**Study Protocoll „Non-HFE Hemochromatosis Registry“**

1. **Background**

Hepatic iron overload is an important risk factor for the progression of chronic liver diseases from fibrosis to cirrhosis [7, 10] and the hallmark of liver disease in patients with hereditary hemochromatosis [1]. Genetic studies have shown that up to 85 % of patients with the clinical diagnosis ‘hemochromatosis’ are homozygous for the common C282Y polymorphism of the HFE gene [4]. Hence, the disease is now referred to as HFE-associated hemochromatosis [1]. Identification of the HFE gene has transformed clinical management for hemochromatosis, enabled earlier diagnosis and probably improved the disease course [3, 9].

However, in addition to HFE-associated hemochromatosis, other genetic conditions, which are clinically and pathologically reminiscent of classical HFE hemochromatosis, have been identified in patients homozygous or compound heterozygous for mutations in TFR2, HJV or HAMP. These disorders are referred to as non-HFE hemochromatosis [2].

Ferroportin disease is another disorder of iron metabolism caused by heterozygous mutations in the SLC40A1 gene [8], In contrast to HFE and non-HFE associated hemochromatosis variants which are typically associated with high transferrin saturation, transferrin saturation is variable and can be low in ferroportin disease. Invariably low transferrin saturation and severe hepatic iron overload is also typical in the autosomal recessive iron disorder aceruloplasminemia [6].

In comparison to HFE associated hemochromatosis, which has an estimated prevalence of 1:1000, non-HFE hemochromatosis, ferroportin disease and aceruloplasminemia are relatively rare, but are increasingly recognized to contribute to the overall burden of iron overload disorders, especially in Southern and Central Europe where the prevalence of HFE mutations is lower. Moreover, uncertainties about the true prevalence and disease management (in contrast to HFE-hemochromatosis) present a significant clinical challenge. The ultimate goal of this Registry is to improve patient care by comprehensively categorizing non-HFE liver iron overload disease across Europe, which will enable an evidence-based revision of clinical guidelines.

The number of patients with genetically confirmed non-HFE hemochromatosis, ferroportin disease or aceruloplasminemia is increasing, because novel sequencing strategies are increasingly used in the diagnostic workup of patients with confirmed or suspected hepatic iron overload. Further, clinical application of full exome sequencing has further increases the number of aberrations found incidentally in genes associated with non-HFE hemochromatosis [5]. Interpretation of such test results and identification of pathogenic mutations can be guided by structured collection and documentation of cases in a registry. Such a registry will help to establish disease penetrance, expressivity of certain mutations as well as clinical presentation and prognosis of different non-HFE variants. Most importantly, it will be the basis of a collaborative effort to elucidate the genetic architecture of these complex diseases.

The aim of the proposed registry is to collect structured information on the clinical presentation, biochemistry, radiology, family history, genetics and histology of patients with non-HFE hemochromatosis, ferroportin disease and aceruloplasminemia. Collecting follow-up information on these patients will help to understand the natural course of each disease variant and determine the effect of specific interventions, and ultimately facilitate more informed clinical guidelines.

**2. Aims**

1. What is the clinical presentation of non-HFE associated hepatic iron overload diseases (non HFE hemochromatosis, ferroportin disease and acoeruloplasminemia)?

2. What is the risk of developing hepatic and non-hepatic complications (heart disease, arthropathy, diabetes, hypogonadism etc.) in non-HFE associated hepatic iron overload diseases over time?

3. Which treatment strategies are used in non HFE associated hepatic iron overload diseases and does treatment affect disease course?

4. What is genetic architecture of non HFE associated hepatic iron overload diseases (i.e. what is the clinical significance of specific mutations)?

5. When is genetic testing beyond HFE genotyping recommended and what are the implications of test results for disease management?

6. How do initial staging, follow up and treatment in patients with HFE and non HFE associated hemochromatosis differ?

Each of these key questions can be directly addressed by the registry. The outcomes will have direct implications for clinical management in terms of diagnostic algorithms for patients with hepatic iron overload, follow-up and treatment.

**3. Collaborating Entities**

Heinz Zoller (coauthor of EASL’s clinical practice guidelines on hemochromatosis) and Hal Drakesmith (secretary of the European Iron Club) have assembled a consortium of 29 collaborators from 17 different institutions including national reference laboratories for iron disorders from 11 different European countries. Each of the participants has a track record in research on hemochromatosis. In addition to the consortium members the registry will be open to other institutions and data entry will be promoted by the European Iron Club, which is the main hub for European iron research with a history going back >40 years. The EIC will host sessions at each annual congress to discuss and disseminate the Registry and educate EIC membership as to its existence, function and goals, in order to maximize inclusivity and case reporting.

The registry will be set up by Florian Kronenberg and his team for the Department of Genetic Epidemiology at the Medical University of Innsbruck, who is experienced in setting up and conducting registry studies and has an according track record. A collection of high-quality data requires a standardized procedure. We will therefore apply electronic case report forms implemented in "Askimed" (<http://www.askimed.com>).

**4. Length of project in months**

The proposed duration of the project is 60 months. Initial setup of the database is expected to be completed within 6 months. Data entry is expected to start in October 2016.

The retrospective study arm will be closed 24 months after database launch. Formal analysis of the retrospective data will be completed in month 30 and presented to the funding body. Retrospective data analysis will also be submitted for peer review and publication.

Prospective follow up will be completed in month 54 to leave 6 months for data curation, analysis and scientific publication.

**5. Methods**

(i) Retrospective assessment of patients with genetically confirmed non-HFE hemochromatosis, ferroportin disease or aceruloplasminemia to determine prognosis, treatment strategies and complications.

(ii) Prospective inclusion of patients with newly diagnosed non-HFE hemochromatosis, ferroportin disease or aceruloplasminemia to determine the incidence of non-HFE hemochromatosis and improve our understanding on the prognosis.

Retrospective assessment aims at determining disease prognosis and collect information on currently used treatment strategies for the different entities. The prospective study design aims at understand the epidemiology of these conditions and at collecting prospective data on disease prognosis and compilcations.

The collection of high-quality data requires a standardized procedure. We will therefore apply electronic case report forms implemented in "Askimed" (http://www.askimed.com). Askimed is an intuitive and self-explaining researcher-supporting software solution that allows a standardized data collection with extensive checking of plausibility as part of the quality control management. It is already in use in the German Chronic Kidney Disease Study (http://www.gckd.de/), the FP7-funded ncRNAPain project (http://www.ncrna-pain.eu/) and in a large study including patients with macular degeneration (AugUR study). All data are securely transferred to a centralized MySQL database. The Medical University of Innsbruck will provide this centralized database for storage of data considering all security issues of importance for such a project. This includes that all interactions with the server and the database are encrypted. Furthermore, Askimed is compliant to the 21 CRF Part 11 regulations (FDA guidelines on electronic records and electronic signatures). The questionnaires will be programmed by the Innsbruck team, a database will be provided and - if requested - the questionnaires can be prepared in the most widely-used languages. The consortium will be owner of the data and access will be allowed according to the rules of the consortium.

**6. Data protection and data aquisition**

Refer to appendix.

**7.** Case report form

Refer to Appendix

**8. References**

[1] EASL clinical practice guidelines for HFE hemochromatosis. Journal of hepatology 2010;53(1): 3-22.

[2] Bardou-Jacquet E, Ben Ali Z, Beaumont-Epinette MP, Loreal O, Jouanolle AM, Brissot P. Non-HFE hemochromatosis: pathophysiological and diagnostic aspects. Clin Res Hepatol Gastroenterol 2014;38(2): 143-154.

[3] Bardou-Jacquet E, Morcet J, Manet G, Laine F, Perrin M, Jouanolle A, et al. Decreased cardiovascular and extrahepatic cancer-related mortality in treated patients with mild HFE hemochromatosis. Journal of hepatology 2014.

[4] Feder JN. The hereditary hemochromatosis gene (HFE): a MHC class I-like gene that functions in the regulation of iron homeostasis. Immunol Res 1999;20(2): 175-185.

[5] Goddard KA, Whitlock EP, Berg JS, Williams MS, Webber EM, Webster JA, et al. Description and pilot results from a novel method for evaluating return of incidental findings from next-generation sequencing technologies. Genetics in medicine : official journal of the American College of Medical Genetics 2013;15(9): 721-728.

[6] Harris ZL. Aceruloplasminemia. J Neurol Sci 2003;207(1-2): 108-109.

[7] Lambrecht RW, Sterling RK, Naishadham D, Stoddard AM, Rogers T, Morishima C, et al. Iron levels in hepatocytes and portal tract cells predict progression and outcomes of patients with advanced chronic hepatitis C. Gastroenterology 2011;140(5): 1490-1500 e1493.

[8] Mayr R, Janecke AR, Schranz M, Griffiths W, Vogel W, Pietrangelo A, et al. Ferroportin Disease: A systematic meta-analysis of clinical and molecular findings. J Hepatol 2010;in press.

[9] Niederau C, Fischer R, Purschel A, Stremmel W, Haussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology 1996;110(4): 1107-1119.

[10] O'Brien J, Powell LW. Non-alcoholic fatty liver disease: is iron relevant? Hepatol Int 2011.

**9. Signatures**

Klinikvorstand Prinicipal Investigator:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Date, Place, Signature Date, Place, Signature