Cholesteryl Ester Transfer Protein and Phospholipid Transfer Protein Have Nonoverlapping Functions in Vivo*

Koichi Kawano, Shu-Cun Qin, Min Lin, Alan R. Tall, and Xian-cheng Jiang†

From the Division of Molecular Medicine, Department of Medicine, Columbia University, New York, New York 10032

Plasma phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) are homologous molecules that mediate neutral lipid and phospholipid exchange between plasma lipoproteins. Biochemical experiments suggest that only CETP can transfer neutral lipids but that there could be overlap in the ability of PLTP and CETP to transfer or exchange phospholipids. Recently developed PLTP gene knock-out (PLTP0) mice have complete deficiency of plasma phospholipid transfer activity and markedly reduced high density lipoprotein (HDL) levels. To see whether CETP can compensate for PLTP deficiency in vivo, we bred the CETP transgene (CETPTg) into the PLTP0 background. Using an in vivo assay to measure the transfer of [3H]PC from VLDL into HDL or an in vitro assay that determined [3H]PC transfer from vesicles into HDL, we could detect no phospholipid transfer activity in either PLTP0 or CETPTg/PLTP0 mice. On a chow diet, HDL-PL, HDL-CE, and HDL-apoprotein AI in CETPTg/PLTP0 mice were significantly lower than in PLTP0 mice (45 ± 2 versus 79 ± 9 mg/dl; 9 ± 2 versus 16 ± 5 mg/dl; and 51 ± 6 versus 100 ± 9, arbitrary units, respectively). Similar results were obtained on a high fat, high cholesterol diet. These results indicate 1) that there is no redundancy in function of PLTP and CETP in vivo and 2) that the combination of the CETP transgene with PLTP deficiency results in an additive lowering of HDL levels, suggesting that the phenotype of a human PLTP deficiency state would include reduced HDL levels.

The plasma lipid transfer proteins, phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP), play a central role in high density lipoprotein (HDL) metabolism. PLTP and CETP belong to a family of lipid transfer/ligand-binding proteins and show sequence homology, related gene structures and probably share a similar protein fold (1). Genetic models have been central to elucidating the role of PLTP and CETP in mouse and human physiology. The importance of CETP in HDL metabolism and reverse cholesterol transport has been elucidated by human genetic deficiency of CETP (2–4), as well as by the introduction of CETP, normally absent from mouse plasma, into mice by transgenesis (5–8). In human CETP deficiency, HDL levels are markedly elevated, whereas the opposite effect was observed in CETP transgenic mice.

Markedly reduced plasma HDL levels in PLTP gene knock-out mice have recently provided the first in vivo evidence for a crucial role of PLTP-mediated lipid transfer in the maintenance of HDL levels (9). PLTP also facilitates free cholesterol influx into HDL (9). This is followed by CE formation by lecithin:cholesterol acyltransferase and, in species such as humans, redistribution of CE to triglyceride-rich lipoproteins by CETP.

Although biochemical experiments indicate that PLTP and CETP have different abilities to transfer neutral lipids between lipoproteins, they also suggest overlap in their ability to transfer or exchange phospholipids. Both lipid transfer proteins have been reported to facilitate phospholipid exchange between the plasma lipoproteins (10, 11). In humans with genetic deficiency of CETP, plasma phospholipid exchange activities are about 50% of normal, suggesting that CETP and PLTP might contribute equally to this activity in human plasma (12). However, PLTP enhances net transfer of phospholipids from phosphatidylcholine (PC) vesicles into HDL, whereas CETP does not (10, 11). This is thought to be analogous to the transfer of very low density lipoprotein (VLDL) phospholipids into HDL, but the ability of CETP to transfer phospholipids from VLDL into HDL has never been tested in vivo. Thus it is possible that there is in vivo redundancy in function. In the present study, this was evaluated by crossing the CETP transgene into the PLTP0 background. If there is redundancy of phospholipid transfer activities, CETP would be expected to ameliorate the low HDL state of PLTP deficiency.

MATERIALS AND METHODS

Animals and Diets Used in This Study—All phenotypic characterization was performed with female wild type (Wt), PLTP0, CETPTg, and CETPTg/PLTP0 mice (in the C57BL/6 background), 10–12 weeks old. Two diets were used: Chow diet (Purina Laboratory Rodent Chow 5001) or chow diet plus 20% hydrogenated coconut oil and 0.15% cholesterol (Research Diets Inc., New Brunswick, NJ).

Lipids and Lipoprotein Measurements—Fasted blood (food was removed at 9:00 a.m., and blood was collected at 4:00 p.m.) was collected for lipoprotein isolation and lipid measurement. Total cholesterol, free cholesterol (FC), and phospholipid (PL) in plasma and lipoproteins were assayed by enzymatic methods (Wako Pure Chemical Industries Ltd., Osaka, Japan). Cholesteryl ester concentration was calculated by subtracting the amount of free cholesterol from the total plasma cholesterol. Lipoprotein profiles were obtained by fast protein liquid chromatography using a Sepharose 6B column as described previously (9). A 200-μl aliquot of pooled plasma (from 7 animals) was loaded onto the column and eluted with TS buffer (50 mM Tris, 0.15 M NaCl, pH 7.5) at a constant flow rate of 0.35 ml/min. An aliquot of 80 μl from each fraction (1 ml) was used for the determination of total cholesterol and phospholipid.

Received for publication, April 25, 2000, and in revised form, June 22, 2000
Published, JBC Papers in Press, July 12, 2000, DOI 10.1074/jbc.M003523200

This paper is available on line at http://www.jbc.org

* This work was supported by National Institutes of Health Grant HL-54591. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Div. of Molecular Medicine, Dept. of Medicine, Columbia University, 630 W. 168th St., New York, NY 10032. Tel.: 212-305-7720; Fax: 212-305-5052; E-mail: xcj1@columbia.edu.

‡ The abbreviations used are: PLTP, phospholipid transfer protein; CETP, cholesteryl ester transfer protein; FC, free cholesterol; PL, phospholipid; Wt, wild type; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; PC, phosphatidylcholine; PAGE, polyacrylamide gel electrophoresis; CE, cholesteryl ester; Tg, transgenic.

Printed in U.S.A.
PLTP Gene Knock-out and CETP Transgenic Mice

RESULTS

PLTP Activity Assays—To evaluate possible redundancy in the in vivo functions of PLTP and CETP, we crossed CETPTg and PLTP0 mice, both in the C57BL/6 background. Plasma PLTP activity analysis using [3H]PC vesicles as donor and HDL as acceptor, showed no PC transfer activity in either PLTP0 or CETPTg/PLTP0 mice (Fig. 1). This activity was detected at similar levels in Wt and CETPTg mice (Fig. 1).

To evaluate the in vivo transfer of phospholipid between plasma lipoproteins, [3H]PC-PLTP was injected intravenously into mice of different genotypes. In CETPTg mice, there was rapid transfer of a substantial portion of PC radioactivity into HDL. In contrast, [3H]PC transfer from VLDL to HDL was almost absent in CETPTg/PLTP0 mice (Fig. 2), similar to results obtained in PLTP0 mice (9). This suggests that in vivo CETP has no PLTP-like phospholipid transfer function, at least based on these assays.

Plasma CETP activity was also measured in these mice, using [3H]HDL-HDL as a donor and excess unlabeled LDL as acceptor. This showed significantly lower activity in CETPTg/PLTP0 mice, compared with CETPTg mice (Fig. 3, 52 ± 6 versus 100 ± 9, arbitrary units, p < 0.001). As expected, CE transfer activity was not detected in either Wt or PLTP0 mice (Fig. 3). To confirm the result, we quantitated CETP mass in CETPTg and CETPTg/PLTP0 mouse plasma by agarose electrophoresis and immunoblot analysis. By this assay, CETP levels in CETPTg/PLTP0 mice were 25% (p < 0.05) lower than in CETPTg mice (Fig. 4).

Plasma Lipoprotein Analysis—The distribution of plasma lipoprotein lipids was determined by fast protein liquid chromatography of pooled plasma samples (Fig. 5). In PLTP0 mice, all HDL lipids were markedly reduced compared with Wt mice. Importantly, in CETPTg/PLTP0 mice, HDL-PL and HDL-CE were significantly decreased compared with PLTP0 mice. Plasma lipoprotein analysis by precipitation showed a significant reduction in total PL, HDL-PL and cholesteryl ester (HDL-CE) (31, 43, and 44%, respectively) in CETPTg/PLTP0 mice versus PLTP0 littermates (Table I). Non-HDL lipids were not significantly altered. All lipid levels, except non-HDL-FC, were significantly decreased in both PLTP0 and CETPTg/PLTP0 mice compared with Wt and CETPTg mice (Table I).

Assessment of apolipoprotein composition of centrifugally isolated lipoproteins by reducing SDS-PAGE gels revealed a significant decrease of apolipoprotein AI in HDL (51 ± 6 versus 100 ± 9, arbitrary units, p < 0.01) (Fig. 6). We noted that apoB100 was decreased in non-HDL from PLTP0 mice compared with Wt mice, as suggested previously (9). Also apoB100 was increased in non-HDL from CETPTg mice compared with Wt mice (Fig. 6), which is probably due to the down-regulation of the LDL receptor (17).
sion has an impact on the accumulation of these particles, 3-month-old female mice were challenged with the high fat diet for 2 weeks. HDL-PL and HDL-CE were significantly decreased in CETPTg/PLTP0 mice compared with PLTP0 mice (18 and 48%, respectively) (Table I). Like the PLTP0 mice, the double mutant mice also showed a dramatic increase in non-HDL-FC and non-HDL-PL levels compared with Wt and CETPTg mice (Table I). However, there was no significant difference between CETPTg/PLTP0 and PLTP0 mice in terms of the accumulation of FC/PL-rich particles. These results suggest that the increased flux of lipid through the plasma compartment on the coconut oil-based high fat diet leads to accumulation of FC- and PL-enriched surface components of triglyceride-rich lipoprotein in the non-HDL fraction in PLTP-deficient mice. However, the expression of the human CETP gene in these mice does not influence the accumulation of such particles.

**DISCUSSION**

Related members of the same gene family often show in vivo redundancy in their functions. As a result, knock-out of one gene may not result in an in vivo phenotype, until combined with deficiency of the related compensating gene. LDL receptor-related protein is an example, where the lipoprotein phenotype only becomes evident after combination with a knock-out of the LDL receptor (18). Even though CETP and PLTP show moderate homology of sequence (19) and similar structural features (1, 20), they show no overlap in their in vivo functions. This was demonstrated in the present study by preparing CETPTg/PLTP0 mice; the expression of CETP did not rescue the low HDL phenotype of PLTP deficiency. In fact the phenotypes were additive, resulting in markedly reduced HDL levels in the CETPTg/PLTP0 mouse. Similarly, the related plasma lipopolysaccharide-binding protein, even though it has some ability to transfer phospholipids in vitro (21), does not compensate for deficiency of PLTP in vivo (9). Thus, these different members of the lipid transfer/lipopolysaccharide-binding gene family have evolved diversified, nonoverlapping functions.

PLTP and CETP were originally shown to have distinct abilities to mediate net transfer of phospholipids from PC vesicles into HDL, even though both could stimulate phospholipid exchange between HDL and LDL or VLDL (10). Both exchange and net transfer activities were stimulated by lipolysis of VLDL (22). The present findings support the original view that the net transfer of phospholipids from vesicles into HDL could be regarded as a model of the transfer of phospholipids from VLDL to HDL during lipolysis in vivo (10). Recent studies in PLTP0 mice have shown that low HDL primarily results from increased catabolism of HDL lipid and protein (23). Thus, the net transfer of phospholipids from VLDL to HDL serves to metabolically stabilize HDL in the circulation. In PLTP0 mice, the HDL is specifically depleted in PC mole-

---

**FIG. 3. CETP Activity Assay.** CE transfer activity was measured by incubating 3 μl of plasma from different mice with [3H]-CE-containing HDL and unlabeled LDL for 4 h and then precipitating the LDL by the heparin/manganese method and counting an aliquot of the supernatant. Results are shown for Wt mice (n = 8), CETPTg mice (n = 6), PLTP0 mice (n = 7), and CETPTg/PLTP0 mice (n = 6).

**FIG. 4. Detection of CETP in CETPTg and CETPTg/PLTP0 mouse plasma.** 10 μl of mouse plasma was run on a sodium barbital/barbital buffered agarose gel and blotted onto a nitrocellulose membrane as described under "Materials and Methods." CETP was detected by blotting with 125I-labeled anti-CETP mAb (TP2). The relative CETP concentration was determined by quantitative scanning using a PhosphorImager.

**FIG. 5. Plasma lipoprotein analysis by fast protein liquid chromatography.** An aliquot (200 μl) of pooled plasma (from six to eight animals) was loaded onto a Superose 6B column and eluted with TS buffer (50 mM Tris, 0.15 M NaCl, pH 7.5). An aliquot of 80 μl from each fraction (1 ml) was used for the determination of free cholesterol, cholesteryl ester, and phospholipid.
TABLE I
Plasma and lipoprotein lipid analysis in Wt, CETPTg, PLTP0, and CETPTg/PLTP0 mice on a chow diet and a high fat high cholesterol diet

| Mice          | Plasma PL | HDL PL | Non-HDL PL | Plasma Chol | HDL CE | HDL FC | Non-HDL CE | Non-HDL FC |
|---------------|-----------|--------|------------|-------------|--------|--------|------------|------------|
| Chow diet     |           |        |            |             |        |        |            |            |
| Wt            | 171 ± 16  | 130 ± 4 | 41 ± 9     | 78 ± 8      | 44 ± 6 | 16 ± 3 | 15 ± 6     | 4 ± 1      |
| CETPTg        | 186 ± 26  | 139 ± 12| 47 ± 6     | 72 ± 9      | 36 ± 7 | 14 ± 2 | 16 ± 7     | 3 ± 2      |
| PLTP0         | 124 ± 16  | 79 ± 9  | 45 ± 7     | 40 ± 5      | 16 ± 5 | 6 ± 3  | 14 ± 4     | 3 ± 1      |
| CETPTg/PLTP0  | 85 ± 11b  | 45 ± 7  | 40 ± 5     | 30 ± 6a     | 9 ± 2b | 5 ± 2  | 13 ± 2     | 3 ± 2      |
| High Fat diet |           |        |            |             |        |        |            |            |
| Wt            | 225 ± 20  | 165 ± 16| 71 ± 8     | 118 ± 15    | 70 ± 9 | 14 ± 4 | 24 ± 8     | 10 ± 5     |
| CETPTg        | 230 ± 36  | 150 ± 19| 80 ± 7     | 111 ± 26    | 62 ± 8 | 11 ± 3 | 30 ± 7     | 8 ± 2      |
| PLTP0         | 210 ± 28  | 77 ± 9  | 133 ± 22   | 120 ± 20    | 23 ± 8 | 8 ± 3  | 36 ± 10    | 55 ± 15    |
| CETPTg/PLTP0  | 202 ± 21  | 63 ± 7  | 139 ± 33   | 115 ± 12    | 12 ± 2 | 6 ± 2  | 39 ± 7     | 59 ± 9     |

*p < 0.05, Wt mice versus CETPTg mice.
*b p < 0.01, PLTP0 mice versus CETPTg/PLTP0 mice.
*c p < 0.05, PLTP0 mice versus CETPTg/PLTP0 mice.

(1) There are two lipid-binding pockets, one in the N terminus and the other in the C terminus. The inability of PLTP to transfer CE and triglyceride could perhaps be related to the distinctive C-terminal helical peptide of CETP, which plays an essential role in neutral lipid transfer activity (26). This peptide may perturb the surface of HDL in a way that facilitates entry of CE molecules into the N-terminal pocket. Recent site-directed mutagenesis studies indicate that the N-terminal lipid-binding pockets of both CETP and PLTP are involved in phospholipid exchange activity (20). Thus, the distinct functions of PLTP and CETP in mediating net phospholipid movement are probably not related to the properties of the lipid-binding pockets. Rather this could reflect different abilities of PLTP and CETP to bind to phospholipid donors in the circulation or to a distinct ability of PLTP to mediate fusion of lipoproteins or lipoproteins and vesicles (27). This latter property is poorly understood but could perhaps involve ternary complex formation.

Crossing human apolipoprotein AITg mice with either human CETPTg or human LCATTg mice, the phenotypic effect of CETP and LCAT becomes much more pronounced, indicating that human CETP and LCAT work much better when they use human-like HDL as their substrate in mice (8, 29). This preference is also shared by PLTP (30). There is a possibility that the inability of CETP to reverse the phenotype of PLTP0 mice may be due to the fact that the HDL in these mice are a suboptimal lipoprotein substrate for CETP. However, it is not likely, because HDL levels in CETPTg/PLTP0 mice are further decreased compared with PLTP0 mice, indicating that HDL from PLTP0 mice still can serve as a CETP substrate.

Several genetically determined low HDL states are now known, including rare apoA-I mutations (31), common nonsense or truncation variants in lipoprotein lipase (32), and, most recently, mutations in ABC1 (homozygous in the rare Tangier Disease and heterozygous in more common familial hypoalphalipoproteinemia) (33–35). Although a human deficiency state of PLTP has not yet been described, the present study suggests that the phenotype would include markedly reduced HDL levels, analogous to the CETPTg/PLTP0 mice. Recent studies in ABC1 knock-out mouse liver using gene expression profiling approaches have shown that the largest change in any hepatic mRNA is up-regulation of PLTP (28). This suggests overlap in the functions of PLTP and ABC1. Thus, ABC1 may initiate nascent HDL formation by interacting with free apoA-I at the cell surface, and the activity of PLTP (transfer of PL from triglyceride-rich lipoproteins) may lead to

2 C. Bruce and A. Tall, unpublished observation.
further increase in the size of HDL particles (23). It is interesting to speculate that compound deficiency states involving low activity variants of PLTP combined with common low activity variants of LPL or ABC1 could result in marked hypoalphalipoproteinemia.

REFERENCES
1. Bruce, C., Beamer, L. J., and Tall, A. R. (1998) Curr. Opin. Struct. Biol. 8, 426–434
2. Brown, M. L., Inazu, A., Hesler, C. B., Agellon, L. B., Mann, C., Whitlock, M. E., Marcel, Y. L., Milne, R. W., Koizumi, J., Mabuchi, H., and Tall, A. R. (1989) Nature 342, 448–451
3. Inazu, A., Brown, M. L., Hesler, C. B., Agellon, L. B., Koizumi, J., Takata, K., Maruhama, Y., Mabuchi, H., and Tall, A. R. (1990) N. Engl. J. Med. 323, 1234–1238
4. Inazu, A., Ando, K., Haraki, T., Yagi, K., Kamon, N., Koizumi, J., Mabuchi, H., Takeda, R., Takada, K., Moriyama, Y., and Tall, A. R. (1994) J. Clin. Invest. 94, 1872–1882
5. Hayek, T., Azrolan, N., Verderey, R. B., Walsh, A., Chajek-Shaul, T., Agellon, L. B., Tall, A. R., and Breslow, L. J. (1993) J. Clin. Invest. 92, 1143–1152
6. Agellon, L. B., Walsh, A., Hayek, T., Moulin, P., Jiang, X., Shelanski, S. A., Breslow, J. L., and Tall, A. R. (1991) J. Biol. Chem. 266, 10796–10801
7. Masucci-Magoulas, L., Plump, A., Jiang, X.-C., Walsh, A., Breslow, J. L., and Tall, A. R. (1996) J. Clin. Invest. 97, 154–161
8. Hayek, T., Chajek-Shaul, T., Walsh, A., Agellon, L. B., Moulin, P., Tall, A. R., and Breslow, J. L. (1992) J. Clin. Invest. 89, 505–510
9. Jiang, X.-C., Bruce, C., Mar, J., Lin, M., Ji, Y., Francone, O. L., and Tall, A. R. (1999) J. Clin. Invest. 103, 907–914
10. Tall, A. R., Ahreus, E., and Shuman, J. (1983) J. Biol. Chem. 258, 2174–2180
11. Lagrost, L., Athias, A., Gambert, P., and Lallemand, C. (1994) J. Lipid Res. 35, 825–835
12. Brown, M. L., Hesler, C., and Tall, A. R. (1990) Curr. Opin. Lipidol. 1, 122–127
13. Jeong, T. S., Schissel, S., Tabas, J., Pownall, H. J., Tall, A. R., and Jiang, X.-C. (1998) J. Clin. Invest. 101, 905–912
14. Morton, R. E., and Zilversmit, D. B. (1983) J. Biol. Chem. 258, 11751–11757
15. Swenson, T. S., Simmons, J. S., Hesler, C. B., Bisgaier, C., and Tall, A. R. (1987) J. Biol. Chem. 262, 16271–16274
16. Hesler, C. B., Swenson, T. L., and Tall, A. R. (1987) J. Biol. Chem. 262, 2275–2282
17. Jiang, X., Masucci-Magoulas, L., Mar, J., Lin, M., Walsh, A., Breslow, J. L., and Tall, A. R. (1993) J. Biol. Chem. 268, 27406–27412
18. Rohmann, A., Gotthardt, M., Hammer, R. E., and Herz, J. (1998) J. Clin. Invest. 101, 689–695
19. Day, J. R., Albers, J. J., DeFronzo, R. A., Gilbert, T. L., Ching, A. F. T., Grant, F. J., O'Hara, P. J., Marcovina, S. M., and Adolphson, L. J. (1994) J. Biol. Chem. 269, 9388–9391
20. Huuskonen, J., Wohlforth, G., Jauhainen, M., Ehnholm, C., Teleano, O., and Oddonen, V. M. (1999) J. Lipid Res. 40, 1123–1130
21. Yu, B., Hilman, E., and Wright, S. D. (1997) J. Clin. Invest. 99, 315–324
22. Tall, A., Krumholz, T., Olvercrona, T., and Deckelbaum, R. J. (1985) J. Biol. Chem. 260, 842–851
23. Qin, S. C., Kanwano, K., Bruce, C., Lin, M., Bisgaier, C., Tall, A. R., and Jiang, X.-C. (2000) J. Lipid Res. 41, 269–276
24. Pattnaik, N. M., and Zilversmit, D. B. (1978) J. Biol. Chem. 253, 2782–2786
25. Beamer, L. J., Carroll, S. F., and Eisenberg, D. (1997) Science 276, 1861–1864
26. Wang, S., Deng, L., Milne, R. W., and Tall, A. R. (1992) J. Biol. Chem. 267, 17487–17490
27. Lussa, S., Jauhainen, M., Metso, J., Somerharju, P., and Ehnholm, C. (1996) Biochem. J. 313, 275–282
28. McNichol, P., Gifford, R. J., Gifford, R. J., de Wet, J., Brocardo, C., Chinmin, G., and Francone, O. L. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4245–4250
29. Francone, O. L., Gong, E. L., Ng, D. S., Fielding, C. J., and Rubin, E. M. (1995) J. Clin. Invest. 96, 1440–1448
30. Jiang, X.-C., Francone, O. L., Bruce, C., Milne, R., Mar, J., Walsh, A., Breslow, J. L., and Tall, A. R. (1996) J. Clin. Invest. 93, 2373–2380
31. Breslow, J. L. (1989) in The Metabolic Basis of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) pp. 1251–1266, McGraw-Hill, New York.
32. Wittrup, H. H., Tybjerg-Hansen, A., and Nordestgaard, D. B. (1999) Circulation 99, 2901–2907
33. Brooks-Wilson, A., Marciel, M., Clee, S. M., Zhang, L. H., Roomp, K., van Dam, M., Yu, L., Brewer, C., Collins, J. A., Molhuizeo, H. O., Louher, O., Ouellette, B. F., Fichter, K., Ashburne-Excoffon, K. J., Sensen, C. W., Scherer, S., Mott, S., Denis, M., Martindale, D., Frohlich, J., Morgan, K., Koop, B., Pimstone, S., Kastelein, J. J., and Hayden, M. R. (1999) Nat. Genet. 22, 336–345
34. Bodzioch, M., Orso, E., Klucken, J., Langmann, T., Bottcher, A., Diederich, W., Drobnik, W., Brlaga, S., Buchler, C., Poroch-Grezuman, M., Kaminski, W. E., Hahmann, H. W., Oette, K., Rothe, G., Aslandis, C., Lackner, K. J., and Schmitz, G. (1999) Nat. Genet. 22, 347–351
35. Rust, S., Ruster, M., Punke, H., Reul, J., Amoura, Z., Pfitz, J. C., Deleuze, J. F., Brewer, H. B., Duverger, N., Denelle, P., and Aumann, G. (1999) Nat. Genet. 22, 352–355