Evaluation of genotyping methods and costs for IL1α polymorphisms in Platelet Rich-Plasma (PRP); viewpoint for therapy on the diabetic foot ulcers

Dear Editor,

we read with great mindfulness the work published by Rossano et al1; the authors affirmed that the genetic screening IL1α polymorphisms could be a useful tool for early identification of the effectiveness of Platelet Rich Plasma (PRP) application in hair follicle regeneration. In this work, the authors have planned experimental panel test by a kit based Real-time PCR allelic discrimination method for IL1α polymorphisms detection1.

This pilot study appeared to be cost-effective in the treatment of androgenic alopecia in both males and females, without remarkable adverse effects, while they were accompanied by a discrete patients’ satisfaction rate. In addition, they encourage the plastic surgeon and the lab manager join in order to evaluate costs and availability of the procedures for PRP production and the appropriate methods to setting IL1α Genotyping. We agree to this affirmation and prospect the use of this procedure in PRP-based therapy in diabetic foot ulcers (DFUs) patients2.

In general, as genomics tests performed widely in clinical laboratories, the evaluation of the best commercially available platforms becomes a noteworthy consideration about the clinical employment of genetic information, particularly in so-called frail patients3.

However, if the detection of IL1α polymorphisms is routinely incorporating into clinical practice, knowledge concerning the predictive value of test which will eventually enable of individual therapy not only for hair follicle regeneration, but also for a plastic surgeon in diabetics patients4.

It is known that peripheral neuropathy is the most common chronic neurological complication of diabetes causing DFUs. Moreover, DFUs still is a puzzling problem for clinicians. Universally accepted detriments to the healing of diabetic foot ulcers include: infection, glyceemic control, vascular supply, smoking, nutrition, deformity, and genetic predisposition to chronic inflammation4.

Several studies examined the application of autologous PRP as additional treatment of foot ulcers in diabetics patients5.

Several approaches to weigh the quality and cost-effectiveness of genetic tests have now offered. Notably, is the Diagnostic Advisory Committee of National Institute for Health and Clinical Excellence (NICE) which excites Health communities to generate data for suitable cost-effective models into healthcare systems6.

Current Genotyping Methods and Costs

The assessments of the Single Nucleotide Polymorphisms (SNPs) could be performed by several platforms, but, it is still laking a core gold standard technique for the daily diagnostic routine (Table I). Hi-tech platforms most broadly used for the detection of well-known SNPs include: (1) PCR-based methods without fluorescent emission as Allele Specific Amplification and RFLP; (2) PCR with fluorescent hybridization probes as FRET-based platforms, Locked Nucleic Acid Probes and Invader assay; (3) PCR-based with intercalating fluorescent dye as High-Resolution Melting; (4) pre-treatment PCR only for template production, as Denaturing-High Performance Liquid Chromatography; sequencing methods as automated Sanger’s; Pyrosequencing; and high-throughput sequencing technologies named “Next Generation Sequencing” (NGS).
The primary purpose of cost-effectiveness analysis is to provide adequate information for decision-makers to allocate capitals in the genetic tests for the healthcare progresses. Overviews of cost-effectiveness studies on genetic assay and platforms in healthcare fields are now available.

Nevertheless, the literature is still low of studies addressing the commercial implication of genomic tests in clinical healthcare. Noteworthy, comparison study showed the cost two validated genotyping methodologies: SNP detection cost (for single assay) was $1.90 (US dollars) by PCR-Pyrosequencing and $ 3.14 by RFLP. In this case, the cost of instrumentation is about $110,000 and $4,000 respectively. It is clear that the better platform is directly related to the number of samples. Besides, when the number of processing sample is little (per patients), and the kind of the tests is great, genotyping cost should be dramatically reduced by “home brew” validated tests. For example, an early outline of pharmacogenomic tests performed on FRET-Assay platforms averaging about €20 per SNP.

The early outline evaluation costs of the detection of inflammatory cytokines gene variants could average about either €5 per polymorphisms by RFLP platform and or about €20 by Allelic Discrimination Real-time PCR (Table I). Thus, for the full proposed panel (five SNP) the cost is averaged €100.

**Conclusion and Future Outlook**

We still need precise evidence that genetic tests offer an added value, concerning benefit in the individual preventative efficacy of the treatment of foot ulcers in diabetes patients. There is an incontestable need for more detailed and extensive studies to establish the cost and effectiveness of genotyping cost of a panel test proposed here (Table II).

Since this inflammatory-related SNPs will be validated in international guidelines, another open question is related to the ability of physicians expertise to interpret the results of this genetic tests.

With new genetic inflammatory-related cytokines markers being validated, clinicians will have different means to best tailor specific foot ulcers therapy based on individual genetic profiles.

Consequently, it is indispensable that pharmaceutical and biotech companies join their financial programs in order to develop low-cost genetics tests for routine diagnostics. Promising, decision-maker might be able to accelerate the translation of genetic technologies into the routine clinical laboratory.

**Table I.** Current platforms for detection known polymorphisms.

<table>
<thead>
<tr>
<th>PCR based class</th>
<th>Genotyping methods to detect known SNP</th>
<th>Instrument mean costs$</th>
<th>Reagent costs per SNP#</th>
<th>Approximate time-labour per SNP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Allele Specific Amplification (ASA)</td>
<td>+</td>
<td>Very low</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>Restriction Fragment Length</td>
<td>+</td>
<td>Very low</td>
<td>Very laborious</td>
</tr>
<tr>
<td></td>
<td>Polymorphism (RFLP)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>FRET probe Allelic Discrimination</td>
<td>++</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>(Hyb Probe® TagMan®,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beacons® Scorpions®)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>High resolution melting (HRM)</td>
<td>++</td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td>IV</td>
<td>Denaturing-High Performance</td>
<td>++</td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>Liquid Chromatography (D-HPLC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conventional Sanger sequencing</td>
<td>++++</td>
<td>Very high</td>
<td>Very fast</td>
</tr>
<tr>
<td></td>
<td>(automated with fluorescent detection),</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyrosequencing</td>
<td>+++</td>
<td>Very high</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Next Generation sequencing (NGS)</td>
<td>++++</td>
<td>Moderate</td>
<td></td>
</tr>
</tbody>
</table>

$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).
Table II. Panel test about inflammation and genetic profile.

<table>
<thead>
<tr>
<th>Genes</th>
<th>rs SNP code</th>
<th>Nucleotide (Codon)</th>
<th>*MAF</th>
<th>Clinical annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1a</td>
<td>17561</td>
<td>4845 C/A (A1145)</td>
<td>0.20 C</td>
<td>CC allele is correlated to low</td>
</tr>
<tr>
<td>IL1b</td>
<td>16944</td>
<td>-511 G/A</td>
<td>0.47 A</td>
<td>AA allele correlate with higher trombotic risk</td>
</tr>
<tr>
<td>IL 6</td>
<td>1800795</td>
<td>-174 V/G</td>
<td>0.19 C</td>
<td>CC allele show high cardiovascular risk</td>
</tr>
<tr>
<td>IL 10</td>
<td>1800872</td>
<td>-592T/G</td>
<td>0.41 T</td>
<td>GG Allele is protective</td>
</tr>
<tr>
<td></td>
<td>1800896</td>
<td>-1082 A/G</td>
<td>0.30 G</td>
<td>AA allele correlate with higher stroke risk</td>
</tr>
</tbody>
</table>

*MAF: Minor Allele Frequency.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References


7) **De Monaco A, D’Orta A, Fierro C, Di Paolo M, Calenti L, Di Francia R.** Rational selection of PCR-Based platforms for pharmacogenomics testing. WCRJ 2014; 1: e391


9) **Aquilante CL, Loehmeyer MT, Langabe TY, Johnson JA.** Comparison of cytochrome P450 2C9 genotyping methods and implications for the clinical laboratory. Pharmacotherapy 2004; 24: 720-726.


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