

Tangier Disease

- Univ.-Prof. em. Dr. med. Gerd Assmann -

Die Identifizierung der molekularen Ursache einer seltenen Fettstoffwechselstörung, der Tangier-Krankheit (engl. Tangier Disease), bei der HDL-Cholesterin im Blut vollständig fehlt und die Entschlüsselung der Struktur der High Density Lipoproteine (HDL) als einer wichtigen Komponente des Fettstoffwechsels und Gegenspieler des LDL-Cholesterins waren wesentliche Aspekte der Forschungstätigkeit von Univ.-Prof. Dr. Gerd Assmann am NIH in Bethesda, USA und Münster.



Auszüge aus dem Interview mit Professor Assmann „Wie alles begann“ geben Aufschluss zu den Hintergründen seiner Forschungstätigkeit bezüglich Tangier-Krankheit und HDL-Cholesterin. Ab Seite 2 finden Sie die daraus hervorgegangenen Publikationen.

Die Zeit am National Institutes of Health (NIH) als Visiting Scientist war sicherlich sehr arbeitsreich, aber auch spannend. Was stand im Fokus Ihrer wissenschaftlichen Arbeit?

„Eine Forschungsstelle am NIH in Washington/Bethesda zu bekommen, war eine ganz große Herausforderung. Man muss zunächst einmal das amerikanische Staatsexamen machen, man musste wiederum ein Stipendium bekommen und damals gab es so genannte Fogarty Fellowships, von denen es drei pro Jahr gab für Deutschland, Österreich und die Schweiz. Ich war der Glückliche, der in Deutschland dieses Stipendium erhielt und als ich dann in Amerika forschen durfte unter der Leitung von Donald Fredrickson, einem international höchst renommierten Fettstoffwechselforscher, war mir klar geworden, dass bestimmte Dinge, die etwas zu tun haben mit den High Density Lipoproteinen (HDL), etwas sein könnten, mit dem ich mich beschäftigen sollte.“

Welche Bedeutung hatte das Rätsel um „Tangier-Disease“ für Sie?

„Tangier-Disease war der Anlass, weshalb ich mich überhaupt mit HDL (High Density Lipoproteine) beschäftigt habe. Dr. Fredrickson hatte mir erläutert, was Tangier Disease ist. Da hatte man kurz bevor ich kam, einen jungen Menschen referiert bekommen, der hatte nicht rote Tonsillen (Mandeln), sondern gelbe und gleichzeitig eine große Milz, eine große Leber und was vor allen Dingen aufgefallen war, der Betreffende hatte null HDL in seinem Blut. Das war eine totale Faszination, die mich 20 Jahre lang nicht losgelassen hat, bevor wir das Rätsel lösen konnten. In der Zwischenzeit ging es um die Frage, die Tertiärstruktur der High Density Lipoproteine aufzuklären. Dazu gab es fünf Publikationen, die internationale Beachtung gefunden haben. Also die Struktur der High Density Lipoproteine war der Forschungsschwerpunkt geworden für mich in der Zeit in Amerika.“

Konnten Sie das Rätsel „Tangier-Disease“ lösen?

„Es war so, dass, wenn man Tangier Disease in Deutschland beforschen wollte, Patienten mit Tangier Disease finden musste. Wie es der Zufall wollte, hatte ich tatsächlich einen solchen Patienten gefunden in der Eifel, der diese Erkrankung hatte, auch seine Schwester. Das war absolute Voraussetzung, um überhaupt darüber forschen zu können. Es hat in der Tat 20 Jahre gedauert, bis wir den molekularen Defekt der Tangier Krankheit identifiziert haben: ein Membrandefekt, ein sogenannter ABCA1-Mangel, der die Ursache für diese Erkrankung war.“

(Das vollständige Interview finden Sie [hier](#))

Publikationsliste (Auszug “Tangier Disease”)

– Prof. Dr. med. Gerd Assmann –

1. Assmann, G.: Tangier-Krankheit. In: Handbuch der inneren Medizin, Fettstoffwechsel, edited by G. Schettler, H. Greten, G. Schlierf, and D. Seidel. Berlin, Heidelberg, New York: Springer-Verlag, 1976.

Die Tangier-Krankheit ist eine seltene autosomal-rezessive Stoffwechselkrankheit. Bei homozygoten Patienten sind α -Lipoproteine (HDL) im Plasma mittels Lipoproteinelektrophorese nicht nachweisbar, lipidchemisch sind Hypocholesterinämie und Hypertriglyceridämie besonders charakteristisch. Die Cholesterinesterspeicherung in Zellen des retikuloendothelialen Systems ist Ursache der grotesken Vergrößerung und Verfärbung der Tonsillen sowie der Veränderung anderer Organe (z.B. Hepatosplenomegalie). Die HDL-Apoproteine A-I und A-II sind immunchemisch in Tangier-Plasma identifizierbar und partiell charakterisiert. Bisherige Untersuchungen haben keinen Hinweis auf eine Apoprotein-Strukturmutation als Ursache der Analphalipoproteinämie erbracht.

2. Assmann, G.: Structure-function relationships of lipoproteins in Tangier disease. pp. 106-10. in: Greten. H., ed.,1976.

Tangier disease is a rare disorder of plasma lipid transport thought to be due to a mutant autosomal gene. The disease was discovered in 1960 (1 - 3) and given its name from Tangier Island which lies off the coast of Virginia in Chesapeake Bay and is the home of the original patients. To date, 20 patients have been discovered, representing 16 kindreds none of whom are known to be related (4). The clinical manifestations include:

1. Low plasma cholesterol and normal or elevated triglyceride concentrations,
2. Absence of the usual high density lipoproteins and abnormal composition of other lipoproteins in plasma,
3. Distinctive enlargement and coloration of the tonsils,
4. Frequently peripheral neuropathy,
5. Lipid deposition in tissues accompanied by enlargement of liver, spleen, or lymph nodes, alterations in the cornea, intestinal mucosa, and possibly blood vessels.

Chemical analysis of tissues has revealed that the lipid deposits consist mainly of cholesteryl esters; histologic and ultrastructural observations have provided evidence that intracellular lipid storage is mainly confined to reticuloendothelial cells (foam cells) in tonsils, bone marrow, skin and jejunal submucosa, Schwann's cells in peripheral nerves and myenteric plexus, and nonvascular smooth muscle cells (5).

In the past much attention has been focused on the nature of the plasma lipoproteins, particularly high density lipoproteins (HDL). It has been demonstrated that patients with this disease have small amounts of plasma HDL, which is similar but not identical with normal lipoproteins of this density class (6).

An average chemical composition for normal HDL is given in Fig. 1. The lipid consists mainly of cholesteryl esters and phospholipid with approximately four moles of phosphatidylcholine per mole of sphingomyelin. About 90 % of the protein moiety of HDL is composed of two major apoproteins which are designated as apo A-I and apo A-II, the remaining 10 % is mainly composed of apo C-I, apo C-II, and C-III.

Recently considerable progress was achieved concerning the primary structure of these apolipoproteins. It could be shown that these apolipoproteins have conformational amphipathic regions (7) which are a structural prerequisite for hydrophobic binding to fatty acid chains of phospholipids and possibly cholesteryl esters (8). It became evident that in the quaternary structure of high density lipoproteins the polar head groups of the phospholipids are all oriented on the outer surface of a spherical micelle and that cholesteryl esters and triglycerides are located within the core of these macromolecules (9 - 11). To what extent the apoproteins are located in the surface region of the micelle or embedded for an appreciable distance in the lipid core of the lipoproteins is still a matter of debate (12).

3. Gheorghiu, T., G. Assmann and H. E. Schaefer: Endoscopic findings in Tangier disease. *Endoscopy* 8: 164-169, 1976

Tangier disease is a rare familial metabolic disorder due to the absence of normal plasma HDL. Cholesteryl esters are stored in various tissues, mostly in reticulo-endothelial foam-cells; enlarged tonsils, (hepato) splenomegaly and polyneuropathy are the principal clinical manifestations. The very unusual endoscopical findings in a recently observed patient might contribute to the detection of this disease: the uneven surface of the liver has a salmon-bright red, strawberry-like color; the enlarged spleen is covered by numerous minute, yellowish, subcapsular "stipples" and some isolated, irregular, yolk-yellow patches; the rectal, and even more the colonic mucosa, presents itself with round, brownish-red spots, diffusely scattered on a yellow-orange back-ground ("cheetah fur-like").

4. **Schaefer, H. E., G. Assmann and T. Gheorghiu: Licht- und elektronenmikroskopische Untersuchungen zur Tangier-Krankheit (sog. An-alpha-lipoproteinämie). Verh. Dt. Ges. Path. 60: 473, 1976**

Verh. Dtsch. Ges. Path. 60, 473 (1976)

37. H. E. SCHAEFER, G. ASSMANN und TH. GHEORGHU (Abteilungen für feinstrukturelle Pathologie und klinische Chemie und Medizinische Klinik Köln)

Licht- und elektronenmikroskopische Untersuchungen zur Tangier-Krankheit (sog. An-alpha-Lipoproteinämie)

Seit ihrer Erstbeschreibung durch D. S. FREDRICKSON und Mitarbeiter (Ann. intern. Med. 55, 1016–1031, 1961) sind 19 homozygote Fälle von Tangier-Krankheit bekannt geworden. In einer neu entdeckten Sippe haben wir zusätzlich 2 homozygote neben 14 heterozygoten Merkmalsträgern nachgewiesen. In der über 7 Generationen zu verfolgenden Aszendenz sind 4 Verwandtenehen 2. bis 6. Grades feststellbar. Der klinisch asymptomatische heterozygote Zustand ist u. a. durch eine Verminderung des Serumcholesterins und der „high density“ (alpha)-Lipoproteine (HDL) charakterisiert. Die homozygoten Propositi (42jähriger Mann, 46jährige Frau) weisen demgegenüber noch niedrigere Cholesterinwerte (45 mg/dl, 80 mg/dl) bei fast fehlendem HDL und eine begleitende Hypertriglyzeridämie (220 mg/dl, 350 mg/dl) auf. – In Biopsien der homozygoten Fälle finden sich feintropfige und kristalline Lipidablagerungen in kennzeichnenden histiozytären Schaumzellen im Knochenmark, vorwiegend periportal in der Leber, in den Marksträngen der Milz, perivaskulär in der Dermis, im Schleimhautstroma von Magen, Duodenum (basal betont) und Colon. Die am stärksten betroffene Colonschleimhaut zeigt rektoskopisch eine buttergelbe Farbe mit punktförmigen braunroten Aussparungen (Lymphfollikel). Auch Leber und Milz sind oberflächlich stippchenförmig gelbweiß gefärbt, ebenso residuales Tonsillengewebe (Tonsillektomie in der Jugend). Die histiozytären Lipidablagerungen verhalten sich anisotrop und sudanophil, lassen aber weitgehend eine sichtbare Reduktion von OsO₄ vermissen. Tropfige Lipidablagerungen dieses osmiophoben Types treten ausgedehnt auch in glatten Muskelzellen der rektalen Muscularis mucosae sowie in Schwannschen Zellen dermalen, hepatischer und rektal intramuskulärer Nerven auf. Die mangelnde Osmiophilie läßt sich mit dem weitgehenden Sättigungsgrad der abgelagerten Cholesterinester (vorwiegend Oleat) erklären. Demgegenüber enthalten von Kupffer'sche Sternzellen (ebenso wie Fettzellen) z. T. osmiophiles Fett, das offenbar mehr höher ungesättigte Fettsäuren enthält und wahrscheinlich endozytotisch aus dem Blut (Hypertriglyzeridämie) aufgenommen worden ist. Elektronenmikroskopisch treten die Tangier-typischen, d. h. osmiophoben Lipidablagerungen überwiegend als membranlose intrazytoplasmatische Vakuolen auf, die teilweise einem sekundären lysosomalen Kontakt und Abbau unterworfen sind. In späten Stadien bleiben Lysosomen mit transparenten Einschlüssen übrig, welche offenbar kristallin ausgefallenen Cholesterin bzw. Cholesterinestern entsprechen. Zwischen membranlosen Lipidvakuolen und dilatierten Cisternen des rauhen endoplasmatischen Reticulum bestehen gelegentlich Kommunikationen, die möglicherweise eine Freisetzung intrazisternal synthetisierter Lipide signalisieren. – Diese Beobachtungen lassen die Deutung zu, daß die Tangiertypischen, relativ osmiophoben Lipide zumindest teilweise aus endogener Synthese der verfettenden Zellen stammen und infolge eines dem Leiden zugrunde liegenden HDL-Apoproteindefektes nicht ausgeschleust werden können. Ein solcher Zusammenhang würde die scheinbare Paradoxie einer Hypcholesterinämie bei intrazellulärer Cholesterinspeicherung zwanglos erklären.

5. Assmann, G., O. Simantke, H. E. Schaefer and E. Smootz: Characterization of high density lipoproteins in patients heterozygous for Tangier disease. J. Clin. Invest. 60: 1025-1035, 1977.

In this study a large family group affected with Tangier disease has been investigated. Besides two homozygous probands, several heterozygous patients have been identified on the basis of quantitative measurements of high density lipoproteins and their constitutive polypeptides. By a variety of quantitative immunological methods, such as one-dimensional Laurell electrophoresis, two-dimensional immunoelectrophoresis, and double-antibody radioimmunoassay, the total amount of apoprotein A-I and apoprotein A-II contained in the serum of heterozygous patients and the distribution of these A apoproteins among serum lipoproteins have been determined. The molar ratio of apoprotein A-I and apoprotein A-II contained in high density lipoproteins of heterozygous patients did not significantly differ from that of control preparations, although the total mass of high density lipoproteins was reduced by approximately 50%. The elution profile of high density lipoproteins from agarose columns and their morphological appearance, as ascertained by electron microscopy, were similar to control preparations. In addition to the quantitative alterations of serum lipoproteins, lipid storage in histiocytes of the rectal mucosa obtained from heterozygous patients has been documented. It is concluded that patients heterozygous for Tangier disease have normal high density lipoproteins in circulation, the total mass of which is reduced by approximately 50%.

6. Assmann, G., P. N. Herbert, D. S. Fredrickson and T. Forte: Isolation and characterization of an abnormal high density lipoprotein in Tangier Disease. J. Clin. Invest. 60: 242-252, 1977.

The nature of the high density lipoproteins has been investigated in five patients homozygous for Tangier disease (familial high density lipoprotein deficiency). It has been established that Tangier high density lipoproteins, as isolated by ultracentrifugation, are morphologically heterogeneous and contain several proteins (Apo B, albumin, and Apo A-II). An abnormal lipoprotein has been isolated from the $d = 1.063-1.21$ g/ml ultracentrifugal fraction by agarose-column chromatography which contains apoprotein A-II as the sole protein constituent. In negative-stain electron microscopy, these lipoproteins appeared as spherical particles 55-75 Å in diameter. By a variety of criteria (immunochemical, polyacrylamide electrophoresis, amino acid composition, and fluorescence measurements), apoprotein A-I the major apoprotein of normal high density lipoproteins and the C apoproteins were absent from this lipoprotein. As demonstrated by (125)I very low density lipoprotein incubation experiments with Tangier plasma, C apoproteins did not associate with lipoproteins of $d = 1.063-1.21$ g/ml. Tangier apoprotein A-II, isolated to homogeneity by delipidation of the apoprotein A-II-containing lipoprotein or Sephadex G-200 guanidine-HCl chromatography of the $d = 1.063-1.21$ g/ml fraction, was indistinguishable from control apoprotein A-II with respect to amino acid composition and migration of tryptic peptides in urea-polyacrylamide electrophoresis. The ability of Tangier apoprotein A-II to bind phospholipid was demonstrated by in vitro reconstitution experiments and morphological and chemical analysis of lipid-protein complexes. It is concluded that normal high density lipoproteins, as defined by polypeptide composition and morphological appearance, are absent from Tangier plasma and that as a consequence, the impairment of C apoprotein metabolism contributes to the hypertriglyceridemia and fasting chylomicronemia observed in these patients.

7. **Assmann, G., E. Smootz, K. Adler, A. Capurso and K. Oette: The lipoprotein abnormality in Tangier disease: quantitation of A apoproteins. J. Clin. Invest. 59: 565-575, 1977**

In this study we have determined by radioimmunoassay and double immunoelectrophoresis the total quantities and distributions of A apoproteins in three adult patients affected with Tangier disease (hereditary alpha-lipoprotein deficiency). Compared with normal plasma, the total quantities of apoproteins A-I and A-II in Tangier plasma were determined to be less than 1% and 5-7%, respectively. In Tangier patients, approximately 90% of the apoprotein A-I sedimented when ultracentrifugations of plasma were carried out at density 1.21 g/ml KBr. By contrast, more than 95% of the apoprotein A-II floated under those conditions. In normal plasma, approximately 90% of both apoproteins A-I and A-II is found in the 1.063-1.21-g/ml KBr density fraction. These findings suggest that complete dissociation of A apoproteins occurs in Tangier plasma. This dissociation of apoproteins was confirmed by double immunoelectrophoresis with monospecific antisera. Immunochemical and electrophoretic experiments did not provide evidence for a structural abnormality of apoprotein A-I in these patients. The results taken together strongly suggest that normal high-density lipoproteins are absent from Tangier plasma.

8. **Schaefer, H. E. and G. Assmann: Die Manifestation der Tangier Krankheit an der portio uteri vaginalis. Verh. Dt. Ges. Path. 61: 401, 1977.** (No abstract available)
9. **Assmann, G., A. Capurso, E. Smootz and U. Wellner: Apoprotein A metabolism in Tangier disease. Atherosclerosis 30: 321-332, 1978** – (No abstract available)

The metabolic defect in Tangier disease has been investigated in two homozygous family members. One patient was pretreated with large amounts of homologous HDL prior to the injection of [¹²⁵I]HDL and the metabolism of the substituted HDL was analyzed by two-dimensional immunoelectrophoresis employing monospecific antisera against apoproteins A-I and A-II. In addition, the specific activities of the A apoproteins have been determined throughout the course of HDL catabolism. Radioactivity confined to apoproteins A-I and A-II decayed from the plasma monoexponentially with a $T_{1/2}$ of 7 h and 17 h, respectively; the specific activity of apoprotein A-I remained unchanged in the course of HDL catabolism, while that of apoprotein A-II constantly decreased.

The cellular concentration of the A apoproteins in the epithelial cells of the jejunal mucosa was determined in cryostat sections of intestinal biopsy specimens obtained from the second homozygous Tangier patient and several control patients. Employing the direct immunofluorescence technique and fluorescein isothiocyanate-labeled antibodies (γ -globulins) against apoprotein A-I and apoprotein A-II, intense specific apoprotein A immunofluorescence was detected within the epithelial cells and the collecting lymph vessels of the intestinal mucosa. The pattern of immunofluorescence in the Tangier and control intestinal mucosae was indistinguishable. Thus, the intravascular depletion of the A apoproteins contrasts with their regular cellular concentration. Our studies link the defect in Tangier disease to apoprotein A-I. The inability of this apoprotein to associate with protein and/or lipid and to become a regular constituent of HDL has been identified as the biochemical defect in this disease. This abnormality is currently best explained by a structural mutation or a specific catabolic defect of apoprotein A-I.

- 10. Assmann, G., G. Schmitz and H. Heckers: The role of high density lipoproteins in lecithin:cholesterol acyltransferase activity: Perspectives from Tangier disease. Scand. J. Clin. Lab. Invest. Suppl. 150: 98-102, 1978.**

Lecithin:cholesterol acyltransferase activity and the lipid composition of VLDL and LDL were examined in five patients homozygous for Tangier disease. The following results were obtained: I. The percentage of cholesterol that was esterified was similar in Tangier and control lipoproteins. II. Linoleic acid was the predominant fatty acid constituent of cholesteryl esters in Tangier plasma. III. Molar cholesterol esterification rates in Tangier plasma were reduced; however, fractional rates of cholesterol esterification were equal to or exceeded those of control plasma. IV. In vitro addition of apoprotein A-I and isolated lipoproteins led to a concentration-dependent increase in the initial rates of cholesterol esterification in Tangier plasma. It is concluded that HDL is not an exclusive substrate for the LCAT reaction, and that cholesterol esterification is not impaired in Tangier plasma.

- 11. Assmann, G.: High Density Lipoproteins (Composition, Structure, Metabolism. Tangier Disease and Role in Atherogenesis). In: II. Eur. Meeting on Metabolism, Anonymous Academic Press, 263-271, 1978**

An excessive concentration of serum low density lipoproteins (LDL) is generally regarded as a primary risk factor in the development of atherosclerosis. By contrast, the concentration of alpha- or high density lipoproteins (HDL) in plasma appears negatively correlated to the risk of acquiring coronary heart disease (CHD). It has been proposed that the coronary risk can be more simply estimated from the plasma HDL cholesterol concentration than from a consideration of other major lipid risk factors and blood pressure (Miller *et al.*, 1976a).

The purpose of this report is to review recent advances in research on HDL structure and metabolism. Particular emphasis will be given to the presumed role of these lipoproteins in atherogenesis.

- 12. Assmann, G. and E. Smootz: High density lipoprotein infusion and partial plasma exchange in Tangier disease. Eur. J. Clin. Invest. 8: 131-135, 1978**

High density lipoprotein (HDL) infusion and partial plasma exchange were undertaken in two patients homozygous for Tangier disease. Serum samples and ultracentrifugally isolated serum fractions were analysed over a period of 7 days post infusion by agarose electrophoresis, two-dimensional immunoelectrophoresis (employing antibodies to HDL, HDL3, Apoprotein A-I, and Apoprotein A-II), Apoprotein A radioimmunoassay, and analytical polyacrylamide electrophoresis. The following observations were made: (a) immediately after HDL substitution the broad-beta band, normally visible upon agarose electrophoresis of Tangier plasma, resolved into a distinct beta and pre-beta band; (b) as HDL was catabolized, an abnormal alpha-migrating lipoprotein was generated which contained Apoprotein A-II as protein constituent; and (c) there was a preferential loss of Apoprotein A-I from HDL and the plasma compartment in the course of HDL catabolism. The results suggest that the defect in Tangier disease resides with enhanced catabolism or defective synthesis of Apoprotein A-I.

13. Assmann, G.: The metabolic role of high density lipoproteins: Perspectives from Tangier Disease. In: High Density Lipoproteins and Atherosclerosis, A.M. Gotto Jr., N.E. Miller and M.F. Oliver, eds., Elsevier / North-Holland Biomedical Press, 1978

THE METABOLIC ROLE OF HIGH DENSITY LIPOPROTEINS: PERSPECTIVES FROM TANGIER DISEASE

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Tangier disease is a rare hereditary disorder of plasma lipid transport characterized by the absence of normal α - or high density lipoproteins (HDL) in plasma and storage of cholesteryl esters in foam cells in many tissues¹. Prominent clinical features include orange-colored tonsils, splenomegaly, and frequently, peripheral neuropathy. Histological and ultrastructural observations have provided evidence that the intracellular lipid storage is mainly confined to histiocytes (foam cells) in tonsils, bone marrow, spleen, skin, and jejunal submucosa; Schwann cells in peripheral nerves and myenteric plexus; and nonvascular smooth muscle cells².

In the past, Tangier disease has been the subject of intensive investigation in our laboratory³⁻⁹. Studies were undertaken to define the biochemical defect in this disease and to unravel the metabolic relationship of extracellular depletion of HDL and intracellular cholesteryl ester storage. The following results were obtained:

1. Compared with normal plasma, the total quantities of the major HDL apoproteins, Apo A-I and Apo A-II, in Tangier plasma were determined by radioimmunoassay to be < 1% and 5 - 7%, respectively. In Tangier plasma, approximately 90% of the Apo A-I sedimented when ultracentrifugations of plasma were carried out at density 1.21 g/ml KBr. By contrast, more than 95% of the Tangier Apo A-II floated under those conditions. In normal plasma, approximately 90% of both Apo A-I and Apo A-II is found in the 1.063 - 1.21 g/ml KBr density fraction. The findings suggested that complete dissociation of A apoproteins occurs in Tangier plasma³.

2. Tangier HDL, as isolated by ultracentrifugation, are morphologically heterogenous and contain several proteins (Apo B, albumin, Apo A-II). An abnormal lipoprotein has been isolated from the $d = 1.063 - 1.21$ g/ml ultracentrifugal fraction by agarose-column chromatography which contains Apo A-II as the sole protein constituent. In negative-stain electron microscopy, these lipoproteins appeared as spherical particles 55 - 75 Å in diameter. It could be demonstrated by a variety

14. a) Gheorghiu, T., G. Assmann, R. Mies, and H. E. Schaefer: [Gastroenterologic diagnosis of hypolipoproteinemias with special emphasis on Tangier disease]. *Verh. Dt. Ges. Inn. Med.* 1050-1053, 1978 – (No abstract available)

b) Gheorghiu, T., G. Assmann, R. Mies and H. E. Schaefer: Zur gastroenterologischen Diagnostik der Hypolipoproteinämien unter besonderer Berücksichtigung des Morbus Tangier. In: *Verhandlungen der Deutschen Gesellschaft für innere Medizin, Anonymous München: J.F. Bergmann Verlag, 1049-1054, 1978*

Hypolipoproteinämien sind autosomal vererbte Stoffwechselerkrankungen, die sich durch mangelhafte oder fehlende Synthese der spezifischen Eiweißkomponenten der Lipoproteine kennzeichnen [1, 2]. In ihrer homozygoten Form sind sie sicher selten, als heterozygoter Defekt jedoch wahrscheinlich häufiger als bisher angenommen. Homozygote weisen typische Plasmalipidveränderungen auf, darunter eine meist ausgeprägte Hypocholesterinämie. Diagnoseweisend ist häufig die Beteiligung des Gastrointestinaltrakts – sei es *klinisch* wie bei der A- β - und der homozygoten Hypo- β -Lipoproteinämie, *endoskopisch* (M. Tangier) oder *histologisch*. Die Organbefunde sind nicht selten nahezu pathognomonisch, so daß z. B. eine Racheninspektion, eine Rektoskopie oder die Dünndarmbiopsie diagnostisch ausschlaggebend sein können.

15. Assmann, G.: Die Tangier Krankheit – Klinik und Pathophysiologie (Vortrag anlässlich der Verleihung des Heinrich-Wieland-Preises am 28.10.1977 in München) [Tangier-disease (author's transl)]. *Klin. Wochenschr.* 57: 53-61, 1979

Tangier disease is a rare autosomal recessive lipid transport disease characterized by the absence of the usual high density lipoproteins from plasma and cholesteryl ester storage in many organs. 25 cases of Tangier disease have been described so long. The predominant clinical symptoms include tonsillar hypertrophy, splenomegaly and peripheral neuropathy. The cholesteryl ester storage is limited to macrophages, Schwann's cells and intestinal smooth muscle cells. Hypocholesterolemia (less than 80mg/dl), hypertriglyceridemia (greater than 200 mg/dl), and the absence of high density lipoproteins in agarose electrophoresis are the major plasma abnormalities. The protein moiety of normal high density lipoprotein consists of apoprotein A-I and apoprotein A-II. In Tangier disease, serum concentrations of these apoproteins are reduced to less than 1% and 5-10%, respectively. Theories concerning the pathogenesis of Tangier disease are only incomplete and unproved up to now; however, a structural abnormality of apoprotein A-I causing an inability to bind to lipid or other proteins (apoprotein A-II) is consistent with several of the recent biochemical findings. The imbalance of cellular cholesterol metabolism caused by the absence of high density lipoproteins as well as the presumed role of these lipoproteins in cholesterol removal from cells are discussed in this article.

16. **Assmann, G.: Tangier disease and the possible role of high density lipoproteins in atherosclerosis. In: Atherosclerosis Reviews, edited by A. M. Gotto and R. Paoletti. New York: Raven Press, 1-28, 1979**

Tangier disease is a rare hereditary disorder of plasma lipid transport. The disease was discovered by Fredrickson et al. (1,2) and given its name from Tangier island, which lies off the coast of Virginia in Chesapeake Bay and is the home of the original patients. As of today, 23 patients have been discovered. The clinical and morphological findings of the individual patients are described in patient reports (1–23) and several review articles (24–27). The clinical manifestations include:

1. Low plasma cholesterol (< 120 mg/dl) and normal or elevated triglyceride concentrations;
2. Absence of the usual high-density lipoproteins (HDL) and abnormal composition of other lipoproteins in plasma;
3. Distinctive enlargement and coloration of the tonsils;
4. Occasional peripheral neuropathy;
5. Lipid deposition in tissues accompanied by enlargement of spleen, liver, or lymph nodes; alterations in the cornea, intestinal mucosa, skin, bone marrow, and other tissues. Chemical analysis of several tissues has revealed that the lipid deposits consist mainly of cholesteryl esters; histological observations have provided evidence that intracellular lipid storage is mainly confined to macrophages (foam cells), Schwann cells, and nonvascular smooth muscle cells (23,26).

The purpose of this chapter is to summarize recent knowledge of the lipoprotein abnormalities in Tangier disease and to hypothesize about the possible metabolic mechanisms leading to extracellular depletion of HDL and intracellular cholesteryl ester storage. Particular emphasis will be given to the presumed role of HDL in triglyceride metabolism. The implications derived from Tangier disease concerning the potential relationship between HDL and atherosclerosis will be discussed in the last section of this article.

17. Schaefer, H. E. and G. Assmann: Cholesteatosis naevi naevocellularis - ein bisher unbekanntes Phänomen bei der Tangier Krankheit. Verh. Dt. Ges. Path. 63: 708, 1979.

Das Krankheitsbild der Tangier-Krankheit, also des hereditären HDL-Defektes äußert sich in einer pathologischen Lipidspeicherung, die ein spezielles Verteilungsmuster einhält. Betroffen sind ubiquitär die Makrophagen, Schwannsche Zellen in den peripheren Hautnerven, gingivale und cutane Fibroblasten sowie speziell die glatten Muskelzellen der Muscularis mucosae coli (SCHAEFER et al., 1976; GHEORGHIU et al., 1976; SCHAEFER und ASSMANN, 1977; ASSMANN et al., 1977). Bisher unbekannt ist eine erhebliche Lipidspeicherung auch in Naevus-Zellen, welche wir bei beiden, bereits früher vorgestellten homozygoten Tangier-Patienten beobachtet haben. Bei diesem Geschwisterpaar (♂ 45 J., ♀ 49 J. alt) sind von den Patienten selbst kaum bemerkt wenige Millimeter große, leicht erhabene und teilweise gering pigmentierte Knötchen in der Haut von Handrücken und Unterarmen aufgetreten, welche histologisch das typische Bild überwiegend corialer Naevuszellnaevi zeigen. Die Naevuszellen fallen durch eine Vakuolisierung ihres Cytoplasmas auf, ein Phänomen, das sich durch seinen mehr grobtropfigen Aspekt deutlich vom Zelltyp des sog. Ballonzellnaevus unterscheidet. Innerhalb der Vakuolen sind sudanophile und stark doppelbrechende und insofern offenbar cholesterinreiche Lipide in Gefrierschnitten nachweisbar. Aufgrund elektronenmikroskopischer Befunde werden solche Lipide offenbar im Rahmen einer zum Teil deutlich ausgeprägten Pinozytose in das Zellinnere eingeschleust. Die zunächst kleinsten Lipidvesikeln konfluieren zu größeren, zunächst nicht membrangebundenen Vakuolen, welche schließlich besonders in der Nachbarschaft des Golgi-Apparates mit Melanosomen fusionieren. Aus diesem Prozeß resultieren Vakuolen, die von solitären oder auch multiplen dreischichtigen Membranen umgeben werden und welche unter dem Bilde verfetteter Melanosomen Melaninpartikel enthalten können. Intraepidermale Melanozyten außerhalb des Naevuszellnaevus lassen meist keinerlei Fettspeicherung erkennen. – Die ungewöhnliche Fettaufnahme der Naevuszellen steht wahrscheinlich mit einer für Tangier-Patienten typischen Lipämie in Zusammenhang, welche auf einem gestörten Chylomikronenmetabolismus beruht. Diese Störung äußert sich u. a. in der Bildung atypischer, diskusartiger Lipoproteinpartikel (ca. $100 \times 500 \text{ \AA}$), welche stapelartig angeordnet im postprandialen Plasma nachweisbar sind und wahrscheinlich infolge verzögerter Cholesterinveresterung durch Lecithin-Cholesterin-Acyltransferase entstehen. Ferner sind in der Adventitia kleiner Hautgefäße sowie im Interstitium der Naevuszellnaevi mutmaßliche Lipoproteinkonglomerate anzutreffen, welche als unmittelbare Quelle für die gesteigerte Lipidaufnahme der Naevuszellen in Betracht kommen. – Grobvakuolär verfettete Naevuszellnaevi scheinen von pathognomonischer Bedeutung für die Diagnostik der Tangier-Krankheit zu sein. Ob ein gleichartiges Phänomen auch bei Lipämien anderer Ursache vorkommen, ist noch unbekannt.

18. **Assmann, G. and H. E. Schaefer: Possible mechanisms of lipid storage in Tangier disease. In: Atherosclerosis V, edited by A. M. Gotto, L. C. Smith, and B. Allen. Berlin, Heidelberg: Springer-Verlag, 666-670, 1980.**

Tangier disease is a rare hereditary disorder characterized by the virtual absence of normal high density lipoproteins (HDL) from plasma and widespread tissue accumulation of cholesteryl esters, mostly cholesteryl oleate (Assmann 1979; Herbert et al. 1978a). Prominent clinical features include enlarged, orange-yellow tonsils, splenomegaly and, frequently, peripheral neuropathy; early atherosclerosis is not a manifestation of the disease. Histological and ultrastructural observations have provided evidence that the intracellular lipid storage is mainly confined to macrophages (tissue histiocytes), Schwann cells of peripheral nerves and myenteric plexus, nonvascular smooth muscle cells of the intestine, nevus cells and, occasionally, mast cells and fibroblasts. Endothelial cells and smooth muscle cells of large and small arteries are not affected by lipid storage. The tissue macrophage is the site of most of the lipid deposition, and the conversion of histiocytic cells to foam cells is the predominant abnormality in affected tissues. The foam cells contain sudanophilic, cytoplasmic lipid droplets and, upon occasion, crystalline material; on examination at room temperature in plane polarized light, the storage material is birefringent and partially exhibits a maltese cross pattern. At body temperature a large proportion of the cholesteryl ester droplets within foam cells are in the smectic liquid crystalline state (Katz et al. 1977). Two major mechanisms may account for the intracellular accumulation of cholesteryl esters in various tissues: phagocytosis of abnormal lipoproteins and/or ineffective removal of cholesterol from cells. These two mechanisms are discussed in the light of present biochemical and morphological evidence.

19. **Herbert, P. N., G. Assmann, A. M. J. Gotto and D. S. Fredrickson: Familial lipoprotein deficiency: abetalipoproteinemia, hypobetalipoproteinemia, and Tangier disease. In: *The Metabolic Basis of Inherited Disease*, edited by Stanbury et al. McGraw-Hill, 589-621, 1982**

1. *The inherited lipoprotein deficiency states are of two major types. One type, exemplified by abetalipoproteinemia and familial hypobetalipoproteinemia, primarily affects the plasma lipoproteins that contain a protein called apolipoprotein B (apo B). These lipoproteins include chylomicrons and very low density lipoproteins (VLDL), which are transporters of triglycerides, and low density lipoproteins (LDL), which are end products of VLDL catabolism and which are transporters of cholesterol. Another deficiency state, exemplified by Tangier disease, involves primarily the lipoproteins containing A apolipoproteins, A-I and A-II (apo A-I and apo A-II). These are the high density lipoproteins (HDL), the functions of which are not established but may include the transport of cholesterol from peripheral cells to the liver.*
2. *Abetalipoproteinemia is characterized clinically by fat malabsorption, ataxic neuropathy, retinitis pigmentosa, and acanthocytosis. The usual mechanisms for transport of triglyceride from the intestine and liver are abolished, and chylomicrons, VLDL, and LDL are absent from the plasma. The defect is presumed (without direct proof) to involve the synthesis of apo B or intracellular assembly of apo B with lipid. The disorder is rare and is inherited as an autosomal recessive trait. A high proportion of cases (~ 50 percent) have resulted from consanguine matings. Obligate heterozy-*
3. *gotes show no clinical manifestations and typically have normal levels of plasma cholesterol and LDL. Vitamin E may prevent many of the morbid consequences in the homozygote.*
3. *Familial hypobetalipoproteinemia is a rare disorder that is distinguished from abetalipoproteinemia on genetic grounds: heterozygotes for familial hypobetalipoproteinemia have low plasma levels of cholesterol and LDL, whereas heterozygotes for abetalipoproteinemia have normal levels. Homozygotes with familial hypobetalipoproteinemia are phenotypically indistinguishable from patients with abetalipoproteinemia except for possibly milder neuromuscular impairment. A high proportion of homozygotes (~ 70 percent) have resulted from consanguine matings. In at least some heterozygotes, the abnormality appears to involve a decrease in LDL (and presumably VLDL) synthesis. Several variant forms of hypobetalipoproteinemia have been described; these may be due to defective synthesis of apo B-containing lipoproteins by either the liver or intestinal mucosa.*
4. *Tangier disease is characterized clinically by hyperplastic orange tonsils, storage of cholesteryl esters in other reticuloendothelial tissues, corneal opacities, and relapsing neuropathy. This rare disorder is inherited as an autosomal recessive trait. Approximately 25 percent of cases have resulted from consanguine mat-*

20. **Rosseneu, M., G. Schmitz and G. Assmann: Association of the Tangier apolipoprotein A-I with lipids. *Arch. Internat. Physiol. Biochim.* 91: B 38-B 39, 1983**
(No abstract available)

21. **Schmitz, G., G. Assmann, S. C. Rall and R. W. Mahley: Tangier disease - defective recombination of a specific Tangier apolipoprotein A-I isoform (pro-apo A-I) with high-density lipoproteins. *Proc. Natl. Acad. Sci. USA* 80: 6081-6085, 1983**

Isoforms of apolipoprotein A-I (apo A-I) from subjects with Tangier disease were characterized, and their ability to recombine with normal high density lipoproteins (HDL) was studied. In contrast to normal serum, in which isoprotein 4 is the dominant species [79 +/- 1.8% (mean +/- SD)], the Tangier serum contained much less total apo A-I (approximately equal to 1% of that in normal serum), and isoproteins 2 and 4 were present in roughly equivalent amounts (35.3 +/- 2.5% and 42.7 +/- 3.6%, respectively). The Tangier isoprotein 2 was shown to correspond to pro-apo A-I, having a six-amino acid amino-terminal extension with the sequence: Arg-His-Phe-Trp-Gln-Gln-. The Tangier isoprotein 4 had the same amino-terminal sequence as normal circulating plasma apo A-I. Its association with normal HDL (70%) was similar to the association of normal apo A-I with HDL (80-90%) in recombination experiments. In marked contrast to this behavior, very little (less than 10%) of Tangier isoprotein 2 (pro-apo A-I) associated with HDL in recombination experiments. These results suggest that the underlying defect in Tangier disease may be a faulty conversion of pro-apo A-I to mature apo A-I, either due to a defect in the converting enzyme activity or to a further specific structural defect in Tangier apo A-I. The failure of Tangier pro-apo A-I to associate with HDL may be at least partially responsible for the HDL deficiency in Tangier subjects.

22. **Rosseneu, M., G. Assmann, M. J. Taveirne and G. Schmitz: Lipid binding properties of the Tangier apolipoprotein A-I and its isoproteins. *J. Lipid Res.* 25: 111, 1984**

The apolipoprotein A-I was isolated from the plasma of normal individuals and of three homozygous patients with Tangier disease by immunoprecipitation. The apoA-I isoforms were further fractionated by isofocusing on polyacrylamide gels. The physicochemical behavior of normal and Tangier apoA-I and of the isoproteins-2 and -4 was studied by monitoring the tryptophanyl fluorescence emission as a function of temperature, pH, and under exposure to guanidinium (guanidine) hydrochloride (GdmCl). Lipid-apoprotein complexes were generated by incubation with dimyristoylphosphatidylcholine and isolated by density gradient ultracentrifugation. Our results show that normal apoA-I and its isoprotein-4 associate with lipids to yield a complex containing 150-200 mol lecithin/mol apoA-I. The isoprotein-2 of normal apoA-I and the isoprotein-4 of Tangier apoA-I generate lipid-rich complexes with lecithin, while the isoprotein-2 of Tangier apoA-I shows only a limited association with lipids. ApoA-I normal and Tangier and their isoproteins-4 undergo a structural transition around 45 degrees C, which is not observed in the lecithin-apoA-I complexes. This transition is accompanied by an increased exposure of the tryptophanyl residues to the solvent. This transition was observed for the isoprotein-2 of apoA-I Tangier both in its lipid-free form and in the presence of lecithin. The pH denaturation of apoA-I and of the isoprotein-4 between pH 9 and 13 and between pH 7 and 2 is accompanied by a similar conformational transition. The transition occurs around pH 10.8 for the native apoproteins and is shifted towards respectively higher and lower pH's as result of the protective action of lipid binding on the protein conformation. Such an effect was not observed with the isoprotein-2 of apoA-I Tangier which is denatured at lower pH's both in its native form and in a lipid-protein mixture. Finally the denaturation of apoA-I by GdmCl indicates that apoA-I normal and Tangier undergo structural changes around 1 M GdmCl, whereas the apoA-I-Tangier-lecithin complex is more susceptible to denaturation than the complex with apoA-I normal. These data suggest that the apoA-I normal and Tangier and their isoproteins-4 are able to associate with lipids although the association between apoA-I Tangier with lecithin is weaker than that of apoA-I normal. The isoprotein-2 of normal apoA-I associates to a greater extent with lipids than the isoprotein-2 of Tangier apoA-I, whose structure differs from that of the isoprotein-4.

23. **Gibbels, E., H. E. Schaefer, U. Runne, J. M. Schroder, W. F. Haupt and G. Assmann: Severe polyneuropathy in Tangier disease mimicking syringomyelia or leprosy - clinical, biochemical, electrophysiological, and morphological evaluation, including electron microscopy of nerve, muscle, and skin biopsies. *J. Neurol.* 232: 283-294, 1985.**

Polyneuropathy in Tangier disease can be divided into three clinical types. The most severe form (type III) with a syringomyelia-like syndrome has been described in three cases only. Here, a fourth case of this type is presented. Because of unusual trophic disturbances even leprosy was suspected. Electrodiagnostic findings, including evoked cerebral potentials in this case, were suggestive of a generalized neuropathy with some degree of primary or secondary demyelination and implied possible impairment of central structures. Sural nerve biopsy, including electron microscopy and quantitative analysis, revealed a predominant reduction of smaller myelinated and unmyelinated fibres. The main morphological feature was the abundance of abnormal non-membrane-bound vacuoles in Schwann cells, mostly of the unmyelinated type, and in some endoneurial fibroblasts, macrophages and perineurial cells. There was no inverse relationship between lipid vacuoles and axons in Schwann cell complexes as suspected by others. An excess of endoneurial collagen as well as an increased fascicular area were obvious. In five skin biopsy specimens of different regions typical vacuoles were noted in Schwann cells, histiocytes, nevus cells, and rarely in perineurial cells.

24. **Schmitz, G., G. Assmann, H. Robenek and B. Brennhäuser: Tangier disease - a disorder of intracellular membrane traffic. *Proc. Natl. Acad. Sci. USA* 82: 6305-6309, 1985.**

The interaction of human high density lipoproteins (HDL) with isolated human monocytes from control and Tangier patients was studied in tissue culture experiments. It was observed that normal monocytes, similar to mouse peritoneal macrophages, bind HDL to a cell surface receptor, internalize the bound HDL particles, transport the internalized HDL intracellularly through the cytoplasmic compartment without significant degradation, and ultimately resecret intact HDL. The cellular interaction of Tangier monocytes with normal HDL was markedly different from control monocytes. HDL binding to Tangier monocytes was moderately increased and cell-associated HDL radioactivity was 6- to 10-fold enhanced in Tangier monocytes. The bulk of the internalized HDL, however, was detected in secondary lysosomes. Only minor amounts of the internalized HDL were resecreted from the Tangier monocytes, and most of this was degraded. These data suggest that the cellular metabolism of HDL is abnormal in Tangier monocytes. It is postulated that Tangier disease may be a disorder of intracellular membrane traffic in which HDL is diverted into the lysosomal compartment and degraded instead of being secreted through its regular transcellular route.

25. **Carlson, L. A., L. Holmquist and G. Assmann: Different substrate specificities of plasma lecithin -cholesterol acyl transferase in fish eye disease and Tangier disease. *Acta Med. Scand.* 222: 345-350, 1987.**

Esterification of plasma free cholesterol is mediated by lecithin:cholesterol acyl transferase (LCAT). The free cholesterol of plasma high density lipoproteins (HDL) is considered to be the preferred substrate for LCAT. It therefore appeared as a paradox that plasma cholesterol esterification, both in vivo and in vitro, is normal in fish eye disease and Tangier disease, two familial conditions with extremely low plasma HDL levels. Fish eye disease plasma, however, was shown to have LCAT activity primarily acting on combined very low (VLDL) and low (LDL) density lipoproteins, denominated beta-LCAT, while it lacked LCAT activity esterifying HDL cholesterol (alpha-LCAT). Here we show that Tangier plasma, in contrast, has both alpha- and beta-LCAT. Thus, in both fish eye and Tangier diseases it is beta-LCAT that explains the apparent normal plasma cholesterol esterification. We also show that Tangier plasma, having alpha-LCAT activity, normalizes the low cholesteryl ester content as well as the abnormally small size of fish eye disease HDL particles during incubation.

26. **Schmitz, G., G. Assmann, B. Brennhäusen and H. J. Schaefer: Interaction of Tangier lipoproteins with cholesteryl ester-laden mouse peritoneal macrophages. *J. Lipid Res.* 28: 87-99, 1987**

Cholesterol efflux was studied from cholesteryl esterladen mouse peritoneal macrophages in the presence of Tangier lipoproteins derived from fasting and postprandial sera of three patients homozygous for Tangier disease (analphalipoproteinemia). The d greater than 1.063 g/ml fractions isolated from fasting patients and 3 hr and 18 hr after an oral fat load were all effective in cellular cholesterol removal. By contrast, the d greater than 1.063 g/ml fractions isolated 6 hr and 12 hr after fat ingestion did not affect net removal of cellular cholesterol. The d greater than 1.21 g/ml protein fractions derived from fasting as well as postprandial sera were all effective in removing cholesterol. D 1.063-1.21 g/ml fractions from fasting Tangier patients contained HDLT. In the corresponding postprandial fractions, in addition to HDLT, apoB-100- and apoB-48-containing lipoproteins were present. Furthermore, the 6 hr and 12 hr postprandial Tangier HDL fractions contained apoB-immunoreactive proteins of lower molecular weight. The abnormal activity of the elastase/alpha 1-antitrypsin proteolytic system and the abnormal fibronectin concentration we found in Tangier plasma suggests a possible relationship to the in vivo degradation of apoB. The peculiar type of membrane-bound lipid droplets in Tangier splenic macrophages points to a lipoprotein source of lipid accumulation which possibly originates from the uptake of chylomicrons or chylomicron-derived particles. It is concluded that cholesteryl ester storage in Tangier macrophages results from an imbalance of cholesterol influx and efflux. In the absence of HDL, the net increase of cholesterol caused by abnormal lipoproteins in certain postprandial states cannot be fully compensated by effective efflux and ultimately leads to macrophage cholesteryl ester accumulation.

27. **Assmann, G., G. Schmitz and H. B. Brewer, Jr.: Familial high density lipoprotein deficiency: Tangier Disease. In: *The Metabolic Basis of Inherited Disease*, edited by C. H. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle. McGraw-Hill, 1267, 1989**
(No abstract available)
28. **Schmitz, G., H. Robenek, B. Brennhäusen and G. Assmann: Abnormal processing of HDL precursors in Tangier monocyte-derived macrophages. In: *NATO Advanced Res. Commun. Series*, edited by C. Sirtori. Plenum Press, 1989**
(No abstract available)
29. **Schmitz, G., H. Robenek, B. Brennhäusen, and G. Assmann: Abnormal processing of HDL precursors in Tangier monocyte-derived macrophages. In: *NATO Advanced Res. Commun. Series*, edited by C. Sirtori. New York: Plenum Press, 1990.**
(No abstract available)

30. Walter, M., U. Gerdes, U. Seedorf and G. Assmann: The high density lipoprotein-induced and apolipoprotein A-I-induced mobilization of cellular cholesterol is impaired in fibroblasts from Tangier disease subjects. *Biochem. Biophys. Res. Comm.* 205: 850-856, 1994

Tangier disease (also known as familial HDL-deficiency) is characterized by very low high density lipoprotein (HDL) plasma levels, splenomegaly, and massive cholesteryl ester accumulation in the cytoplasm of various cell types. Since this phenotype may in part be caused by a defect in the pathway mediating cholesterol efflux from peripheral cells, we investigated the HDL3-mediated mobilization of cholesterol synthesized de novo from [14C]-mevalonolactone in cultivated fibroblasts from two patients with Tangier disease. Our results indicate that the HDL3-induced translocation of [14C]-cholesterol from intracellular pools to the plasma membrane and its subsequent secretion into the extracellular medium was approximately 50% less in the cells from the patients than in controls. The same result was also obtained with artificial apolipoprotein A-I-containing phospholipid vesicles. By contrast, no significant difference in HDL3-induced cholesterol efflux was observed when plasma membrane was labeled with exogenous [14C]-cholesterol. We conclude that inefficient cholesterol efflux in Tangier disease is primarily caused by impaired HDL3-induced activation of cholesterol translocation from intracellular pools to the plasma membrane.

31. Walter, M., S. Kerber, C. Fehtrup, U. Seedorf, G. Breithardt and G. Assmann: Koronarangiographie und intravaskuläre Ultraschalluntersuchung bei einem 60jährigen Patienten mit familiärer HDL-Defizienz (Tangier-Krankheit). *Z. Kardiol.* 83: 381-385, 1994

Zusammenfassung: Wir berichten über die Koronarangiographie und intravaskuläre Ultraschalluntersuchung bei einem 60jährigen Patienten mit familiärer HDL-Defizienz, der trotz einer fast kompletten HDL-Defizienz und einer massiven Schaumzellbildung in retikuloendothelialen Geweben nur geringgradige atherosklerotische Veränderungen aufwies. Die Koronarangiographie zeigte nur diskrete Wandveränderungen einzelner Gefäßsegmente, die intravaskuläre Sonographie zeigte den normalen Gefäßwandaufbau der peripheren Gefäße mit nur einer umschriebenen arteriosklerotischen Läsion in der A. illaca.

32. Assmann, G., A. von Eckardstein and H. B. J. Brewer: Familial high density lipoprotein deficiency: Tangier disease. In: *The Metabolic and Molecular Basis of Inherited Disease*, edited by C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle. New York: McGraw-Hill, 2053-2072, 1995

1. **Tangier disease is characterized by severe deficiency or absence of normal high-density lipoproteins (HDL) in plasma and results in the accumulation of cholesteryl esters in many tissues throughout the body. These include tonsils, liver, spleen, lymph nodes, thymus, intestinal mucosa, peripheral nerves, and probably the cornea.**
2. **The major clinical signs are hyperplastic orange tonsils, splenomegaly, and relapsing neuropathy. HDL deficiency and low plasma cholesterol concentration accompanied by normal or elevated triglyceride levels in combination with hyperplastic orange-yellow tonsils and adenoidal tissue are pathognomonic. Despite HDL deficiency, there is only a minimal increase in risk for myocardial infarction.**
3. **Plasma apo A-I concentration is extremely low (<3 percent that of controls), and the small amount of HDL in Tangier plasma differs from normal HDL, particularly with respect to apolipoprotein content. In addition, chylomicron remnants and VLDL are highly abnormal.**
4. **Obligate heterozygotes have no clinical manifestation and are characterized biochemically by half-normal serum concentrations of HDL cholesterol, apo A-I, and apo A-II.**
5. **The molecular basis of Tangier disease is as yet unknown, but it likely involves an autosomal gene affecting a pathway in intracellular lipid transfer processes that is intimately related to the metabolism of HDL.**
6. **There is no specific treatment for Tangier disease.**

33. Huang, Y., A. Von Eckardstein, S. Wu, C. Langer and G. Assmann: Generation of pre- β 1-hdl and conversion into β -hdl evidence for disturbed hdl conversion in tangier disease. *Arterioscler. Thromb. Vasc. Biol.* 15: 1746-1754, 1995

HDL encompasses several apoA-I-containing particles that differ by size and show pre-beta- or alpha-mobility on agarose gel electrophoresis: pre-beta 1-LpA-I, pre-beta 2-LpA-I, pre-beta 3-LpA-I, alpha-LpA-I2, and alpha-LpA-I3. The quantitatively minor subclass pre-beta 1-LpA-I serves as an initial acceptor of cell-derived cholesterol. In this study, we generated a pre-beta 1-LpA-I-like particle in vitro by the incubation of biotinylated apoA-I with cholesterol-loaded macrophages. Both native pre-beta 1-LpA-I and in vitro-generated pre-beta 1-LpA-I were indistinguishable from lipid-free apoA-I by two-dimensional nondenaturing polyacrylamide gradient gel electrophoresis but exhibited a different size upon gel filtration. In vitro-generated biotin-pre-beta 1-LpA-I took up twofold to threefold more [3 H]cholesterol from labeled fibroblasts during a 1-minute pulse incubation than lipid-free apoA-I. The in vitro conversion of biotin-pre-beta 1-LpA-I was investigated in the presence of plasmas of healthy probands and patients with Tangier disease, with apoA-I deficiency, and with lecithin-cholesterol acyltransferase (LCAT) deficiency. Incubation of biotin-pre-beta 1-LpA-I with plasmas either from normoalphalipoproteinemic probands or from a patient with apoA-I deficiency generated a biotinylated particle with the size and electrophoretic mobility of alpha-LpA-I2. This conversion was sensitive to heating at 56 degrees C but not to the removal of calcium. Inhibition of LCAT by dithiobisnitrobenzoic acid led to the formation of alpha-LpA-I3 instead of alpha-LpA-I2.

34. von Eckardstein, A., Y. Huang, S. Wu, H. Funke, G. Nosedá and G. Assmann: Reverse cholesterol transport in plasma of patients with different forms of familial HDL deficiency. *Arterioscler. Thromb. Vasc. Biol.* 15: 691-703, 1995.

HDLs encompass structurally heterogeneous lipoproteins that fulfill specific functions in reverse cholesterol transport. Two-dimensional nondenaturing gradient gel electrophoresis (2D-PAGE) of normoalphalipoproteinemic plasma and subsequent immunoblotting with anti-apoA-I-antibodies differentiates pre-beta 1-LpA-I, pre-beta 2-LpA-I, pre-beta 3-LpA-I, alpha-LpA-I2, and alpha-LpA-I3. Immunodetection with anti-apoE antibodies differentiates gamma-LpE and alpha-LpE. Pulse-chase incubations of plasma with [3 H]unesterified cholesterol ([3 H]UC)-labeled fibroblasts and subsequent 2D-PAGE revealed that cell-derived [3 H]UC is taken up by pre-beta 1-LpA-I and gamma-LpE. From these initial acceptors, [3 H]UC is transferred to LDL via pre-beta 2-LpA-I \rightarrow pre-beta 3-LpA-I \rightarrow alpha-LpA-I. Some UC is esterified in pre-beta 3-LpA-I, and some is esterified in alpha-LpA-I after its retransfer from LDL. In this study we investigated the effect of various forms of familial HDL deficiency on reverse cholesterol transport. Plasma samples of patients with various forms of HDL deficiency are characterized by the lack of specific HDL subclasses. ApoE-containing HDLs, including gamma-LpE, are present in all kinds of HDL deficiency. However, all forms of LpA-I are absent in apoA-I-deficient plasma, pre-beta 3-LpA-I and alpha-LpA-I from the plasma of patients with Tangier disease (TD), and pre-beta 3-LpA-I and large alpha-LpA-I from the plasma of patients with lecithin:cholesterol acyltransferase (LCAT) deficiency and fish-eye disease (FED). After a 1-minute pulse with labeled fibroblasts, efflux of [3 H]UC into HDL-deficient plasmas decreased, compared with normal plasma, by 49% (apoA-I deficiency), 36% (TD), 21% (LCAT deficiency), and 28% (FED). In apoA-I deficiency, only gamma-LpE takes up cell-derived [3 H]UC. In the three other HDL-deficiency states, cell-derived [3 H]UC is initially taken up by both pre-beta 1-LpA-I and gamma-LpE. The four HDL deficiencies are also characterized by differences in the esterification of cell-derived [3 H]UC. No esterification occurs in LCAT-deficient plasma. In FED plasma, [3 H]UC is esterified in LDL. In apoA-I deficiency and TD, however, [3 H]UC is esterified in lipoproteins free of apoA-I and apoB. In the two latter cases, the transfer of [3 H]cholesteryl ester to LDL is enhanced compared with normal plasma. The lack of specific HDL subclasses and the consequent changes in reverse cholesterol transport pathways differently affect net mass efflux of cholesterol from fibroblasts into HDL-deficient plasma.

35. Funke, H., H. Wiebusch, S. Rust and G. Assmann: Molecular genetics approach to lipoprotein metabolism disorders. In: HDL deficiency and atherosclerosis, edited by G. Assmann. Dordrecht: Kluwer Academic Publishers, 1-15, 1995. (No abstract available)

1

Molecular genetics approach to lipoprotein metabolism disorders

H. FUNKE, H. WIEBUSCH, S. RUST and G. ASSMANN

INTRODUCTION

In past decades epidemiological studies have identified several risk factors for early-onset coronary artery disease (CAD¹⁻³). Among these factors disorders of lipoprotein metabolism have a leading role. The PROspective Cardiovascular Münster (PROCAM) study, carried out in the northwest of Germany, has demonstrated that among all single-parameter biochemical markers the concentration of plasma lipids has the highest predictive value.

There are different degrees of association between the incidence of myocardial infarctions and the three main forms of lipoprotein metabolism disorders, hypercholesterolaemia, hypertriglyceridaemia and high density lipoprotein (HDL) reduction. In addition, it is not unusual that these disorders occur in combination. Yet another complicating factor is that these easy-to-determine variations in plasma lipids do not have a common aetiological determinant. This can be illustrated by the fact that, e.g., a hypercholesterolaemia can in one case be caused largely by dietary mistakes, whereas in another case the only aetiological factor is an inherited gene defect.

Even though the results from the PROCAM study and other prospective studies have clearly demonstrated a close link between lipoprotein metabolism and the risk of developing CAD, this knowledge is often difficult to translate into individual risk and the correct strategy for treatment. One possibility to improve risk prediction for the individual may be the breakdown of risk factors into aetiological distinct entities. The formation of phenotype subgroups and a differentiated risk assessment might thus be possible based on underlying genetic defects.

36. von Eckardstein, A., Y. Huang and G. Assmann: Role of high density lipoprotein subclasses in reverse cholesterol transport. In: HDL Deficiency and Atherosclerosis, edited by G. Assmann. Dordrecht, Boston, London: Kluwer Academic Publishers, 17-23, 1995. (No abstract available)

Role of high density lipoprotein subclasses in reverse cholesterol transport

A. VON ECKARDSTEIN, Y. HUANG and G. ASSMANN

INTRODUCTION

Several epidemiological and clinical studies have demonstrated the inverse correlation between the plasma concentration of high density lipoprotein (HDL) cholesterol and the risk of myocardial infarction (reviewed in ref. 1). The ability of HDL to protect the vessel wall from atherosclerosis has usually been explained by the reverse cholesterol transport model (reviewed in ref. 2) in which HDL mediates the flux of excess cholesterol from peripheral cells to the liver. HDL-cholesterol levels are determined by environmental and genetic factors. The influence of genes on the variation of HDL-cholesterol levels has been estimated to account for up to 50%, but only rare defects in the genes of apolipoprotein (apo)A-I and lecithin:cholesterol acyltransferase (LCAT) could be made responsible for familiarly low HDL-cholesterol levels³. Despite the virtual absence of HDL, several homozygotes for apoA-I deficiency, LCAT deficiency, and fish-eye disease, but also patients with Tangier disease or unclassified forms of HDL deficiency did not present with premature atherosclerosis³⁻⁶. Family histories of these patients did not indicate any increased prevalence of coronary heart disease (CHD) events, although heterozygotes for various defects in the genes of apoA-I and LCAT, as well as obligate heterozygotes for Tangier disease, usually have HDL-cholesterol levels below the 10th percentile of sex- and age-matched controls^{3,5,6}. These clinical observations have questioned a direct anti-atherogenic role of HDL. HDL, however, include structurally and functionally heterogeneous lipoproteins which can be differentiated on the basis of density, size, charge and apolipoprotein composition⁷. During recent years, experiments of our and other investigators' laboratories have yielded a large body of evidence show-

37. Seedorf, U., P. Brysch, T. Engel, S. Scheek, M. Raabe, M. Fobker, T. Szyperski, K. Wüthrich, N. Maeda and G. Assmann: Intracellular cholesterol transport. In: HDL Deficiency and Atherosclerosis, edited by G. Assmann. Dordrecht, Boston, London: Kluwer Academic Publishers, 43-52, 1995. (No abstract available)

Intracellular cholesterol transport

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Intracellular trafficking of cholesterol and various other sterols is important for cholesterol homeostasis of cells and the entire organism. Understanding the mechanisms involved in regulated, target-specific intracellular cholesterol transport is of great impact for understanding the pathogenesis of atherosclerosis and a number of inherited lipid storage disorders, such as Niemann–Pick disease (type C), Zellweger disease and Tangier disease. Since, in humans, only the liver contains the enzymes necessary for cholesterol degradation, all other tissues in the body have to export excess cholesterol to this organ in a pathway called reverse cholesterol transport. It is obvious that this pathway represents an important mechanism protecting cells and tissues from cholesterol overloading, a phenomenon observed in cells of the arterial wall during atherogenesis. The initial steps consisting of a target-specific flux of cholesterol from intracellular pools to the plasma membrane are only poorly understood at present. It is generally accepted that intracellular trafficking of cholesterol and other sterols is not mediated by non-specific diffusion but requires target-specific transport mechanisms. Earlier studies performed in our and other laboratories indicated that the bulk flow of *de-novo* synthesized cholesterol from the endoplasmic reticulum to the plasma membrane is a fast, energy-requiring, vesicular process that proceeds independently of the secretory protein pathway¹. Specific transport mechanisms have also been suggested for the transfer of cholesterol from the membranes of secondary lysosomes to the endoplasmic reticulum, the intracellular site of cholesterol esterification catalysed by the enzyme acyl-CoA cholesterol acyl transferase (ACAT), and the delivery of sterols to mitochondria and peroxisomes². The latter pathways are primarily required for the synthesis of

38. von Eckardstein, A., Y. Huang and G. Assmann: Uptake transfer and esterification of cell-derived cholesterol in plasma of patients with familial HDL deficiency. *Z. Gastroenterol* 36: 143-144, 1996 (No abstract available)

- 39. Walter, M., H. Reinecke, U. Gerdes, J. R. Nofer, G. Höbbel, U. Seedorf and G. Assmann: Defective regulation of phosphatidylcholine-specific phospholipases C and D in a kindred with Tangier disease. Evidence for the involvement of phosphatidylcholine breakdown in HDL-mediated cholesterol efflux mechanisms. *J. Clin. Invest.* 98: 2315-2323, 1996.**

The negative correlation between coronary heart disease and plasma levels of HDL has been attributed to the ability of HDL to take up cellular cholesterol. The HDL3-induced removal of cellular cholesterol was reported to be impaired in fibroblasts from patients with familial HDL deficiency (Tangier disease, TD). In addition, we have recently shown that HDL3 stimulates the hydrolysis of phosphatidylcholine (PC) in cholesterol-loaded fibroblasts. To investigate whether this cell signaling pathway is involved in cholesterol efflux mechanisms, we compared the HDL3-induced PC hydrolysis in normal fibroblasts and in fibroblasts from a TD kindred, in whom the HDL3- and apolipoprotein A-I (apo A-I)-induced mobilization of cellular cholesterol was found to be reduced by 50%. The HDL3-induced formation of phosphatidic acid (PA) via PC-specific phospholipase D (PC-PLD) was markedly reduced by 60-80% in these cells, whereas the formation of diacylglycerol (DG) via PC-specific phospholipase C (PC-PLC) was two- to threefold enhanced. Defective regulation of PC-PLC and PC-PLD was similarly observed in response to apo A-I and endothelin, but not in response to the receptor-independent stimulation of PC hydrolysis by PMA. A Tangier-like PA and DG formation pattern could be induced in normal cells after preincubation with pertussis toxin, suggesting the involvement of a G-protein. The impaired mobilization of radiolabeled cellular cholesterol in TD cells could completely be overcome by increasing the PA levels in the presence of the PA phosphohydrolase inhibitor propranolol. Conversely, the inhibition of PA formation in the presence of 0.3% butanol as well as the inhibition of DG formation in the presence of the PC-PLC inhibitor D 609 reduced the mobilization of cellular cholesterol both in normal and in TD cells. Our data indicate that the coordinate formation of PA and DG via PC-PLD and PC-PLC is essential for efficient cholesterol efflux. The molecular defect in this TD kindred appears to affect an upstream effector of protein kinase C responsible for the G-protein-dependent regulation of PC-specific phospholipases.

- 40. Rust, S., M. Walter, H. Funke, A. von Eckardstein, P. Cullen, H.Y. Kroes, R. Hordijk, J. Geisel, J. Kastelein, H.O.F. Molhuizen, M. Schreiner, A. Mische, H.W. Hahmann and G. Assmann: Assignment of Tangier disease to chromosome 9q31 by a graphical linkage exclusion strategy. *Nat. Gen.* 20, 96-98, 1998**

A low level of high density lipoprotein (HDL) cholesterol is a strong predictor of ischaemic heart disease (IHD) and myocardial infarction. One cause of low HDL-cholesterol is Tangier disease (TD), an autosomal codominant inherited condition first described in 1961 in two siblings on Tangier Island in the United States of America. Apart from low HDL-cholesterol levels and an increased incidence of atherosclerosis, TD is characterized by reduced total cholesterol, raised triglycerides, peripheral neuropathy and accumulation of cholesteryl esters in macrophages, which causes enlargement of the liver, spleen and tonsils. In contrast to two other monogenic HDL deficiencies in which defects in the plasma proteins apoA-I and LCAT interfere primarily with the formation of HDL (refs 7-10), TD shows a defect in cell signalling and the mobilization of cellular lipids. The genetic defect in TD is unknown, and identification of the Tangier gene will contribute to the understanding of this intracellular pathway and of HDL metabolism and its link with IHD. We report here the localization of the genetic defect in TD to chromosome 9q31, using a genome-wide graphical linkage exclusion strategy in one pedigree, complemented by classical lod score calculations at this region in a total of three pedigrees (combined lod 10.05 at D9S1784). We also provide evidence that TD may be due to a loss-of-function defect.

41. **von Eckardstein, A., A. Chirazi, S. Schuler-Lüttmann, M. Walter, J.J.P. Kastelein, J. Geisel, J. T. Real, R. Miccoli, G. Nosedo, G. Höbbel and G. Assmann: Plasma and fibroblasts of Tangier disease patients are disturbed in transferring phospholipids onto apolipoprotein A-I. *J. Lipid Res.* 39: 987-998, 1998.**

Plasmas of patients with Tangier disease (TD) lack lipid-rich alpha-HDL which, in normal plasma, constitutes the majority of high density lipoprotein (HDL). Residual amounts of apolipoprotein (apo)A-I in TD plasma occur as lipid-poor or even lipid-free prebeta-HDL. By contrast to normal plasma, TD plasma does not convert prebeta-HDL into alpha-HDL. Moreover, fibroblasts of TD patients were found to be defective in secreting cholesterol or phospholipids in the presence of lipid-free apoA-I. We have therefore hypothesized that both defective conversion of prebeta-HDL into alpha-HDL and defective lipid efflux from TD cells onto lipid-free apoA-I result from a disturbance in phospholipid transfer occurring in both cellular and extracellular compartments. To test this hypothesis we established an assay that measures the activity of plasma, cells, and cell culture media to transfer radiolabeled phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) from vesicles onto apoA-I, apoA-II, albumin, or reconstituted HDL. Plasmas, HDL, and lipoprotein-depleted plasma of normolipidemic probands as well as cell homogenates and culture media of normal fibroblasts were active at 37 degrees C but not at 4 degrees C in transferring radiolabeled PC, PI, and PE dose- and time-dependently onto either lipid-free apoA-I or reconstituted HDL. Transfer of glycerophospholipids onto apoA-II was much lower than onto apoA-I; transfer onto albumin was close to background. Compared to ten normolipidemic plasmas and four apoA-I-deficient plasmas, plasmas of six TD patients were significantly reduced by 40-50% in their glycerophospholipid transfer activities. Compared to eight normal fibroblast cell lines, homogenates and culture media of four TD fibroblast cell lines were reduced by 40-50% and 30-35%, respectively, in their activity to transfer PC, PI, or PE onto apoA-I. Our data suggest that in TD the same mechanism underlies both defective conversion of prebeta-HDL into alpha-HDL and impaired efflux of cellular lipids, namely a defective phospholipid transfer.

42. **von Eckardstein, A., Y. Huang, J.J.P Kastelein, J. Geisel, J.T. Real, J.A. Kuivenhoven, R. Miccoli, G. Nosedo and G. Assmann: Lipid-free apolipoprotein (apo) A-I is converted into alpha-migrating high density lipoproteins by lipoprotein-depleted plasma of normolipidemic donors and apo A-I-deficient patients but not of Tangier disease patients. *Atherosclerosis* 138: 25-34, 1998**

Plasma of patients with Tangier disease (TD) is devoid of alpha-LpA-I (apolipoprotein A-I-containing lipoprotein), which in normolipidemic plasma constitutes the majority of high density lipoprotein (HDL). The residual amounts of apolipoprotein A-I (apo A-I) in TD plasma have electrophoretic prebeta1-LpA-I mobility. We have previously demonstrated that TD plasma does not convert prebeta1-LpA-I into alpha-LpA-I. In this study we found that plasmas of normolipidemic controls, apo A-I-deficient patients and patients with fish-eye disease, but not plasmas of six TD patients, convert biotinylated lipid-free apo A-I into alpha-LpA-I. Supplementation of plasma with free oleic acid or fatty acid free albumin neither inhibited conversion activity in normal plasmas nor reconstituted it in TD plasma. In normal plasma the conversion activity was assessed in HDL and in the lipoprotein-free fraction. The latter fraction, however, generated larger particles only in the presence of exogenous phospholipid vesicles. To obtain particles with alpha-mobility, these vesicles had to contain phosphatidylinositol and/or cholesterol. Lipoprotein-depleted TD plasma did not convert lipid-free apo A-I into alpha-LpA-I even in the presence of exogenous vesicles with phospholipids or cholesterol. Taken together we conclude that disturbed transfer of glycerophospholipids onto apo A-I or prebeta1-LpA-I prevents maturation of HDL and thereby possibly causes deficiency of HDL cholesterol in patients with TD. Moreover, the lack of alpha-LpA-I in TD plasma together with its failure to convert exogenous apo A-I into an alpha-migrating particle provide specific tests for the diagnosis of TD.

43. **Assmann, G. and A. von Eckardstein: Tangier Disease. In: *Lipoproteins in Health and Disease*. Edited by Hodder & Stoughton Ltd. Kent, pp 767-781, 1999**
(No abstract available)

44. **Remaley, A.T., S. Rust, M. Rosier, C. Knapper, L. Naudin. C. Broccardo, K.M. Peterson, C. Koch, I. Arnould, C. Prades, N. Duverger, H. Funke, G. Assmann, M. Dinger, M. Dean, G. Chimini, S. Santamarina-Fojo, D.S. Fredrickson, P. Deneffe and H.B. Brewer Jr.: Human ATP-binding cassette transporter 1 (ABC1): Geomic organization and identification of the genetic defect in the original Tangier disease kindred. *Proc. Natl. Acad. Sci* 22: 12685-12690, 1999.**

Tangier disease is characterized by low serum high density lipoproteins and a biochemical defect in the cellular efflux of lipids to high density lipoproteins. ABC1, a member of the ATP-binding cassette family, recently has been identified as the defective gene in Tangier disease. We report here the organization of the human ABC1 gene and the identification of a mutation in the ABC1 gene from the original Tangier disease kindred. The organization of the human ABC1 gene is similar to that of the mouse ABC1 gene and other related ABC genes. The ABC1 gene contains 49 exons that range in size from 33 to 249 bp and is over 70 kb in length. Sequence analysis of the ABC1 gene revealed that the proband for Tangier disease was homozygous for a deletion of nucleotides 3283 and 3284 (TC) in exon 22. The deletion results in a frameshift mutation and a premature stop codon starting at nucleotide 3375. The product is predicted to encode a nonfunctional protein of 1,084 aa, which is approximately half the size of the full-length ABC1 protein. The loss of a Mnl1 restriction site, which results from the deletion, was used to establish the genotype of the rest of the kindred. In summary, we report on the genomic organization of the human ABC1 gene and identify a frameshift mutation in the ABC1 gene of the index case of Tangier disease. These results will be useful in the future characterization of the structure and function of the ABC1 gene and the analysis of additional ABC1 mutations in patients with Tangier disease.

45. **Rust, S., M. Rosier, H. Funke, J. Real, Z. Amoura, J-C. Piette, J.-F Deleuze, H.B. Brewer, N. Duverger, P. Denèfle and G. Assmann: Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nat. Gen.*22: 352-355, 1999.**

Tangier disease (TD) was first discovered nearly 40 years ago in two siblings living on Tangier Island. This autosomal co-dominant condition is characterized in the homozygous state by the absence of HDL-cholesterol (HDL-C) from plasma, hepatosplenomegaly, peripheral neuropathy and frequently premature coronary artery disease (CAD). In heterozygotes, HDL-C levels are about one-half those of normal individuals. Impaired cholesterol efflux from macrophages leads to the presence of foam cells throughout the body, which may explain the increased risk of coronary heart disease in some TD families. We report here refining of our previous linkage of the TD gene to a 1-cM region between markers D9S271 and D9S1866 on chromosome 9q31, in which we found the gene encoding human ATP cassette-binding transporter 1 (ABC1). We also found a change in ABC1 expression level on cholesterol loading of phorbol ester-treated THP1 macrophages, substantiating the role of ABC1 in cholesterol efflux. We cloned the full-length cDNA and sequenced the gene in two unrelated families with four TD homozygotes. In the first pedigree, a 1-bp deletion in exon 13, resulting in truncation of the predicted protein to approximately one-fourth of its normal size, co-segregated with the disease phenotype. An in-frame insertion-deletion in exon 12 was found in the second family. Our findings indicate that defects in ABC1, encoding a member of the ABC transporter superfamily, are the cause of TD.

46. **Schuler-Lüttmann, S., Y. Zhu, M. Hoffmann, W. März, G. Feussner, H. Wieland, G. Assmann and A. von Eckardstein: Cholesterol efflux from normal and Tangier disease fibroblasts into normal, HDL-deficient, and apoE-deficient plasmas. *Metabolism* 49: 770-777, 2000.**

Tangier disease (TD) fibroblasts have defective cholesterol release in the presence of lipid-free apolipoproteins. We compared normolipidemic probands and patients with apolipoprotein A-I (apoA-I) deficiency, apoE deficiency, or TD in terms of the plasma capacity to induce the efflux of [3H]-cholesterol from normal and TD fibroblasts and to esterify this cell-derived cholesterol. Compared with normal fibroblasts, TD fibroblasts released a significantly smaller fraction of [3H]-cholesterol into normal, high-density lipoprotein (HDL)-deficient, and apoE-deficient plasmas. Supplementation of apoE-deficient plasma with exogenous apoE normalized the cholesterol efflux from normal cells but did not fully restore the reduced cholesterol efflux from TD fibroblasts. Compared with control plasma, HDL- and apoE-deficient plasmas had a significantly reduced activity to esterify cell-derived cholesterol. Cholesterol derived from TD fibroblasts was less available for esterification in either patient or normal plasmas than cholesterol derived from normal cells. The esterification defect of TD cell-derived cholesterol was more pronounced in patient plasmas than in control plasma. We conclude that (1) apoA-I and, to a lesser degree, apoE are important determinants of the cholesterol efflux and esterification capacity of plasma, (2) TD fibroblasts have a reduced capacity to release cholesterol into the plasma, and (3) TD cell-derived cholesterol is less available for esterification in plasma than cholesterol from normal fibroblasts. The absence of distinct apoA-I- or apoE-containing subclasses aggravates the defective efflux and esterification of cholesterol derived from TD cells.

47. **Assmann, G., A. von Eckardstein and HB Brewer: Familial anaphalipoproteinemia: Tangier disease. In: Scriver, Beaudet, Sly, Valle, (eds.): *The Metabolic and Molecular Bases of Inherited Disease*, 8th ed., McGraw-Hill, New York, 2937-2960, 2001**
(No abstract available)

48. **Fobker, M., R. Voss, H. Reinecke, C. Crone, G. Assmann and M. Walter: Accumulation of cardiolipin and lysocardiolipin in fibroblasts from Tangier disease subjects. *FEBS Lett.* 500: 157-162, 2001.**

Tangier disease (TD) is an inherited disorder of lipid metabolism characterized by very low high density lipoprotein (HDL) plasma levels, cellular cholesteryl ester accumulation and reduced cholesterol excretion in response to HDL apolipoproteins. Molecular defects in the ATP binding cassette transporter 1 (ABCA1) have recently been identified as the cause of TD. ABCA1 plays a key role in the translocation of cholesterol across the plasma membrane, and defective ABCA1 causes cholesterol storage in TD cells. Not only cholesterol efflux, but also phospholipid efflux was shown to be impaired in TD cells. By use of thin layer chromatography, high performance liquid chromatography and time-of-flight secondary ion mass spectrometry, we characterized the cellular phospholipid content in fibroblasts from three homozygous TD patients. The cellular content of the major phospholipids was not found to be significantly altered in TD fibroblasts. However, the two phospholipids cardiolipin and lysocardiolipin, which make up minute amounts in normal cells, were at least 3-5-fold enriched in fibroblasts from TD subjects. A structurally closely related phospholipid (lysobisphosphatidic acid) has recently been shown to be enriched in Niemann-Pick type C, another lipid storage disorder. Altogether these data may indicate that the role of these phospholipids is a regulatory one rather than that of a bulk mediator of cholesterol solubilization in sterol trafficking and efflux.

49. **Lorkowski, S., M. Kratz, C. Wenner, R. Schmidt, B. Weitkamp, M. Fobker, J. Reinhardt, J. Rauterberg, EA. Galinski, G. Assmann and P. Cullen: Tangierkrankheit und Expression des ATP-Bindungskassettentransporters G1 (ABCG1). In: *Die Prävention atherosklerotischer Prozesse*. Heinle, H., Schulte, H. and M. Hanefeld, eds. Deutsche Gesellschaft für Arterioskleroserecherche, Tübingen, 216-221, 2001**
(No abstract available)

50. **Utech, M., G. Hobbel, S. Rust, H. Reinecke, G. Assmann and M. Walter: Accumulation of RhoA, RhoB, RhoG, and Rac1 in fibroblasts from Tangier disease subjects suggests a regulatory role of Rho family proteins in cholesterol efflux. *Biochem. Biophys. Res. Commun.* 280: 229-236, 2001**

Tangier disease (TD) is an inherited disorder of lipid metabolism characterized by very low high density lipoprotein (HDL) plasma levels, cellular cholesteryl ester accumulation and reduced cholesterol excretion in response to HDL apolipoproteins. Molecular defects in the ATP binding cassette transporter 1 (ABCA1) have recently been identified as the cause of TD. ABCA1 plays a key role in the translocation of cholesterol across the plasma membrane, and defective ABCA1 causes cholesterol storage in TD cells. However, the exact relationship of many of the biochemical and morphological abnormalities in TD to ABCA1 is unknown. Since small GTP-binding proteins are important regulators of many cellular functions, we characterized these proteins in normal and TD fibroblasts using the [α - 32 P]GTP overlay technique and Western blotting of SDS and isoelectric focusing gels. Our results indicate that GTP-binding proteins of the Rho family (RhoA, RhoB, RhoG, Rac-1) are enriched in fibroblasts from TD patients. The accumulation of small G proteins may have potential implications for the TD phenotype and the regulation of cholesterol excretion in TD cells.

51. **Nofer, JR., G. Herminghaus, M. Brodde, E. Morgenstern, S. Rust, T. Engel, U. Seedorf, G. Assmann, H. Bluethmann and B.E. Kehrel: Impaired platelet activation in familial high density lipoprotein deficiency (Tangier disease). *J. Biol. Chem.* 279: 34032-34037, 2004.**

ATP binding cassette transporter A1 (ABCA1) is involved in regulation of intracellular lipid trafficking and export of cholesterol from cells to high density lipoproteins. ABCA1 defects cause Tangier disease, a disorder characterized by absence of high density lipoprotein and thrombocytopenia. In the present study we have demonstrated that ABCA1 is expressed in human platelets and that fibrinogen binding and CD62 surface expression in response to collagen and low concentrations of thrombin, but not to ADP, are defective in platelets from Tangier patients and ABCA1-deficient animals. The expression of platelet membrane receptors such as GPVI, α 2 β 1 integrin, and GPIIb/IIIa, the collagen-induced changes in phosphatidylserine and cholesterol distribution, and the collagen-induced signal transduction examined by phosphorylation of LAT and p72syk and by intracellular Ca²⁺ mobilization were unaltered in Tangier platelets. The electron microscopy of Tangier platelets revealed reduced numbers of dense bodies and the presence of giant granules typically encountered in platelets from Chediak-Higashi syndrome. Further studies demonstrated impaired release of dense body content in platelets from Tangier patients and ABCA1-deficient animals. In addition, Tangier platelets were characterized by defective surface exposure of dense body and lysosomal markers (CD63, LAMP-1, LAMP-2, CD68) during collagen- and thrombin-induced stimulation and by abnormally high lysosomal pH. We conclude that intact ABCA1 function is necessary for proper maturation of dense bodies in platelets. The impaired release of the content of dense bodies may explain the defective activation of Tangier platelets by collagen and low concentrations of thrombin, but not by ADP.