Tamoxifen pharmacogenetics and pharmacokinetics in early breast cancer

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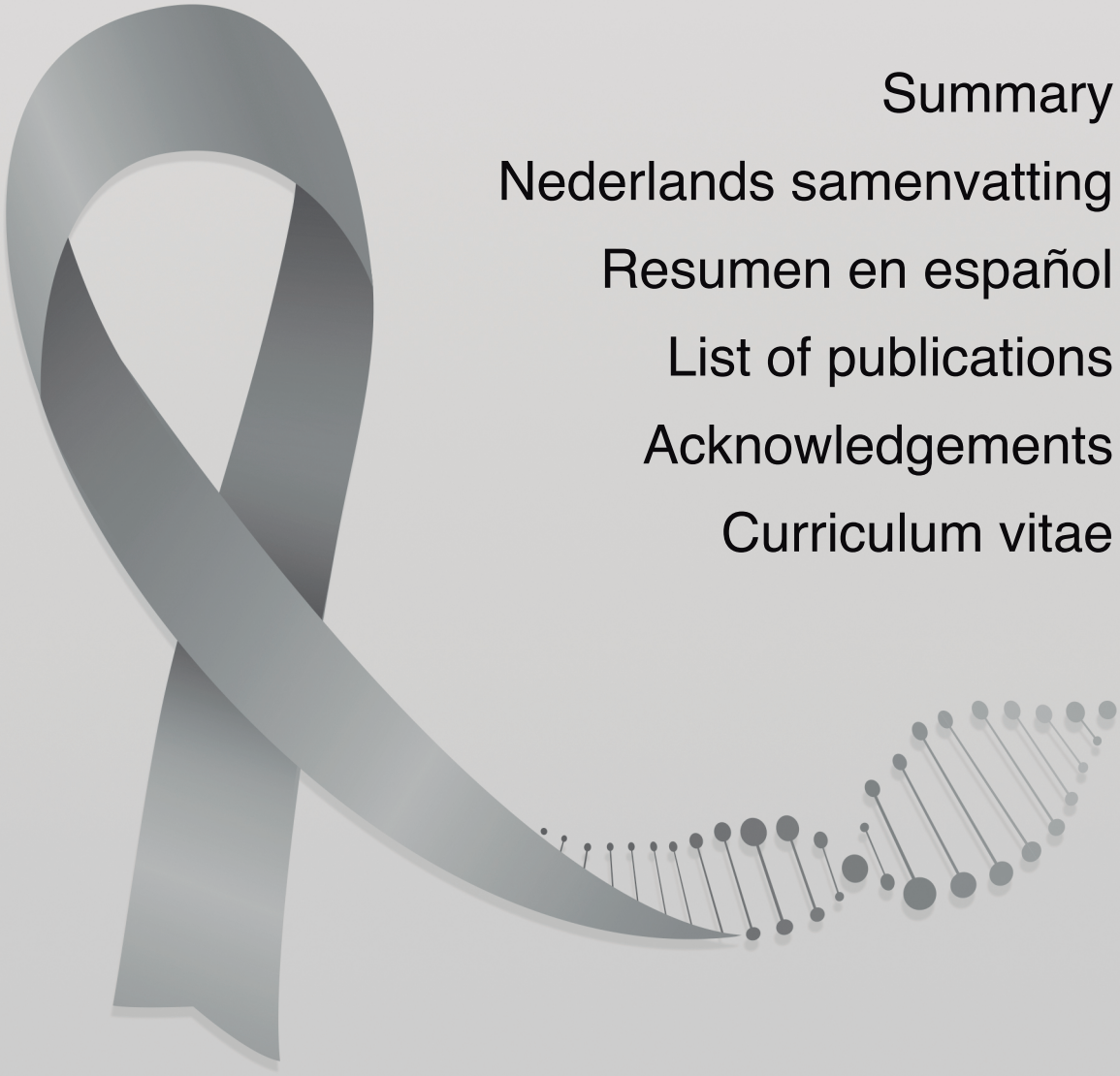
11 CHAPTER



Summary

Nederlands samenvatting

Resumen en español

List of publications

Acknowledgements

Curriculum vitae

Summary

For more than 40 years, the selective estrogen receptor modulator tamoxifen has been the cornerstone of the endocrine therapy for hormone receptor-positive breast cancer patients. However, a wide variability in response to therapy is still observed since disease recurrence happens in nearly 30 % of breast cancer patients. Tamoxifen has a complex metabolism and it is mainly metabolized by CYP2D6 enzyme, among others, into endoxifen, the most active metabolite of tamoxifen. CYP2D6 enzyme is encoded by the highly polymorphic *CYP2D6* gene. To date, there are many variants that encode for fully functional or non-functional alleles, or variations with decreased enzymatic activity. At the same time, individuals can be classified in different *CYP2D6* phenotypes according to the combinations of these variants. Commonly, patients can be categorized in four *CYP2D6* phenotypes: ultrarapid metabolizer (UM), normal metabolizer (NM; which previously was known as extensive metabolized (EM)), intermediate metabolizer (IM) and poor metabolizer (PM).

In the search of a manner in order to individualize endocrine therapy with tamoxifen, *CYP2D6* genotyping was proposed as a potential tool. In fact, the association between clinical survival outcomes and the role of *CYP2D6* polymorphisms in breast cancer patients receiving tamoxifen has been an ongoing discussion and many studies have been published claiming both negative and positive associations. In theory, *CYP2D6* PM and IM patients reach lower endoxifen concentrations due to the decreased or nearly absent CYP2D6 enzymatic activity. Consequently, these individuals are supposed to have a higher probability of breast cancer relapse due to the lower anti-estrogenic exposure (e.g. lower endoxifen concentrations) compared to NM (formerly EM).

This thesis focusses on assessing the influence of diverse genetic variations involved in tamoxifen metabolism, defined as concentrations and metabolic ratios (MRs), and their impact on clinical survival outcome in early-breast cancer patients treated with tamoxifen. Additionally, we also investigated the effect of relevant polymorphisms tamoxifen metabolic pathway on the explained variability between patients.

Tamoxifen metabolism and efficacy: beyond *CYP2D6* genotyping

Tamoxifen is a drug with a complex metabolic pathway in which many enzymes are implicated. *CYP2D6* genotyping has been proposed as a manner to guide tamoxifen efficacy. However, the main limitation of this predictor is the fact that *CYP2D6* only partially explains the observed inter-variability of tamoxifen metabolism. Consequently, other factors that might influence tamoxifen metabolism are also relevant in order to improve of knowledge about tamoxifen inter-variability. In **chapter 2**, a review summarizing the genetic and non-genetic factors that affect tamoxifen metabolismis presented.

In another attempt to learn more about the inter-variability of tamoxifen, we examined in **chapter 3** the association between rs5758550 enhancer with *CYP2D6*2* allele. Generally, *CYP2D6*2* is considered a fully functional variant, but it only might occur due to the presence of rs5758550. To investigate this hypothesis, we "reclassified" *CYP2D6*2* individuals from a large cohort of breast cancer patients who received tamoxifen in a *CYP2D6* predicted phenotype with a decreased activity, according to the presence or absence of the rs5758550 enhancer. However, the explained inter-variability after this "reclassification" did not improve (e.g. endoxifen concentrations R^2: 0.43 *versus* 0.413), whereas no differences in mean concentration levels of tamoxifen and its metabolites (p-value>0.05) were found when comparing *CYP2D6*2* patients with and without the rs5758550 SNP. These results indicate that the rs5758550 enhancer does not lead to improved prediction of the concentrations or explained inter-patient variability of tamoxifen and its metabolites concentrations.

Chapter 4 describes the impact of *CYP3A4*22* and *CYP3A5*3* and their combined effect on tamoxifen and metabolites concentrations and MRs. While *CYP3A4*22* leads to a diminished enzymatic activity, *CYP3A5*3* is described as non-functional allele. Interestingly, *CYP3A4*22* carriers reached significant higher concentrations of tamoxifen, NDM-tamoxifen and 4-hydroxy-tamoxifen, whereas a tendency towards increased endoxifen levels was observed (p-value: 0.088). In contrast, the non-functional *CYP3A5*3* allele marginally contributed to improving the inter-patient variability of tamoxifen and its metabolites concentrations and MRs. Our outcomes indicate that *CYP3A4*22* and *CYP3A5*3* have only a small effect on tamoxifen metabolism.

In **chapter 5**, we analysed for the first time the role of rs6839 and rs1042157 of 3'- untranslated region of *SULT1A1* on tamoxifen metabolism and relapse-free survival (RFSt). To this end, patients were categorized according to the combined known effect of rs6839 and rs1042157 on SULT1A1 enzymatic activity. These groups were defined as low, medium and high SULT1A1 enzyme activity. Interestingly, statistical significant differences were observed when comparing mean concentration levels and MRs of tamoxifen and its metabolites between all the groups (high, intermediate and low activity). In this study, the low activity group had a borderline improved survival outcome (HR: 0.297; 95 % CI: 0.088-1.000; p-value: 0.05). These results applied to the 3'- untranslated region of *SULT1A1*, and to the best of our knowledge, no other studies showed this association or even analysed these genetic variants and clinical outcome. **Chapter 6** explores the effect of *CYP2C19*2* and *CYP2C19*17* genotypes on tamoxifen metabolism and clinical outcome. In this case, no differences on mean concentrations or MRs of tamoxifen and its metabolites were found, when comparing both genotypes alone. Also, we did not find any statistical differences in terms of RFSt. These results suggest that both genotypes might play a small role on tamoxifen metabolism, but their impact might not be clinically relevant.

In **chapter 7**, we evaluated the role of therapeutic drug monitoring based on endoxifen concentration levels by using threshold values from the literature (5.9 ng/ml, 5.2 ng/ml and 3.3 ng/ml). Additionally, we performed a logistic regression in order to estimate the probability of recurrence and contrasted it with endoxifen concentrations. In this study, no statistical significant effect was observed (Odds ratio (OR): 0.971 (95 % CI: 0.923-1.021, p-value: 0.248). Accordingly, these findings might indicate, therefore, a limited value of therapeutic drug monitoring based on endoxifen concentration levels by using these reported threshold values to guide tamoxifen efficacy.

In **chapter 8**, outcomes from the prospective CYPTAM study are presented. A total of 667 early-breast cancer patients from Belgium and The Netherlands treated with adjuvant tamoxifen were recruited. The main outcome in this study showed no associations between *CYP2D6* genotype (UMs/EMs *versus* hetEM/IMs/PMs) and RFSt. At the same time, we did not find any type of association between endoxifen concentrations and RFSt.

In **chapter 9,** we used another approach in order to identify new genetic polymorphisms associated with endoxifen concentrations and RFSt. To this end, we performed a genome-wide association study (GWAS) for which 608 Dutch patients from the CYPTAM study were analysed. Interestingly, the majority of the genetic polymorphisms were located on *TCF20* and *WBP2NL* genes. In this GWAS, we found two variants that were significantly associated with poorer clinical outcome (RFSt), of which rs77693286 and rs6790761 were situated on *LPP* gene (HR: 18.3).

Tamoxifen: the future of a contradiction in pharmacogenetics

Tamoxifen has been prescribed for many decades in breast cancer patients. Despite of its accidental discovery, tamoxifen is a singular example of a successful drug development. However, a decisive manner to personalize tamoxifen therapy still lacks. While *CYP2D6* genotyping or endoxifen concentrations have been proposed as ways to individualize tamoxifen treatment, the current evidence suggest that both predictors might not be the expected answer. In any case, tamoxifen has proved to be an excellent drug and additional research should be performed in order to continue the search for other potential predictors in order to anticipate tamoxifen efficacy.