Heterogenomics in autoimmunity towards personalized medicine

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Chapter 5. Summary and Discussion

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A genomic view of subtypes in rheumatoid arthritis: Towards personalized medicine
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The development of high-throughput techniques such as microarray analysis holds great promise for the unraveling of complex multifactorial diseases. After a decade of technical and analytical improvements, the technology and algorithms for data analysis have been shown to be robust and reproducible across properly designed and controlled experiments, and across different research groups. Encouraging results have been generated using microarray technology for the identification of predictors for disease outcome and metastasis and underlying pathways in breast cancer and lymphoma (1;2).

The results described in this thesis are focused on the autoimmune diseases RA and MS and highlight the importance of molecular redefinition of these diseases based on differential gene expression profiles, which provides a framework for further detailed investigation.

5.1 Genomics for early diagnosis of disease

5.1.1 Rheumatoid Arthritis (RA)

RA is one of the most common chronic inflammatory diseases for which no cure is available. Because of the destructive nature of the disease it would be highly desirable to start treatment as early as possible. Recognition of the preclinical phase allows a timely start of treatment with the ultimate goal of primary prevention. Several reports have described the appearance of autoantibodies against citrullinated proteins and RF years before the clinical symptoms of arthritis (3;4). In addition, a recent study showed that arthritis development in prospectively followed arthralgia patients is dependent on ACPA status and that autoantibodies can be present years without causing disease (5). Thus, autoantibodies may represent changes in the immune system that induce early pathogenesis. However, since not all autoantibody positive individuals are likely to develop arthritis we hypothesized that additional factors/processes are required or some individuals may have a protective immune response profile. To test this hypothesis we analyzed the peripheral blood gene expression profiles of autoantibody positive
arthralgia patients without arthritis. In chapter 2 we identified four different molecular subgroups of autoantibody positive arthralgia patients associated with future development of arthritis. Interestingly, one subgroup of patients was associated with protection against arthritis development and characterized by an increased expression of genes involved in B-cell activity. The other subgroups were associated with development of arthritis and displayed increased expression levels of genes involved in IFN-mediated immunity, hematopoiesis and cytokine/chemokine mediated signaling. These results provide a framework for the discovery of biomarkers to predict the development of arthritis in autoantibody positive individuals at risk for RA.

The increased IFN response activity might have its origin in a disturbed response to an environmental trigger and/or an abnormality in genes involved in type I IFN biology. Subsequently, the increased expression of genes involved in IFN-mediated immunity may affect the overall reactivity of the immune system and thus can predispose the individual to autoimmunity. This altered immunoreactivity is directed to particular antigens, i.e. citrullinated antigens, which affect B- and T-cell recognition of epitopes and autoantibody production. Genes involved in cytokine/chemokine mediated signalling may act on the progression of autoimmunity by modulating the immune response and altering cell trafficking to target tissues. Accordingly, Hueber and colleagues reported an increase in the levels of monocyte chemoattractant protein-1 (MCP-1) in ACPA positive patients before disease onset (6). Knowing that immune cell trafficking is a crucial process in the initiation of disease, it is tempting to speculate that the increased expression of B cell genes in individuals who did not develop arthritis is a reflection of a blockade in the migration of B cells to the target tissue.

On the basis of our data, we propose that the activity of individuals’ genes affect susceptibility to RA at three levels. First, some genes affect the overall reactivity of the immune system and thus can predispose the individual to autoimmunity. Second, this altered
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immunoreactivity is directed to particular antigens and third, still other genes act on the progression of autoimmunity to target tissues to modulate immune attack. Our results imply that, among others IFN-mediated immunity and cell trafficking specify the processes relevant to progression to arthritis besides autoantibody positivity.

Additional specific processes that contribute to progression towards RA might involve epitope spreading. Mouse models of autoimmune arthritis and demyelination have shown the occurrence of epitope spreading to citrullinated antigens (7). In line with these results, a recent study showed a limited ACPA isotype usage in healthy relatives compared to RA patients. The authors speculate that epitope spreading and expansion of the isotype repertoire might be necessary for development of RA. It is tempting to speculate that the pathogenicity of the autoantibodies is related to the immune profile of the autoantibody positive individual. It will be interesting to determine if there is a relation between the different gene expression profiles in autoantibody positive arthralgia patients that we described and the fine specificity and isotype usage of their autoantibodies.

Perhaps our results in autoantibody positive arthralgia patients can be extrapolated to other diseases wherein autoantibodies are involved. In that case the production of autoantibodies in individuals who have a skewed immune response profile may lead to epitope spreading, immune activation, cell trafficking and development of disease. Therefore the combined analysis of different autoantibodies together with gene expression profiles may be predictive for future development of different autoimmune diseases. Future studies are needed to test this hypothesis.

5.1.2 Multiple Sclerosis (MS)

The prevalence of MS is much lower than RA and diagnosis of disease is generally at a younger age, which makes large studies using blood donors like those performed in RA more
difficult. Unfortunately, preclinical markers like RF and ACPA have not yet been identified in MS patients. However, a recent nested case-control study of US military personnel (63% male) who have serum samples stored in the Department of Defense Serum Repository showed that the presence of anti-MOG (myelin oligodendrocyte glycoprotein) IgM-/IgG+ antibodies measured by ELISA was associated with a moderately increased risk of developing MS (8). Interestingly, since this association was lost after adjustment for antibody titers to Epstein-Barr nuclear antigens (EBNA), the association may in part reflect cross-reactivity between MOG and EBNA.

5.2 Genomics for patient subclassification

MS and RA are both complex chronic inflammatory diseases with unknown etiology and a multifactorial background. Gene expression profiling of peripheral blood cells of RA and MS patients revealed a remarkable variation in gene expression levels leading to subclassification of patients depending on their molecular signature.

5.2.1 Heterogenomics in RA

A large-scale gene expression profiling study of synovial tissues from patients with erosive RA revealed considerable heterogeneity among different patients (9;10). It has been demonstrated that different arthroscopic biopsies taken from one joint result in gene expression signatures that are more similar within the joint of one patient than between patients (11). A systematic characterization of the differentially expressed genes highlighted the existence of at least two molecular distinct forms of RA tissues. One group revealed abundant expression of clusters of genes indicative of an ongoing inflammation and involvement of the adaptive immune response. This subgroup is referred to as the RA high inflammation group. Further analyses of the genes involved in the high inflammation tissues
provide evidence for a prominent role of genes indicative for an activated STAT-1 pathway. These findings were confirmed at the protein level (12;13)

Tsubaki and colleagues demonstrated that tissue heterogeneity within RA can already be observed in the early phase of RA (14). In this study, gene expression profiles were analyzed from synovial lining tissues from 12 patients with early RA (duration of less than 1 year after diagnosis) and 4 with long-standing RA (duration of more than 3 year after diagnosis). As seen in the previous study using biopsies from long-standing RA patients, the early RA patients could be divided in at least two different groups based on their gene expression profiles.

In chapter 3 we confirmed and further extended the molecular heterogeneity at tissue level using synovial tissue biopsies obtained during arthroscopic surgery. In addition, the subclassification of synovial tissues based on gene expression profiles matched the subclassification based on immunohistochemical data using protein markers for infiltrating cells and inflammatory cytokines. The high inflammatory tissues were characterized by a high expression of genes involved in many inflammation-mediated processes such as immunity and defense and T and B cell mediated immunity. In contrast, the low inflammatory tissues displayed increased expression of genes involved in amongst others developmental processes and neurogenesis. We demonstrated the clinical relevance of tissue subtyping. RA patients with a high inflammatory subtype had a higher disease activity score (DAS) together with a shorter disease duration compared to patients with a low inflammatory tissue type. Since data on normal healthy synovial tissue was not available we were not able to investigate how these biological activities relate to “normal” levels.

The existence of molecular heterogeneity in rheumatoid synovial tissues fits a model proposed by Firestein and Zvaifler (15), who suggested two independent phases that drive destruction of bone and cartilage; an antigen-driven immune cell mediated process and a
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subsequent antigen-independent stromal cell driven process. This model is extended by Kirwan who proposed the existence of two pathogenic mechanisms in which synovial inflammation will lead to joint pain, joint swelling and cartilage thinning, whereas synovial hyperplasia will lead to joint swelling and erosions (16). Accordingly, we showed that the high inflammatory tissue type is accompanied with a higher tender joint count. Since our patient groups also differ in disease duration, the observed tissue heterogeneity may also reflect two different phases of joint destructions; an acute antigen-dependent inflammatory cell type phase and a chronic stromal cell type driven phase. The transition from immune-mediated inflammation to a more autonomous, immune-independent process may occur several years after onset of clinical signs and symptoms. Longitudinal studies are needed to test if these pathogenic mechanisms are running in parallel or in time.

5.2.1.1 Heterogenomics of peripheral blood cells from RA patients

While gene expression analysis of synovial tissue samples of affected organs offers insights into the genes that are instrumental in patient stratification and primarily involved in disease activity and pathogenesis, this approach is not feasible in studies of large cohorts of patients. Due to the systemic nature of RA and the communication between the systemic and organ specific compartments, we and others have studied whole blood and/or peripheral blood mononuclear cells to obtain disease-related gene expression profiles. The peripheral blood is especially suitable to analyze gene expression profiles that can be used as biomarker to select patients for improved diagnosis as well as for patient centric therapy approach.

In chapter 3 we performed gene expression profiling analysis on whole blood cells, which revealed clear and significant differences between RA patients (n=35) and healthy individuals (n=15). The microarray data confirmed previous observations of increased expression of the calcium-binding protein S100A8 and S100A12. Application of gene set analysis algorithms to
identify gene sets that reflect a distinct pathway or biological process revealed an increased expression of immune defense genes, including type I IFN-response genes, suggesting that this pathway is activated systemically. Most interestingly, the increased expression of immune defense genes was characteristic of not all but approximately half of the patients (17). In addition, a combined cluster analysis of RA and control samples together with samples from a viral infection model revealed that the gene expression profile of a subgroup of RA patients was reminiscent to that of poxvirus-infected macaques (18). Based on these findings we conclude that activation of the immune response, with among the gene sets a type I IFN signature, defines a subgroup of RA patients. Similar findings were reported by Olsen and colleagues who studied differentially expressed genes between early (disease duration less than 2 years) and established RA (with an average disease duration of 10 years) (19). Genes involved in immune/inflammatory processes and genes related to cell proliferation and neoplasia were expressed at lower levels in early arthritis. About a quarter of the early arthritis genes overlapped with an influenza-induced gene set. This finding led the authors to suggest that the early arthritis signature may partly reflect the response to an unknown infectious agent. Together these results are supportive for differential expression of IFN response gene activity in RA, which could be the consequence of either an exogenous or endogenous stimulatory agent.

5.2.1.2 Linking tissue heterogeneity to peripheral blood profiles

In chapter 3.3 we explored the presence of blood based markers associated with tissue inflammation. Whereas the synovial tissues clearly demonstrated the presence of a high and low inflammatory tissue type, this difference was not found to be linked to the paired peripheral blood gene expression level. These results imply that the reflection of tissue inflammation in the peripheral blood cell compartment is not that obvious. We confirmed
other studies by showing that patients with a higher DAS and higher level of tissue inflammation display increased levels of CRP combined with a higher ESR and more thrombocytes. The lack of molecular peripheral blood cell activation markers measured at transcriptional level in combination with increased CRP, ESR and thrombocytes in the blood maybe partially explained by the fact that CRP is an acute phase protein produced in the liver, thrombocytes have negligible amounts of RNA and the finding that the frequency of white blood cells were not different between the two groups. In addition, we should keep in mind that we analyzed the gene expression profiles of only one affected joint which may explain the difficulties in finding correlating blood markers since inflammatory processes may be different between joints.

Using a pathway level analysis approach we found that the peripheral blood cells of patients with a high inflammatory tissue type displayed increased expression of genes involved in protein biosynthesis. Conversely, peripheral blood cells of patients with a low inflammatory tissue type showed an enriched expression of genes involved in developmental processes. Further studies are needed to elucidate the role of these biological processes in tissue inflammation. Figure 1 summarizes the heterogenomics of RA.
Figure 1. Discovery of RA subtypes in peripheral blood cells and synovial tissue

Schematic overview of the discovery of RA subtypes in peripheral blood cells and synovial tissue. Hierarchical clustering of differentially expressed genes in PB cells and synovial tissues from RA patients highlighted the existence of at least two molecular distinct forms of RA. It is hypothesized that two different pathogenic mechanisms are playing a role in RA. The first is an immune cell mediated destruction of cartilage via activation of matrix metalloproteases such as MMP3 whereas the second is an immune independent mechanism possible mediated by tissue remodeling and growth factors. Patients with a high inflammatory tissue type display increased CRP, ESR and thrombocyte counts. In addition, a subgroup of patients has increased expression levels of type I IFN response genes. (Adapted and changed from Future Rheumatology June 2006 Vol. 1, No. 3, Pages 311-322, with permission of Future Medicine Ltd)
5.2.2 Heterogenomics in MS

Since it is highly difficult to obtain from large study groups brain tissue during the course of disease it is not easy to elucidate the role of tissue heterogeneity in the pathogenesis of disease. Because of the autoimmune nature of the disease and the migration of immune cells to the brain, we hypothesized that the disease processes are reflected in the peripheral blood compartment which we are able to study during disease.

In chapter 3.4 we performed gene expression profiling in peripheral blood (PB) cells of MS patients which revealed an elevated expression of a spectrum of genes involved in immune defense e.g. TLR signaling when compared to healthy controls. Most strikingly, our results demonstrated evidence for a remarkable heterogeneity between the RRMS patients. This was primarily based on differential expression of type IFN response genes. Moreover, the expression signatures of half of the MS patients were similar to those of virus-infected macaques (20). Collectively, these findings provide a basis for patient stratification in RRMS and may support the hypothesis that viral antigens play an important role in the pathogenesis MS. However, since endogenous antigens such as DNA or RNA containing immune complexes can also activate certain TLR signaling routes leading to increased transcription of type I IFN induced genes (21), further studies are needed to elucidate the origin of the type I IFN induced gene expression signature. Similar results have been generated by Yamaguchi and colleagues who also showed a dysregulated expression of IFNβ response genes in treatment naïve RRMS patients (22).

5.3 Common denominators in autoimmunity; what’s the role of IFN?

Upregulation of IFN-induced genes has now been observed in PB cells and/or target tissue of (a subset of) patients with different autoimmune diseases e.g. RA, SLE, SSc, SS, MS, and
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type 1 diabetes. These findings suggest that an activated IFN gene expression program may be a common denominator in autoimmune diseases in general.

The initial activation of the type I IFN gene activity most likely depends on both the genetic background as well as encountered environmental (endogenous and/or exogenous) factors of the individual. Individuals harboring a certain genetic profile of e.g. risk alleles for genes involved in IFN biology may than generate increased levels of IFN-response genes. It is speculated that the IFN-response program could be associated with the activation of immature DCs, which regulate deletion of autoreactive lymphocytes. Subsequently, IFN-matured DCs may activate autoreactive T cells leading to autoreactive B cell development and a first level of autoimmunity (23). Loss of tolerance for self antigens may then lead to autoantibody production.

In the case of SLE, autoantigen/autoantibody complexes may trigger pathogen recognition receptors (such as TLRs) that induce IFNα production and thereby perpetuates the IFN response program. Consequently, a vicious circle is established of increased type I IFN-response activity and autoreactivity against self antigens resulting in autoimmune disease (Figure 2). Breaking this circle resulting in downregulation of the type I IFN-response gene activity could be a therapeutic approach for SLE. Indeed, recent results from a phase I clinical trial showed reduction in clinical disease activity and downregulation of type I IFN-response genes in SLE patients treated with an anti-IFNα mAb (24). Conversely, type I IFN treatment is beneficial in MS patients and in animal models of arthritis.
Figure 2. The possible role of IFN in autoimmunity

Both genetic and environmental factors may lead to an increased expression of type I IFN-response genes. The increased activity of type I IFN-response genes may lead to accelerated DC activation and a break of peripheral tolerance resulting in activation of autoreactive T and B cells. The subsequent immune response against self-antigens will lead to autoantibody production. In SLE the autoantibody production may lead to immune complex formation which can activate immune cells to produce more type I IFN eventuating in a vicious circle of immune activation and IFN production.

Besides a role for the IFN response program as common denominator in autoimmune diseases other gene expression profiles have been reported that are shared by autoimmune diseases. In particular Maas and colleagues (25) studied the overlap of gene expression profiles between different diseases. They identified 95 genes that were increased and 117 genes that were decreased in PBMC of all patients with RA, SLE, type I diabetes and MS. These genes were involved in e.g. inflammation, signaling, apoptosis, ubiquitin/proteasome function and cell cycle. Hierarchical cluster analysis on the basis of gene signatures in PBMC revealed that RA and SLE patients were intermixed with one another. Moreover, they reported that from the genes that were differentially expressed between PBMCs from patients and unrelated unaffected individuals, the gene expression profile of 127 genes was shared between patients with autoimmune diseases and unaffected first-degree relatives. This commonality between affected and unaffected first degree relatives suggests a genetic basis for these shared gene
expression profiles. Accordingly, they showed that these genes are clustered in chromosomal domains supporting the hypothesis that there is some genetic logic to this commonality (26).

5.4 Pharmacogenomics

Given the destructive nature of autoimmune diseases it would be highly desirable to predict in an early stage the most beneficial treatment for the patients at risk. If we rely solely on clinical or radiographic/MRI manifestations we will probably be responding too late in order to maximize protection. Ideally, it would be desirable to make predictions on the success before the start of therapy. Ultimately, this may lead to a personalized form of medicine, whereby a specific therapy will be applied that is best suited for an individual patient.

We applied genomics for studying the pharmacodynamics of IFNβ and anti-TNF therapy and for predicting the response to therapy. The term pharmacogenomics emerged in the late 1990s and is associated with the application of genomics in drug development. Pharmacogenomics is defined as: “The investigation of variations of DNA and RNA characteristics as related to drug response”. In this thesis we focused on transcriptomics which investigates RNA characteristics.

5.4.1 Pharmacogenomics of anti-TNF treatment in RA patients

TNF antagonists are approved worldwide for the treatment of various rheumatic diseases. Clinical experience indicates that there are ‘responders’ as well as ‘non responders’, however clear criteria for such classification are still lacking. For RA, treatment is only effective for approximately two-third of the patients (27) which has attracted interest in the pharmacology and mechanism of action.

Until now a few pharmacogenomics studies have been published to gain insight in the pharmacodynamics and to identify genes predictive of responsiveness to TNF blockers. Thus
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far, studies aimed at identifying biomarkers before the treatment to predict the response to anti-TNF treatment in RA have not revealed consistent results. The discrepancies are partly explained because of differences in study design, experimental platform and patients included. In addition, universal criteria for defining the clinical response to treatment are currently lacking. Therefore, additional studies using large cohorts of patients and more stringent response criteria are necessary.

In chapter 4.1 we describe the pharmacogenomics of Infliximab treatment in RA patients (n=15) before and 1 month after the start of infliximab. These results revealed a similar change in the expression of a pharmacogenomic response gene set in the peripheral blood compartment of all patients treated irrespective of clinical response. This study indicates that all RA patients exhibit an active TNF response program contributing to disease pathogenesis (28). However, since not every RA patient shows a good clinical response to anti-TNF treatment we hypothesize that in these non-responder patients additional biological programs besides TNF are involved in disease pathogenesis. The relative contribution of these TNF-independent pathways may determine clinical response to treatment.

Although TNF antagonists have been shown to be effective for the treatment of several inflammatory diseases including RA, several clinical complications have been reported. Patients treated with TNF antagonists may develop lupus-like symptoms and increased dsDNA autoantibody levels. It was hypothesized that these complications were the result of anti-TNF induced increase in type I IFN activity since SLE is characterized by increased expression levels of IFN-response genes in PB and because SOJIA patients treated with TNF antagonist have increased expression levels of IFN-response genes (29). However, as described in chapter 4.2, gene expression studies in whole blood of RA patients before and 1, 2, 3 and 4 months after the start of TNF-blockade (infliximab) revealed a variable effect on
the expression of IFN-response genes upon treatment. Therefore, the positive effect of TNF blockade on IFN is not consistently observed in RA. The anti-TNF induced increase in type I IFN gene activity was not associated with the extent of IFN-response gene activity at baseline. Interestingly, the anti-TNF induced increase in type I IFN-response gene activity was associated with poor clinical response to treatment as assessed by EULAR criteria. Although the anti-TNF induced increase in IFN-response gene activity might be an epiphenomenon related to the effect of TNF blockade, it is tempting to speculate on a role of increased IFN bioactivity in the deteriorating clinical effects (as discussed in chapter 5.3). Figure 3 summarizes our main findings with respect to pharmacogenomics of TNF blockade in RA.

**Figure 3. Pharmacogenomics of anti-TNF treatment in RA**

By analyzing the change in gene expression profiles upon antagonizing the function of TNF we demonstrated that all RA patients exhibit an active TNF response program contributing to disease pathogenesis. The results described in chapter 4 favour a model for the parallel presence of TNF-dependent and TNF-independent pathways (named “others”) in the individual RA patient. Clinical response to TNF blockade might be determined by the relative contribution of the TNF-independent pathways to disease. Moreover, poor response to treatment is associated with TNF blockade induced expression of type I IFN response genes.
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5.4.2 Pharmacogenomics of IFNβ treatment in MS

Interferons (IFN) were the first agents to show clinical efficacy in treatment of MS, and prolonged treatment is still the best available therapy. Because MS is a chronic disease with an unpredictable clinical course it remains difficult to assess clinical responder status at an individual patient level. Despite availability of objective methods for determining disease activity such as the measurement of MRI parameters, e.g. CNS atrophy measures or T1 gadolinium enhancing or the appearance of new T2 lesions (30-32) it is still extremely difficult to precisely define the state of responsiveness after a short period of treatment. This emphasizes the importance of finding pharmacological response markers that could aid in the assignment of clinical responders and non-responders of IFNβ therapy.

In chapter 4.3 we described the change in gene expression profiles in PB after IFNβ therapy, or in other words: the pharmacogenomics of IFNβ treatment in PB. The heterogeneous expression of type I IFN induced genes in untreated MS patients was confirmed and we investigated how this signature was related to treatment response. As anticipated (22), type I IFN response genes were increased upon treatment. Interestingly, it was observed that the extent of the pharmacological response correlates negatively with the baseline expression of a specific set of IFN response genes. Furthermore, these ex vivo results could be predicted in vitro using IFNβ stimulated PB cells isolated prior to treatment. Overall, we concluded that the inter-individual variation in response to IFNβ therapy is an intrinsic property of the PB cell compartment and unrelated to treatment regimens and interfering serum proteins such as NAbs.

Because MS is a chronic disease with an unpredictable disease course it is highly difficult to determine the clinical response to treatment at the individual patient level. Due to the small sample size of our study we lacked power to address the question of an association with the clinical response to treatment. Nonetheless, these data imply that the type I IFN induced gene
expression signature prior to treatment determines the pharmacological difference between patients and could serve a role as biomarker for clinical response to IFNβ. Currently, experimental studies are carried out with larger study groups and well defined patient samples to investigate the clinical relevance of the observed results.

The inter-individual differences in IFNβ responsiveness may be the result of genetic variation in the IFNβ biology. Other mechanisms that could account for differential responsiveness to IFNβ include variation in activity of inhibitory transcription factors. Evidence exists that crosstalk with other cytokine-activated pathways, could cause tachyphylaxis to type I IFNs. Although type I IFNs have an essential function in mediating innate immune responses against viruses, they may already be produced at very low levels in the absence of viral infections (33) in serum of a subset of MS patients. Since e.g. IFNα is known to desensitize further responses to IFNs (62), the presence of low endogenous IFNs could block IFNβ-induced signals (34;35). Future studies aimed at unraveling these possibilities should shed new light on the mechanism of variation in IFN response in relation to the endogenous IFN signature. Figure 4 describes the future activities towards a personalized medicine approach for IFNβ in RRMS.

Figure 4. Steps towards a personalized medicine approach for IFNβ therapy in RRMS
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5.5 Conclusion

Taken together, the work described in this thesis has provided novel information about molecular heterogeneity in relation to disease development, disease subclassification, disease pathogenesis and response to treatment. Accordingly, evidence is accumulating that large-scale gene expression profiling will contribute considerably to our understanding of the molecular and biological basis of the well-recognized but as yet poorly defined heterogeneity of diseases such as MS and RA.

Clearly, these developments open the way for a “redefinition” of diseases like RA and MS. The molecular markers (biomarkers) that distinguish patients from one another can be used for early diagnosis, prognosis, determining drug efficacy and risk management. The transition of this explorative type of research to integrated clinical diagnostics will require extensive optimization of the technological procedures. Moreover, the assignment and implementation of useful and reliable classifiers requires rigorous standardization and several levels of validation.

Hence future treatment will be tailored to the molecular and biological features of an individual patient. This will lead to personalized medicine, i.e. the prescription of therapeutics that is best suited for an individual patient.
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