Low Plasma Concentrations of Retinol-Binding Protein in Individuals with Mutations Affecting Position 84 of the Transthyretin Molecule

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Retinol-binding protein (RBP), the principal carrier for vitamin A, is known to form a complex with transthyretin (TTR) for transport in plasma. Individuals from a kindred with the amino acid substitution of serine for isoleucine at position 84 (Ser84) of the TTR molecule show substantial reduction in plasma concentrations of RBP. In the present study, we measured plasma RBP in individuals from several kindreds, demonstrating 17 different point mutations within the TTR gene. In each case, these mutations caused single amino acid substitutions at various positions throughout the TTR molecule. Of all the individuals examined, only those with mutations causing amino acid substitutions at position 84 of the TTR molecule (Ser84 and Asn84) demonstrated substantial decreases in plasma concentrations of RBP. These results suggest that the isoleucine at position 84 on the TTR molecule may be critically involved in mediating RBP binding. Further, these findings demonstrate the importance of considering TTR gene mutations when clinically evaluating patients with low RBP.

Indexing Terms: familial amyloidotic polyneuropathy/enzyme-linked immunosorbent assay/pretalbinum

Retinol-binding protein (RBP), a 184-amino-acid-residue 21-kDa protein (1), is synthesized in hepatocytes and plays a role in the transport of vitamin A alcohol (retinol). Ordinarily, RBP binds to transthyretin (TTR) for transport in plasma, and thus is found primarily as a complexed holoprotein (2). Upon delivery of vitamin A to the target membrane, RBP dissociates from TTR (3). RBP has a very short half-life in blood, suggesting that RBP not bound to TTR is subject to rapid glomerular filtration (4, 5). Thus, under normal circumstances, plasma concentrations of RBP would be expected to correlate with the concentrations of TTR.

Plasma concentrations of RBP are often evaluated clinically as an index of nutritional status because numerous clinical conditions are known to influence circulating RBP. For example, oliguric renal failure produces increased plasma RBP, whereas liver dysfunction, hyperthyroidism, and other forms of renal disease produce abnormally low concentrations of plasma RBP (6). Further, Benson and Dwulet have shown substantial reductions in plasma RBP in affected members of an Indiana/Swiss kindred with familial amyloidotic polyneuropathy (FAP) (7). The affected members of this kindred were also found to have low plasma concentrations of TTR, a condition characteristic of advanced stages of FAP. In addition, about half the offspring from affected members of this kindred demonstrated marked decreases in RBP, whereas the other half did not. The offspring with low RBP were found to have normal (or only moderately reduced) TTR concentrations, indicating that low TTR could not solely account for the reduced concentrations of RBP in these individuals.

Since the time of this initial report, a variant TTR protein containing the amino acid substitution of serine for isoleucine at position 84 (Ser84) was found to be responsible for FAP in this kindred (8). A specific point mutation in the TTR gene was later identified as the cause of this variant protein (9). Because DNA diagnosis was not available at the time of the initial report of the kindred, the authors did not demonstrate whether reductions in RBP concentrations, without abnormal reductions in TTR, cosegregate with the Ser84 TTR variant.

At least 50 different point mutations in the TTR gene have been reported, most of which are responsible for various forms of hereditary amyloidosis in different kindreds (for review, see 10). However, a thorough study of RBP concentrations in patients with TTR variants other than Ser84 has not been conducted. An in-depth examination of these other TTR variants may shed light on the formation of the TTR:RBP complex.

The goals of the present study were twofold. First, we sought to examine the extent to which lowered concentrations of plasma RBP cosegregate with the Ser84 gene mutation in members of the Indiana/Swiss FAP kindred, before the onset of clinical symptomology. Second, we sought to examine the effect of other amino acid substitutions, at various positions throughout the TTR molecule, on plasma concentrations of RBP. To this end, we measured RBP in members of several kindreds, who demonstrated a total of 17 different point mutations in the TTR gene.

Materials and Methods

RBP Concentrations in Members of the Indiana/Swiss Ser84 Kindred

RBP and TTR concentrations in plasma from 19 children of affected members of the Indiana/Swiss FAP kindred were determined. Of those 19 individuals at risk, 9 were positive for the Ser84 TTR gene mutation by DNA analysis; the other 10 were negative. Diagno-
sis of TTR gene mutations was performed by restriction fragment length polymorphism analysis, with previously published procedures (11). All Ser84-positive individuals in this study were found to be heterozygous for the TTR gene mutation. For all individuals in this study, blood was collected before the onset of the clinical symptomology of amyloidosis.

Plasma RBP was measured with an ELISA developed in our laboratory. Plasma samples were diluted 1:20 000 and 1:40 000 with 0.05 mol/L carbonate–bicarbonate buffer (pH 9.6) to a final volume of 100 μL, and coated onto a 96-well polystyrene microtiter plate. After incubation overnight at 4 °C, the plates were blocked with 30 g/L bovine serum albumin (BSA) in phosphate-buffered saline (PBS), again overnight at 4 °C, and then incubated for 2 h at 37 °C with rabbit anti-human RBP antibody (Dako, Carpenteria, CA) diluted 1:1000. After washing, plates were incubated with 2000-fold-diluted alkaline phosphatase-conjugated anti-rabbit immunoglobulins (Hyclone, Logan, UT) for 1 h at 37 °C. Color was developed by incubation with a phosphatase substrate (Sigma, St. Louis, MO) for 15 min, and the absorbance was read at 410 nm with an MR600 microplate reader (Dynatech, Alexandria, VA). Pooled human serum, the RBP content of which was determined by nephelometric immunoassay (Behring Diagnostics, Somerville, NJ) was used for assay calibration. To establish the accuracy of this ELISA, we measured RBP in 24 human plasma samples both by this ELISA and by a previously reported radial immunodiffusion assay (CalBiochem, San Diego, CA) (7). The values obtained by these two methods were positively correlated (Pearson r = 0.94, n = 24).

Plasma TTR concentrations were also determined with an ELISA developed in our laboratory. Anti-human TTR antibody (The Binding Site, San Diego, CA) was diluted 2000-fold to a final volume of 100 μL and used to coat 96-well microtiter plates as above. After being blocked with 30 g/L BSA, the plates were incubated with plasma samples, diluted 1:100 000, for 3 h at room temperature. After washing, the plates were incubated for 2 h at room temperature with alkaline phosphatase-conjugated anti-human TTR (The Binding Site), followed by color development as above. Pooled human serum was used for assay calibration. To establish the accuracy of this ELISA, we measured TTR in 24 human plasma samples both by this ELISA and by radial immunodiffusion (CalBiochem) (7). The values obtained by these two methods were positively correlated (Pearson r = 0.92, n = 24).

Effect of Several TTR Gene Mutations on RBP Concentration

Plasma RBP and TTR concentrations were determined for individuals in several kindreds with TTR-related hereditary amyloidosis, representing 17 different TTR gene mutations (Table 1). In addition to members of the Indiana/Swiss Ser84 kindred, one individual from a newly identified Hungarian kindred with the Ser84 TTR variant, and one individual with the amino acid substitution of asparagine for isoleucine at position 84 (TTR Asn84; 20) were studied. The control group consisted of 40 healthy individuals, all of whom tested negative for TTR gene mutations. Plasma RBP and TTR were measured in these individuals with the ELISA procedures described above.

Results

The mean plasma concentrations of TTR and RBP in the Ser84-negative siblings were 346 mg/L (range 255–445) and 51 mg/L (range 39–68), respectively, and correlated significantly in these individuals (Pearson r = 0.82). Mean plasma concentrations of TTR and RBP in the siblings positive for the Ser84 TTR gene mutation were 266 mg/L (range 215–315) and 18 mg/L (range 14–22), respectively, also significantly correlated (Pearson r = 0.78).

Between-groups comparisons of plasma TTR and RBP concentrations in the Ser84-positive and -negative siblings by Student’s t-tests were not significant (P >0.05) for TTR; however, the individuals carrying the Ser84 TTR mutation had significantly lower (P <0.01) plasma RBP concentrations than their mutation-negative siblings (Fig. 1).

Mean plasma TTR and RBP in the controls were 371

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**Table 1. TTR variants and associated clinical features of kindreds examined in this study.**

<table>
<thead>
<tr>
<th>Amino acid substitution</th>
<th>No. in group</th>
<th>Clinical features</th>
<th>Reference</th>
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<tbody>
<tr>
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<td>Asymptomatic</td>
<td>Unpubl.</td>
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<tr>
<td>Arg for Cys 10</td>
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<td>Unpubl.</td>
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<tr>
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<td>Polyneuropathy</td>
<td>14</td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>His for Leu 58</td>
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<tr>
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<td>5</td>
<td>Cardiomyopathy, carpal tunnel syndrome</td>
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<tr>
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<td>18</td>
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<tr>
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<td>3</td>
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<td>19</td>
</tr>
<tr>
<td>Ser for ile 84I (Indiana/Swiss)</td>
<td>9</td>
<td>Vitreous opacity, cardiomyopathy, carpal tunnel syndrome</td>
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<td>ile for Val 122</td>
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mg/L (range 235–465) and 58 mg/L (range 32–72), respectively. The RBP concentrations were significantly greater than in members of the Indiana/Swiss kindred with TTR Ser84 (P < 0.001 by Student’s t-test), but the TTR concentrations were not significantly different (Fig. 2). RBP was also decreased in the Hungarian Ser84 individual (18 mg/L), but the plasma TTR was not abnormally low (240 mg/L). Compared with the control group, the individual with the Asn84 TTR gene mutation showed decreased plasma RBP (19 mg/L) and a moderate reduction in plasma TTR (224 mg/L). Statistical tests were not performed in these latter two cases, given n = 1 in each group.

Discussion

In this study, we found significant decreases in plasma RBP concentrations in Ser84 gene-carriers of the Indiana/Swiss FAP kindred, relative to non-Ser84 gene carriers and normal controls. No individual with the Ser84 TTR variant had an RBP concentration > 30 mg/L, and none of the individuals in our study without TTR gene mutations had RBP < 30 mg/L. Notable reductions in plasma RBP were also evident in a TTR Ser84-positive individual from the Hungarian kindred, as well as an individual with TTR Asn84. Because the plasma TTR concentrations in these individuals were not below the expected range for normal healthy adults, decreases in plasma TTR cannot solely account for the low RBP values.

No significant reductions in plasma RBP concentrations were found in individuals with any of the other TTR gene mutations examined. Some individuals (e.g., His69, His74) appeared to have slightly increased RBP; however, these increases were not statistically significant.

RBP concentrations have previously been examined in individuals with the Met30 TTR gene mutation (FAP Type I). Saraiva et al. reported finding no abnormal decreases in RBP in members of a Met30 kindred (23). Shoji and Nakagawa reported finding low RBP in another Met30 kindred (24); however, these reductions were dramatic only in clinically affected members with correspondingly low concentrations of plasma TTR. These results suggest that decreased RBP is not present in Met30 gene carriers antecedent to the onset of clinical symptomology. Apparently, reduction in plasma RBP concentrations, without substantial decreases in plasma TTR, may be specific to mutations affecting position 84 of the TTR molecule. This is consistent with the hypothesis that specific amino acid substitutions at position 84 of the TTR molecule alter the active binding site for RBP. The preservation of a correlation between TTR and RBP concentrations in Ser84 individuals may be a result of RBP binding to the

Fig. 1. Plasma RBP and TTR concentrations in members of the Indiana/Swiss FAP kindred. (*) Individuals positive for TTR Ser84; (○) individuals negative for TTR Ser84.

Fig. 2. Mean ± SD plasma RBP in individuals with TTR gene mutations: Ser84I, members of the Indiana/Swiss kindred; Ser84H, a member of the Hungarian TTR Ser84 kindred. *, statistically significant decrease in RBP (P < 0.001).
normal TTR allele product in these heterozygous subjects.

Although x-ray crystallographic examinations of the TTR molecule and of the TTR-RBP complex are ongoing, the specific surface(s) on the TTR molecule involved in RBP binding is not fully understood at this time. Crystallographic studies indicate overall structural homology between normal TTR and Ser84 TTR (25), but protein binding kinetics have revealed a marked decrease in RBP affinity for recombinant Ser84 TTR, compared with that for recombinant normal TTR (26). This finding is well supported by our study of in vivo conditions.

Plasma RBP concentration is often measured as an index of nutritional status. Our present findings demonstrate the importance of considering TTR gene mutations at position 84 for patients with low RBP. For example, Matsuo et al. reported a Japanese kindred wherein affected members clinically presented with low plasma RBP, low circulating retinol, and normal TTR concentrations (27). The authors suggested an autosomal dominant pattern of inheritance in this kindred, and proposed that affected individuals might be heterozygous for a mutation in the gene encoding RBP—although no such mutation was known at the time of the report. As we have demonstrated, mutations in the TTR gene affecting position 84 may result in the clinical profile described by Matsuo et al., and thus should be investigated as a candidate cause in such cases.

In conclusion, our study indicates that the isoleucine at position 84 of TTR plays an integral role in RBP binding by the transthyretin molecule and demonstrates that RBP concentrations may be reduced in individuals with Ser84 and Asn84 TTR before the onset of clinical symptomology.

We thank Martha Skinner for graciously providing several samples for analysis in this study. This work was supported by Veterans Affairs Medical Research (MR1S 583-0888), and grants from RR-00750 (GCRC), US Public Health Service, NIDDK-42111, AG 10608, National Institute of Arthritis Metabolism and Digestive Diseases (AM20582), The Arthritis Foundation, and the Marion E. Jacobson Fund. R.P.W. is supported, in part, by the graduate training fund, Indiana University School of Medicine.

References