INTERNATIONAL JOURNAL OF LEPROSY

Volume 46, Number 4
Printed in the U.S.A.

INTERNATIONAL
JOURNAL OF LEPROSY
And Other Mycobacterial Diseases

VOLUME 46, NUMBER 4 OCTOBER-DECEMBER 1978

Serum Pseudocholinesterase Variants in Mexican-Born Patients with Lepromatous Leprosy [1]

Thomas H. Rea and Won G. Ng [2]

Study of apnea induced by succinylcholine led to recognition of variants in serum pseudocholinesterase (synonyms: plasma cholinesterase, serum cholinesterase) ([5]). At present, serum pseudocholinesterase is considered to be regulated genetically by two loci, E_1 and E_2. Four allelic genes have been recognized at E_1 locus ([10]), one coding for the usual (u) enzyme or E_1^u, one coding the atypical enzyme (a) or E_1^a, one coding for the fluoride resistant (f) variant or E_1^f and, finally, the silent (s) gene or E_1^s associated with extremely low activity. Measurement of serum enzyme activity with a cholinesterase substrate will identify an individual homozygous for the silent gene, $E_1^s E_1^s$, if the absence of activity is not due to organophosphorus poisoning or other drug intoxication. However, in order to distinguish other variant forms of the enzyme, specific inhibitors of pseudocholinesterase must be used in measurement of enzyme activity.

At certain concentrations, dibucaine was found to inhibit the atypical enzyme to a lesser extent than the normal enzyme ([8]). Similarly, fluoride inhibits the fluoride resistant variant to a lesser extent than the normal enzyme ([7]). The extent of enzyme inhibition is expressed in terms of a number, i.e., percent inhibition. Thus the terms Fluoride Number or Dibucaine Number have been employed. Individuals homozygous for the usual gene, $E_1^u E_1^u$ (97% of most populations) have a Dibucaine Number greater than 70, i.e., considerable inhibition. Individuals heterozygous for atypical enzymes, $E_1^u E_1^a$ (3% of most populations) or $E_1^u E_1^f$ (usually rare) have both gene products and show only partial inhibition, Dibucaine Number from 40 to 70. Individuals homozygous for atypical enzymes (0.03% of most populations) have a Dibucaine Number of less than 40, or little inhibition. The f variant is identified by substituting fluoride for dibucaine. The E_2 locus gene product (the C_5 variant) is identified by electrophoresis on starch gels ([6]). The C_5 variant is associated with relatively high serum pseudocholinesterase activity.

[1]Received for publication 14 March 1978.

[2]T. H. Rea, M.D., Associate Professor of Medicine, Section of Dermatology, University of Southern California, School of Medicine, Los Angeles County/University of Southern California Medical Center, Los Angeles, and Assistant Director, Department of Dermatology, Los Angeles County/University of Southern California, School of Medicine, Los Angeles; W. G. Ng, Ph.D., Assistant Professor of Pediatrics, University of Southern California, School of Medicine, Los Angeles, and Division of Medical Genetics, Children's Hospital of Los Angeles, Los Angeles, California, U.S.A.

Therefore, it is not associated with apnea, but its contribution to total serum pseudocholinesterase activity must be considered when making genetic interpretation of data on families.

Because susceptibility to and mode of expression of leprosy are probably determined by genetic factors ([17]), serum pseudocholinesterases have been measured in leprosy in an effort to clarify the nature of genetic influences in leprosy. Thus, Thomas and Job ([18]) measured serum pseudocholinesterase activity and Dibucaine Number in 390 Indian leprosy patients and in 343 Indian controls. Esterase activity was similar in patients and controls but Dibucaine Numbers were statistically significantly decreased, i.e., less than 70, in tuberculoid and lepromatous patients. The decrease was particularly striking in lepromatous patients. A subsequent study by Thomas *et al* ([19]), was confirmatory. Agarwal *et al* ([1]) in a study from Ethiopia found that the distribution of serum Dibucaine Numbers was similar in 150 controls and in 206 leprosy patients whether classified as tuberculoid, borderline, or lepromatous. In a study of 580 Africans with leprosy and 1,034 controls, Whittaker, *et al* ([21]) did not find the $E_1^u E_1^a$ phenotype. However, the fluoride variant, $E_1^u E_1^f$, was present in 11% of controls and 6% of leprosy patients, an insignificant difference. Of particular interest was the low incidence of the $E_1^u E_1^f$ phenotype, three percent in 312 patients with tuberculoid leprosy, a statistically significant difference, compared with controls.

We have studied serum pseudocholinesterase variants in 29 Mexican-born lepromatous leprosy patients, and 30 Mexican-born controls.

MATERIALS AND METHODS

Mexican-born patients were selected as lepromatous, following the criteria of Ridley and Jopling ([14]) and Ridley and Waters ([15]). By histologic criteria, patients were either polar lepromatous or subpolar lepromatous. By clinical criteria patients were polar lepromatous and had no sharply defined skin lesions of tuberculoid or borderline type and no nerve trunk palsies. Controls were Mexican-born, dermatology clinic patients judged to be in good health.

Serum pseudocholinesterase activity was determined by a spectrophotometric procedure employing benzoylcholine chloride as the substrate. The methods used for identification of atypical variant and fluoride variant were according to Kalow and Genest ([8]) and Harris and Whittaker ([7]), respectively. The enzyme activity is expressed in empirical units as O.D. change per minute at 240 nm with plasma diluted 1:200 and a time factor of 1000.

RESULTS

The results are summarized in Table 1. There is no statistically significant difference in serum pseudocholinesterase activity between lepromatous leprosy patients and controls. Furthermore, the two heterozygous

TABLE 1. *Summary of serum pseudocholinesterase activity and Dibucaine Numbers in patients and controls.*

	Polar lepromatous leprosy (29)[a]	Controls (30)[a]
Serum pseudocholinesterase activity:		
Range	18.7-38.1	17.5-32.2
Mean	27.58	25.81
S.D.	5.30	3.78
Dibucaine Number:		
>70%	27	30
70-40%	2[a]	0
<40%	0	0

[a] $E_1^a E_1^u$ phenotype. Numbers in parentheses represent number of subjects.

atypical variants in the patient group do not represent a statistically significant increase over the controls.

DISCUSSION

Studies of the relationship between leprosy and serum pseudocholinesterase variants suggest that the two are linked genetically, not causally. Thus, Thomas and Job ([18]) found a statistically significant incidence of low Dibucaine Numbers in leprosy, especially in the lepromatous form (but did not distinguish between the atypical E_1^a, and fluoride, E_1^f, variants). However, Whittaker *et al* ([21]) found no atypical variants in leprosy patients but did find a statistically significant lower incidence of the fluoride variant in tuberculoid patients. In contrast, Agarwal, *et al* ([1]) found no evidence of a relationship between pseudocholinesterase variants and leprosy. Similarly, we have found no relationship between variants and lepromatous leprosy.

This diversity of findings appear to parallel those studies of prevalence rates of HLA-A and HLA-B histocompatibility antigens in leprosy. Two studies showed significant evidence of associations ([9, 20]); three showed equivocal evidence of an association ([2, 4, 11]); and three showed no evidence of an association ([12, 13, 16]). Subsequently, De Vries *et al* ([3]), measuring HLA-A and HLA-B haplotype inheritance, presented evidence that susceptibility to leprosy was linked to the major histocompatibility region, even though linkage to a specific HLA-A or HLA-B antigen evidently was not demonstrable. In explication, De Vries *et al* ([3]) reasoned that the diversity of previous findings could be understood as differing pressures for genetic disequilibrium in different populations. This attractive idea could also reconcile the diverse findings concerning leprosy and pseudocholinesterase variants. However, at present there is no evidence to suggest if the hypothetical linkage between leprosy and pseudocholinesterase variants lies within or outside of the major histocompatibility region.

SUMMARY

No difference in the distribution of serum pseudocholinesterase variants could be found in lepromatous leprosy patients as compared with controls. The variety of reported relationships of pseudocholinesterase variants in leprosy suggests that only in some populations is a locus regulating pseudocholinesterase genetically linked to a hypothetical locus regulating susceptibility to leprosy.

RESUMEN

Se compararon los niveles de las variantes de pseudocolinesterasa sérica en pacientes con lepra lepromatosa, con aquellos encontrados en controles sanos. No se encontraron diferencias. La diversidad de correlaciones publicadas entre las variantes de pseudocolinesterasa y lepra, sugiere que sólo en algunas poblaciones, un locus regulador de pseudocolinesterasa está lígado genéticamente al locus hipotético que regula la susceptibilidad a la lepra.

RÉSUMÉ

Aucune différence dans la distribution des variants de la pseudocholinesterase sérique n'a pu être mise en évidence chez les malades souffrant de lèpre lépromateuse, lorsqu' on les compare à des témoins. La diversité des relations qui ont été rapportées concernant des variants de pseudo-cholinestérase dans la lèpre suggère que ce n'est que dans certaines populations qu'il existe un locus réglant la pseudo-cholinestérase, et qui serait lié génétiquement à un locus hypothétique qui déterminerait la susceptibilité à la lèpre.

Acknowledgments. This work was supported in part by the Michael J. Connell Foundation, Los Angeles, California. We wish to thank Helen Fry for technical assistance.

REFERENCES

1. Agarwal, D. P., Goedde, H. W., Schloot, W., Flatz, G. and Rohde, R. A note on atypical serum cholinesterase and genetic factors in leprosy. Hum. Hered. **2** (1973) 370-373.
2. Dasgupta, A., Mehra, N. K., Ghei, S. K. and Vaidya, M. C. Histocompatibility antigens in leprosy. Tissue Antigens **5** (1975) 85-87.
3. De Vries, R. R. P., Nijenhuis, L. E., Lai A. Fat, R. F. M. and Van Rood, J. J. HLA-linked genetic control of host response to *Mycobacterium leprae*. Lancet **2** (1976) 1328-1330.
4. Escobar-Gutierrez, A., Gorodezky, C. and Salazar-Mallen, M. Distribution of some of the HL-A system lymphocyte antigens in Mexicans. Vox. Sang. **25** (1973) 151-155.

5. EVANS, F. T., GRAY, P. W. S., LEHMAN, H. and SILK, E. Sensitivity to succinylcholines in relation to cholinesterase. Lancet **1** (1952) 1229-1230.
6. HARRIS, H., HOPKINSON, D. A. and ROBSON, E. B. Two dimensional electrophoresis of pseudocholinesterase components in human serum. Nature (Lond) **196** (1962) 1296-1298.
7. HARRIS, H. and WHITTAKER, M. Differential inhibition of human serum cholinesterase with fluoride: recognition of two phenotypes. Nature (Lond) **191** (1961) 496-498.
8. KALOW, W. and GENEST, K. A method for the detection of atypical forms of human cholinesterase: determination of dibucaine numbers. Can. J. Biochem. Physiol. **35** (1957) 339-346.
9. KREISLER, M., ARNAIZ, A., PEREZ, B., CRUZ, E. F. and BOOTELLO, A. HL-A antigens in leprosy. Tissue Antigens **4** (1974) 197-201.
10. LEHMANN, H. and LIDDELL, J. Genetic variants of human pseudocholinesterase. *In:* Progress in Medical Genetics, A. G. Steinberg and A. G. Bearn, eds., New York: Grune and Stratton, 1964, vol. 3, pp 75-105.
11. NAKAJIMA, S., KOBAYASHI, S., NOHARA, M., SATO, S., OZAWA, S., TAKEDA, H. and SEKIGUCHI, S. HLA antigen and susceptibility to leprosy. Int. J. Lepr. **44** (1976) 551-552.
12. REA, T. H., LEVAN, N. E. and TERASAKI, P. I. Histocompatibility antigens in patients with leprosy. J. Infect. Dis. **134** (1976) 615-618.
13. REIS, A. P., MAIA, F., REIS, V. F., ANDRADE, I. M. and CAMPOS, A. A. S. HL-A antigens in leprosy. Lancet **2** (1974) 1384.
14. RIDLEY, D. S. and JOPLING, W. H. Classification of leprosy according to immunity: a five-group system. Int. J. Lepr. **34** (1966) 255-273.
15. RIDLEY, D. S. and WATERS, M. F. R. Significance of variations within the lepromatous group. Lepr. Rev. **40** (1969) 143-152.
16. SMITH, G. S., WALFORD, R. I., SHEPARD, C. C., PAYNE, R. and PROCHAZKA, G. J. Histocompatibility antigens in leprosy. Vox. Sang. **28** (1975) 42-49.
17. SPICKETT, S. G. Genetic mechanisms in leprosy. *In:* Leprosy in Theory and Practice, 2nd edit., R. G. Cochrane and T. F. Davey, eds., Baltimore: Williams and Wilkins, 1964, pp 98-124.
18. THOMAS, M. and JOB, C. K. Serum atypical pseudocholinesterase and genetic factors in leprosy. Br. Med. J. **3** (1972) 390-391.
19. THOMAS, M., JOB, C. K. and KURIAN, P. V. Susceptibility to leprosy and serum atypical pseudocholinesterase. Int. J. Lepr. **44** (1976) 315-318.
20. THORSBY, E., GODAL, T. and MYRVANG, B. HL-A antigens and susceptibility to diseases. II. Leprosy. Tissue Antigens **3** (1973) 373-377.
21. WHITTAKER, M., LOWE, R. F. and ELLIS, B. P. B. Serum cholinesterase variants in African leprosy patients resident in Rhodesia. Hum. Hered. **26** (1976) 372-379.