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Letter to the Editor

Evaluation of genotyping methods and costs for MTHFR, CBS and MTRR polymorphisms in patients with vitiligo

Dear Editor,

We read with great appreciation the work published by Benincasa et al[1]. The authors affirmed that the genetic screening for methylenetetrahydrofolate reductase (MTHFR) C677T, cystathionine β-synthase enzyme (CBS) I278T and methionine synthase reductase (MTRR) A66G polymorphisms could be a useful tool for preliminary identification of the vitiligo in patients with vitiligo familiarity. In this review, the authors have used a commercial kit based on Restriction Fragment Length Polymorphism (RFLP) method for the three polymorphisms detection. They achieve the issue to evaluate costs and availability of appropriate methods to setting molecular diagnostics of genotyping with detection of the polymorphisms MTHFR C677T, CBS I278T and MTRR A66G. We support this affirmation.

Generally, as genomic tests performed widely in clinical laboratories, the evaluation of the best commercially available platform becomes a noteworthy consideration about the clinical employment of genetic information. Nowadays, genetic tests are performed by the academic ultra-specialized labor custom service laboratories that use certified commercial kits (when available). In Europe, the field of diagnostic products is regulated by "*in vitro Diagnostic*" (IVD) policy, without a distinction between commercial products and diagnostics service. In both cases, clinical laboratories may develop tests in-house ("home-brew") and validate them by submitting standardized results to outside referenced laboratories in the context of International External Quality Assurance (EQA) programs[2,3].

Payment and refund for genetic testing are another issue of great importance that is already creating controversy among healthcare providers. It will be stimulating to consider whether insurers will evaluate genetic testing to be cost-effective. However, if the detection of the polymorphisms MTHFR C677T, CBS I278T and MTRR A66G is routinely incorporating into clinical practice, knowledge concerning the predictive value of tests will eventually enable individual therapy (Table I)[4].

Some methods to evaluate the quality and cost-effectiveness of genetic tests are now available. Noteworthy is the authoritative Diagnostic Advisory Committee of the National Institute for Health and Clinical Excellence (NICE), which stimulates Health Company and governance communities to create data for fitting economic models into healthcare system[5].

Current Genotyping Methods

The qualitative assessment of the MTHFR C677T, CBS I278T and MTRR A66G Single Nucleotide Polymorphisms (SNPs) could be performed by several allelic discrimination methods. To date, no standard gold method for the daily diagnostic routine was established (Table II).

In general, the most widely used platforms for genotyping of known SNPs include: (I) PCR with fluorescent hybridization probes as FRET-based platforms, locked nucleic acid probes and invader assay; (II) PCR-based methods without fluorescent emission as allele

Corresponding Author: Salvo Di Martino, Ph.D; e-mail: salvo-dimartino@libero.it

Table I. Genotyping panel assay for genetic predisposition to vitiligo.

SNP code	Genetic variant	MAF*	Clinical annotation
rs1801133	MTHFR C677T	A = 0.3 in Europe population (ExAc study)	MTHFR deficiency; Gastrointestinal stromal tumor; Cyclophosphamide response-toxicity/ADR; Carboplatin response efficacy; Neural tube defects, methotrexate response
Rs5742905	CBS I278T	G = 0.001 in Europe population (1000Genomes study)	Mild clinical homocystinuria pyridoxine-responsive; Hyperhomocystinemia, Thrombotic, CBS-related
Rs10380	MTRR A66G	T = 0.0 in Europe population (GnomAD Exomes study)	Gastrointestinal stroma tumor; Disorders of intracellular cobalamin metabolism; Association with the risk of cancers (breast, colon, prostate, pancreatic)

*Minor Allele Frequence.

specific amplification and RFLP; (III) PCR-based with intercalating fluorescent dye as high-resolution melting; (IV) Pre-treatment PCR only, as denaturing-high performance liquid chromatography and (V) sequencing methods either as automated *Sanger*'s sequencing or high-throughput sequencing technologies "next generation sequencing" (NGS).

Genotyping Costs

The primary intention of cost analysis is to provide adequate information for decision-makers to allocate funds in the genetic tests for the healthcare advances. Overviews of cost-benefit studies on genetic assay and platforms in healthcare fields are now available[6]. However, the literature is still low of studies addressing the economic implication in clinical healthcare of genomics tests. Significant survey to compare the cost of two methodologies validated for genotyping variations in the cytochrome P450 subtype 2C9 gene: the cost/sample for single SNP detection was $1.90 (US dollars) by PCR-Pyrosequencing and $3.14 by RFLP[7]. In this case, the instrumentation cost is averaged $100,000 and $5,000, respectively. It is clear that the better platforms are directly correlating to many samples. Furthermore, when the number of processing sample is little, the genotyping cost should be dramatically reduced by "homebrew" validated tests. For example, an early outline of pharmacogenomics tests performed on FRET-Assay platforms averaging about €20 per SNP[8]. The initial context evaluation costs of the detection of MTHFR C677T, CBS I278T and MTRR A66G gene variants could average about €5,00 per polymorphisms by RFLP platform (Table II).

Conclusions

We still need to highlight that genetic tests offer an added value, regarding relative cost and benefit. Moreover, there is more genomic expertise to interpret the results of these genetic tests efficiently[9-10].

The usefulness of genetic markers in clinical practice depends on improving the diagnostic prediction or endorsement ameliorative treatments strategy[11]. To date, about fifty different genetic loci that contribute to vitiligo risk have been discovered thanks to genome-wide association studies (GWAS). Some of these loci also contribute to other autoimmune diseases, epidemiologically associated with vitiligo. At many of these vitiligo susceptibility loci the corresponding relevant genes have now been identified, and for some of these genes the specific DNA sequence variants that contribute to vitiligo risk are also now known[12]. Thanks

Table II. Current platforms for detection VDR polymorphisms.

Genotyping methods to detect known SNP	Instrument mean costs[§]	Reagent costs per SNP[$]	Approximate time-labor per SNP[#]
Allele Specific Amplification (ASA)	+	Very low	Moderate
Restriction Fragment Length Polymorphism (RFLP)	+	Very low	Very laborious
FRET probe Allelic Discrimination (Hyb Probe® TaqMan®, Beacons® Scorpions®)	++	Moderate	Moderate
High resolutionmelting (HRM)	++	Low	Moderate
Conventional Sanger sequencing (automated with fluorescent detection)	++	Low	Moderate
Next Generation sequencing (NGS)	++++	Very high	Very fast
Denaturing-High Performance Liquid Chromatography (D-HPLC)	++++	Moderate	Very fast

[§]Approximate instrumentation list price were scored as + (< 10000€); ++ (< 50000€); +++ (< 100000€), ++++ (> 100000€); [$]Reagent costs scored as very low (< 5€), low (< 10€), cheap (< 30€), high (< 50€), very high (> 50€); [#]Time-labour refers input needed to perform a single test of multiple samples. It were scored as very fast (< 1 hour), fast (< 4 hours), moderate (< 1 day), laborious (< 2 days) very laborious (> 2 working day).

to the identification and validation of new genetic markers, physicians will have new ways and means to tailor specific therapy to individual genetic profiles[13].

Therefore, it is crucial that biotechnology companies plan their future investments to develop accurate and low-cost genetics tests for routine diagnostics in vitiligo.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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S. Di Martino[1,2], T. Muto[3], V. de Lucia[4]

[1]Pathology Unit, Santa Maria della Pietà Hospital, Nola (ASL NA 3 SUD), Naples, Italy
[2]Scientific Directorate, CETAC Research Center, Caserta, Italy
[3]Hematology and Cellular Immunology (Clinical Biochemistry), A.O. dei Colli Monaldi Hospital, Naples, Italy
4Second Division of Neurology, Campania University Luigi Vanvitelli, School of Medicine, Naples, Italy