Dear Editor,

We read with great appreciation the work published by Benincasa et al. 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.

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.

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.

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.

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...
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. 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. 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. 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.

The usefulness of genetic markers in clinical practice depends on improving the diagnostic prediction or endorsement ameliorative treatments strategy. 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. Thanks

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

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

*Minor Allele Frequency.
to the identification and validation of new genetic markers, physicians will have new ways and means to tailor specific therapy to individual genetic profiles. 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.

References