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United States Patent [19]

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Wetterau, II et al.

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[54] **NUCLEIC ACIDS ENCODING MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN**

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[21] Appl. No.: **117,362**

[22] Filed: **Sep. 3, 1993**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 15,449, Feb. 22, 1993, abandoned, which is a continuation-in-part of Ser. No. 847,503, Mar. 6, 1992, abandoned.

[51] **Int. Cl.**⁶ **C12Q 1/70**; C07H 21/02; C12N 15/79; C12N 5/10

[52] **U.S. Cl.** **435/6**; 435/240.2; 435/320.1; 935/77; 935/78; 536/23.1

[58] **Field of Search** 435/6, 240.2, 320.1; 536/23.5

References Cited

U.S. PATENT DOCUMENTS

4,772,549 9/1988 Frossard 435/6

FOREIGN PATENT DOCUMENTS

WO9305778 2/1993 WIPO .

OTHER PUBLICATIONS

C. C. Shoulders et al. *Human Molecular Genetics*, vol. 2, No. 12, pp. 2109-2116, 1993.

D. Sharp et al., *Nature*, vol. 365, pp. 65-69, Sep. 2, 1993. Bulleid & Freedman, *Nature* 335, 649-651 (1988). "Defective co-translational formation of disulphide bonds in protein disulphideisomerase-deficient microsomes".

Koivu et al., *J. Biol. Chem.* 262, 6447-6449 (1987). "A Single Polypeptide Acts Both as the β Subunit of Prolyl 4-Hydroxylase and as a Protein Disulfide-Isomerase*".

Kane & Havel in *the Metabolic Basis of Inherited Disease*, Sixth Edition, 1139-1164 (1989). "Disorders of the Biogenesis and Secretion of Lipoproteins Containing The B Apolipoproteins".

Schaefer et al., *Clin. Chem.* 34, B9-B12 (1988). "Genetics and Abnormalities in Metabolism of Lipoproteins".

Drayna et al., *Nature* 327, 632-634 (1987). "Cloning and sequencing of human cholesteryl ester transfer protein cDNA".

Pihlajaniemi et al., *EMBO J.* 6, 643-649 (1987). "Molecular cloning of the β -subunit of human prolyl 4-hydroxylase. This subunit and protein disulphide isomerase are products of the same gene".

Yamaguchi et al., *Biochem. Biophys. Res. Comm.* 146, 1483-1492 (1987). "Sequence of Membrane-Associated Thyroid Hormone Binding Protein From Bovine Liver: Its Identity with Protein Disulphide Isomerase".

Edman et al., *Nature* 317, 267-270 (1985). "Sequence of protein disulphide isomerase and implications of its relationship to thioredoxin".

Kao et al., *Connective Tissue Research* 18, 157-174 (1988). "Isolation of cDNA Clones and Genomic DNA Clones of β -Subunit of Chicken Prolyl 4-Hydroxylase*".

Wetterau, J. et al., *Biochem.* 30, 9728-9735 (1991). "Protein Disulfide Isomerase Appears Necessary To Maintain the Catalytically Active Structure of the Microsomal Triglyceride Transfer Protein".

Morton, R. E. et al., *J. Biol. Chem.* 256, 1992-1995 (1981). "A Plasma Inhibitor of Triglyceride and Cholesteryl Ester Transfer Activities".

Wetterau, J. et al., *Biochem.* 30, 4406-4412 (1991). "Structural Properties of the Microsomal Triglyceride-Transfer Protein Complex".

Wetterau, J. et al., *J. Biol. Chem.* 265, 9800-9807 (1990). "Protein Disulfide Isomerase Is a Component of the Microsomal Triglyceride Transfer Protein Complex".

Wetterau, J. and Zilversmit, D. B., *Chem. and Phys. of Lipids* 38, 205-22 (1985). "Purification and Characterization of Microsomal Triglyceride and Cholesteryl Ester Transfer Protein From Bovine Liver Microsomes".

Wetterau, J. and Zilversmit, D. B., *Biochimica et Biophysica Acta* 875, 610-617 (1986). "Localization of intracellular triacylglyceroc and cholesteryl ester transfer activity in rat tissues".

Wetterau, J. and Zilversmit, D. B., *J. Biol. Chem.* 259, 10863-10866 (1984). "A Triglyceride and Cholesteryl Ester Transfer Protein Associated with Liver Microsomes".

Wetterau, J., Grant Application entitled: "Intracellular Triglyceride Transport and Metabolism".

Presentation Materials, Aspen Bile Acid/Cholesterol Conference, Aug. 15, 1992.

Wetterau, J. R., et al., *Science*, vol. 258, 999-1001, Nov. 6, 1992, "Absence of Microsomal Triglyceride Transfer Protein in Individuals with Abetalipoproteinemia".

(List continued on next page.)

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[57] **ABSTRACT**

Nucleic acid sequences, particularly DNA sequences, coding for all or part of the high molecular weight subunit of microsomal triglyceride transfer protein, expression vectors containing the DNA sequences, host cells containing the expression vectors, and methods utilizing these materials. The invention also concerns polypeptide molecules comprising all or part of the high molecular weight subunit of microsomal triglyceride transfer protein, and methods for producing these polypeptide molecules. The invention additionally concerns novel methods for preventing, stabilizing or causing regression of atherosclerosis and therapeutic agents having such activity. The invention concerns further novel methods for lowering serum lipid levels and therapeutic agents having such activity.

OTHER PUBLICATIONS

Archibald, J. L., et al., *Journal of Medicinal Chemistry*, vol. 14, No. 11, pp. 1054-1059.

Cortizo, L. et al., *J. Med. Chem.*, 34, pp. 2242-2247, 1991.

Hall, I. H. et al., *Pharmaceutical Research*, vol. 9, No. 10, pp. 1324-1329, 1992.

Hall, I. H., et al., *Pharmacological Research Communications*, vol. 19, No. 12, pp. 839-858, 1987.

Murthy et al., *Eur. J. Med. Chem. —Chim. Ther.*, vol. 20, No. 6, pp. 547-550, 1985.

Derwent Abstract No. 93-117225/14 for WO93/05778.

FIG. 1

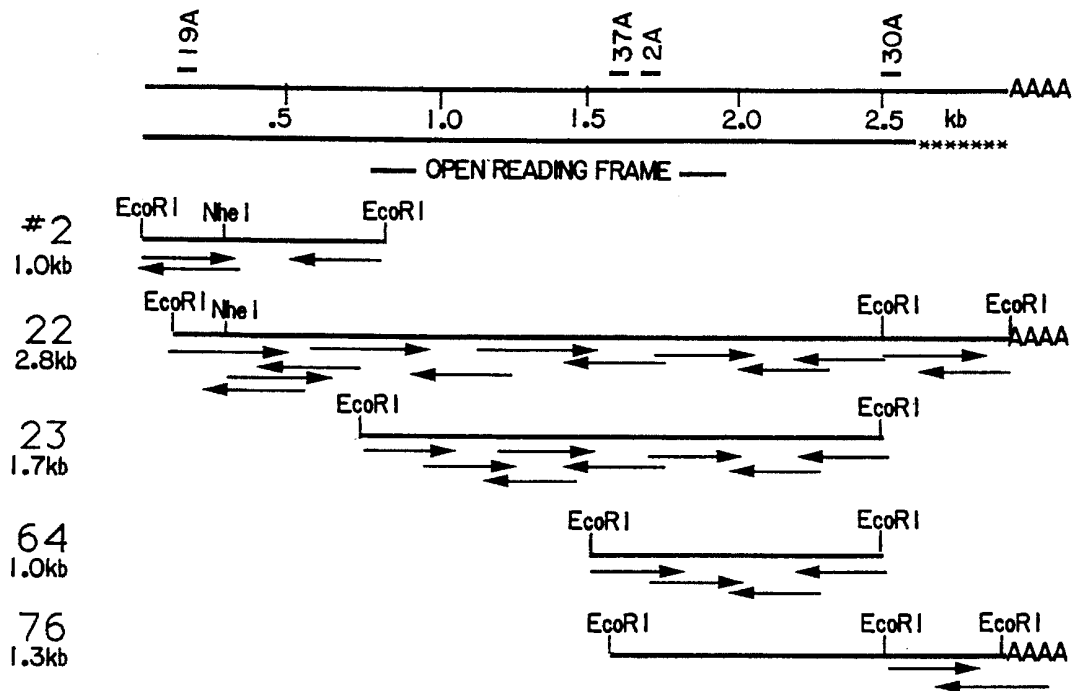


FIG. 2

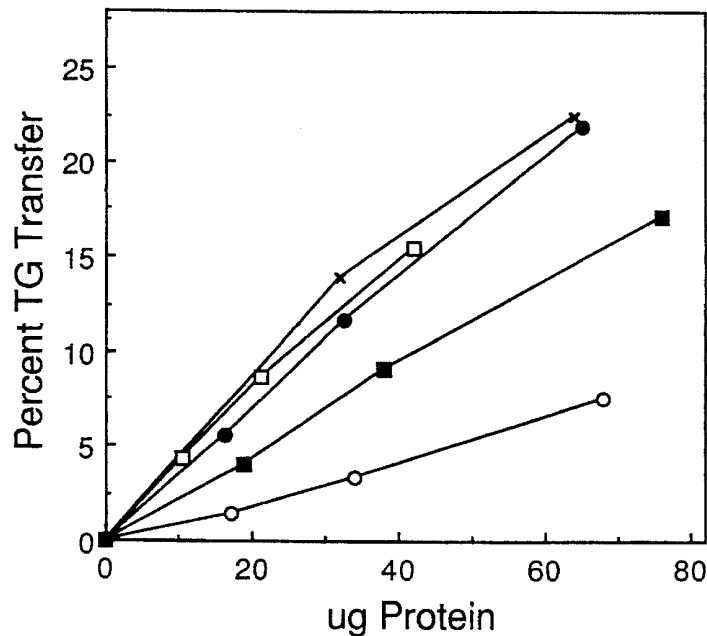


FIG. 3

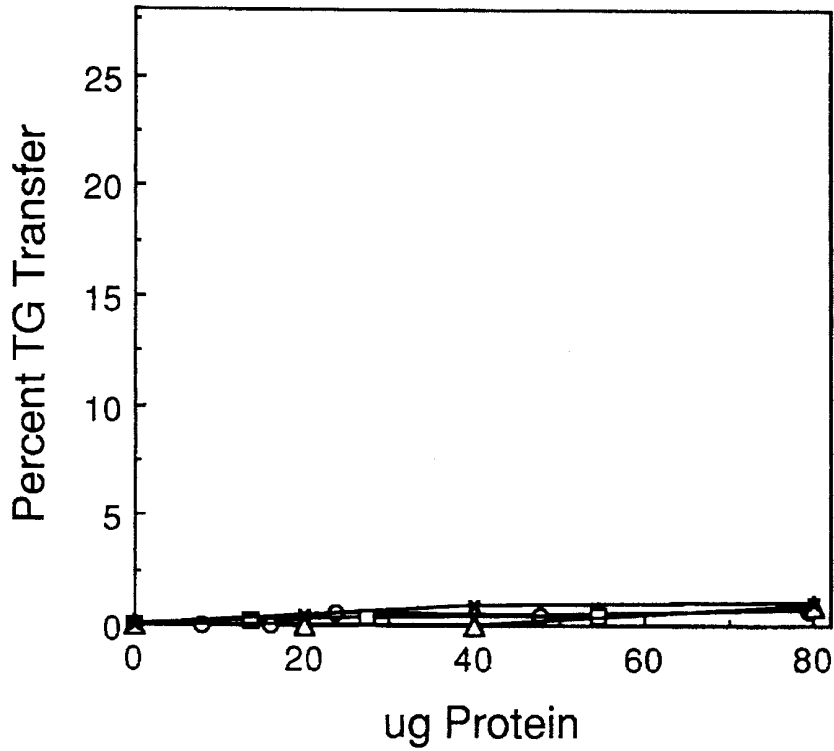


FIG. 4

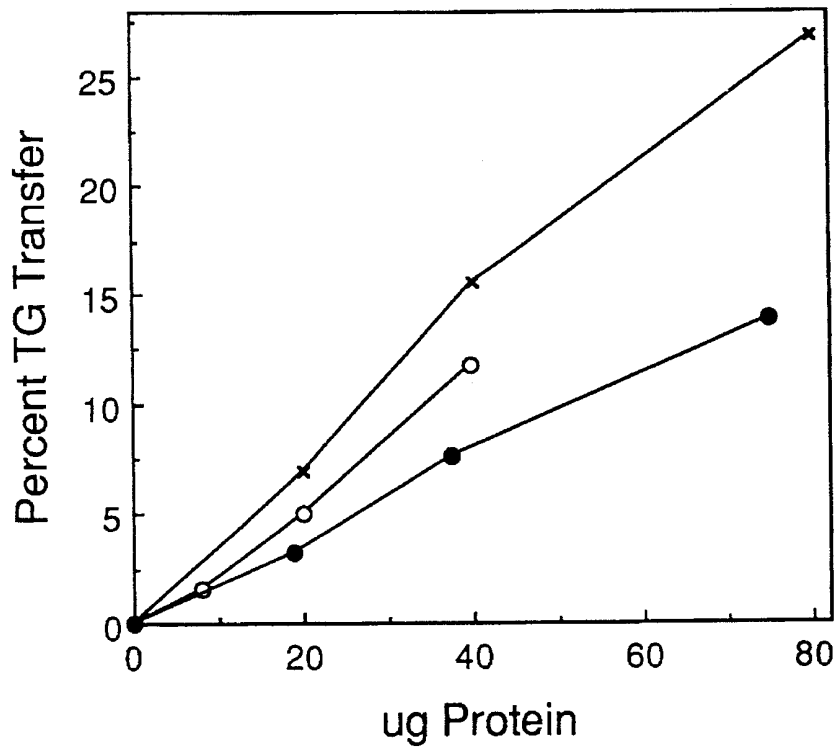


FIG. 5

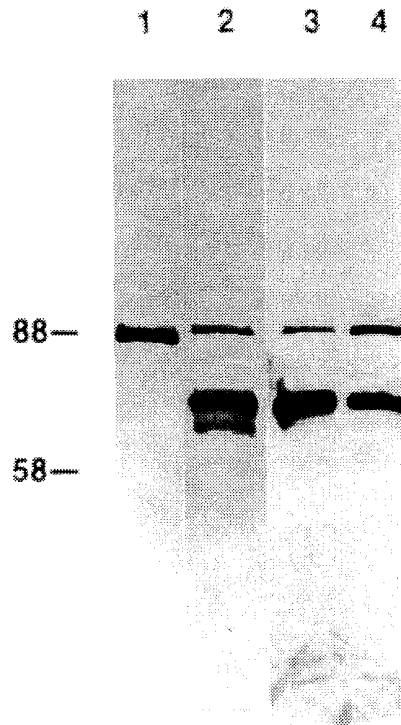
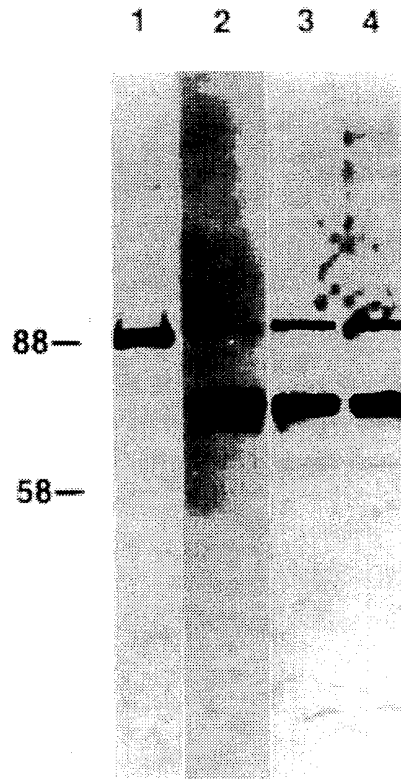


FIG. 6



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