Subject Review:

Adrenoleukodystrophy

BRIAN P. O'NEILL and HUGO W. MOSER

SUMMARY: *Adrenoleukodystrophy (ALD) is an X-linked degenerative disease characterized by progressive demyelination and adrenal insufficiency. Several phenotypes are described. In post-mortem tissues there is an accumulation of saturated or mono-unsaturated very long chain fattyacids (VLCFA) in the cholesterol ester fraction of adrenal cortex and cerebral white matter. The accumulated fatty acids are unbranched with carbon chain length between 23 and 32 with most containing 25 or 26 carbons. Determination of VLCFA in readily accessible tissues such as skin fibroblasts and plasma allows for reliable detection of patients and carriers.*

RÉSUMÉ: *La surrénaloleulodystrophie est une maladie dégénérative liée au chromosome X et caractérisée par une démyélinisation progressive et une insuffisance surrénalienne. Nous décrivons plusieurs phénotypes. Dans le tissu post-mortem nous trouvons une accumulation d'acides gras à très longue chaîne (VLCFA) saturés ou mono-nonsaturés, dans la fraction d'esters de cholestérol du cortex surrénalien et de la substance blanche cérébrale. Les acides gras accumulés sont sans chaîne latérale avec des chaînes de carbone entre 23 et 32, le plus souvent 25 ou 26 atomes de carbone. La détermination des VLCFA dans les fibroblastes ou le plasma permet la détection fiable des patients et des porteurs.*

From the Department of Neurology, Mayo Clinic and Mayo Foundation, Rochester, MN (Dr. O'Neill) and the John F. Kennedy Institute and Department of Neurology, The Johns Hopkins Medical Institutions, Baltimore, MD (Dr. Moser).

Requests for reprints to: Dr. B.P. O'Neill, Department of Neurology, Mayo Clinic and Foundation, Rochester, MN 55901

Adrenoleukodystrophy (ALD) is a progressive neurologic disorder thought due to a defect of fatty acid metabolism inherited as an X-linked trait. First described by Siemerling and Creutzfeldt (1923), ALD is manifested by adrenal atrophy and demyelination of central and peripheral myelin. Adrenal cortical cells and macrophages in brain white matter contain characteristic trilaminar cytoplasmic inclusions (lamellar lipid profiles, LLP) suggesting a lipid storage disorder (Ghatak et al, 1981; Powers et al, 1980). ALD is currently classified as a dysmyelinating disease or leukodystrophy. Very long chain, unbranched, saturated or mono-unsaturated fatty acids (VLCFA) of carbon length 20 to 30 accumulate in cholesterol esters and gangliosides of cerebral white matter and adrenal cortex (Moser et al, 1980b).

Several phenotypes of ALD have been described (Table 1). These are: 1) progressive cerebral degeneration of young males often with cortical blindness (Schaumburg et al, 1975); 2) progressive spinal cord degeneration in adult males (Griffin et al, 1977; Schaumburg et al, 1977); 3) an intermediate form in juvenile or adult males with both cerebral and spinal involvement (Weiss et al, 1980); 4) a connatal form in male infants (Ulrich et al, 1978; Manz et al, 1980); 5) X-linked Addison's disease without neurologic disease (O'Neill et al, 1982b); and 6) spinal cord disease in female carriers (O'Neill et al, 1982c). For this review, we use the term adrenoleukodystrophy or ALD to refer collectively to these varying manifestations.

The duration of illness or the therapy of adrenal insufficiency correlates poorly with the extent of pathologic change (Blaw, 1970). In typical ALD, demyelination is usually widespread, symmetric, and predominant in the posterior quadrant of the centrum semi-ovale but sparing a narrow strip of subcortical white matter (Schaumburg et al, 1975). This predilection for parieto-occipital white matter may account for the early cortical blindness, the EEG slowing posteriorly and the lack of cortically-evoked EEG responses. It also correlates with the distinctive CT image of decreased attenuation of the cerebral white matter beginning in this region and extending anteriorly (O'Neill and Forbes, 1981; Lane et al, 1978). Microscopically, the lesions are those of severe demyelination with a perivascular mononuclear cell infiltrate (Schaumburg et al, 1975). Typically, this infiltrate is intense at the advancing edge of the lesion and correlates with contrast enhancement on CT scanning (Furuse et al, 1978). The CT scan is usually normal or nonspecifically abnormal in the myeloneuropathy variant where pathologic involvement of the brain is minimal (O'Neill and Forbes, 1981).

The mechanism of the myelin loss is unknown. The LLP are seen only in brain macrophages as membrane-bound inclusions. Although occasionally seen in Schwann-cells, these inclusions have not been described in oligodendroglia. Their presence in brain macrophages is thought to reflect removal of myelin debris because the intensity of the macrophage response is most marked in regions of active demyelination (Schaumburg et al, 1975; Ghatak et al, 1981).

In most cases of ALD, clinical and laboratory evidence of hypoadrenalism can be demonstrated at some time during the illness (Blaw, 1970). Adrenal symptoms may be present for as long as ten years before the appearance of

TABLE I
PHENOTYPES OF ADRENOLEUKODYSTROPHY

	Typical ALD	Myeloneuropathy (AMN)	Mixed	Connatal (Neonatal)	Addison's Disease (AD)	Symptomatic Heterozygote
Sex	Male	Male	Male	Male	Male	Female
Age at onset	4-8 years	2nd-3rd decades	1st-2nd decades	soon after birth	5-10 years	3rd decade
Duration	1-10 years	> 2 decades	1-10 years	1-5 years	—	—
Neurologic manifestations	Cortical blindness, deafness, progressive dementia	Spastic paraparesis, peripheral neuropathy	Seizures, dementia, spastic paraparesis, peripheral neuropathy	Psychomotor retardation, failure to thrive	None	Spastic paraparesis
Endocrine manifestations	Mild-moderate adrenal insufficiency	Moderate adrenal insufficiency, azoospermia, hypotestosteronemia	Moderate adrenal insufficiency, hypotestosteronemia	None; adrenal cortical atrophy	Moderate adrenal insufficiency	None
CT scan	Posterior quadrant hypodensity, ± enhancement	Normal or non-specific	Posterior quadrant hypodensity	Frontal-temporal hypodensity, enhancement	Normal	Normal

neurologic disease (Schaumburg et al, 1976). In one family, Addison's disease was seen in men of several generations without evidence of neurologic disease (O'Neill et al, 1982b). On pathologic examination, the adrenals are atrophic, the cut section revealing a normal medulla surrounded by a thin cortical rim, mainly the zona glomerulosa (Schaumburg et al, 1975). Microscopically, there is a mixture of cells including ballooned cortical cells containing non-membrane-bound LLP (Schaumburg et al, 1972; Powers et al, 1980; Ghatak et al, 1981). Similar microscopic changes have been noted in testes which correlates to reports of azoospermia and diminished testosterone in adults (Schaumburg et al, 1977).

The pattern of familial incidence suggests that ALD is inherited as an X-linked trait. The combination of neurologic and endocrine disease has been demonstrated only in men and no example of male-to-male transmission is known. Heterozygous females may have mild myelopathy (O'Neill et al 1981), and occasionally severe myelopathy, but a progressive cerebral syndrome has not been described (Moser et al, 1980b). In one study of 15 women proven to be carriers by clinical and biochemical criteria (O'Neill et al, 1982), neurological signs were present in 10. Since no carrier has been examined pathologically, the full extent of involvement is unknown. Reports of well-studied carriers did not demonstrate endocrinopathy (Davis et al, 1979; O'Neill et al, 1981). A progressive neurologic disease with adrenal failure in women has been reported, but this appears to be an immune disease different from ALD (Pilz and Schiener, 1973; Dyck et al, 1981).

Restricted expression of the phenotype in women may be explained by the Lyon hypothesis of inactivation of the X-chromosome (Lyon, 1961). The ease of detection of myelopathy by examination may partly explain the frequency of appearance of disease in carriers. The "reserve" in the normal adrenal gland may similarly explain why mild hypoadrenalism is not also encountered even though adrenal pathology may exist. If the ALD trait is transmitted as an X-linked recessive, then according to the Lyon hypothesis, carriers should be found to have two populations of cells. Fibroblasts cloned from skin biopsies of ALD carriers were found to have morphological and biochemical characteristics typical of normal or ALD cells. Inactivation of the X-chromosome appeared to favor the mutant gene, partly explaining the high prevalence of neurologic disease in carriers (Migeon et al, 1981).

Different phenotypes occur in the same kindred, suggesting interaction of the abnormal gene with host and environmental factors. A hormonal influence on phenotypic expression has not been examined. Some of the accumulated VLCFA may be of dietary origin (Kishimoto et al, 1980), suggesting that environmental factors may influence gene expression. Whether different phenotypic expression reflects one or more mutations remains speculative until the molecular defect in ALD is clarified (Rosenberg, 1981).

The accumulation of VLCFA, particularly hexacosanoate (C26:0), a saturated 26 carbon unbranched fatty acid, appears to be unique to ALD. These fatty acids may account for up to 40% of the total fatty acid of the cholesterol esters and gangliosides of the cerebral cortex and the adrenals (Igarashi et al, 1976; Menkes and Corbo, 1977; Ramsey et al, 1979). Similar accumulations of VLCFA have been demonstrated in cultured skin fibroblasts (Moser et al, 1980a), culture muscle cells (Askanas et al, 1979) and plasma (Moser et al, 1981). These observations suggest that ALD is

related to a generalized metabolic defect present in all cells but which has functional effects in only certain tissues.

The striking increase of VLCFA in the cholesterol ester fraction suggested a defect of a specific cholesterol esterase. However, the activities of mitochondrial-, microsomal- and myelin-associated cholesterol esterases from post-mortem ALD brain are normal, as is cholesterol lignocerate hydrolysis in ALD fibroblasts (Moser et al, 1980b). These results do not exclude a defect of an as yet unidentified cholesterol esterase, but other observations argue against it. VLCFA accumulation is observed in other lipid classes such as gangliosides, sphingomyelin and free fatty acids (Igarashi et al, 1976; Kawamura et al, 1978; Moser et al, 1980b). Plasma cholesterol esters are also normal (Igarashi et al, 1976). These observations are more compatible with the hypothesis that the basic defect involves the metabolism of VLCFA per se. The accumulation of these fatty acids in the cholesterol ester fractions of brain white matter and adrenal cortex would then be a secondary phenomenon.

Fatty acid degradation occurs through one of several oxidation pathways. A ω-oxidation system is likely of minor importance (Antony and Landau, 1968). Phytanic acid is degraded by α-oxidation and is deficient in Refsum's disease (Steinberg, 1978). Another mechanism degrades α-hydroxy fatty acids to odd-numbered fatty acids. These are not increased in ALD (Kishimoto et al, 1979). A specific β-oxidation system resides in the peroxisome and is active towards VLCFA (Osmundsen et al, 1979). In ALD the abnormality involves mainly 25 and 26 carbon length fatty acids with no alteration of those of 20 and 22 carbons in length. The metabolic defect in ALD may reside in a β-oxidation system specific for these VLCFA (Moser et al, 1980b).

Diagnostic assays using readily-accessible tissues have been developed. Cultured skin fibroblasts accumulate VLCFA and the increase may be expressed as $C_{26:0}/C_{22:0}$, the ratio of hexacosanoate to behenic ($C_{22:0}$) acid, a 22 carbon fatty acid not increased in ALD. Increased ratios of up to 10 fold over normal and neurologic disease controls have been demonstrated in ALD. No false-positives have been described and nearly 90% of heterozygotes are detected (Moser et al, 1980a). Little differences are noted in the ratios derived from patients with the various phenotypic expressions (Moser et al, 1980a, O'Neill et al, 1982a; O'Neill et al, 1982b). The similar accumulation of VLCFA from the cultured medium in these forms may in part reflect the proliferative advantage of the mutant cell (Migeon et al, 1981).

A significant increase of plasma VLCFA, particularly 25 and 26 carbon-linked fatty acids, and of the $C_{26:0}/C_{22:0}$ ratio is demonstrable in ALD patients and carriers (Moser et al, 1981). Using gas-liquid chromatography with capillary columns, accurate values are obtained with as little as 1 ml of plasma. Elevated values have been found in asymptomatic male siblings of ALD patients who may represent presymptomatic ALD. Interpretation of values for heterozygotes as false-negative may be diminished if both plasma and fibroblast assays are performed since several obligate heterozygotes with normal or borderline plasma values had abnormal values in fibroblasts (Moser et al, 1981; O'Neill et al, 1982c).

In summary, ALD is an X-linked degenerative disease characterized by progressive demyelination and adrenal insufficiency. Determination of VLCFA in readily accessible tissues such as skin fibroblasts and plasma allows for reliable detection of patients and carriers.

REFERENCES

ANTONY, G.J., LANDAU, B.R., (1968). Relative contributions of α-, β-, and ω-oxidative pathways to in vitro fatty acid oxidation in rat liver. J Lipid Res., 9:267-269.

ASKANAS, V., McLAUGHLIN, J., ENGEL, W.K., ADORNATO, B.T., (1979). Abnormalities in cultured muscle and peripheral nerve of a patient with adrenomyeloneuropathy. N Engl J Med, 301:588-590.

BLAW, M.E., (1970). Melanodermic type leukodystrophy (adrenoleukodystrophy). In: Vinken PJ, Bruyn GW, eds. Handbook of Clinical Neurology: Leucodystrophies and Poliodystrophies. Amsterdam: North Holland Publishing Company, 10:128-133.

DAVIS, L.E., SNYDER, R.D., ORTH, D.N., NICHOLSON, W.E., KORNFELD, M., SEELINGER, D.F., (1979). Adrenoleukodystrophy and adrenomyeloneuropathy associated with partial adrenal insufficiency in three generations of a kindred. Am J Med, 66:342-347.

DYCK, P.J., YAO, J.K., KNICKERBOCKER, D.E., HOLMAN, R.T., GOMEZ, M.R., HAYLES, A.B., LAMBERT, E.H., (1981). Multi-system neuronal degeneration, hepatosplenomegaly and adrenal cortical deficiency associated with reduced tissue arachidonic acid. Neurology (Ny), 31:925-934.

FURUSE, M., OBAYASHI, T., TSUJI, S., MIYATAKE, T., (1978). Adrenoleukodystrophy: a correlative analysis of computed tomography and radionuclide studies. Radiology, 126:707-710.

GHATAK, N.R., NOCHLIN, D., PERIS, M., MYER, E.C., (1981). Morphology and distribution of cytoplasmic inclusions in adrenoleukodystrophy. J Neurol Sci, 50:391-398.

GRIFFIN, J.W., GOREN, E., SCHAUMBURG, H., ENGEL, W.K., LORIAUX, L., (1977). Adrenomyeloneuropathy: a probable variant of adrenoleukodystrophy. I. Clinical and endocrinologic aspects. Neurology (Minneap), 27:1107-1113.

IGARASHI, M., SCHAUMBURG, H.H., POWERS, J., KISHIMOTO, Y., KOLODNY, E., SUZUKI, K., (1976). Fatty acid abnormality in adrenoleukodystrophy. J Neurochem, 26:851-860.

KAWAMURA, N., MOSER, A.B., MOSER, H.W., OGINO, T., SUZUKI, K., SCHAUMBURG, H., MILUNSKY, A., MURPHY, J., KISHIMOTO, Y., (1978). High concentration of hexacosanoate in cultured skin fibroblast lipids from adrenoleukodystrophy patients. Biochem Biophys Res Comm, 82:114-120.

KISHIMOTO, Y., MOSER, H.W., KAWAMURA, N., PLATT, M., PALLANTE, S.L., FENSELAU, C., (1980). Adrenoleukodystrophy: evidence that abnormal very long chain fatty acids of brain cholesterol esters are of exogenous origin. Biochem Biophys Res Comm, 96:69-76.

LANE, B., CARROLL, B.A., PEDLEY, T.A., (1978). Computerized cranial tomography in cerebral diseases of white matter. Neurology (Minneap), 28:534-544.

LYON, M.F., (1961). Gene action in the X-chromosome of the mouse (*Mus musculus* L.). Nature, 190:372-373.

MANZ, H.J., SCHUELEIN, M., McCULLOUGH, D.C., KISHIMOTO, Y., EIBEN, R.M., (1980). New phenotypic variant of adrenoleukodystrophy: pathologic, ultrastructural, and biochemical study in two brothers. J Neurol Sci, 45:245-260.

MENKES, J.H., CORBO, L.M., (1977). Adrenoleukodystrophy: accumulation of cholesterol esters with very long chain fatty acids. Neurology (Minneap), 27:928-932.

MIGEON, B.R., MOSER, H.W., MOSER, A.B., AXELMAN, J., SILLENCE, D., NORUM, R.A., (1981). Adrenoleukodystrophy: evidence for X linkage, inactivation, and *in vitro* selection favoring mutant allele in heterozygous cells. Proc Nat Acad Sci USA, 78:5066-5070.

MOSER, H.W., MOSER, A.B., KAWAMURA, N., MURPHY, J., SUZUKI, K., SCHAUMBURG, H., KISHIMOTO, Y., (1980a). Adrenoleukodystrophy: elevated C_{26} fatty acid in cultured skin fibroblasts. Ann Neurol, 7:542-549.

MOSER, H.W., MOSER, A.B., KAWAMURA, N., MIGEON, B., O'NEILL, B., FENSELAU, C., KISHIMOTO, Y., (1980b). Adrenoleukodystrophy: studies of the phenotype, genetics and biochemistry. Johns Hopkins Med J, 147:217-224.

MOSER, H.W., MOSER, A.B., FRAYER, K.K., CHEN, W., SCHULMAN, J.D., O'NEILL, B.P., KISHIMOTO, Y., (1981). Adrenoleukodystrophy: increased plasma content of saturated very long chain fatty acids. Neurology (Ny), 31:1241-1249.

O'NEILL, B.P., FORBES, G.S., (1981). Computerized tomography and adrenoleukomyeloneuropathy: differential appearance in disease subtypes. Arch Neurol, 38:293-298.

O'NEILL, B.P., MARMION, L.C., FERINGA, E.R., (1981). The adrenoleukodystrophy complex: expression in four generations. Neurology (Ny), 31:151-156.

O'NEILL, B.P., MOSER, H.W., MARMION, L.C., (1982a). Adrenoleukodystrophy: elevated C_{26} fatty acid in cultured skin fibroblasts and correlation with disease expression in three generations of a kindred. Neurology (Ny), 32:540-543.

O'NEILL, B.P., MOSER, H.W., SAXENA, K.M., (1982b). Familial X-linked Addison disease as an expression of adrenoleukodystrophy (ALD): elevated C_{26} fatty acid in cultured skin fibroblasts. Neurology (Ny), 32:543-547.

O'NEILL, B.P., MOSER, H.W., SAXENA, K.M., MARMION, L.C., (1982c). Adrenoleukodystrophy (ALD). Neurological disease in carriers and correlation with very long chain fatty acid (VLCFA) concentrations in plasma and cultured skin fibroblasts. Neurology (Ny), 32(2):A216-217.

OSMUNDSEN, H., NEAT, C.E., NORUM, K.R., (1979). Peroxisomal oxidation of long chain fatty acids. FEBS Lett, 99:292-296.

PILZ, P., SCHIENER, P., (1973), Kombination von Morbus Addison und Morbus Schilder bei einer 43 jährigen Frau. Acta Neuropathol (Berl), 26:357-360.

POWERS, J.M., SCHAUMBURG, H.H., JOHNSON, A.B., RAINE, C.S., (1980). A correlative study of the adrenal cortex in adrenoleukodystrophy - evidence for a fatal intoxication with very long chain saturated fatty acids. Invest Cell Pathol, 3:353-376.

RAMSEY, R.B., BANIK, N.L., DAVISON, A.N., (1979). Adrenoleukodystrophy brain cholesteryl esters and other neutral lipids. J Neurol Sci, 40:189-196.

ROSENBERG, R.N., (1981). Biochemical genetics of neurologic disease. N Engl J Med, 305:1181-1193.

SCHAUMBURG, H.H., RICHARDSON, E.P., JOHNSON, P.C., COHEN, R.B., POWERS, J.M., RAINE, C.S., (1972). Schilder's disease: sex-linked recessive transmission with specific adrenal changes. Arch Neurol, 27:458-460.

SCHAUMBURG, H.H., POWERS, J.M., RAINE, C.S., SUZUKI, K., RICHARDSON, E.P., (1975). Adrenoleukodystrophy: a clinical and pathological study of 17 cases. Arch Neurol, 32:577-591.

SCHAUMBURG, H.H., POWERS, J.M., RAINE, C.S., JOHNSON, A.B., KOLODNY, E.H., KISHIMOTO, Y., IGARASHI, M., SUZUKI, K., (1976). Adrenoleukodystrophy: a clinical, pathological and biochemical study. Adv Exp med Biol, 68:379-387.

SCHAUMBURG, H.H., POWERS, J.M., RAINE, C.S., SPENCER, P.S., GRIFFIN, J.W., PRINEAS, J.W., BOEHME, D.M., (1977). Adrenomyeloneuropathy: a probable variant of adrenoleukodystrophy. II. General pathologic, neuropathologic and biochemical aspects. Neurology (Minneap), 27:1114-1119.

SIEMERLING, E., CREUTZFELDT, H.G., (1923). Bronzekrankheit und sklerosierende encephalomyelitis (diffuse sklerose). Arch Psychiat Nervenkrank, 68:217-244.

STEINBERG, D., (1978). Phytanic acid storage disease: Refsum's syndrome. In: Stanbury JB, Wyngaarden JB, Frederickson DS, eds. The Metabolic Basis of Inherited Disease. 4th edit. New York: McGraw-Hill; 688-706.

ULRICH, J., HERSCHKOWITZ, N., HEITZ, P., SIGRIST, T., BAERLOCHER, P., (1978). Adrenoleukodystrophy: preliminary report of a connatal case: light- and electron microscopical, immunohistochemical and biochemical findings. Acta Neuropathol, 43:77-83.

WEISS, G.M., NELSON, R.L., O'NEILL, B.P., CARNEY, J.A., EDIS, A.J., (1980). Use of adrenal biopsy in diagnosing adrenoleukomyeloneuropathy. Arch Neurol, 37:634-636.