INDIVIDUALIZED DRUG RESPONSE RELATED TO GENETIC VARIATIONS OF CYTOCHROME P450 ISOFORMS AND OTHER ENZYMES

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Abstract

Gene polymorphism and single nucleotide polymorphism (SNPs) called "snips" might play a pivotal role in the future of the clinical therapy. Although not much is known about the distribution of SNPs in population and about the gene polymorphism of the drug-metabolizing enzymes, their investigation could represent an important tool in order to determine the precise therapy for maximum efficacy. SNPs represent a DNA sequence variation of a single nucleotide, variation that could determine and alter the genome sequence. If just one nucleotide (A - adenine, C - citosine, G - guanine, T - thymine) is changed this will definitely bring a change in the DNA sequence. In order to improve public health, since the map of the genome was created, scientists thought about new SNPs maps, which will bring a new vision in diagnosis, biological markers, drug therapy, and human response to disease. This review brings some insights in the knowledge of gene polymorphism regarding their impact on drug therapy and disease.

Rezumat

Polimorfismul genelor şi polimorfismul unui singur nucleotid (SNPs) ar putea juca un rol central în viitorul terapiei. Deşi distribuţia SNPs în populaţie şi polimorfismul genelor enzimelor cu rol în metabolismul medicamentelor nu sunt foarte cunoscute, investigarea acestora ar putea reprezenta un instrument important pentru determinarea tratamentului adecvat pentru o maximă eficacitate. SNPs reprezintă variaţia unei secvenţe de ADN a unui singur nucleotid, variaţie care ar putea determina şi modifica secvenţa genomului. În cazul în care doar unul dintre nucleotide (A - adenină, C - citozină, G - guanină, T - timină) este schimbat aceasta va duce cu siguranţă la o schimbare în secvenţa ADN-ului. În scopul îmbunătăţirii sănătăţii publice, harta genomului fiind deja creată, oamenii de ştiinţă s-au gândit la hărţi noi, ale SNPs-urilor, care vor aduce o nouă viziune în privinţa diagnosticelor, markerilor biologici, tratamentului medicamentos, şi în general a reacţiilor omului la boli. Acest articol abordează unele aspecte legate de nivelul actual al cunoştiinţelor privind polimorfismul genelor şi a impactului lor asupra tratamentului medicamentos şi a bolilor în general.

Keywords: Gene polymorphism, SNPs, cytochrome P450, therapy, dose, efficacy.

Introduction

Pharmacogenetics/pharmacogenomics is the study of genes influences on the individual response to a drug. Pharmacogenetics generally refers to the study of variations in a single gene, whereas pharmacogenomics is the study of variations in multiple genes. It is a combined notion that includes the role of genetics in pharmacology.

Pharmacogenetics is a new scientific discipline emerged from the fusion of pharmacology, genetics and biochemistry. Based on studies from the past decades, this unique discipline indicates that a substantial amount of variability in drug response is genetically determined and highlights the concept “therapy with the right drug at the right dose in the right patient"; moreover, it will play an integral role in disease assessment, drug discovery and development, and selection of the type of drug.

In fact, this discipline can revolutionize medical therapeutics, by challenging pharmaceutical industry with the concepts of "individualized drug therapy" focusing on "small patient populations as target" [20].

Thus, determining an individual's unique genetic profile in respect to disease risk and drug response will have a profound impact on understanding the pathogenesis of disease, and it may enable truly personalized therapy. In this way, old notions such as "genetic polymorphism" or "genetic variability" were brought up to debate.

Subsequently, enabled by high-throughput technologies in DNA analysis new notions such as “single nucleotide polymorphism" were necessary [18].

Single nucleotide polymorphism and individual variations of drug response

A mutation in the DNA sequence - genetic polymorphism - is present in at least 1% of the population. Based on these polymorphic enzymes there are four distinctive phenotypes: extensive (normal) metabolizers (EMs), poor metabolizers (PMs), intermediate metabolizers (IMs) and ultrarapid metabolizers (UMs).

Genetic variability is different from genetic diversity (or genetic variation) in a way that the former measures how much the trait or the genotype will tend to vary whereas the latter measures the number of the actual variation of species in a population. Compared with genetic diversity, genetic variability is more difficult to measure. At the molecular level, genetic variability may be measured by determining the rate of mutation.

A major contributor to the variation in genetic profile are the single nucleotide polymorphisms (SNPs), which are highly abundant throughout

the genome, and both current and future methodologies have the potential to screen millions of SNP genotypes in one analysis.

SNPs represent genetic variations in individuals; in order to be called SNPs, they must occur in at least 1% of population.

With the completion of the human genome project, about 12 million true SNPs have been identified to date [17]. SNPs can be present in the coding part (cSNPs) but also in the non-coding part of the genome. Testing and identifying SNPs would help to establish a personalized therapy, to avoid some side effects and to administer the right drug and the right dose[8].

Evidence based on gene variations involved in drug metabolism and the role of cytochrome P450 and other enzymes

The pharmacology of a drug is subjected to inherited variability in metabolism. This inherited variability could mean:

- the drug site of action is predictably affected by the genotype or phenotype;
- the drug metabolism could be predictably affected by the genotype of the drug-metabolizing enzymes;
- the drug effects could be the result of genetic variations not only of the metabolizing enzymes but also of the transporters, receptors, and/or ion channels.

Based on these facts we can use the classification made by Gardiner S.J. *et al.* regarding the genetic variations influence on the metabolic biotransformation. They divided the metabolic biotransformation pathways into: cytochrome P450-mediated reactions and others [6].

Cytochrome P450 in drug metabolism

The cytochrome P450 (CYP450) represents a large group of enzymes located mainly in liver. A small part of these enzymes can be found in intestines, lungs or other organs. These enzymes share few characteristics: all are bound to membranes within a cell (which provide the term of cyto) and contain a heme pigment (the origin of the term chrome and P) that absorbs light at a wavelength of 450 nm when exposed to carbon monoxide. The cytochrome P450 enzymes are involved in the metabolism of a large range of xenobiotics, from drugs to environmental carcinogens [6]. The cytochrome P450 is involved mainly in the first phase of drug metabolism but is catalyzing a large number of chemical reactions on an almost unlimited variety of substrates. These properties, added to the fact that P450s occur in families and subfamilies with different degrees of

sequence similarity make them the ideal starting materials for genetic research and testing. The cytochrome P450 enzymes are designated by a family number, a subfamily letter, a number for an individual enzyme within the subfamily, and an asterisk followed by a number and a letter for each genetic (allelic) variant.

As a result of different alleles and genotypes, we can observe a series of phenotypic consequences such as: absent, diminished, qualitatively altered or enhanced activity of the CYP enzymes. Inspite the fact that there are more than 50 CYP enzymes, only a few of them are responsible for abnormal pharmacological response, as 6 of them are responsible for the metabolization of over 90% of all known drugs: CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5. Each enzyme is termed as isoform since each derives from a different gene. This is why a variation of one enzyme has no influence over the activity of others. Clinical relevance of genetic variability in CYP genes depends of a variety of factors like: patient's clinical status, drug's therapeutic index, number of enzymes involved in the drug's metabolism, smoking, other concomitant administered drugs [25]. Till now studies proved well-documented polymorphisms for CYP2D9**,** CYP2D6, CYP2C9, and CYP2C19.

Close relations between CYP isoenzymes and drug adverse effects have been documented. Poor metabolizers (PM)- those persons with two variant alleles resulting in inactive or absent enzyme, seem to suffer adverse effects of drugs known to be metabolized by the isoenzymes they lack [25].

Based on a systematic review of the literature from 1991 to 2007, Sistonen *et al.* concluded that altered activity variants of CYP2C9, CYP2C19, and CYP2D6 occur globally in all geographic regions, reaching extremely high frequencies in some populations, each of the CYP genes studied (CYP2C9, CYP2C19, and CYP2D6) showing a distinct geographic pattern of variation population substructure that can strongly affect the variation seen in pharmacogenetic loci. [19]

A special attention has been paid to CYP2D9 as it has been discovered that a polymorphism of this enzyme is affecting its capacity of oxidation for over 25% of all drugs. Now we know that 7% of the white population and between 2 and 7% of the black population are poor metabolizers of drugs dependent of this enzyme.

CYP2D6 is known to be responsible for the metabolisation of at least 65 usual drugs. The rate of drug metabolism influenced by CYP2D6 can be divided in four phenotypic subpopulations: persons with a poor, an intermediate, an extensive, or an ultra rapid ability to metabolize. Five to 10 percent of white people have a poor ability to metabolize, as do 1 to 2

percent of Southeast Asians. Substrates for CYP2D6 isoenzymes are: antidepressants **(**amitriptyline, clomipramine, desipramine, doxepin, fluoxetine, imipramine, nortriptyline, paroxetine, venlafaxine), antipsychotics **(**haloperidol, perphenazine, risperidone, thioridazine), beta blockers **(**metoprolol, penbutolol, propranolol, timolol), narcotics (codeine, tramadol). [21, 24]

CYP2C19 variation is responsible for the poor metabolizing activity for more than 20% of the Asian population. Two to 3% of white people and 4% of black people have poor metabolism due to this enzyme variations [25]. At the present moment there are 21 alleles of this enzyme discovered. Substrates for CYP2C19 isoenzymes are: clomipramine, diazepam, imipramine, omeprazole, and propranolol.

CYP2C9 has 30 different alleles, most of them associated with the decreased metabolism of the respective substrates. The highest variation for this enzyme is shown for *3 allele. Some examples of high frequency: the Spanish population 16.2%, 3.3% for Chinese and 4.5% for Japanese. Two percent of Chinese individuals are carriers of the *CYP2C9*13* allele. Substrates for CYP2C9 isoenzymes are: nonsteroidal anti-inflammatory drugs, phenytoin, s-warfarin, torsemide.

Despite the large list of drugs affected by cytochrome P450 family, there are few examples about how cytochrome P450 gene polymorphism influences the therapy response.

Because the management of the therapy with oral anticoagulants is an important issue and because of their narrow therapeutic range it may cause bleeding complications or recurrent thrombosis, pharmacogenetics should bring some help for clinicians by providing some empirical doses for individual genotype combinations [2, 22].

Response to warfarin

It is of interest to remind that genetics influence the patient response to warfarin. [4,19]. The degree to which warfarin reduces vitamin K synthesis depends on its dose but also on patient's vitamin K epoxide reductase complex subunit 1 genotype. Not only variations of the vitamin K epoxide reductase complex subunit 1 genotype but also CYP2C9 genotypes influence the pharmacokinetics and pharmacodynamics of this drug. Patients who present variants of both genotypes are at increased risk of major bleeding. The studies show that patients metabolize warfarin differently. They experience an increased bleeding risk and subsequently need lower doses of drug, therefore in 2007 FDA (Food and Drug Administration) announced marketing approval of a genetic test which will identify people that are more sensitive to warfarin.

Response to statins therapy

Another example represents the patient response to statins therapy and also the rate of incidence of myopathy after statins.[16] The apolipoprotein E (ApoE) gene represents a response predictor for statins therapy. ApoE is a major binding protein for very-low-density lipoprotein and intermediate-density lipoprotein cholesterol. Some allele variants of ApoE, such as apoE-[varepsilon]4 is associated with a poor response to statins and a high incidence of cardiovascular disease, while the apoE-[varepsilon]2 had the greatest reduction in serum low-density lipoprotein (LDL) cholesterol after statins treatment. [5, 14] Atorvastatin, lovastatin, and simvastatin are metabolized by CYP3A5, but pravastatin or fluvastatin, are less dependent on CYP3A. Kivisto *et al.* [12] showed that lipoprotein cholesterol concentrations after 1 year of treatment with atorvastatin, lovastatin, or simvastatin was ~25% greater in individuals who express this enzyme compared with patients who do not express it. Also, it is well known that statin-induced side effects can interfere with therapy. The study noted STRENGTH (Statin Response Examined by Genetic Haplotype Markers) proved that single nucleotide polymorphisms impair statin metabolism. The researchers genotyped the participants for polymorphisms in the genes CYP2D6, CYP2C8, CYP2C9, CYP3A4, and SLCO1B1. SLCO1B1 (solute carrier organic anion transporter family member 1B1 SLCO1B1) encodes the organic anion-transporting polypeptide (OATP1B1), which has been shown to regulate the hepatic uptake of statins. But, they found that the reduced function of SLCO1B1*5 allele impairs statin clearance and is associated with simvastatin-induced myopathy with creatine kinase (CK) elevation [17, 23]. These results led to a big concern for clinicians and patients regarding the safety of statin therapy. In order to enable safer clinical practice and better healthcare decisions, these identified genetic markers will allow patients at higher risk of myopathy to be identified before treatment regimens are chosen [23].

The analgesic effect of codeine depends on metabolic activation by the enzyme CYP2D6, which is known to exhibit genetic polymorphisms that result in decreased production of active opioid. Codeine is a prodrug; it becomes an active analgesic only when it is converted into morphine during its metabolization by CYP2D6 enzymes. There are studies which have shown that the effects of codeine — analgesic, respiratory, psychomotor, and miotic — are markedly attenuated in people with poor activity of CYP2D6. On the other hand, people with ultrarapid metabolism, produce greater amounts of morphine from codeine and therefore may experience

exaggerated pharmacologic effects in response to regular doses of codeine. Similar effects but less dramatic, have been described in patients with ultrarapid metabolism of CYP2D6 in response to usual doses of hydrocodone or oxycodone, which are other opioids requiring CYP2D6-mediated activation. These reports clearly illustrate the effect of *CYP2D6* genetic polymorphisms on the action of codeine, ranging from virtually no effect in patients with poor metabolism to severe toxic effects in those with ultrarapid metabolism [7, 11].

Beta-blockers are an important cardiovascular class of drugs. Because they are used in a wide range of diseases such as hypertension, ischemic disease, heart failure, as well as after myocardial infarction, the variable response to their use was investigated [1]. The beta-blocker metoprolol is metabolized by the enzyme cytochrome CYP2D6. There are studies that suggest that variation of this gene, respectively a polymorphism related to a decreased activity of this enzyme, would result in a significant clinical response such as bradycardia in patients treated with metoprolol [3, 24]. Also, because this cytochrome CYP2D6 is mainly involved in the metabolism of metoprolol but not in the metabolism of carvedilol, propranolol, or timolol, there is a strong relationship between this gene and the dosage of metoprolol, but not between this gene and the dosage of carvedilol, propranolol and timolol.

Important debates rised from the PRINC trial (Plavix Response in Coronary Intervention), regarding the dosage and the individualized therapy with clopidogrel [7]. They investigated the relationship among the dosage, the response and the polymorphism in the CYP2C19, involved in clopidogrel metabolism. The response came for those patients carrying alleles CYP2C19*2 and *4, patients with decreased ability to create the active metabolite of clopidogrel; these patients required higher doses than 600 mg and also higher maintenance dosage. At this dose of 600 mg, these patients showed reduced platelet inhibition [7]. King *et al.* [11] discussed about a personalized therapy with clopidogrel, therapy that would need both genetic tests and *ex vivo* platelet aggregation testing (such as the VerifyNow P2Y12 platelet function test (Accumetrics, Inc., San Diego, California); he proposed another thienopyridine instead of clopidogrel, namely the use of prasugrel. His proposal came because prasugrel has a more direct metabolic path, a more rapid onset of action and is more potent than clopidogrel.

There is a strong association between CYP2C9 allelic variants and phenytoin dose requirement. Both mutant alleles, CYP2C9*2 and CYP2C9*3, are associated with markedly impaired metabolic capacity for

many CYP2C9 substrates compared to the wild-type, CYP2C9*1, resulting in raised serum drug levels upon a given dose. For patients carrying at least one mutant CYP2C9 allele, the mean phenytoin dose required to achieve a therapeutic serum concentration is about 37% lower than the mean dose required by wild-type individuals. A low maintenance dose (< 200 mg/day) is enough for 47% of carriers, while 58% of normal required a high dose (> 300 mg/day) for an effective serum level.

Other enzymes in drug metabolism

In 2002, in the medical literature it was published an article about the enzyme involved in the nitroglycerin (GTN) bioactivation [1]. The enzyme, called mitochondrial aldehyde dehydrogenase-2 (ALDH2) is responsible for the formation of NO, the metabolite needed for GTN efficacy. At that moment it was suggested that ALDH2 polymorphism might play a role in GTN responsiveness and therapeutic efficacy. Recently, it was shown that a polymorphism of the ALDH2 (the replacement Glu504Lys in the exon 12 - replacement which means enzyme inactivation) is associated with the lack of efficacy of sublingual GTN in Asian (Chinese) population. This result is very important in the Asian population because approximately 30-50% possess the inactive mutant of ALDH2 [13].

Gene chips to detect drug response variations

In order to evaluate the patient response to a drug it is very comfortable that prior to prescribing, for example, the AmpliChip Cyp450 test to be done. This test allows the clinician to identify a poor, intermediate, extensive or ultra rapid metabolizer of the substrate of enzymes such as CYP2D6 and CYP2C19 [15]. CYP2D6 and CYP2C19 genes represent two key genetic regions encoding the enzymes of the cytochrome P450 complex. Other tests such as GeneChip System—Affymetrix or NanoChip Molecular Biology Workstation—Nanogen, Inc. can be also used [10].

Also, because of the importance of warfarin genotypes and anticoagulant therapy response The ParagonDx and Idaho Technology assays, The Third Wave assay or The AutoGenomics assay were used and compared. Finally, Nanosphere Verigene Warfarin Metabolism Nucleic Acid Test was the genetic test approved by FDA for market release.

Another way in which gene detection could help to choose the right treatment comes from the identification of human epidermal growth factor receptor 2 (HER-2). Because HER2-positive breast cancers are more aggressive than other forms of breast cancer and are less responsive to hormone therapies, using tests such as immunohistochemistry (IHC),

fluorescence *in situ* hybridization (FISH) or chromogen *in situ* hybridization (CISH) for detecting the HER-2 status will help the oncologist to establish the treatment plan, to predict drug response and to select patients for trastuzumab treatment [9].

Conclusions

Pharmacogenetics is the study of genes influence on an individual's response to drugs. The field seems to be very new but it is half a century old. Because new perspectives came into field we can clearly see that an alteration in gene expression or inactivation may be correlated with pharmacological function or therapeutic response. In the future, primary care physicians will have to perform some routine genetic tests before prescribing drugs; they will have to do this in order to identify the responders, the poor responders and the non-responders to a certain drug.

Individualized drug therapy based on genetic tests will open new opportunities for the pharmaceutical market. With the advance of the technology, new SNPs will be identified, cheaper tests will appear on market and also some insurance coverage will be decided.

The old concept "one-size-fits-all" suffers a major challenge because pharmacogenomics promises to take the guesswork out of developing and prescribing safe and effective drugs. So, the answer for the rhetoric question "could this lead to individualized therapy?" is yes. If the DNA micro arrays (or DNA chips or gene chips) will be used for DNA sequencing, doctors will have access to a rapid and affordable tool before prescribing.

Many adverse reactions are due to genetic variants. In order to avoid them, prescreening will be done before prescribing. By reducing the occurrence of adverse effects to a drug the cost of patient's treatment will be reduced.

On the other hand, for the time being, the prohibitive price of the existing gene tests (ex. genotyping in the case of warfarin use is about 500$) rise barriers in front of pharmacogenetics research.

References

1. Azuma J., Nonen S., Chronic heart failure: beta-blockers and pharmacogenetics, *Eur J Clin Pharmacol.* Jan. 2009,65(1), 3-17.
2. Beinema M., Brouwers J.R., Schalekamp T., Wilffert B., Pharmacogenetic differences between warfarin, acenocoumarol and phenprocoumon, *Thromb Haemost*. Dec. 2008, 100(6), 1052-7.
3. Bijl M.J., Visser L.E., van Schaik R.H. *et al*, Genetic variation in the CYP2D6 gene is associated with a lower heart rate and blood pressure in beta-blocker users, *Clin Pharmacol Ther.* Jan. 2009, 85(1), 45-50.
4. Caldwell M.D., Berg R.L., Zhang K.Q. *et al.*, Evaluation of genetic factors for warfarin dose prediction, *Clin Med Res.,* Mar. 2007, 5(1), 8-16.

5. Suciu M., The role of nitric oxide (NO) and statins in endothelial dysfunction and atherosclerosis, *Farmacia*, 2009, 57(2), 131-140.
6. Gardiner S.J., Begg E.J., Pharmacogenetics, drug-metabolizing enzymes, and clinical practice, *Pharmacol Rev*., Sep. 2006, 58(3), 521-90.
7. Gladding P., Webster M., Zeng I. *et al*, The antiplatelet effect of higher loading and maintenance dose regimens of clopidogrel: the PRINC (Plavix Response in Coronary Intervention) trial, *JACC Cardiovasc Interv*. Dec. 2008, 1(6), 612-9.
8. Jain K.K., Basic technologies for developing personalized medicine in: Textbook of personalized medicine, Springer, 2009, 15-19.
9. Kaneko S., Gerasimova T., Butler W.M. *et al*, The Use of FISH on breast core needle samples for the presurgical assessment of HER-2 oncogene status, *Exp Mol Pathol*. Aug. 2002, 73(1), 61-6.
10. Keen-Kim D., Grody W.W., Richards C.S., Microelectronic array system for molecular diagnostic genotyping: Nanogen NanoChip 400 and molecular biology workstation, *Expert Rev Mol Diagn.* May 2006, 6(3), 287-94.
11. King S.B. 3rd, Momary K.M., Thienopyridines: Time for Personalized Therapy?, *JACC Cardiovasc Interv.* Dec. 2008, 1(6), 718-9.
12. Kivistö K.T., Niemi M., Schaeffeler E., *et al*, Lipid-lowering response to statins is affected by CYP3A5 polymorphism, *Pharmacogenetics*, Aug. 2004, 14(8), 523-5.
13. Li Y., Zhang D., Jin W. *et al*, Mitochondrial aldehyde dehydrogenase-2 (ALDH2) Glu504Lys polymorphism contributes to the variation in efficacy of sublingual nitroglycerin, *J Clin Invest*. Feb. 2006, 116(2), 506-11.
14. Mega J.L., Morrow D.A., Brown A., Cannon C.P., Sabatine M.S., Identification of genetic variants associated with response to statin therapy, *Arterioscler Thromb Vasc Biol*. Sep. 2009, 29(9), 1310-5.
15. Rebsamen M.C., Desmeules J., Daali Y. *et al*, The AmpliChip CYP450 test: cytochrome P450 2D6 genotype assessment and phenotype prediction, *Pharmacogenomics J*. Feb. 2009, 9(1), 34-41.
16. Rossi J.S., McLeod H.L., The pharmacogenetics of statin therapy: when the body aches, the mind will follow, *J Am Coll Cardiol*. Oct. 2009, 54(17), 1617-8.
17. SEARCH Collaborative Group, Link E., Parish S., Armitage J. *et al*, SLCO1B1 variants and statin-induced myopathy - a genomewide study, *N Engl J Med*. Aug. 2008, 359(8), 789-99.
18. Sherry S.T., Ward M., Sirotkin K., dbSNP-database for single nucleotide polymorphisms and other classes of minor genetic variation, *Genome Res* Aug. 1999, 9(8), 677-9.
19. Sistonen J., Fuselli S., Palo J.U., Chauhan N., Padh H., Sajantila A., Pharmacogenetic variation at CYP2C9, CYP2C19, and CYP2D6 at global and microgeographic scales, *Pharmacogenet Genomics*. Feb. 2009, 19(2), 170-9.
20. Smart A., Martin P., The promise of pharmacogenetics: assessing the prospects for disease and patient stratification, *Stud Hist Philos Biol Biomed Sci*., Sep. 2006, 37(3), 583-601.
21. Cucuiet S., Dogaru G., Bild V.N., Dogaru M.T., Modulation of tramadol antinociception by ketamine and baclofen in mice, *Farmacia*, 2008, 56(6), 675-691.
22. Stehle S., Kirchheiner J., Lazar A., Fuhr U., Pharmacogenetics of oral anticoagulants: a basis for dose individualization, *Clin Pharmacokinet*. 2008, 47(9), 565-94.
23. Voora D., Shah S.H., Spasojevic I. *et al*, The SLCO1B1*5 genetic variant is associated with statin-induced side effects, *J Am Coll Cardiol*., Oct. 2009, 54(17), 1609-16.
24. Prasacu I., Mircioiu C., Sandulovici R., Enache F., Release of metoprolol from solid dosage forms. Choice and validation of theoretical model, *Farmacia*, 2009, 57(1), 89-98.
25. Ward M.B., Sorich M.J., McKinnon R.A., Cytochrome P450 Part 2: Genetics of Inter-Individual Variability. *J Pharm Pract Res*, 2008, 38, 226-9.

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