity. Although this is partially true, there are some evident dissociations between genotype, phenotype and clinics instead (Figure 1). In fact, the presence of some interleukin 6 responsive elements in the sequence of the gene determines a substantial increase of the plasma concentration in response to inflammation (21). Likewise, sex hormones and physical activity also up-regulate gene transcription, renal failure can produce accumulation in plasma of Lp(a)-immunoreactive material because Lp(a) is also cleared by the kidneys, whereas some diets (e.g., alcohol consumption) and liver failure are associated with a substantial decrease of the plasma concentration since this organ is the main site of apolipoprotein(a) synthesis (21,22). Since the definitive Lp(a) particle is assembled in plasma by a covalent association between apolipopro-




**Slika 1.** Čimbenici koji utječu na koncentraciju lipoproteina(a).

**Figure 1.** Major determinants of the plasma concentration of lipoprotein(a).

očitim paradoksom (23). Međutim, dosljedno se izvještava o tome da je aterogenost Lp(a) usko povezana (podređena) s istodobnom prisutnošću dodatnih čimbenika rizika (odnosno hiperkolesterolemijom i hiperhomocisteinemijom), koji bi na koncu mogli razotkriti ili pojačati njegov aterogeni i anti-fibrinolitički potencijal (24).

## Zaključne napomene

Glavno područje translacijske medicine je nastojanje da se premosti jaz između eksperimentalnih istraživanja i kliničke prakse, te da se razrade i objave novi podaci koji bi mogli pomoći u prevenciji, dijagnostici i liječenju bolesti (25-27). Sa završetkom HGP mnogi istraživači sad ispituju kako to geni i proteini međusobno djeluju da bi stvorili druge proteine. Nažalost, poveznica između genotipa, fenotipa i kliničkih podataka nije uvijek očita, pa je to veći izazov ispitivati u kojoj će mjeri veza između gena i okoline utjecati na upravljano liječenje bolesnika. No, nema nikakve sumnje da će saznanja koja se danomice stječu u prediktivnim znanostima obilježenim zajedničkim dometkom -omika (genomika, proteomika, lipidomika, metabolomika, citomika) pokrenuti i poticati širok spektar istraživanja, što će omogućiti da se rasvijetli nekoliko faza na genetsko-podstanično-molekularno-biokemijskoj razini. Tada će biti moguće prevladati tradicionalni mehanistički pristup bolesti i razviti personalizirani okvir za klasificiranje bolesnika u različite (čak pojedinačne) podskupine, svaka od njih sa zajedničkim ali jedinstvenim značajkama (25-28). S izumom epigenetske tehnologije velikog kapaciteta zasnovane na čipovima (npr. ChIP-on-chip i ChIP-seq), genetsko će se testiranje proširiti dalje od genske ekspresije i istraživati aktivnost genske regulacije, čime će se prevladati nedostaci svojstveni tradicionalnom genetskom testiranju i omogućiti istodobno mjerenje relativnih razina ekspresije tisuća pojedinih gena.

Daljnje prepreke odnose se na financijska, etička, regulatorna i praktična pitanja te na kraju, iako jednako važno, na nedostatak djelotvorne komunikacije između laboratorijskih stručnjaka i kliničara u cilju najboljeg mogućeg iskorištenja i tumačenja laboratorijskih mogućnosti (29,30). Priklonili bismo se dvama idealnim pristupima koji istražuju višestruke odnose između genetike, biokemije i kliničkih nalaza. Studije genetske udruženosti pružaju izglede za mapiranje uzročnih gena umjerenih učinaka, ali su ograničene, jer se istodobno može ispitivati tek manji broj gena. Za razliku od ovih, genomske studije udruženosti (engl. *genome-wide association*, GWA) uskoro će otkriti novi scenarij za razumijevanje ljudske biologije, fiziologije i patologije (31). DNA čipovi (engl. *microarray*) se izrađuju pomoću visoko brzinske robotike, što omogućava usporednu izraženost velikog mnoštva gena i studije za otkrivanje gena. Pokus sa samo jednim DNA čipom može istraživačima pružiti podatke o tisućama gena istodobno,

tein(a) and the apolipoprotein B moiety of a low density lipoprotein, the plasma concentration of the latter strongly influences Lp(a) levels (21). Finally, concerning the atherogenicity of Lp(a), there is a debate as to whether high Lp(a) levels are compatible with longevity, so that this phenomenon would turn out as an apparent paradox (23). It has been consistently reported, however, that the atherogenicity of Lp(a) is tightly linked (subordinated) to the concomitant presence of additional risk factors (namely, hypercholesterolemia and hyperhomocysteinemia) that would ultimately unmask or enhance its atherogenic and anti-fibrinolytic potential (24).

## Concluding remarks

The major scope of translational medicine is seeking to close the gap between experimental research and clinical practice, and to develop and deliver new information that may assist prevention, diagnosis, and treatment of disease (25-27). With the completion of the HGP, many researchers are now looking at how genes and proteins interact to form other proteins. Unfortunately, the link between the genotype, the phenotype and the clinics is not always obvious, and it is even more challenging to address how much the link between gene and environment will impact on the managed care of patients. There is no doubt, however, that ongoing developments within predictive sciences which share the suffix -omics (genomics, proteomics, lipidomics, metabolomics, cytomics), will raise and stimulate a broad spectrum of research, allowing us to elucidate several steps at the genetic-subcellular-molecular-biochemical level that might enable us to overcome the traditional mechanistic approach to disease and to develop a personalized framework to stratify patients into different (even individual) subsets, each with common, but unique, characteristics (25-28). With the advent of high throughput microarray-based epigenetic technology (e.g., ChIP-on-chip and ChIP-seq), genetic testing will go beyond gene expression to explore gene regulation activity, thereby overcoming the inherent shortcomings of traditional genetic testing and providing the simultaneous measurement of the relative expression levels of thousands of individual genes.

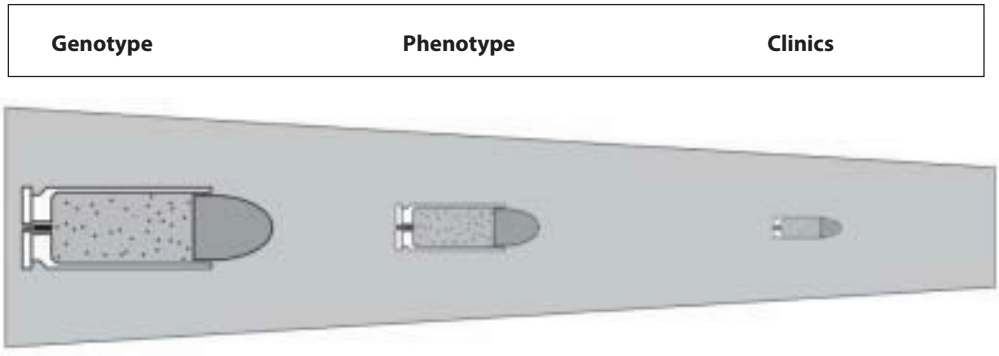
Further hurdles come from financial, ethical, regulatory and practical barriers, and the last but not the least, the lack of effective communication between laboratory professionals and clinicians for the best possible use and interpretation of laboratory resources (29,30). Two ideal approaches can be advocated to investigate the multifaceted relationship between genetics, biochemistry and clinics. Genetic association (GA) studies offer a great opportunity for mapping causal genes with modest effects, but are limited because only a small number of genes can be studied at a time. In contrast, genome-wide association (GWA) studies will soon disclose a new scenario

što predstavlja golem iskorak koji je, međutim, vrlo zahtjevan za izvedbu na obje strane (laboratorijskoj i kliničkoj). Kako se studije GWA uglavnom temelje na komercijalnim čipovima jednonukleotidnih polimorfizama za koje je sveobuhvatna pokrivenost genoma opći kriterij procjene (32), glavna prepreka je ponovno omjer troškova i koristi, jer je postupak i dalje preskup i klinički upitan. Kako bi se spriječio predvidiv i katastrofičan scenarij ograničavajuće regulacije genetskog testiranja diljem svijeta (od ljekarna do „virtualnih", nekontroliranih sredstava na webu) (33), laboratorijsko savjetovanje će biti prvi korak slijedom kojega će se proizvođače upućivati na razvijanje najkorisnijih i predvidivih čipova, dok će kliničare upućivati prema najprobranijoj primjeni ovih moćnih sredstava.

U zaključku, postavlja se slijedeća zagonetka: kakav je stvaran odnos između genotipa, fenotipa i kliničkih podataka? I k tome, što je još provokativnije, hoće li analiza genotipa zamijeniti fenotipsko testiranje u rutinskoj laboratorijskoj praksi? Zasad nema jednoznačnog odgovora na ova pitanja. Genetsko testiranje doista ima velik unutarnji potencijal za identificiranje rizičnih brakova, neinvazivno prenatalno određivanje spola ploda i preimplantacijsku genetsku dijagnostiku. Ono isto tako pomaže u predviđanju, dijagnosticiranju i liječenju patologija, ali se ne može smatrati „magičnom kuglom" u gore spomenutim patologijama, kao ni u drugim poremećajima, jer se predvidivost genetskog testiranja postupno smanjuje tijekom ovog translacijskog procesa (Slika 2.). Razmotriti treba i neka važna etička pitanja. U ovom trenutku je veza između genetskog testiranja i kliničkih podataka slaba kod nekih patologija, tako da bi procjena rizika mogla velik broj bolesnika izložiti lažnom ohrabrenju ili pak pretjeranoj zabrinutosti. Nadajmo se da će nam daljnja znanstvena i tehnološka postignuća pomoću u razumijevanju stvarne uloge, korisnosti i učinkovitosti genetskih analiza, očekujući da one još neće zamijeniti fenotipsko testiranje koje ostaje temeljnim uporištem laboratorijske prakse u godinama koje dolaze.

in the understanding of human biology, physiology and pathology (31). DNA microarray, or DNA chips are fabricated by high-speed robotics, allowing massively parallel gene expression and gene discovery studies. An experiment with a single DNA chip can provide researchers information on thousands of genes simultaneously, a dramatic increase in throughput, which is, however, very challenging to be handled from both sides (the laboratory and the clinic). Since GWA studies mostly rely on commercial single-nucleotide polymorphisms (SNPs) chips, for which a common evaluation criterion is global coverage of the genome (32), a major hurdle is again the cost-to-benefit ratio, in that it still remains prohibitively expensive and clinically questionable to genotype all the samples by a genome-wide genotyping array. To prevent the predictable and catastrophic scenario of a worldwide down-regulation of genetic testing (from pharmacies to "virtual", uncontrolled facilities on the Web) (33), laboratory counseling will be foremost, instructing manufacturers to develop the most useful and predictive arrays, and guiding clinicians towards the most discretionary use of these powerful tools.

The concluding enigma is thereby: which is the real relationship between the genotype, phenotype and clinics? And, even more provocatively, would genotype analysis replace phenotypic testing in routine laboratory practice? There is no unequivocal answer to these questions as yet. Indeed, genetic testing has the great inherent potentials of identifying risk marriages, noninvasive prenatal fetal sex determination and preimplantation genetic diagnosis. It also helps predict, diagnose and treat pathologies, but it cannot be considered the "magic bullet" in the above mentioned pathologies as in other disorders because the predictivity of genetic testing gradually decreases throughout this translational process (Figure 2). Some important ethical issues should also be considered. At this point in time the link between genetic testing and clinics is labile for several pathologies, so that risk asses-




**Slika 2.** Slaba veza između genotipa, fenotipa i kliničkih podataka zbog smanjenih prediktivnih vrijednosti rezultata genetskog testiranja.

**Figure 2.** The labile link between genotype, phenotype and clinics due to the decreasing predictivity of genetic testing.

**Adresa za dopisivanje:**

Giuseppe Lippi
Sezione di Chimica Clinica
Dipartimento di Scienze Morfologico-Biomediche
Università degli Studi di Verona
Ospedale Policlinico G.B. Rossi,
Piazzale Scuro, 10
37134 - Verona
Italy
e-pošta: ulippi@tin.it, giuseppe.lippi@univr.it

sment might expose a large number of patients to false reassurances or excessive concerns. Hopefully, further scientific and technological advancements will help us understand the real role, usefulness and efficacy of genetic analyses, with the expectation that it will not yet replace phenotypic testing, which will remain a cornerstone of laboratory practice for several years to come.

**Corresponding author:**

Giuseppe Lippi
Sezione di Chimica Clinica
Dipartimento di Scienze Morfologico-Biomediche
Università degli Studi di Verona
Ospedale Policlinico G.B. Rossi,
Piazzale Scuro, 10
37134 - Verona
Italy
e-mail: ulippi@tin.it, giuseppe.lippi@univr.it

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