Research Article

Genetic Variability of RXRB, PPARα, and PPARγ in Wegener’s Granulomatosis

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Received 29 August 2008; Revised 4 December 2008; Accepted 5 January 2009

Recommended by Mostafa Badr

A major genomic region involved in Wegener’s granulomatosis includes the gene for retinoid receptor beta (RXRB) which forms heterodimers with peroxisome proliferator-activated receptors (PPARs). It is unclear whether this association directly arises from the RXRB allele(s) or via a linked variation. In order to reveal any hitherto unknown and potentially disease-relevant variation of the RXRB gene, we have genotyped four tagging SNPs of this genomic region and have directly sequenced selected WG patients and controls representing disease-associated haplotypes. Additionally, we have genotyped 2 SNPs each in the genes for PPARα and PPARγ (PPARA and PPARG). Hence, we confirmed the strong association of the RXRB locus with WG but could not reveal any novel variation in RXRB. None of the PPARα and PPARγ SNPs showed association with WG. Moreover, no epistatic effect was seen between RXRB and PPARA/PPARG alleles. These results do not support an etiopathological role of PPAR in WG. Analyses of further genes functionally linked to RXRB may provide additional data useful to evaluate the RXRB association found in WG.

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1. Introduction

Wegener’s granulomatosis (WG) is a form of small vessel vasculitis belonging to the group antineutrophil cytoplasmic antibody- (ANCA-) associated vasculitides (AAV). WG usually presents with granulomatous lesions of the upper respiratory tract and often develops into generalized vasculitis with multiple organ involvement in later disease stages. In WG ANCA typically recognize proteinase 3, delineating this diagnostic entity from other AAV forms like Churg Strauss syndrome or microscopic polyangiitis, in which ANCA are usually directed against myeloperoxidase (reviewed in [1]). Current concepts of its etiopathology are incomplete, but WG is accounted to the large and heterogenous group of complex disorders arise from a mostly elusive interplay of environmental and genetic factors.

A major genomic locus for WG was identified on chromosome 6p21.3, including (among others) the genes for HLA-DPB1*, RXRB, and RING1 [2]. The linkage disequilibrium (LD) pattern of this genomic region is complex with larger blocks of strong LD alternating with regions of high recombination rates. There is evidence that the association of this locus with the disorder may arise from more than one variation, making 6p21.3 a quantitative trait locus for WG. While a strong association is demonstrable with the HLA-DPB1*0104 allele, a (partly) independent association was detected for the defined region including potential WG candidate genes RING1 and RXRB [3]. In addition certain alleles of RXRB, the gene encoding retinoid X receptor beta, are highly significantly associated with WG susceptibility [4].

Peroxisome proliferator-activated receptors (PPAR) are group of nuclear receptors mediating the effects of peroxisome proliferators on gene transcription. Binding of PPAR to regulatory DNA sequences requires the heterodimerization with RXR. PPAR mediated effects are complex and include modulation of inflammatory processes. PPARα is a ligand for leukotriene B4, and PPARα deficient mice show a prolonged response to inflammatory stimuli [5]. PPARα also appears to be involved in downregulation of the activity of cyclooxygenase 2 (COX2) and nuclear factor kappa-b (NFkB). Likewise, PPARγ exhibits anti-inflammatory properties. It is upregulated in activated macrophages and inhibits
the expression, for example, of the inducible nitric oxide synthase [6].

Genetic polymorphisms of PPAR have been investigated in numerous conditions, predominantly in metabolic disorders like type 2 diabetes or atherosclerosis [7–9]. There is evidence for accelerated atherosclerosis in WG patients [10], suggesting that both conditions may share some (genetic) risk factors.

Given the pleiotropic role of PPAR in the regulation of (vascular) inflammation, we have hypothesised that genetic predisposition to WG may arise from the interaction of certain genetic variations of RXRB and PPAR. We have, therefore, screened the RXRB gene for novel, potentially WG-specific variations. Moreover, we have genotyped two single nucleotide polymorphisms in each of the genes for PPARα and PPARγ in large panels of WG cases and healthy controls. Finally, we searched for epistatic effects of certain alleles of RXRB and PPARα or PPARγ.

2. Patients and Methods

2.1. Subjects. All patients included in this study were diagnosed with WG according to the criteria of the American College of Rheumatology and the Chapel Hill consensus conference [11]. They were selected at the interdisciplinary vasculitis centre at the University of Luebeck/Rheumaklinik Bad Bramstedt. All patients were asked for their ancestry and reported German decent for at least two generations. Healthy German blood donors were used as controls and ancestry was evaluated equal to the patient group.

For the RXRB locus we genotyped the same patients (n = 282) and controls (n = 380) that were previously analyzed for the 6p21.3 locus [3]. In order to increase statistical power, the SNP in the PPAR genes were analyzed in expanded sample comprising 462 WG cases and 701 controls.

Ethical principles for medical research involving human subjects as defined in the Declaration of Helsinki have been followed. The study design was approved by the local ethics committee at the University of Luebeck, Germany (No. AZ 06-087).

2.2. Genotyping. Four types SNPs rs9277935, rs2072915, rs2744537, and rs1547387 (see Figure 1) were selected from the HapMap database to serve as tagSNPs for a region of 10 kb including the RXRB gene. With this selection all HapMap SNPs within this region are efficiently tagged (r² > 0.9). rs2072915 and rs2744537 are located within the 3’ untranslated region (UTR) of RXRB, while rs9277935 is located approximately 1 kb 3’ of the last RXRB exon. rs1547387 is located 5’ of RXRB, in exon 5 of the SLC39A7 gene, in which it constitutes a silent SNP (Ser209Ser).

rs9277935, rs2072915, and rs2744537 were genotyped via PCR-RFLP techniques and a commercially available TaqMan genotyping assay (Applied Biosystems) was used for rs1547387.

For rs1800206 (Val162Leu) in PPARα also a commercially available TaqMan assay was used, while rs6008259 (3’ UTR of PPARα), rs1801282 (PPARG, Ala12Pro), and rs3856806 (PPARG, His449His) were genotyped using newly designed primers and probes (see Table 1).

2.3. Direct Sequencing of RXRB. The entire RXRB gene (all 10 exons and exon/intron boundaries) was directly sequenced in 5 WG patients typed homozygous for the associated RXRB haplotype (see below; primer sequences available on request). For comparison 5 healthy controls homozygous for the inversely associated (i.e., protective) haplotype were also sequenced.

2.4. Statistical Analysis. Genotypes were recorded in linkage format. Association for each single marker was tested by using chi square tests on contingency tables. A P value <.05 was considered significant. LD between each SNP of a respective locus, and haplotype block frequencies were calculated by using Haplovie 4.1 [12].

Interaction between RXRB and PPARα/PPARγ SNPs were calculated with GAIA (http://gump.qimr.edu.au/GAIA/gaia.html; [13]). This application uses a logistic regression model which tests for pairwise locus/locus interactions between genes. We applied an additive interaction model for each pair of SNPs testing the significance of the interaction model terms over and above any main effects.

3. Results and Discussion

The overall call rate for all 8 SNPs in patients and controls was 93.60%. None of the SNPs revealed significant deviation from Hardy-Weinberg equilibrium. Two of the RXRB SNPs (rs9277935 and rs2744537) showed highly significant association with WG (see Table 2). Accordingly, the GTTC haplotype (calculated from all four SNPs of this locus) was significantly more common in WG while the TTGC haplotype was overrepresented in controls (see Table 2).
Table 1: Oligonucleotides and restriction enzymes used for genotyping.

| Gene   | SNP     | Forward (F) and reverse (R) primer (5′ > 3′) | Allele specific probes | Restriction enzyme | Fragment lengths |
|--------|---------|---------------------------------------------|------------------------|--------------------|-----------------|
| RXRB   | rs9277935 | F: TGCCCCCTTGTA- GTCTCCAC | —                      | BtgI               | T-Allel: 208 bp  |
|        | T/G     | R: CCTCCACCTGTG- CCCCCTAA |                        |                    | G-Allel: 34 + 174 bp |
|        | rs2072915 | F: ACATCTCCACCA- GCCCCTTC | —                      | MboI               | T-Allel: 325 bp  |
|        | T/A     | R: GTCCCTCCCCCA- GCACAAAG |                        |                    | A-Allel: 224 + 101 bp |
|        | rs2744537 | F: TTCTCAAGCTCA- TTGGTGAC | —                      | BsaI               | T-Allel: 451 bp  |
|        | T/G     | R: CCGATTTCCACT- CTTCAAGAT |                        |                    | G-Allel: 326 + 125 bp |
|        | rs1547387 | Information not provided by the manufacturer | —                      |                    | —               |
|        | C/G     | Information not provided by the manufacturer | —                      |                    | —               |
| PPARA  | rs1800206 | Information not provided by the manufacturer | —                      |                    | —               |
|        | G/C     | —                                            |                        |                    | —               |
|        | rs6008259 | F: CCCCTGTGTCGAA- CCAGATCC | —                      | —                  | —               |
|        | G/A     | R: CCTGAATGGCAC- AGGGTACAT | A: YY-CCTGTGTTGTCC- CCAACGCC | —                  | —               |
|        | rs1801282 | F: TTATGGGTGAAA- CTTGGGAGATT | —                      | —                  | —               |
|        | G/C     | R: TTGTGATATGTGTTGACAGCAT | C: YY-TCTATTTTCAGCC- AAAGGCTTTTC | —                  | —               |
|        | rs3856806 | F: CCAGAAAAATGAC- AGACCTGAAC | —                      | —                  | —               |
|        | T/C     | R: GGAGGGGTTGGA- GAAGTCATG | C: YY-ATTGTCAAGGAA- CACGTGGAGC | —                  | —               |

(1) A 17 mer nucleotide (GTAAAACGACGGCCAGT) was added 5′ to the forward primer to increase fragment length differences between the two alleles.

Based on the SNP data we have then selected 5 WG patients homozygous for the GTTC haplotype for direct sequencing of the RXRB gene. For comparison 5 control subjects homozygous for the TTGC haplotype were also analyzed. Yet, no novel sequence variation was detected, a finding which is consistent with previous results from Szyld et al. [4], who had screened the RXRB gene in WG patients without consideration of the haplotype structure of this locus. Results from the initial tagging SNP genotyping were confirmed in the ten included individuals for the two exonic tagging SNPs (rs2744537 and rs2072915). Two other exonic SNPs (rs1152296 in exon 10 of RXRB and rs6531 in exon 7) were identified and perfectly segregated with the previously identified risk/nonrisk haplotypes.

Both associated RXRB SNPs (rs9277935 and rs2744537) are in strong LD with HLA-DPB1 as well as with rs311728 and rs213208 (see Figure 2) which had revealed the highest (and partly HLA-DPB1 independent) association with WG in a previous study [3]. Moreover, according to the HapMap data rs2744537 is in strong LD with rs6531 (a synonymous SNP in exon 7 of RXRB) which was significantly associated with WG earlier [4]. As for the other associated SNPs in this genomic region, it is therefore hard to differentiate which of the SNPs (or even a yet unknown variation of this locus) constitutes the primary WG risk factor. A potential approach to overcome this problem is the analysis of factors related to the different proteins encoded in the 6p21.3 region. We have therefore investigated SNPs in the genes for PPARα and PPARγ which are functionally closely linked to RXRB.

The two SNPs in PPARγ were in weak to moderate LD ($D’ = 0.64, r^2 = 0.40$) while the two SNPs in PPARα were virtually unlinked ($D’ = 0.47, r^2 = 0.06$). Therefore, no haplotype frequencies were calculated for these genes. Both SNPs in PPARγ showed very similar allele frequencies in cases and controls not revealing any significant differences (see Table 2). The two SNPs in PPARα both revealed a
Table 2: Allele and haplotype frequencies.

| Gene  | SNP/haplotype | Allele | Frequency in controls | Frequency WG patients | P value | OR (95% CI) |
|-------|---------------|--------|-----------------------|-----------------------|---------|-------------|
|       |               |        |                       |                       |         |             |
|       | rs9277935     | G      | 0.76                  | 0.89                  | 6.65 × 10⁻⁹ | 2.55 (1.86–3.56) |
|       |               | T      | 0.24                  | 0.11                  |         |             |
| RXRB  | rs2072915     | T      | 0.71                  | 0.75                  | .157    | 1.20 (0.93–1.55) |
|       |               | A      | 0.29                  | 0.25                  |         |             |
|       | rs2744537     | T      | 0.73                  | 0.57                  | 6.63 × 10⁻⁹ | 0.49 (0.39–0.63) |
|       |               | G      | 0.27                  | 0.43                  |         |             |
|       | rs1547387     | C      | 0.87                  | 0.85                  | .34     | 0.85 (0.62–1.18) |
|       |               | G      | 0.13                  | 0.15                  |         |             |
| RXRB* | GTTC          |        | 0.24                  | 0.40                  | 1.15 × 10⁻⁹ | 2.11 (1.66–2.69) |
|       | TTGC          |        | 0.19                  | 0.07                  | 9.73 × 10⁻¹⁰ | 0.32 (0.22–0.47) |
|       | other         |        | 0.43                  | 0.47                  | .157    | 1.18 (0.94–1.47) |
|       | rs1800206     | C      | 0.93                  | 0.95                  | .076    | 1.40 (0.96–2.05) |
| PPARA |               | G      | 0.07                  | 0.05                  |         |             |
|       | rs6008259     | G      | 0.80                  | 0.83                  | .069    | 0.81 (0.65–1.02) |
|       |               | A      | 0.20                  | 0.17                  |         |             |
| PPARG | rs1801282     | C      | 0.85                  | 0.85                  | .74     | 0.96 (0.75–1.23) |
|       |               | G      | 0.15                  | 0.15                  |         |             |
|       | rs3856806     | C      | 0.86                  | 0.85                  | .70     | 0.95 (0.75–1.22) |
|       |               | T      | 0.14                  | 0.15                  |         |             |

OR: Odds ratio,
CI: confidence interval,
*haplotype frequencies calculated from all four RXRB SNPs.

decreased frequency of the minor allele but these differences did not reach significance level (see Table 2). No significant pairwise epistatic effect for any of the investigated SNPs, that is, neither between PPARA and PPARG nor between PPARA (or PPARG, resp.) and RXRB or HLA-DPB1. Taken together, we cannot provide evidence for a genetically based involvement of PPARα and PPARγ in the etiopathology of WG. Moreover, the strong WG association with the 6p21.3 locus is unlikely to be based on any coding variation of the RXRB gene. Future studies will therefore have to focus on regulatory elements of this area (e.g., cis acting elements or micro RNAs).

4. Conclusions

These results do not support a direct etiopathological role of RXRB and/or PPAR in WG. Analyses of further genes functionally linked to RXRB, for example, retinoic acid receptors or vitamin D receptors, may provide additional data useful to evaluate the RXRB association found in WG.

Acknowledgment

This work was supported by the Deutsche Forschungsgemeinschaft [KFO 170].

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