Summary

Fanconi anemia (FA) is a rare, hereditary disorder characterized by congenital abnormalities, bone marrow failure (BMF), endocrine abnormalities and a high risk of developing malignancies, mainly acute myeloid leukemia (AML) and head and neck squamous cell carcinoma (HNSCC). FA is caused by mutations in one of the 20 currently known FA genes. The proteins encoded by these genes act together in the FA pathway that organizes repair of DNA damage. FA patients have an increased risk of developing cancer, but are, at the same time, hypersensitive to chemotherapy and radiotherapy. Treatment possibilities for cancer are therefore limited. In the past 15 years improved diagnostics and innovations in stem cell transplantation (SCT) have dramatically extended the lifespan of FA patients. To date, more than 80% of the FA patients reach adulthood and require transition from pediatric to adult healthcare. The improved outcome in FA has consequences for the organization of healthcare for FA patients and necessitates improved approaches for diagnosis and adequate treatment of other FA related problems, such as the development of solid tumors and endocrine disorders.

In Chapter 2 we described the phenotype and molecular analysis of a family with FANCD2 mutations. A 9 year-old girl was diagnosed with FA after presenting with developmental delay, hearing problems, growth failure and microcephaly. Mutation analysis revealed biallelic mutations in the FANCD2 gene (c.458T>C and c.2715+1G>A). Three years earlier her younger brother developed T cell acute lymphoblastic leukemia (T-ALL) and died of SCT-related complications. The father, who carried the c.2715+1G>A mutation, developed testicular seminoma at the age of 36. The mother was diagnosed with an SLE-like connective tissue disease and carried the c.458T>C mutation. The brother was found to be a heterozygote carrier of the c.458T>C mutation. Heterozygosity was found in both diagnostic and remission T-ALL bone marrow samples. Sequencing of DNA of the testicular tumor tissue also showed presence of both FANCD2 alleles. Therefore, the T-ALL and testicular tumor seemed not to be caused by loss of the wild type allele. The FANCD2 mutation c.458T>C has previously been reported in an FA patient who developed T-ALL. SLE has not been described associated with FA or FA mutation carriers. However, sequence variants in other DNA repair disorders have been implicated in the pathogenesis of SLE. In conclusion, the clinical and molecular data of this family implies possible novel associations between heterozygous mutations in FANCD2, malignancies and autoimmune disease.
In Chapter 3 we described the epidemiology, genotype, phenotype and course of disease of a large cohort of Dutch FA patients. To date, 137 Dutch FA patients have been identified of whom 84 are currently alive (estimated prevalence 1:208,000, estimated birth prevalence 1:57,000). Mutation analysis, performed in 118 patients, confirmed that the FA-C subtype is most prevalent in the Netherlands (39%), followed by FA-A (34%). The Dutch founder mutation c.67delG in the FANCC gene was found homozygous in 32 patients and compound heterozygous in 14 patients. When focusing on the homozygous c.67delG patients, we noticed that these patients did not show major congenital abnormalities. 61% of this patient group developed progressive BMF and 48% received a SCT at a median age of 12.3 years. Long-term follow-up showed that 26% of the adult homozygous c.67delG patients developed a solid tumor, mainly SCC, at a median age of 36.0 years. Ten of 32 patients died at a median age of 26.1 years. Cause of death was SCC (n = 4), SCT-related (n = 3), BMF (n = 2) and unknown (n = 1). Cumulative survival was 54% at the age of 35 years.

In conclusion, the majority of Dutch patients belong to the FA-C subtype. Patients with homozygous c.67delG mutations in the FANCC gene have in general no major congenital abnormalities, suggesting a milder phenotype. However, they do develop life-threatening BMF and solid tumors.

In Chapter 4 we reported the characteristics and results of all SCTs that have been performed in FA patients in the Netherlands. Between 1972 and 2014, 68 patients were transplanted at a median age of 8.2 years. Fludarabine (FLU)-based conditioning was associated with increased engraftment. Haplo-identical SCT and ex vivo T cell depletion increased the risk of graft failure. FLU significantly decreased early mortality, whereas older age and acute graft-versus-host-disease (GVHD) were associated with increased early mortality. Late mortality was mainly caused by SCC in the 2nd and 3rd decade after SCT. A 5-year overall survival of 87.8% was found in patients treated with FLU compared to 59.3% in the non-FLU group. One-year overall survival in adult patients was 50%. Since 2007, 22 patients were treated with the current Dutch non-irradiation and busulfan-free conditioning regimen. Stable engraftment after first SCT was achieved in 19 patients. Acute GVHD grade was seen in 11% and none of the patients developed chronic GVHD. Five-year overall survival after matched related donor SCT (n=8) and alternative donor SCT (n=14) was 100% and 85.7%, respectively.

In conclusion, for Dutch FA patients who currently require SCT, the prospects have improved markedly. This improvement is associated with the introduction of FLU. Nevertheless, outcome in adult patients is still suboptimal. The current Dutch conditioning regimen can be used successfully in matched related and alternative donor SCT, but not in haplo-identical donor SCT.
In Chapter 5 we determined the prevalence of loss of heterozygosity (LOH) in brushed cells of the oral epithelium of FA patients and analyzed the association of LOH with clinical characteristics and HNSCC. Between May 2005 and November 2009, 141 non-transplanted FA patients were sampled. LOH was found in 14 of 141 (9.9%) FA patients at a median age of 25.5 years. Age was a significant predictor of LOH. Longitudinal sampling of FA patients showed that LOH persisted in all consecutive samples. Five patients developed HNSCC during the study at a median age of 39.6 years. Of interest, 4 of these 5 patients developed SCC of the gingiva. LOH was significantly associated with HNSCC. A technical limitation of the non-invasive LOH assay, which became apparent in this study, was the inability to use it in transplanted FA patients since presence of donor DNA interfered with LOH analysis. This donor DNA was most likely derived from donor leukocytes present in the oral cavity. In conclusion, noninvasive screening using a LOH assay on brushed samples of the oral epithelium has a promising outlook in patients with FA. However, assays need to be adapted in case of SCT, because of contaminating donor DNA.

In Chapter 6 we presented the results of a survey, conducted in collaboration with the Dutch FA patient organization, to evaluate healthcare for FA in the Netherlands. Since FA is a rare disorder, mainly attended to by specialized pediatricians and not well known in general pediatric and adult healthcare, we hypothesized that FA patients may not receive optimal care despite the availability of Dutch guidelines for diagnosis and management of FA. The survey showed that the majority of patients currently receive suboptimal care. In addition, the transition process from pediatric to adult healthcare proves to be difficult. From the patient’s perspective optimization of healthcare can be best achieved through expert multidisciplinary FA teams both for pediatric and adult patients.

Overall, the work presented in this thesis has led to a detailed description of the Dutch FA cohort, the insight that the Dutch non-irradiation and busulfan-free conditioning regimen can be used successfully in SCT for children with FA, but not in haplo-identical SCT, the potential of non-invasive genetic cytology but also the awareness that donor DNA after SCT influences non-invasive genetic assays, and the realization that care for FA, particularly the transition to adult healthcare, needs to be improved.