Susceptibility Genes and Progression in Age-Related Maculopathy: A Study of Single Eyes

Astrid Farwick,1,2 Jürgen Wellmann,1 Monika Stoll,2 Daniel Pauleikhoff,3 and Hans-Werner Hense1

PURPOSE. The specific role of single-nucleotide polymorphisms (SNPs) in the progression of age-related maculopathy (AMD) is not clearly understood. The present study was conducted to investigate whether variants in three susceptibility genes are differentially associated with progression to early and late AMD.

METHODS. The Münster Ageing and Retina Study (MARS) cohort includes 722 patients with different stages of AMD. Participants were reexamined after a median of 2.6 years. The association of SNPs in the CFH, ARMS2, and C3 genes with AMD progression was evaluated in 1435 single eyes by using multivariate logistic regression models with generalized estimating equations.

RESULTS. CFH-rs1061170 was significantly related to the development of early AMD (OR = 1.94, 95% confidence interval [CI], 1.1–3.9) for heterozygous, and OR = 2.9 (95% CI, 1.7–5.1) for homozygous allele carriers) but not to late AMD. In contrast, ARMS2-rs10490924 was associated only with progression to advanced disease (OR = 1.2 [0.7–2.1] and OR = 2.1 [1.1–3.9], respectively). The variant C3-rs2230199 showed no relation with AMD progression.

CONCLUSIONS. The findings indicate that AMD progression is differentially affected by genotypic variants. Probably, aging processes of the human retina predispose to AMD onset in the presence of genetic variation in the complement system, which alters immunoregulatory and inflammatory responses. The specific functional role of ARMS2-rs10490924 remains as yet unknown, but it appears to mainly affect the progression to late AMD stages. (Invest Ophthal Mol Vis Sci. 2010;51: 731–736) DOI:10.1167/iovs.09-3953

Age-related macular degeneration (AMD) is a multifactorial degenerative disease of the retina, mainly affecting the macula lutea. The alterations underlying AMD lead to severe vision impairment such that, due to its high prevalence in industrialized countries, AMD is considered to be the most common cause of legal blindness in the Western world.1,2

The disease is characterized by different phenotypic stages, and the morphologic changes observed at the retina are clinically used for stratification into stages of increasing AMD severity.1,3,4

Although the research on the natural history of AMD has undergone substantial progress within the past years, its details of its pathogenesis remain unclear. Several studies have highlighted the complex etiology of AMD and have pointed out that, apart from the relation to old age, there is a major role of genetic variants in the pathogenesis of the disease.5,6 In particular, common single-nucleotide polymorphisms (SNPs) in the genes for complement factor H (CFH),7,8,12 ARMS2 (formerly labeled LOC387715),11–13 and HTRA114,15 show strong and consistent associations with the presence of AMD. More recently, additional studies have revealed that polymorphisms in genes coding for other complement components, such as component 2 (C2), factor B (CFB),17–20 and component 521,22 are also involved in the pathogenesis of AMD.

To date, most researchers have investigated the association between genetic variation and AMD in cross-sectional or case-control studies of prevalent cases of AMD. However, prospective studies are necessary to identify causal factors and sort out their differential impact on progression stages. A limited number of prospective studies have analyzed so far how major susceptibility polymorphisms influence AMD progression. In most of these studies, progression to late AMD as the study endpoint,25–28 whereas onset and progression to early AMD was mostly neglected. In a recent case-control analysis of the Münster Ageing and Retina Study (MARS) baseline examination, we observed that CFH-rs1061170 is more strongly related to prevalent cases of early AMD, whereas both rs1061170 in the CFH and rs10490924 in the ARMS2 gene, were more common in prevalent late AMD.26

To further clarify these processes, we prospectively analyzed progression to early and late AMD over a median of 2.6 years in the MARS cohort. In contrast to most previous prospective studies, the rate of progression in single eyes was chosen as the unit of analysis in our report, thus including two observations from each study participant when gradable fundus photos from both eyes were available. We chose this approach to integrate the full information obtained for both eyes, and the relation to variants of the CFH, ARMS2, and C3 genes was investigated in statistical models that accounted for the AMD baseline status in both eyes.

MATERIAL AND METHODS

Study Population

The MARS is a longitudinal study designed to identify medical, environmental and genetic factors with implications for the pathogenesis and progression of AMD. Study participants were examined at baseline (June 2001–October 2003, MARS-I) and during the first follow up examination (November 2004–September 2006, MARS-II). All partici-
plicants were of Caucasian origin. The first follow-up was attended after a median of 2.6 years by 828 study participants (85.5% of all eligible) of whom 722 had gradable fundus photographs in both eyes at baseline and at least one eye at risk for progression, that is, without morphologic signs of late AMD. In addition, DNA had been extracted successfully for genetic analyses in each participant. The recruitment and research protocols were reviewed and approved by the Institutional Review Board of the University of Münster, and written informed consent was obtained from all study participants, in compliance with the Declaration of Helsinki.

**Examination Procedures and Progression Assessment**

A comprehensive description of examination procedures and fundus photography methods have been described in detail elsewhere. All baseline and follow-up examinations were conducted by specifically trained staff members whose performance was evaluated against that of observers from the Rotterdam Study. The follow-up visits involved the same examination program as MARS-I, for example, with the same fundus camera, and it was performed by the same investigators. Furthermore, we performed regular semiannual quality checks in 25 pairs of eyes, to assess intra- and interobserver reliability during the study. We further compared the standard of MARS-I—who had been initially certified against staff from the Rotterdam study—with the technicians grading fundus photographs in MARS-II. The weighted $k$ was consistently 0.88 or better for the classification used in this report.

The presence and severity of retinal lesions were graded in each eye in accordance with the Rotterdam Study and the range of fundus signs was stratified into five severity stages. Similar to Despriet et al., we grouped participants in stage 0 (no sign of age-related maculopathy or hard drusen) and stage 1 (either soft distinct drusen <125 μm or pigment epithelium changes) together as no or minimal AMD; those with stages 2 and 3 (soft indistinct drusen or reticular drusen >125 μm, with or without pigmentary irregularities, or soft distinct drusen with pigmentary irregularities) as early AMD, and those in stage 4 (atrophy geography or choroidal neovascularization) as late AMD. Analogous to Despriet et al., we also defined two types of progression. Early progression was present when eyes with no or minimal signs of AMD at baseline had progressed to early AMD at follow-up. Finally, late progression occurred when eyes with no or minimal AMD or with ‘early AMD’ at baseline progressed to late AMD in MARS-II.

**DNA Extraction, SNP Selection, and Genotyping**

We extracted DNA from peripheral blood by standard methods. Individuals were genotyped for the polymorphisms within *CFH* (rs1061170), *C3* (rs2230199), and *ARMS2* (rs10490924), by using SNP genotyping assays (Taqman; Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. Genotyping efficacy was >96%.

**Statistical Analyses**

The analyses of this report are based on the progression of AMD observed in single eyes; that is, individuals with two gradable eyes contributed two observations to our analyses. Of note, the presence and progression of disease in one eye is strongly associated with disease in the other eye. Quantification of the magnitude of increased risk in the contralateral eye thus provides prognostic information that cannot be disregarded when studying the influence of genetic variants on AMD progression rates in single eyes. Models containing the information of the contralateral eye are probably unbiased in their point estimates of odds ratios (provided that the model specification is correct); however, the correlation between the progression rates of the paired eyes has to be accounted for to avoid invalid precision of the estimates. To obtain corrected standard errors, approaches based on generalized estimating equations have been proposed (PROC GENMOD; SAS, ver. 9.2; SAS Cary, NC). We used logistic regression models with incident early or late progression as the outcome and the genetic variants as the predictor. Next, we adjusted the models for age, sex, and smoking history—that is, ever smoking and time since smoking cessation. Finally, we adjusted further for the AMD stage at baseline, using stages in the same and in the contralateral eye for those at risk for progression to late stages. For eyes at risk for early progression—showing, by definition, no or minimal AMD signs at baseline—the adjustment for AMD stage at baseline was omitted.

We defined genotypes as categorical variables with three levels and used Haploview to verify that the observed genotype distributions were in Hardy-Weinberg equilibrium (HWE; performed with STATA 9.0, Stata Corp. College Station, TX, in addition to SAS 9.2). We used logistic regression models with incident early or late progression as the outcome and the genetic variants as the predictor. Next, we adjusted the models for age, sex, and smoking history—that is, ever smoking and time since smoking cessation. Finally, we adjusted further for the AMD stage at baseline, using stages in the same and in the contralateral eye for those at risk for progression to late stages. For eyes at risk for early progression—showing, by definition, no or minimal AMD signs at baseline—the adjustment for AMD stage at baseline was omitted.

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**RESULTS**

There were 722 study participants at risk for AMD progression in at least one eye. Nine eyes (3 right eyes, 6 left eyes) of these participants were not gradable in MARS-II, leaving 1435 single eyes for progression analyses. Table 1 shows how AMD stages

### Table 1. Subject Characteristics at Baseline, Grouped According to AMD Stage in the Right or Left Eye

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Right Eyes (n = 719)</th>
<th>Left Eyes (n = 716)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AMD Stage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No or Minimal</td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>n (%)</td>
<td>386 (53.7)</td>
<td>221 (30.7)</td>
</tr>
<tr>
<td>Mean age, y</td>
<td>69.1</td>
<td>71.6</td>
</tr>
<tr>
<td>Proportion female, %</td>
<td>60</td>
<td>63</td>
</tr>
<tr>
<td>Proportion ever smokers, %</td>
<td>36</td>
<td>39</td>
</tr>
<tr>
<td><strong>CFHrs1061170</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT, %</td>
<td>30.3</td>
<td>22.0</td>
</tr>
<tr>
<td>CT, %</td>
<td>51.6</td>
<td>43.5</td>
</tr>
<tr>
<td>CC, %</td>
<td>18.1</td>
<td>34.6</td>
</tr>
<tr>
<td><strong>ARMS2rs10490924</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG, %</td>
<td>53.4</td>
<td>35.7</td>
</tr>
<tr>
<td>GT, %</td>
<td>38.1</td>
<td>47.9</td>
</tr>
<tr>
<td>TT, %</td>
<td>8.5</td>
<td>16.4</td>
</tr>
<tr>
<td><strong>C3rs2230199</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC, %</td>
<td>66.0</td>
<td>52.6</td>
</tr>
<tr>
<td>CG, %</td>
<td>29.7</td>
<td>39.9</td>
</tr>
<tr>
<td>GG, %</td>
<td>4.3</td>
<td>7.5</td>
</tr>
</tbody>
</table>

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**Statistical Analyses**

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**RESULTS**

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in the right and left eyes related to the cofactors at baseline. No or minimal AMD was slightly more common in left eyes, whereas both eyes were similar with regard to mean age, sex, and smoking history, as well as the frequency of genetic variants across the three stages of AMD.

Of the 791 eyes with no or minimal AMD at baseline, 31.8% progressed to early AMD, 2.0% to late AMD, whereas 19.2% of the 416 eyes with early AMD progressed to late AMD; 228 single eyes already had late AMD at baseline, whereas the fellow eye was still at risk of progression (Table 2).

Genotyping data were available for CFH-rs1061170 in 1397 (97.3%) single eyes, for ARMS2-rs10490924 in 1391 (96.9%) eyes, and for C3-rs2230199 in 1384 (96.1%) single eyes.

Table 3 shows the results for the risk of early progression. It became apparent that CFH-rs1061170 is very strongly and significantly related to early progression, even when adjustment was made for cofactors and AMD stage at baseline. By contrast, the associations of ARMS2-rs10490924 with early progression were much weaker and not statistically significant. The findings for C3-rs2230199 were weak and statistically imprecise.

By contrast, the associations with progression to the late stages showed a different picture. Table 4 demonstrates that, of the three genotypic variants considered in this study, ARMS2-rs10490924 was the only one to exhibit a significant relation to late progression in model 1. However, although the risk elevation was attenuated but still marked and statistically significant after adjusting for age, sex, smoking history, and baseline AMD stage in the same eye (OR = 2.02; P = 0.02), it disappeared when further controlling for the AMD stage of the contralateral eye (OR = 1.64; P = 0.12). CFH-rs1061170, on the other hand, showed only a very modest association with late progression which was removed completely by adjusting for AMD stages at baseline. Likewise, no effect was observed for C3-rs2230199 and late progression. We noted further that ever smoking was significantly associated with progression to the advanced disease stages (P = 0.002) but not with early progression.

### Discussion

It has now been well established that AMD is a complex, multifactorial disease with a prominent genetic component despite disease onset late in life.1 The research on the genetic etiology underwent substantial progress within the last years mainly using cases of prevalent AMD, although the evaluation of the impact of genotypic variants for disease progression was less common. In this report from the prospective MARS cohort, we identify strong and differential associations between genotypic risk variants and the advancement of AMD.

The results of MARS are difficult to compare to those from other studies because the latter commonly use patients as the unit of analysis. However, it is well known that the morphologic changes occurring in pairs of eyes do not progress synchronously in both eyes but at differential rates.1,2,3,32 Although the progression rate in one eye (or, for that matter, the AMD stage achieved in prevalent cases) is predictive of the prognosis in the contralateral eye, the precise temporal relation and determinants of this interrelation have never been clearly established.32 Therefore, we felt that single eyes were the more appropriate unit of analysis when aiming to evaluate the dynamics of AMD. On the other hand, proper account for the prognostic information contained in the AMD stage of the contralateral eye at the beginning of the risk period is necessary and best achieved by adjustment with GEE models.29 In fact, slow progressors may be more easily identified in analyses based on single eyes. Thus, we believe that despite the lack of direct comparability with other reports, our study results are equally valid, while maximizing the entire information with

<table>
<thead>
<tr>
<th>Gene</th>
<th>rsSNP ID</th>
<th>Single Eyes at Risk (n)</th>
<th>Variant</th>
<th>Non-progressors (%)</th>
<th>Progressors (%)</th>
<th>Risk for Early AMD OR (95% CI), Adjusted for Age, Sex, Smoking History</th>
<th>Risk of Early AMD OR (95% CI), Adjusted for Age, Sex, Smoking History, and AMD Stage in the Fellow Eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFH</td>
<td>rs1061170</td>
<td>754</td>
<td>TT</td>
<td>37</td>
<td>22</td>
<td>1.84 (1.23–2.75); P = 0.003</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TC</td>
<td>50</td>
<td>53</td>
<td>3.12 (1.84–5.27); P &lt; 0.0001</td>
<td>2.93 (1.70–5.05); P = 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CC</td>
<td>13</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>512</td>
<td>246</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARMS2</td>
<td>rs10490924</td>
<td>758</td>
<td>GG</td>
<td>58</td>
<td>48</td>
<td>1.36 (0.94–1.95); P = 0.10</td>
<td>1.28 (0.88–1.85); P = 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GT</td>
<td>36</td>
<td>41</td>
<td>1.96 (1.05–3.65); P = 0.04</td>
<td>1.61 (0.82–3.15); P = 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TT</td>
<td>6</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>507</td>
<td>243</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>rs2230199</td>
<td>750</td>
<td>CC</td>
<td>67</td>
<td>64</td>
<td>1.12 (0.77–1.61); P = 0.56</td>
<td>1.02 (0.73–1.54); P = 0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CG</td>
<td>29</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GG</td>
<td>4</td>
<td>6</td>
<td>1.33 (0.52–3.37); P = 0.55</td>
<td>1.34 (0.50–3.60); P = 0.56</td>
</tr>
</tbody>
</table>

Statistical power needed to detect a doubling of risk among homozygous: CFH, 99%; ARMS2, 79%; C3, 62% (α = 0.05).
Table 4. Genetic Variants in Three Genes and the Risk of Progression to Late AMD

<table>
<thead>
<tr>
<th>Gene</th>
<th>rsSNP ID</th>
<th>Risk of Late AMD OR (95% CI), Model 1: Adjusted for Age, Sex, Smoking History</th>
<th>Risk of Late AMD OR (95% CI), Model 2: as Fellow Eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFH</td>
<td>rs1061170</td>
<td>1.0 (0.63–2.40); P = 0.70</td>
<td>1.0 (0.63–2.40); P = 0.70</td>
</tr>
<tr>
<td></td>
<td>rs10490924</td>
<td>1.0 (0.63–2.40); P = 0.70</td>
<td>1.0 (0.63–2.40); P = 0.70</td>
</tr>
<tr>
<td></td>
<td>rs2230999</td>
<td>1.0 (0.63–2.40); P = 0.70</td>
<td>1.0 (0.63–2.40); P = 0.70</td>
</tr>
</tbody>
</table>

Statistical power to detect a doubling of risk among homozygous: CFH, 98%; ARMS2, 82%; and C3, 52%; (c = 0.05).

High discriminatory power for the description of the dynamics of AMD.

Multiple studies have confirmed marked associations between SNPs in the CFH- and ARMS2 gene, more recently also the C3 gene, and the progression to advanced stages of AMD.25–28 However, the investigation of whether the relationship between genotypic variants and the progression to early disease is different from that with progression to late AMD has frequently been neglected. Our analyses seem to corroborate a hypothesis formulated in a previous analysis of prevalent AMD cases in the MARS baseline examination.26 There, we had observed an association between CFH-rs1061170 and the presence of early AMD, while ARMS2-rs10490924 seemed mainly related to prevalent late AMD.26 On stratifying between progression to early and late stages in our cohort, we now show that CFH-rs1061170 was indeed mostly involved in early progression, that is, the onset of drusen and pigmentary abnormalities, while the influence of ARMS2-rs10490924 was mainly detectable for the progression to neovascularization and/or atrophy. Our findings concerning the association between the C3 variant and early or late progression were inconclusive and lacked statistical significance. Associations of C3 with the development of early AMD have been addressed only in a report from the Rotterdam Study.28 However, these authors had to pool prevalent and incident cases and patients from a further case-control study to produce a result with some statistical significance. Contrary to findings of others, our results do not indicate a risk-increasing effect of C3 with regard to late-stage progression.25,33 We note, however, that our results are based on different study design, low statistical power and a short follow-up period.

A specific methodological aspect deserves mentioning. Table 1 reveals that the impact of ARMS2-rs10490924 was massively reduced by adjusting for baseline AMD stage in the same and the companion eye. This finding should not be taken to imply that the genotypic variant is causally less relevant once ophthalmic morphology is taken into account. Rather, a cross-classification of AMD status and genotypes at baseline revealed that carriers of the ARMS2 SNP were significantly more common among patients who already had advanced AMD at the onset of our follow-up, when compared with carriers of the CFH or C3 variant. This reflects the fact that in the MARS cohort, a study sample of prevalent cases with different AMD stages and of controls, the ARMS2 gene was already enriched in those who had late AMD in one eye. Thus, the prognostic information conferred by late AMD in the one eye for those still at risk for late progression in the companion eye captures much of the predictive power contributed by the genotype, including that having both types of information in one model results in a competition for the explanation of the same causal component, that is, overadjustment. An attenuation of the partial model attribution of genotype may therefore not be misinterpreted as refutation of a causal role. In contrast, it is well in accordance with our hypothesis of a specific and pronounced causal role of ARMS2 in progression of AMD to the clinically most relevant stages.

It is now widely assumed that the different morphologic forms of AMD are the result of a complex interplay between genetic and environmental risk factors.5–6,34 However, the three genes in the present study have various functions and are involved in different biological processes and/or pathways. CFH, for example, is the major inhibitor of the alternative complement cascade and has been associated with drusen formation.37,38 Cleavage products of C3, the central component of the complement cascade, have been found in drusen and seem to promote choroidal neovascularization.42 Recently, an animal study provided new insights into the development of AMD late in life by high-
lighting the role that normal aging processes of the RPE may have for disease onset.45 The authors observed that the complement cascade was activated in the RPE/choroid of normal mice as they aged, indicating that this tissue became immunologically active. They proposed that these age-related changes provide the background for the causation of AMD and that it is due to errors in regulation of immunologic activity. Genotypic variation in the genes of the complement system must be an influential factor in this causative process. In contrast, the function of the ARMS2 gene is still poorly understood,6,12 although it has recently been reported that expression of ARMS2 is present in mitochondria of cells of the human retina.53

On the basis of different biological functions, expression patterns, and previous observations, we hypothesize a step model for the pathogenesis of AMD. Thus, dysfunction of immunoregulatory mechanisms, probably triggered by common aging processes, in concurrence with genotypic variation of the complement system and/or oxidative stress will presumably result in substantial changes in the retinal pigment epithelium (RPE), leading to the formation of drusen. This is also in accordance with the pathogenesis model of Kanda et al.44 who suggested that genetic variations in the complement system predispose the aging RPE to develop maculopathy. Contrasting the Kanda model, our data lead us to propose that disease onset is mainly triggered by genetic variations and normal aging, whereas environmental factors like enduring oxidative stress—for example, due to smoking or light exposure—may compound early signs of AMD and affect predominantly the progression of the disease.29

Polymorphisms in the ARMS2 gene and their presently incompletely understood functional role in mitochondrial pathways (e.g., oxidative stress) seem to initiate or aggravate the progression process.24 Findings of Schmidt et al.45 as well as Schauemberg et al.24 point toward a crucial role of ARMS2-rs10490924 especially for neovascularization, which was markedly intensified by smoking. These findings further support the hypothesis that ARMS2 and cigarette smoking act in closely related pathways on AMD development and progression, maybe via oxidative stress.

Future studies are needed to differentiate between the clinical forms of late AMD and disentangle the complex underlying pathobiology.

Strengths of the present study include the prospective study design, a pragmatic sample of clinical patients with different stages of disease better suited to study progression risk over a fairly short follow-up than population-based samples, the employment of a standardized digital fundus grading system, and a high completeness of follow-up in this elderly study group.

Potential limitations of our approach lie in the lack of comparability with most other studies as we opted for single eyes as the unit of analysis. All SNPs in our study sample were in HWE, especially in the group free of AMD at baseline. Furthermore, the distribution of the minor allele frequencies (MAFs) was similar to that in other prospective studies.23–25,28 The short follow-up period of 2.6 years was too short to safely identify a sufficient number of new cases especially with late AMD. In other studies included longer follow-up periods, probably to determine late stage progression with sufficient power.23,24 Likewise, in population-based studies, the advancement to early and particularly late stages must be observed for longer periods to achieve the necessary statistical power.

In summary, our study provides new insights into the complex etiology of AMD by identifying a prominent role for the CFH gene in the progression to early AMD and for the ARMS2 gene in progression to late AMD. In combining findings of prevalent and incident associations between specific genotypes, environmental risk factors and the different features of AMD, our findings indicate a crucial role of inflammation in the onset of AMD, whereas the functional contribution of the ARMS2 gene to the development of advanced disease stages remains to be further elucidated. Future work on the interaction of genotypic variation, environmental factors, and biological aging processes appear particularly promising.

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357:553–561.

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357:553–561.

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