SUMMARY/SAMENVATTING

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Hemoglobinopathies (HBP) are the most common autosomal recessive genetic disorder world-wide and in particular in the African continent, India, Far East, the Mediterranean and Middle Eastern countries including Oman. HBP can be the consequence of mutations causing structural abnormality in the hemoglobin molecule (abnormal hemoglobins) or a reduction/ abolishment in the overall synthesis of the hemoglobin components (thalassemia), leading to anemia. Carriers are usually asymptomatic but carrier couples are at 25% risk of getting a severely affected child.

One of the main challenges faced by most Arab countries and by Oman in particular are the high prevalence rates of HBP [mainly sickle cell disease (SCD) and beta thalassemia major (TM)]. The prevalence is high in these areas due to the positive selection in the presence of malaria tropica. Moreover, due to consanguinity, a socio-cultural habit, the incidence of severely affected children is high in Oman. Around 10 % of Omanis are SCD carriers, 3 % carry a defect causing β-thalassemia and at least half of the population are carriers of α-thalassemia. Although the disease could be treated, there is no definite cure until now except for a matched bone marrow transplant. Public health authorities have focused not only on state of the art management and patient care but also on prevention. National premarital clinics aiming at identifying partners at risk prior reproduction and offering genetic counselling and prevention have been working in the country for the last decade. The focus of this thesis is to study the molecular spectrum of HBP and the associated genetic determinants to work towards the development of prevention strategies for severe HBP's in Oman.

In order to develop and improve risk assessment during pre-matrimonial counselling we have defined the molecular spectrum of the disease all around the country. In Chapter 4, we present a first study on 87 un-related Omanies either heterozygous or homozygous for beta thalassemia mainly coming from four different regions in Oman. We found 11 beta determinants with at least 56% of the cases with heterozygosity or homozygositiy for the common alpha-thalassemia deletions; alpha 3.7kb and/or alpha 4.2kb. We have further extended our analysis to reveal a broader spectrum of beta globin gene mutations in Chapter 5, studying larger number of subjects (n=446) of different tribal origin, covering all the seven regions in the country. Thirty-two different beta mutations were identified with 11 being described for the first time among the Omani population. We then analysed the alpha globin gene defects in details in Chapter 7. A total of (n=634) subjects were divided into seven groups based on their hematological readings and were analysed at the molecular level. Twenty-one different alpha defects were categorised of which 15 were described for the first time among Omanies with two defects presumed to be new. We further reported in Chapter 6 two new cases among the Omani. The first is an alpha variant found in a consanguineous couple that probably resulted in a severe fetal hemolytic anemia while the second is a novel β-globin gene promoter mutation associated with borderline/slightly elevated HbA_2, indicating a very mild de novo β^+ thalassemia mutation. The high heterogeneity of common, rare and novel beta- and alpha- globin genes defects observed among Omanies (Chapters 4,5,6 and 7), outlines the historic migration pattern and the mixed ethnicity among the population and emphasize the necessity of implementing DNA testing during pre-marital screening for accurate risk prediction and genetic counselling

especially when both beta and alpha defects coexist making genotype prediction more complex and herewith providing the state of the art for prenatal diagnosis in the future.

In Chapter 8, we looked at the interfering effect of factors such as delta gene defect or iron deficiency that may influence HbA_2 measurement in the Omanies and herewith the diagnosis of beta thalassemia trait. This is particularly important during beta-thalassemia carrier screening because coexisting delta thalassemia defect with a beta thalassemia carrier status can normalise HbA_2 level and preclude a correct diagnosis. For that, we investigated 33 cases with low HbA_2 levels. Ten different defective delta alleles of which two are reported for the first time in literature were categorised in 20 subjects. The characterization of the delta-gene mutation spectrum is bound to make premarital screening and genetic counseling more reliable in the Omani population screened for beta thalassemia.

Patients with sickle cell disease (SCD) may show a strong variability in morbidity and response to therapy from case to case, depending from the often complex genotypes and haplotypes. The classical associated determinants were investigated in Chapters 9,10 and 11. In Chapter 9, the haplotype/sub-haplotype and phenotype in SCD patients (n=125) was investigated. A total of 11 different haplotype combinations were identified with the Asian haplotype being the most common and associated with a milder clinical form. In Chapter 10, a cohort of (n=52) SCD patients treated with hydroxyurea (HU) were tested for their response to HU based on their XmnI polymorphism. Patients homozygous or heterozygous for Xmn I (T/T or T/C) showed better response and improvement in clinical phenotype than patients bearing the (C/C) genotype. In Chapter 11, we assessed if presence or absence of alpha thalassemia in the same 125 SCD patients with identical beta genotype and haplotypes can ameliorate disease severity. We found that alpha thalassemia improves the overall hematological conditions but amelioration of the general disease severity is only noticed when compared in cohorts of the same haplotype. We conclude from these correlative studies that neither the haplotype or sub-haplotype nor the XmnI polymorphism nor alpha-thalassemia alone appears to be fully associated with the variable clinical phenotypes in SCD and that presumably other external factors can play a role in the different expression of the disease. Nevertheless, identifying genetic determinants is necessary for prognostic purposes, accurate diagnosis and planning for the best-tailored treatment to the affected children.

In Chapter 12, we tested the application of Ion Torrent PGM as a diagnostic ultra high-thoughput sequencing method for beta globin gene during beta thalassemia screening. We scanned a total of 297 Omani cases using a barcoded uni-directional sequence methodology and reliably identified beta-thal mutations in hundreds of patients simultaneously. Our results show that ion torrent can replace Sanger sequencing in the future and is a powerful diagnostic method to detect HBP carriers and carriers of other common genetic disorders in a national screening setting and that these molecular methods may become more practical if financially affordable.

Pre-matrimonial counselling, although thorough, is the only formal option available in Oman to couples at risk. This leaves only two options for couples whenever a presumed genetic risk has been suspected: to change the choice of partner or to continue with the marriage and hope for a healthy child from each pregnancy. Therefore the most effective prevention

method for HBP disorders thus far seems to be also in Oman, is prenatal diagnosis followed by the option of pregnancy termination. In Chapter 13 we investigated the attitude of 35 Omani couples at risk towards prenatal diagnosis and medical abortion. Although the majority would have accepted prenatal diagnosis if the service was available in the country, pregnancy termination was greatly influenced by the Islamic view as interpreted in the country by the main religious Muftee. However, prenatal diagnosis may be eventually considered in Oman with improved public awareness and once the public health authorities have reached a sensible agreement with religious authorities, as has been the case in other Muslim countries.

In conclusion, while providing tools for a better care and a better insight on the management of these severe diseases in Oman, our results will hopefully facilitate the prevention of HBP in the country.