Genomic stratification by HLA-DRB4 - Expression A strategy to identify predictors of rheumatoid arthritis

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Introduction: There is increasing evidence that both genetic and molecular factors can influence the clinical behavior of rheumatoid arthritis (RA). HLA-DRB4 is one of the most critical genes in disease-modifying anti-RA drugs (DMARDs) and thus the strategy of this study was to determine the most promising therapy for RA. In the HLA-DRB4- patients subgroup, the relative expression of the HLA-DRB4 with sensitivity and specificity rates of 83.3% and 100% were obtained, the separation of R, MR, and NR in the HLA-DRB4 - RA patient subgroup (Fig. 4D) resulted in a 100% sensitivity and specificity. Technical validation by qPCR with the 10 best candidates confirmed AUC-values up to 0.97 for prediction in the HLA-DRB4- group and reached AUC up to 0.89 in HLA-DRB4+ patients (data not shown).

Materials and Methods:
Whole blood PAxGene samples from 52 patients with early RA were obtained in the HITHARD and in the ArthroMark study. Exclusively, patients who had received no or only low doses of steroids were analyzed. Clinical characteristics assessed at baseline and after 16 weeks included RF, ACNA, DAS28 and EULAR response or classification in good (GR), moderate (MR) and non-responders (NR). Total RNA was globally refined and processed according to standard protocols for hybridization to Affymetrix HG-U133 Plus 2.0 microarrays. Biostatistical methods included MASS.0 and RMA algorithms with limma, lasso and Wilcox tests. For functional interpretation, expression of candidate genes was tested in various blood cell types and stimulation conditions using own and GEO reference transcriptions. Marker selection was validated by qPCR with commercial primers and four different housekeeping genes (β-actin, y-actin, GAPDH, HPRT).

Results: Assuming impact by immunogenetic or genetic characteristics on pathomechanisms and treatment outcome, patients were grouped either by gender, RF, ACNA, SE or the haplotype-specific HLA transcripts DRB4 (209728_at) and DQA1 (203290_at). In each subgroup, responders and non-responders were compared and genes ranked by frequency of change call and fold change. For each subgroup comparison, the best 100 genes increased and 100 decreased in responders were selected. Scoring differential expression of all 200 genes for each HLA-DRB4- patient revealed a high correlation with clinical response. Moderate responders represented independent samples and were located between responders and non-responders (Fig.1).

Validation of HLA-DRB4- candidate genes by “leave-one-out” excluded each patient at least once and generated 12 different combinations based on MASS.0/BioRelis algorithms (I). Each validation gene set overlapped with the complete group comparison set by 66%-80% for increased and 67%-82% for decreased in responders. Of the complete group comparison set, 96.5% (range: 91-100%) were ranked within the top 250 transcripts of any validation comparison. Overall, validation comparisons revealed correct classification of responders in 96% (range: 83%-100%) and non-responders in 73% (range: 57%-86%). Different biostatistical approaches favored MASS.0 algorithms for marker selection (Fig. 3/Cl). Comparison between good responders and non-responders in unselected patients revealed insufficient discriminatory power. Generating different subgroups, defined by gender, status of RA, ACNA, SE, HLA-DRB4 or DQA1 identified molecular patterns with a strong distinction between GR and NR in HLA-DRB4 subgroups. All other subgroups did not improve pattern selection. In HLA-DRB4+ patients, genes increased in GR were related to phagocytes whereas bone marrow activation (innate), whereas genes increased in NR were associated with lymphocyte (adaptive) activity (Fig. 2, Fig. 3A). In HLA-DRB4+ patients, patterns of adaptive immunity were related to non-response but frequently combined also with innate patterns (Fig. 2, Fig. 3B). After separation of the patients into HLA-DRB4+ (n=29) and HLA-DRB4- (n=16) subgroups 2 predictive gene panels (n=16 each) were identified. Hierarchical cluster analyses using these marker gene panels resulted in a clear discrimination of R and NR and revealed sensitivity and specificity rates of 100% in the HLA-DRB4- patient subgroup (Fig. 4A) and sensitivity of 92.9% and 100% in the HLA-DRB4+ RA patient subgroup (Fig. 4B).

Conclusion: An early outcome prediction of successful therapy, in particular with MTX as anchor drug in RA even after initiation of the disease, will be possible for an individual medication. Identification of specific predictive response marker genes such as CD11c for anti-TNF monotherapy (2, 3) leads to a substantial interest to define predictive biomarkers for MTX monotherapy (4, 5), as well. Combination of these therapy specific markers might be helpful to calculate the outcome of the standard combination therapy with anti-TNF/MTX. Combined genetic, genomic cellular stratification revealed transcriptional patterns that indicate different molecular pathomechanisms in RA depending on response to MTX therapy. Interestingly, the patient subgroups responding to MTX was dominated by phagocyte but not lymphocyte activity (Fig. 2), which may indicate an important contribution of innate immune triggers in RA.

Figure 1: Association of clinical and molecular response criteria in HLA-DRB4- patients

Figure 2: Increase-patterns prediction for MTX outcome and identified by HLA-DRB4-dependent genetic stratification

Figure 3: Clustering of candidate gene in HLA-DRB4- RA subgroup

Figure 4: Hierarchical clustering of the top 16 genes predictor for MTX response with change rate of 0.100 decrease between responders and non-responders of HLA-DRB4 (A,B) and HLA-DRB4 patients (C,D) without (A,C) and with moderate responders (B,D). In HLA-DRB4 patients, discrimination was achieved with 100% sensitivity and specificity and without with moderate responders. In HLA-DRB4- patients without moderate responders, sensitivity was 100% and specificity 92.9%. With moderate responders, sensitivity of 82.3% and specificity of 100% for correct prediction was achieved.