Background

• Pharmacogenetic information is accumulating rapidly and is beginning to show consistent reproducible results for an increasing number of genetic markers for drug response.

• Several consortia have published guidelines to aid physicians and pharmacists with the interpretation and clinical translation of pharmacogenetic test results.

• An increasing number of medical centers have acquired clinical genotyping facilities.

• Among the first medical centers to implement pharmacogenetics there are many highly specialized care centers with complex patient populations.

• These patients may present some unexpected challenges as is exemplified by the following case description.

Case

• Female patient, 20 years old.

• Admitted to the LUMC for living related kidney transplantation.

• Adequate tacrolimus exposure early after transplantation is essential.

• Prior to kidney transplantation, patient is preemptively genotyped for CYP3A5*3 (rs776746) and CYP3A5*6 (rs10264272).

• Genotyping: in duplicate by two independent techniques, to minimize the risk of potential errors.

• Genotyping results from Pyrosequencing and Taqman were conflicting.

• Pyrosequencing \(\rightarrow\) CYP3A5*1/*3

• TaqMan \(\rightarrow\) CYP3A5*3/*3

• Further research: second blood sample and consulting the nephrologist

Results Case

• 1992: 1st allogeneic stem cell transplantation to treat beta-thalassemia major. Transplant rejected.

• 2009: 2nd allo-SCT from a second donor. Result is a mixed hematopoietic chimera (28% autologous, 72% donor).

• Saliva samples from patient and donor were collected and genotyped for the CYP3A5*3 and CYP3A5*6.

• The donor was autocalled CYP3A5*3/*3.

• The patient was autocalled CYP3A5*1/*3.

• This genotype is in line with the relatively low tacrolimus trough level (5.5 \(\mu\)g/L).

• AUC levels of 110 \(\mu\)g*hours/L were achieved with a tacrolimus dose of 8 mg twice daily.

Discussion

• This case illustrates
  - The challenging aspects of pharmacogenetic testing
  - The importance of proper quality control mechanisms
  - Selection of the proper source of DNA to determine the genotype in Tx patients
  - To determine germline DNA in SCT patients: buccal swabs should be used.

  - Pharmacogenetic testing in solid organ transplantation recipients should be handled with great care.

  - Taking the transplant type, the metabolic pathway, mechanism of action and toxicity of the applied drugs into consideration.

Tacrolimus and kidney transplantation

• Patients receive standard quadruple immunosuppressive regime: basiliximab, tacrolimus, mycophenolate, prednisolone.

• Adequate tacrolimus exposure early after transplantation is essential.

• Tacrolimus is metabolized into active and inactive metabolites by CYP3A4 and CYP3A5.

• Patients carrying at least one copy of the CYP3A5*1 allele require a significantly increased tacrolimus dose to attain therapeutic blood concentrations.

- All patients undergoing a kidney transplantation in the LUMC from 2009 onward are preemptively genotyped for the CYP3A5*3 (rs776746) and CYP3A5*6 (rs10264272) polymorphisms

References

5. Thack, C., Prange-Koos, G., Freiberg-Richter, J., Bornhäuser, M. & Ehninger, G. Buccal swabs but not mouthwash samples can be used to obtain pretransplant DNA fingerprints from recipients of allogeneic bone marrow transplants 775-777

Figure 1. Genotyping results of different samples based on pyrosequencing (A-D) and the TaqMan assay (E-G).

1A-D Pyrosequencing results.

Results for patient blood sample (A), for plasmid CYP3A5*1/*3 control (B), saliva sample of patient (C) and saliva sample of donor (D). A-peak indicating presence of the CYP3A5*1 allele, G-peak indicating presence of the CYP3A5*3 allele. Pyrosequencing results from patients' blood sample (A) showed inconsistencies in peak proportion between the A and the G peak compared to the results obtained with plasmid control (B). Saliva sample from the patient is auto-called CYP3A5*1/*3 (C) and the sample of the donor is auto-called CYP3A5*3/*3 (D).

1E-G TaqMan results.

Blue dots indicate samples called as CYP3A5*3/*3; Green dots indicate samples called as CYP3A5*1/*3; Red dots indicate samples called as CYP3A5*1/*1. Encircled dots are results obtained with DNA from the patient (triplo). Squared dots are results obtained with DNA from the donor. Conflicting results obtained with the first (E) and second (F) blood sample. Saliva sample from the patient is called CYP3A5*3/*3 and the sample of the donor is auto-called CYP3A5*3/*3 (G).