Review of methods for TPMT biomarker genotyping in the personalisation of thiopurine therapy

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INTRODUCTION

Thiopurines, such as azathioprine, are used to treat leukaemias, Crohn's disease, rheumatoid arthritis, and as immunosuppressants after organ transplantation. Thiopurines are metabolised by thiopurine S-methyltransferase (TPMT) and impaired TPMT enzyme activity results in accumulation of thiopurines and toxic effects on bone marrow. TPMT-deficient patients are poor metabolisers and can be treated successfully and safely with reduced azathioprine doses if TPMT metaboliser status is identified by pharmacogenetic testing.[1,2] Azathioprine has a 'Testing recommended' drug label annotation by the United States Food and Drug Administration.[3]

AIM

To review and compare genotyping methods for the identification of the TPMT pharmacogenetic biomarker in relation to azathioprine therapy

METHOD

- **Twenty-three research articles in the English language published between 1997 and 2017 describing TPMT biomarker genotyping methods/assays in relation to azathioprine therapy were identified using PubMed, HyDi, ResearchGate and Google scholar and were reviewed.**
- **Product information of the genotyping assays was reviewed and company representatives were contacted when further information was required.**
- **Ten method characteristics were identified for comparative analysis of each genotyping method/assay (Table 1).**

RESULTS

- **Twelve genotyping methods were identified; 10 Molecular and 2 Biochemical-Analytical (Table 2).**
- **All 12 methods have high sensitivity and specificity (≥90%).**
- **Concordance with DNA sequencing (reference method) was 100% for all methods.**
- **The most common genomic DNA source is blood, however pyrosequencing allows use of biopsy samples and soft tissue samples.**
- **ARMS-PCR, Real-time PCR and the Strip assay have integrated controls in the test kits used.**
- **All methods except High Resolution Mutation Analysis have high reproducibility and all methods are reported to have high resolution and robustness.**
- **Time for analysis ranges from 0.33 to 9 hours and estimated cost per test ranges from €3 to €90 (Table 2).**

CONCLUSION

This review demonstrates that for the methods considered, sensitivity, specificity, concordance, reproducibility, resolution, and robustness are high, and time and cost are variable. The possibility for non-invasive testing and presence of integrated controls are limited. The need for development of rapid, point-of-care genotyping methods necessary for timely implementation of pharmacogenetic testing in practice to improve patient safety is recognised.

Table 1. Characteristics evaluated for genotyping methods/assays

Sensitivity	Specificity
Genomic DNA source	Presence of Integrated Controls
Reproducibility	Resolution
Concordance	Robustness
Estimated cost per test	Time for genotyping

Table 2. Comparison of time for analysis and cost of TPMT genotyping methods reviewed

Test method		Time for analysis (Hours)	Estimated cost/test (€)
Molecular	High Resolution Mutation Analysis	2	Not reported
	Pyrosequencing	3.5	9
	Reverse Hybridisation Probe	1	15
	DNA Microchip Technology	3	90
	Single-strand Conformation Polymorphism	9	Not reported
	Southern Blot Hybridisation	4	
	Amplification Refractory Mutation System (ARMS)-PCR	4	3
	Restriction Fragment Length Polymorphism-PCR	3	3.5
	Real-Time PCR	1.17	45
	Strip Assay	6	Not reported
Biochemical-Analytical	Denaturing High Performance Liquid Chromatography	0.33	
	Mass Spectrometry	1	

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