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Letter to the Editor

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 al[1]; the authors affirmed that the genetic screening IL1a 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 detection[1].

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) patients[2].

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 patients[3].

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 patients[4].

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, glycemic control, vascular supply, smoking, nutrition, deformity, and genetic predisposition to chronic inflammation[4].

Several studies examined the application of autologous PRP as additional treatment of foot ulcers in diabetes patients[5].

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 systems[6].

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).

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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[7,8].

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 RFLP9. 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 SNP10.

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[11-12].

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[13].

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.

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

Table II. Panel test about inflammation and genetic profile.

Genes[#]	rs SNP code	Nucleotide (Codon)	*MAF	Clinical annotation
IL1a	17561	4845 C/A (A114S)	0.20 C	CC allele is correlated to low
IL1b	16944	-511 G/A	0.47 A	AA allele correlate with higher trombotic risk
IL 6	1800795	-174 C/G	0.19 C	CC allele show high cardiovascular risk
IL 10	1800872	-592T/G	0.41 T	GG Allele is protective
	1800896	-1082 A/G	0.30 G	AA allele correlate with higher stroke risk

*MAF: Minor Allele Frequence.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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