Expert Review Article

Population Pharmacogenomics and Personalized Medicine Research in Hungary: Achievements and Lessons Learned

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Abstract: There have been a number of notable strides in Hungary in the field of population pharmacogenomics. This paper aims to summarize and share the recent experiences in population genomics and personalized medicine in Hungary with leaders of the Genomic National Technology Platform. The present day Hungary differs from other populations in the region as Hungary was established some 1100 years ago, with founders of the ancestral Hungarian population originating from the east side of the Urals. Additionally, the Roma population of about 700,000 represents the largest ethnic minority living in Hungary. In a series of investigations, we found significant differences between the Hungarian and Roma populations in clinically relevant pharmacogenomics targets such as *VKORC1* and *CYP2C9* genes. Pharmacogenomics applications are also of interest from the standpoint of biomarker-guided drug discovery in Hungary which we highlight briefly in this paper. Regulatory, ethical and economic aspects of genomics are other dimensions crucial for efficient transition of basic genomics discoveries from laboratory to the clinic. Importantly, Hungary has a Parliamental Act for regulation of genetic diagnostic and research test procedures, and for regulation of biobanks since 2008. Diagnostic molecular pharmacogenomics tests are reimbursed from the same insurance budget as with the other molecular biology based tests in Hungary. Personalized medicine diagnostics require further considerations on how best to integrate and reimburse them in routine healthcare as this new field evolves in Hungary.

Keywords: *CYP2C9* and *VKORC1* variations, Hungarians and Roma, personalized therapy, population pharmacogenomics, warfarin.

1. INTRODUCTION

Hungary is presently a developing full member of the European Union but belonged to the former Eastern-European block some 20 years ago with an associated past legacy of narrow economic resources in medical research and development. However, there have been a number of notable recent strides in Hungary in the field of population pharmacogenomics and personalized medicine.

Hungary has two special conditions that are noteworthy in a context of population genomics. First, Hungary differs from the western European populations in the founders of the ancestral Hungarian population that originated from the east side of the Ural Mountains some 1100 years ago [1, 2]. Second, in Hungary there are minorities such as the Roma population that maintained their identity forming relatively closed populations. Inter-ethnic differences in drug response and optimal dose requirements are well established in pharmacogenomics and personalized medicine. Roma population of 700,000 currently residing in Hungary represent the largest minority group, although the Roma populations also exist in other parts of the world. Population genetics/genomics of the Roma has received little research attention to date. Several lines of evidence support the idea that the Roma population originated from Northern India [3-9], and hence, might have different population genetic structure than the Hungarian majority population and other European populations.

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Geographic patterns of genetic variation, including variation at the drug metabolizing enzyme loci and molecular drug targets collectively indicate that geographical structuring of inter-individual variation in drug response may occur frequently. The geographic distribution of certain variants has highlighted the possible importance of average differences in drug response across populations [10]. For this reason we need to take into account not only the differences between individuals' genetic make-up, but also the differences in human genetic variation among populations of different origin [11].

Indeed, the frequencies and distribution of pharmacogenomic polymorphisms vary greatly among the human populations [10, 12]. Recent studies report differences between African and non-African population groups in the structure of sequence variation in the human genome [13]. Another example that received recent research attention is the inter-ethnic differences in the dosing of the anti-coagulant agent warfarin [14]. This paper aims to summarize and share our recent experiences in population genomics and personalized medicine in Hungary with leaders of the Genomic National Technology Platform (GNTP).

2. HUNGARIAN POPULATION ANCESTRY

Several Hungarian subpopulations and other minorities live in Hungary who were to some extent isolated populations even decades ago. Their population genetic structure has not been previously investigated with the modern molecular methods. In particular, the Roma population is not well studied all around the world including in Hungary.

2.1. The Hungarian Majority

The founders of the Hungarian state are the "Magyars" (ancestral Hungarians), who settled in the Carpathian Basin at the end of the 9th century, after two millennia of migration from the east side of the Ural Mountains [15]. The Carpathian Basin region had been settled for thousands of years before the Magyars' arrival, by Dacians, Romans, Sarmatians, Goths, Huns, Avars, Slavs, and others; at the time of the Hungarian settlement the majority of the indigenous population was Slavic.

In order to study the genetic structure in populations living in the Carpathian Basin from these early origins, single nucleotide polymorphisms (SNPs) of mitochondrial DNA, Y chromosomal binary marker investigations, and microarray based SNP studies had been performed. The analysis of the maternal lineage shows that the linguistic isolation of Hungarian populations in the Carpathian Basin did not lead to significant genetic isolation. Mitochondrial DNA sequences of 27 ancestral samples (10-11th centuries), 101 present day Hungarian, and 76 Szekler (isolated minority in Transylvania) samples from Transylvania were analyzed [16]. The data from the latter analyses were compared with the genetic sequences of 7752 individuals representing 57 European and Asian populations, including Finno-Ugric populations. Only two of the 27 ancestral Hungarian samples were unambiguously Asian; the rest belong to one of the Eurasian haplogroups. These analyses collectively suggest that the ancestral population from the 10th to 11th centuries was genetically heterogeneous, and some Asian genetic contribution is seen in the Hungarian ancestral population.

The analysis of the maternal lineage also shows that the linguistic isolation of the Hungarian-speaking populations in the Carpathian Basin has not lead to significant genetic isolation. Gene flow from neighbouring regions and migrations has affected the Hungarian gene pool: maternal lineages in the modern Hungarian gene pool bear the imprints of populations who have been living in the region for centuries. In modern day Hungary, likely there is a dominating effect of populations already living in the Carpathian Basin, with influence from the Balkans and West Eurasia, while in the Seklers the genetic effect of Eastern and Southern Europeans is more visible.

A mtDNA based approach using 55 random DNA samples collected from the general Hungarian population, only three samples (5% of the total) was found to have the M haplogroup that has unequivocally Asian origin [1]. Further, in another study on a pedigree with A7445G mutation associated deafness, the U4b Asian haplogroup could be identified [17].

Paternal lineages, which usually give a higher geographical resolution than maternal, have also been studied. Total of 22 biallelic polymorphisms were characterized in the Y chromosome in 100 men from modern day Hungary and 97 Seklers. The results were compared with data from other European populations as studied by Semino *et al.* [18], together with the phylogeographic context of the Y chromosome pool of the populations [19]. One specific Y-chromosomal base substitution (Tat, T→C) a relatively recent event (95% CI: 3140-6200 years) is a valuable marker in Finno-Ugric population studies [20]. The *C* allele of the Tat polymorphism is widespread in all Uralic-speaking populations, except that it is absent or extremely rare among the modern day Hungarian-speaking populations [2, 18, 20-22].

In another study analyzing a total of 6501 Y chromosomes of 81 populations, including samples collected from 106 unrelated Hungarian males, there was however no statistical difference in the prevalence of the E-M78 and E-V13 haplogroups between Hungarians and the populations in the neighbouring regions [23]. More recently, in a study of more than 270,000 SNPs in 3112 individuals across 16 European countries, no unique genotype could be revealed in the Hungarian samples [24].

2.2. The Roma Population in Hungary

Roma (Gypsy/Romani) people originated in the Northern part of the Indian subcontinent. They began their migration about 1000 years ago. The reason for their diaspora remains an enigma, since in absence of the written history the origin and early history of the Romani people are still not clear. Roma emigrated from India towards the North-West no earlier than the 11th century. Notably, 47.3% of Romani males carry the Y chromosome haplogroup of H-M82, which is rare outside of the Indian subcontinent. Mitochondrial haplogroup M, most common in the Indian population and rare outside Southern Asia, is present in nearly 30% of Romani people [25]. A more detailed study of the Roma in Po-

land shows this as an M5 lineage, which is specific to India [26].

Roma populations live in many countries throughout the world and are well known for their preserved traditions. Currently, the total Roma population size is estimated to be about 12 million in the world [3, 4]. Hungary is the fourth in Europe with respect to the size of its Roma population, which is estimated about 700,000 people [4]. On the other hand, the Roma represents an understudied population in human genetics despite its size as the largest minority within Hungary [27-31].

3. POPULATION PHARMACOGENOMICS RESEARCH IN HUNGARY: A CASE STUDY OF *CYP2C9* AND *VKORC1*

Advances in genomics technologies resulted in a paradigm shift in everyday practice of oncology and pathology in Hungary. This shift started a decade ago with the introduction of the *HER-2* diagnostics of breast cancer. Pharmacogenomics has been subsequently applied, for example, in the context of acute lymphoblastic leukemia (ALL) [32, 33], lung, breast and colorectal cancers, melanoma and oncology more generally [34-46]. Further research is however needed to discern the population genetic/genomic substructure of drug efficacy and safety in these therapeutic indications. Due to the population pharmacogenomics focus of the present paper, we herein highlight several recent studies in Hungary where different subpopulations (Hungarian and Roma) were surveyed with respect to candidate genes that have pharmacokinetic and pharmacodynamic significance. Specifically, two candidate genes – *CYP2C9* and *VKORC1* – have been examined in detail in our population studies in Hungary as outlined below.

3.1. CYP2C9 Genetic Variation in Hungarian and Roma Populations

It has been reported that the metabolism of S-enantiomers of coumarin derivates was significantly decreased by the presence of both the *CYP2C9*2* (R144C) and *CYP2C9*3* (I359L) alleles of the *CYP2C9* gene [47-51]. It has been estimated that about 7-10% of the interindividual variation in coumarin dose could be explained by *CYP2C9*2* and *CYP2C9*3* variant alleles [52]. Genotype-adjusted dosing of warfarin may thus result in faster anticoagulation and reduced toxicity [53, 54].

The *CYP2C9*2* was the most commonly identified variant allele in the Hungarian general population whereas in the Roma population the *CYP2C9*3* was more common [55]. Notably, we found a significant 1.8-fold increase in *CYP2C9*3* prevalence in the Roma population compared to the Hungarian samples which have important therapeutic implications ($p<0.001$). According to the variants of *CYP2C9* gene analyzed in our studies, the proportion of extensive metabolizers was higher in the Hungarians ($p<0.005$), while poor metabolizer subjects were more frequent in the Roma population ($p<0.03$). Hence, Roma population might be at an increased risk of drug-induced side effects due to diminished drug elimination, compared to the Hungarian population [55].

The results of our study were also compared to the findings in other populations of different geographic origin. The prevalence of *CYP2C9*2* and *CYP2C9*3* alleles observed in our study of healthy Hungarian subjects were very close to that found in other Caucasian populations [56-65], but higher than that in Asians [61, 66-70]. We found a significant difference in allele frequencies of the *CYP2C9*2* and *CYP2C9*3* variants in the Roma group versus the Indian population ($p<0.04$) [71]. We observed that the *CYP2C9*3* allele frequency in the Roma group differed and other Caucasian populations; moreover the presence of this allele in the Roma sample was found to be the highest in the literature [56, 58, 61-64]. It has been suggested that the *CYP2C9*3* allele confer the largest reduction in metabolic activity *in vitro*, as compared to *CYP2C9*1* [72]. Hence, the higher frequency of the *CYP2C9*3* allele (0.155) in the Roma population compared to other populations warrants further translational clinicalresearch [56-79].

3.2. VKORC1 Genetic Variation in Hungarian and Roma Populations

Genetic variation in the vitamin K epoxide reductase complex subunit 1 gene (*VKORC1*) results in different pharmacogenetic phenotypes: resistance or sensitivity to coumarin derivates, or in a rare bleeding disorder known as the multiple coagulation factor deficiency type 2 (VKCFD2) [80, 81]. *VKORC1* genotype was found to display interethnic variability and to determine 25-40% of the individual coumarin dose requirement [52, 82, 83]. There are four important haplotypes of *VKORC1* gene [84]. The *VKORC1*1*, the putative ancestral haplotype, is characterized by the presence of ancestral allele of G1639A, G9041A, and C6009T polymorphisms. The *VKORC1*2* haplotype is characterized by the presence of 1639A, G9041, and C6009 SNP combination. The *VKORC1*3* haplotype is characterized by the 9041A, G1639, and C6009 SNPs. The *VKORC1*4* haplotype is characterized by the presence of 6009T, G1639, and G9041 combination [85]. The *VKORC1**2 [84, 86] haplotype belongs to the low-dose haplotype group (A) and the *VKORC1**3 [86-88] and *VKORC1**4 [86, 88] belong to the high-dose haplotype group (B). The characterization both of the low-dose and high-dose defining haplotypes of the *VKORC1* gene is clinically helpful because it can inform the clinicians on under- and over-anticoagulation states.

In the Hungarian population samples, the haplotypes, in decreasing frequency, were *2 (39%), *3 (37%), *4 (21%) and *1 (3%), while in the Roma population samples the frequencies were *3 (46%), *2 (30%), *4 (19%), and *1 (5%). The statistical analysis revealed significant difference in the prevalence rate of *VKORC1*2* and *VKORC1*3* haplotypes between the Roma and the general Hungarian population ($p<0.005$). In addition frequency differences were observed between the Hungarian and the Roma population in *VKORC1* (A) ($p<0.004$) and (B) ($p<0.03$) haplotype groups.

Between the Hungarian and European populations, however, we could observe a difference only in the frequency of the *VKORC1*1* haplotype ($p<0.02$). The distribution of *VKORC1*2*, *VKORC1*3*, *VKORC1**4 haplotypes in the general Hungarian samples was consistent with that of the European Caucasians [84]. By contrast, a significant difference

was found in the *VKORC1*1* and the *VKORC1*2* haplotype frequencies between the Roma and the European populations reported in the literature [84]. Interestingly, the *VKORC1*3* frequency in Roma was the highest among the previously studied populations, including higher than that in Africans. The *VKORC1*1* was found in the Roma population in higher frequency than that in the European population [84]. The Roma population differs from other European populations in the distribution of the *VKORC1* haplotype group A ($p<0.004$) [84]. Taken together, these findings inform the future population pharmacogenomics studies in Hungary and comparative evaluation with other global populations.

4. ADVANCES IN BIOMARKER-GUIDED DRUG DISCOVERY IN HUNGARY

An important goal of pharmacogenomics research is to identify and validate biomarkers that can usefully inform rational drug discovery and development. To this end, the University of Pécs has become the lead pharmacogenomics network center in Hungary. We observed that pharmacogenomics might have good uptake by end users such as clinicians. Pharmacogenomics tests ordered by the clinicians increased from just seven in 2006 to 287 in 2009. Development of upstream drug discovery and development oriented biomarkers might require, however, public and provate partnerships. In this section, we summarize briefly some of the experiences from within the Hungarian biotechnology industry in relation to pharmacogenomics and biomarker discovery and development. While the use of pharmacogenomics in the industry represents a host of diverse applications with regional and international differences, the discussion that follows reflects primarily the experiences from the Vichem, a Hungarian biotechnology company focusing on signal transduction therapy with kinase inhibitors.

Kinase inhibitors are in the front line of modern drug research. Genomics and proteomics approaches by Vichem have contributed towards the development of novel patentable kinase inhibitor chemical structures that effectively and specifically inhibit validated kinase targets in cancer cells [89, 90]. A second application of molecular diagnostics was the focused preclinical drug development of bioassays which model subgroups of human tumors. A third application of genomics and other molecular diagnostics in drug discovery and development in Hungarian biotechnology industry was to help expedite validation of the molecular mechanism of action of the lead compounds [91, 92] and the development of companion diagnostics for personalized use of therapeutic candidates.

Instead of pre-selecting one kinase target in one tumor type, or a single potential predictive biomarker, the approach developed by Vichem was to identify a panel of multiple molecular markers ("mutation array") as a novel companion pharmacodiagnostic (PharmaID-PDx^{TM}) assay, which specifically informs the efficacy of a new drug. Some pertinent examples are the success of imatinib in case of c-KIT and platelet-derived growth factor receptor (*PDGFR*) mutations [93], gefitinib in case of *EGFR* mutations [94] or panitumumab/cetuximab in *KRAS* mutation negative tumors [95]. It is also becoming clear, however, that singular biomarkers will not be sufficient. For example, in case of panitumumab/cetuximab, there is evidence suggesting the merit of combining the *KRAS* mutation test with other mutation tests such as *BRAF* or phosphoinositide-3-kinase, catalytic, alpha polypeptide (*PIK3CA)* [95].

Owing to the large cancer genome sequencing projects in the biotechnology industry, potential genetic alterations in kinase driven signaling pathways were identified. For example, Ding *et al*. sequenced coding exons of 623 candidate cancer genes in 188 lung adenocarcinomas [96]. Tests on cell lines carrying these mutations or mutation combinations might help to identify markers to predict the efficacy of a certain kinase inhibitor. The cell line panels represent the most important molecular genetic alterations identified in the latter study.

We would like to emphasize that while the knowledge of a drug target might help us understand why a certain set of markers are predictive of drug effects, the biomarker development is not necessarily limited to knowledge on demonstrated or putative mechanism(s) of drug action. Further, a given drug may not be a complete and selective kinase inhibitor but it oftentimes has multiple targets all of which might be considered to develop novel biomarkers. In other words, focusing on the drug specific diagnostic panels instead of molecular target specific diagnostics may in the long run bring about greater insights and benefits for biomarker discovery and development towards personalized and rational drug development.

4.1. Highly Selective Next Generation EGFR Inhibitors

The epidermal growth factor receptor is one of the most widely validated molecular targets in human tumors that are highly prevalent such as lung and colorectal cancers. There are several first generation drugs already on the clinic that target the EGFR. Notably, their clinical development was however previously delayed. Based on the experiences with the first generation drugs there is an opportunity to develop more successful second generation EGFR inihibitors. Compounds which inhibit specifically the lung cancer cell lines harboring tumor specific *EGFR* mutations are being developed presently by the Hungarian biotechnology industry. This provides an opportunity to personalize EGFR based therapeutics while decreasing the risk of side effects due to unintended effects on other targets.

4.2. Drugs Selectively Targeting the *KRAS* Mutant Tumors

KRAS mutation testing is becoming a routine diagnostic procedure in all colon cancer and lung cancer patients. This is driven by its negative predictive value for EGFR-inhibitors currently in the clinic. *KRAS* mutation is present in nearly 40% of the colon cancers, 20% of the lung cancers and 70% of the pancreatic cancers. These patients tend to have worse prognosis in the absence of selectively targeted drug therapy. Compounds that selectively inhibit the cell lines carrying *KRAS* mutations are also being developed in Hungary. However, it should be emphasized that considerable discovery and translation research is still necessary before their clinical potential for personalized medicine can be discerned.

5. REGULATION AND REIMBURSEMENT OF GENETICS/GENOMICS RESEARCH AND TESTS IN HUNGARY

In Hungary, the Parliamentary Act on the protection of human genetic data and the regulation of human genetic studies, research and biobanks entered into force on July 1, 2008. The Act applies to the genetic sampling procedure for diagnostic human genetic tests, the human genetic research performed within the Republic of Hungary, and the processing of genetic data irrespective of the place of sampling. Genetic samples and data anonymised even before the Parliamentary Act came into force may be treated according to the Act for human genetic research and study.

Hungary also has special organizational structures to elaborate on the ethical guidelines and to review research programs. Each research protocol intending to involve human beings usually has to be reviewed by two levels of ethics committees: the national, and the regional levels. At the national level, organized into the Health Science Council (ETT; founded in 1951) there are three different National Research Ethics Commissions: the Scientific and Research Ethics Commission (TUKEB), the Clinical Pharmacology Ethics Commission (KFEB), and Human Reproduction Commission (HRB). In addition, the Health Ministry decree 23/2002 (V/9) on biomedical research involving human beings established 12 regional research ethics boards (REKEB) throughout the country. It depends on the nature of the research whether the protocol has to be reviewed on a national or only on a regional level. Thus, according to the the Parliamentary Act of Hungary, the review of research protocols that is carried out by the different levels of ethics commissions includes an evaluation both from a scientific and from an ethical point of view.

In Hungary the cost of pharmacogenetic tests are paid in full by the Hungarian National Health Insurance Fund to insured individuals. In the Hungarian healthcare, the molecular pharmacogenomics tests are reimbursed from the same budget as the other molecular biology based tests. The financing of the genetic tests is the method-based compensation. The budget available for the genome-based diagnostic tests is governmentally limited, undergoes modifications yearly.

6. A SURVEY OF THE GENETIC NATIONAL TECHNOLOGY PLATFORM

The Personalized Medicine Working Group of the National GNTP of Hungary performed in 2009 a questionnaire based interview about the definition of personalized medicine (PM) and perceptions on the concept of PM. 42 leading clinicians, scientists and academicians filled the questionnaire nationally. All of the respondents expressed a view that the pharmacogenomics-based PM will transcend the current blockbuster method of drug development and therapeutics, 64% had the opinion that the multiplex biomarkers will predict and guide treatment of early chronic disorders, and 59% indicated that PM will reform the Hungarian healthcare within the next 10 years. Most of the interviewed persons agreed that the recent rapid advances in genomics and molecular biology are beginning to reveal a large number of potential novel genomic biomarkers for disease susceptibility (59%), or differential response to treatment (63%). Such markers can serve, as stated by the respondents, as the basis of new genome based diagnostics tests for identifying and/or confirming disease, assessing an individual's risk of disease (70%), identifying patients who will benefit from particular interventions, or tailoring dosing regimens to individual variations in drug metabolism and response (70%). Most of the respondents (91%) did not think that the PM will decrease the number of new molecules receiving marketing authorization for clinical use. It was a common opinion that PM will improve the efficacy and safety of the drugs. On the other hand, only 30% estimated that PM will increase the cost of the healthcare.

Insofar as challenges to PM, several views were expressed in our survey in Hungary. These included concerns relating to techonology (33%), regulatory issues (47%), reimbursement (58%), physician and patient education (38%), economics of genetic testing (42%) and privacy issues (27%) Only 26% of our sample of Hungarian physicians and scientists predicted that PM will be applicable in the current day-to-day practice of medicine. In the future they presumed however that PM will be most applicable in oncology (49% of the respondents), neurology-psychiatry (13%), and in autoimmune disorders (31%). We caution the reader however that our preliminary survey does not necessarily represent the views of the entire population of clinicians and scientists in Hungary. Further population based surveys are likely to inform the perceptions on PM and pharmacogenomics in Hungary and other global regions.

CONCLUSIONS

Each country and population apply pharmacogenomics in different contexts while the challenges also differ among countries. This paper aimed to share our recent experiences in population based pharmacogenomics in Hungary including in the Roma population, the largest minority in Hungary. As the Roma represents a hitherto understudied population, our observations on selected candidate genes (of relevance for warfarin pharmacokinetics and pharmacodynamics) might inform future research on PM [55, 85]. This is timely because pharmacogenomic information was recently recommended by the US Food and Drug Administration (FDA) to be included in the warfarin product label [97].

Population pharmacogenomics and personalized medicine are not only driven nor enabled solely by biotechnologies. They also demand sound regulatory, legal, ethical and policy frameworks; The 2008 Parlimentary Act of Hungary is one step in this direction. Personalized medicine diagnostics require further considerations on how best to integrate and reimburse them in routine healthcare as this new field evolves in Hungary.

ABBREVIATIONS

5-FU	=	Fluorouracil
ABCB1	=	Human multidrug resistance 1
ABCG2	=	ATP-binding cassette sub-family G member 2

ALL	=	Acute lymphoblastic leukemia
c-KIT	=	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
CYP2C9	=	Cytochrome P450 family 2 subfamily C polypeptide 9
DPD	=	Dihydropyrimidine dehydrogenase
EGFR	=	Epidermal Growth Factor Receptor
FDA	=	Food and Drug Administration
FLT3-ITD	=	fms-related tyrosine kinase 3
GJA1	=	Gap junction protein, alpha 1
GJB2	=	Gap junction protein, beta 2
GNTP	=	Genomic National Technology Platform (Hungary)
HER2	=	Human epidermal growth factor receptor 2
HRB	=	Human Reproduction Commission
KFEB	=	Clinical Pharmacology Ethics Commission
KRAS	=	Kirsten rat sarcoma viral oncogene homolog
MDS	=	Multidimensional scaling
NPM1	=	Nucleophosmin
NSCLC	=	Non-small cell lung cancer
OMIM	=	Online Mendelian inheritance in man
PDGFR	=	Platelet-derived growth factor receptor
PDGFRA	=	Platelet-derived growth factor receptor, alpha polypeptide
PM	=	Personalized medicine
REKE	=	Regional research ethics boards
SLC22A5	=	Solute carrier family 22
SNP	=	Single nucleotide polymorphism
TS	=	Thymidylate synthase
TUKEB	=	National Research Ethics Commissions
VEGFR	=	Vascular endothelial growth factor receptor
VKORC1	=	Vitamin K epoxide reductase complex subunit 1

CONFLICT OF INTEREST

The following authors are affiliated with the Vichem (György Kéri), KPS (István Peták) and Richter (György Németh). Other outhors did not declare conflict of interest.

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