

Excretion of Catecholamines in Relatives of Patients with Familial Neuroblastoma¹

Engelbert Plöchl, Hans Käser, and Hannes Klein

Children's Hospital of Salzburg [E. P.] and Institute of Pathology of Salzburg [H. K.], A-5020 Salzburg, Austria, and Institute for Clinical and Experimental Cancer Research, University of Berne, CH-3004 Berne, Switzerland [H. K.]

SUMMARY

The urinary excretion of catecholamines in 13 close relatives of two distantly related patients with congenital neuroblastoma was investigated. One of these relatives was a child with already known ganglioneuroblastoma. The catecholamines noradrenaline and dopamine were determined. Vanilmandelic acid and homovanillic acid also were determined and numerous other catecholamine metabolites were measured. In contrast to some previous reports in the literature, no pathological excretion patterns were found in the healthy family members.

INTRODUCTION

Since the 1st reports (9, 16) on increased excretion of catecholamines in the urine of neuroblastoma patients were published, quantitative determinations of these substances and their metabolites have been shown to be highly significant in diagnosing the presence of malignant sympathetic tissue (10). Serial examinations are helpful in appraising maturation of the tumor, guiding the need and dosage of radiation and the effect of chemotherapy (15). In recent years some authors have reported increased excretion of these metabolic products also in apparently healthy relatives of neuroblastoma patients (8), especially in families with several cases (2, 6, 7, 21). Therefore, we were interested in investigating this problem in a family with familial neuroblastoma (12).

METHODS

Twenty-four-hr urine samples were collected from 13 close relatives (Relatives 18, 24, 25, 26, 27, 28, 29, 30, 33, 34, 35, 36, and 37) marked in the family tree of the 2 newborns who had died of neuroblastoma (Relatives 32 and 38). The catecholamines (dopamine, noradrenaline, adrenaline) and their most important metabolites (3,4-dihydroxyphenylacetic acid, 3-methoxytyramine, normetanephrine, metanephrine, 3-methoxy-4-hydroxyphenylethanol, 3-methoxy-4-hydroxyphenylglycol, homovanillic acid, vanilmandelic acid) present in these urines were separated and visualized by paper chromatographic (5) and circular thin-layer

chromatographic methods (11) in order to obtain evidence of their occurrence and their quantities. Furthermore dopamine, noradrenaline, vanilmandelic acid, and homovanillic acid were determined quantitatively by column chromatographic-fluorimetric or photometric methods (10). Creatinine content was measured by the method of Owen *et al.* (17).

CASE REPORTS

The genealogical tree (Chart 1) shows the relationship of 2 children (Relatives 32 and 38) who died shortly after birth from a neuroblastoma that was confirmed at autopsy. In this family there is another child with histologically verified ganglioneuroblastoma (Relative 37). Congenital anomalies or café au lait spots are not known in this family. Retrospectively, there was no evidence for symptoms associated with elevated catecholamine levels during pregnancy in the mothers of the 2 deceased newborns with congenital neuroblastoma (19). The most important data on the confirmed and suspected patients of neuroectodermal tumors are summarized as follows.

Relative 32 is a newborn male who died 2 hr after birth. The autopsy (No. 711/67) revealed a large neuroblastoma in the left suprarenal gland with fatal bleeding into the abdominal cavity; metastases were not demonstrable (22).

Relative 38 is a newborn male who was admitted to the hospital because of poor medical condition after a home delivery 3 weeks before term. He died 8 hr after birth. The autopsy (No. 868/72) showed a neuroblastoma of both suprarenals (18). On the right side the suprarenal gland was almost completely destroyed; on the left side small tumor nodules were found in the medulla. Diffuse and nodular infiltration was seen in the liver. Small nodular metastases also were present in the skin. The skeleton was tumor free. Histological examination revealed an immature type of neuroblastoma, classified as sympathogonioma. Bilateral neuroblastoma, however, has a poor prognosis. This corresponds well with the staging of Evans *et al.* (3) who excluded bilateral primary tumor at Stage 4-S.

Relative 37 is a girl in whom a tumor was noticed supposedly since the age of 2 in the right posterior adjacent to the lower thoracic vertebrae. Three years later an attempt was made to extirpate this mass. After the removal of a piece of tumor (histological findings: No. 5634/73, ganglioneuroma), it was found that the neoplastic process extended deeply. For this reason in a 2nd operation a thoracotomy was per-

¹ Supported in part by the Swiss National Foundation for Scientific Research.

Received February 18, 1975; accepted September 11, 1975.

formed (Pediatric Surgical Department of the Landeskrankenanstalten Salzburg; Head: Dr. H. Henkel). This revealed an intrathoracic portion of a tumor, which, however, could be only partially removed because it had grown firmly adherent to the surrounding tissues. The intra- and extrathoracic extension and the type of spread through the thoracic wall give the impression of a primarily malignant and invasive tumor. Histologically, it resembled a ganglioneuroma with a number of foci of sympathogonia; it was classified as ganglioneuroblastoma (Nos. 6307 and 6308/73). Therefore, one could regard it as a form of matured neuroblastoma (6). Subsequently, radiation of the field of operation was carried out. The child was free of complaint but, as could be seen from the thoracic follow-up X-ray, the portion of tumor left behind remained unchanged.

Relative 29 is a male who had a slowly growing tumor in the right side of the neck since birth (no histological examination), which disappeared after radiotherapy. Since then he has been free of symptoms.

Relative 31 is a female infant who was admitted to the hospital because of unclear fever, hepatosplenomegaly, and severe anemia. Her peripheral blood lymphocyte count was low (2060/cu mm). This finding could indicate a poor prognosis in a patient with neuroblastoma (1). The girl was discharged at the request of the parents before elucidation of the disease and died shortly later at the age of 2 years. No autopsy was performed.

Relative 22 is a female who died at the age of 2 years. According to her father's statements the girl had a strikingly large abdomen and was very pale.

No useful information could be obtained from the remaining members of the family who died during the 1st year of life (Relatives 4, 6, 7, 9, 10, 12, and 19).

RESULTS

The 2-dimensional paper chromatographic and the circular thin-layer chromatographic separations of the catechola-

mines and their metabolites showed no deviations from the normal in any of the urines examined. The results of the quantitative determinations are shown in Table 1. As can be seen, the quantities found in mg/24 hr, as well as the concentrations of vanilmandelic acid, homovanillic acid, noradrenaline, and dopamine expressed in $\mu\text{g}/\text{mg}$ of creatinine, lie below the upper limits of normal for the age (Table 2).

DISCUSSION

Almost all authors who have studied catecholamine excretions in familial neuroblastoma noticed an increased or high normal excretion pattern of 1 or more metabolites in 1 or more healthy family members.

Probably all vanilmandelic acid levels in the families studied by Wagget *et al.* (20) are normal, and in the families studied by Hardy and Nesbit (7) even the level of 6.5 mg/24 hr obtained in the father ranges at the upper limit of normal. In the report of Wong *et al.* (21) the vanilmandelic acid

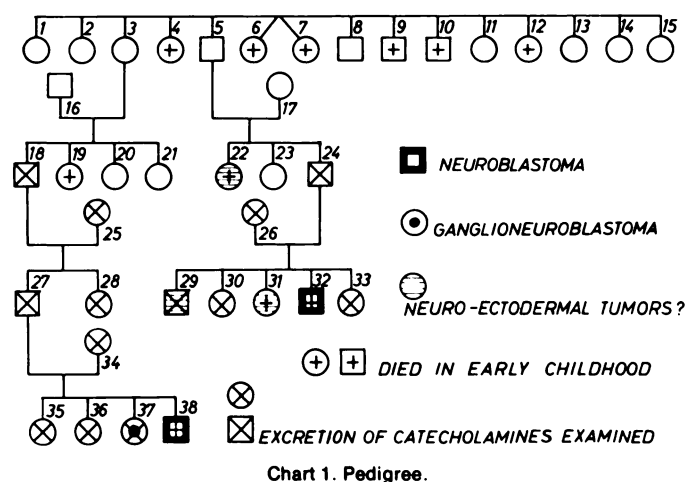


Chart 1. Pedigree.

Table 1
Excretion of vanilmandelic acid, homovanillic acid, noradrenaline, and dopamine in the nearest relatives of the 2 newborn children who died of neuroblastoma

Family examination no.	Age at examination (yr)	Vanilmandelic acid		Homovanillic acid		Noradrenaline		Dopamine	
		mg/24 hr	$\mu\text{g}/\text{mg}$ creatinine	mg/24 hr	$\mu\text{g}/\text{mg}$ creatinine	$\mu\text{g}/24$ hr	$\mu\text{g}/\text{mg}$ creatinine	$\mu\text{g}/24$ hr	$\mu\text{g}/\text{mg}$ creatinine
18	66	3.2	3.6	3.6	4.1	26.1	0.029	278	0.31
25	61	2.9	3.0	3.6	3.7	25.9	0.027	117	0.12
28	35	6.2	7.0	5.2	5.9	32.5	0.037	233	0.26
27	36	2.5	2.3	4.5	4.2	24.1	0.031	211	0.19
34	29	4.8	3.5	4.4	3.2	51.3	0.038	360	0.26
35	8 ⁶ / ₁₂	3.1	7.6	5.1	12.7	14.3	0.035	131	0.32
36	6 ⁶ / ₁₂	1.7	6.6	2.2	8.3	6.0	0.023	74	0.28
37	4 ⁸ / ₁₂	1.5	9.8	2.7	17.9	9.5	0.061	170	1.10
24	37	5.4	3.5	6.2	4.1	70.9	0.046	304	0.19
26	41	4.5	5.3	4.1	4.8	34.9	0.041	138	0.16
29	11 ⁹ / ₁₂	3.0	5.9	3.3	6.5	31.5	0.061	211	0.41
30	10 ⁶ / ₁₂	2.4	5.6	3.8	9.0	27.3	0.064	176	0.41
33	2 ⁷ / ₁₂	1.8	10.0	3.2	17.6	10.8	0.058	255	1.38

Table 2
Highest level (mean + 2 S.D.) of excretion of vanilmandelic acid, homovanillic acid, noradrenaline, and dopamine in relation to age

Age (yr)	Vanilmandelic acid		Homovanillic acid		Noradrenaline		Dopamine	
	mg/24 hr	μg/mg creatinine	mg/24 hr	μg/mg creatinine	μg/24 hr	μg/mg creatinine	μg/24 hr	μg/mg creatinine
2-5	2.2	11.0	4.8	25.2	23.4	0.165	256	1.35
5-10	3.6	7.8	6.9	17.6	32.2	0.117	345	0.97
10-15	4.8	6.6	8.8	11.8	35.2	0.072	366	0.77
20-40	6.5	5.6	11.9	10.0	76.5	0.040	459	0.29
40-60	6.3	5.4	11.7	9.6	41.4	0.039	412	0.49
60-80	5.9	6.9	9.6	10.4	34.5	0.045	354	0.58

values in the father may be a little high at 7.4 and 11.5 mg/24 hr. However, levels of vanilmandelic acid can easily be slightly above normal for various reasons (stress, lability of the autonomous nervous system) and therefore do not by any means indicate a neuroblastoma. In order to prove such a tumor, still other metabolites must be increased, dopamine at least. In the case reported by Griffin and Bolande (6), the mother's and father's vanilmandelic acid values are above the stated normal, but again dopamine is normal or lacking. No doubts can be raised about the case reported by Chatten and Voorhess (2). Here the urinary vanilmandelic acid and dopamine levels were clearly high in the asymptomatic mother of 4 children with neuroblastoma. In a follow-up of this mother an originally unnoticed posterior mediastinal mass was discovered (4).

In view of these reports it was of particular interest to us to be able to investigate thoroughly the relatives of a family with tumors of the sympathetic nervous system. Because of the large family size and good cooperation, we were in a position to examine 13 relatives for the excretion of numerous catecholamines and catecholamine metabolites. All of them with 1 exception (Relative 37) were in good health. Abnormal excretion of 3,4-dihydroxyphenylacetic acid, 3-methoxytyramine, normetanephrine, metanephrine, 3-methoxy-4-hydroxyphenylethanol, 3-methoxy-4-hydroxyphenylglycol, homovanillic acid, and vanilmandelic acid was not observed in any of the subjects by paper chromatographic and circular thin-layer chromatographic methods. In addition, quantitative determinations of vanilmandelic acid, homovanillic acid, noradrenaline, and dopamine (Tables 1 and 2) yielded values corresponding to the age in all the persons investigated. Consequently, the presence of catecholamine-producing neurogenous, sympathetic tumor tissue can be excluded in all the members of the family examined. According to the 2-mutation model of neuroblastoma development (13, 14), it would be of great interest to recognize individuals with the 1st mutation who are predisposed to the tumor. However, our results do not support the suggestion that examination of catecholamines could mark the gene carrier.

ACKNOWLEDGMENTS

We are grateful to Dr. A. Stohl and Rev. J. Messner, priest in Obertrum, for their assistance in establishing the family tree.

REFERENCES

1. Bill, A. H., and Morgan, A. Evidence for Immune Reactions to Neuroblastoma and Future Possibilities for Investigation. *J. Pediat. Surg.*, 5: 111-116, 1970.
2. Chatten, J., and Voorhess, M. L. Familial Neuroblastoma. Report of a Kindred with Multiple Disorders, Including Neuroblastomas in Four Sibblings. *New Engl. J. Med.*, 277: 1230-1236, 1967.
3. Evans, A. E., D'Angio, G. J., and Randolph, J. A Proposed Staging for Children with Neuroblastoma. *Cancer*, 27: 374-378, 1971.
4. Gerson, J. M., Chatten, J., and Eisman, S. Familial Neuroblastoma—A Follow-up. *New Engl. J. Med.*, 290: 1487, 1974.
5. Gjessing, L. R. Studies of Functional Neural Tumors. VI Biochemical Diagnosis. *Scand. J. Clin. Lab. Invest.*, 16: 661-669, 1964.
6. Griffin, M. E., and Bolande, R. P. Familial Neuroblastoma with Regression and Maturation to Ganglioneurofibroma. *Pediatrics*, 43: 377-382, 1969.
7. Hardy, P. C., and Nesbit, M. E., Jr. Familial Neuroblastoma: Report of a Kindred with a High Incidence of Infantile Tumors. *J. Pediat.*, 80: 74-77, 1972.
8. Helson, L., Blasco, P., and Murphy, M. L. Familial Neuroblastoma. *Clin. Res.*, 17: 614, 1969.
9. Isaacs, H., Medalie, M., and Politzer, W. M. Noradrenaline-Secreting Neuroblastoma. *Brit. Med. J.*, 1: 401-404, 1959.
10. Käser, H. Biochemische Diagnostik des Phäochromocytoms, des Neuroblastoms und anderer neuro-ektodermaler Neoplasien. *Helv. Paediat. Acta, Suppl.* 29, 1972.
11. Käser, H. Circular Thin-Layer Chromatography for Diagnosis and Follow-up of Neural Crest Tumors. *J. Chromatog.*, 82: 127-136, 1973.
12. Klein, H., and Plöchl, E. Familiäres Neuroblastom der Nebennieren beim Neugeborenen. *Münch. Med. Wochschr.*, 116: 1163-1168, 1974.
13. Knudson, A. G., Jr. The Genetics of Childhood Cancer. *Cancer*, 35: 1022-1026, 1975.
14. Knudson, A. G., Jr., and Strong, L. C. Mutation and Cancer: Neuroblastoma and Pheochromocytoma. *Am. J. Hum. Genet.*, 24: 514-532, 1972.
15. Liebner, E. J., and Rosenthal, I. M. Serial Catecholamines in the Radiation Management of Children with Neuroblastoma. *Cancer*, 32: 623-633, 1973.
16. Mason, G. A., Hart-Mercer, J., Millar, E. J., Strang, L. B., and Wynne, N. A. Adrenaline-Secreting Neuroblastoma in an Infant. *Lancet*, 2: 322-325, 1957.
17. Owen, J. A., Iggo, B., Scandrett, F. J., and Stewart, C. P. The Determination of Creatinine in Plasma or Serum and in Urine: A Critical Examination. *Biochem. J.*, 58: 426-437, 1954.
18. Plöchl, E., and Klein, H. Neuroblastom beider Nebennieren beim Neugeborenen. *Fortschr. Med.*, 92: 259-262, 1974.
19. Voüte, P. A., Jr., Wadman, S. K., and Van Putten, W. J. Congenital Neuroblastoma. Symptoms in the Mother during Pregnancy. *Clin. Pediat.*, 9: 206-207, 1970.
20. Wagget, J., Aherne, G., and Aherne, W. Familial Neuroblastoma: Report of Two Sib Pairs. *Arch. Disease Childhood*, 48: 63-66, 1973.
21. Wong, K. Y., Hanenson, I. B., and Lampkin, B. C. Familial Neuroblastoma. *Am. J. Disease Children*, 127: 415-416, 1971.
22. Zimmermann, G. Angeborenes Neuroblastoma embryonale sympathicum der linken Nebenniere. *Münch. Med. Wochschr.*, 110: 333-337, 1968.