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Relative Efficiencies of Plasma Catechol Levels and Ratios for Neonatal Diagnosis of Menkes Disease

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Abstract

Background—Menkes disease is an X-linked recessive neurodevelopmental disorder resulting from mutation in a copper-transporting ATPase gene. Menkes disease can be detected by relatively high concentrations of dopamine (DA) and its metabolites compared to norepinephrine (NE) and its metabolites, presumably because dopamine-beta-hydroxylase (DBH) requires copper as a co-factor. The relative diagnostic efficiencies of levels of catechol analytes, alone or in combination, in neonates at genetic risk of Menkes disease have been unknown.

Methods—Plasma from 44 at-risk neonates less than 30 days old were assayed for DA, NE, and other catechols. Of the 44, 19 were diagnosed subsequently with Menkes disease, and 25 were unaffected.

Results—Compared to unaffected at-risk infants, those with Menkes disease had high plasma DA ($P < 10^{-6}$) and low NE ($P < 10^{-6}$) levels. Considered alone, neither DA nor NE levels had perfect sensitivity, whereas the ratio of DA:NE was higher in all affected than in all unaffected subjects ($P = 2 \times 10^{-8}$). Analogously, levels of the DA metabolite, dihydroxyphenylacetic acid (DOPAC), and the NE metabolite, dihydroxyphenylglycol (DHPG), were imperfectly sensitive, whereas the DOPAC:DHPG ratio was higher in all affected than in all unaffected subjects ($P = 2 \times 10^{-4}$). Plasma dihydroxyphenylalanine (DOPA) and the ratio of epinephrine (EPI):NE levels were higher in affected than in unaffected neonates ($P = 0.0015$; $P = 0.013$).

Conclusions—Plasma DA:NE and DOPAC:DHPG ratios are remarkably sensitive and specific for diagnosing Menkes disease in at-risk newborns. Affected newborns also have elevated DOPA and EPI:NE ratios, which decreased DBH activity alone cannot explain.

Keywords

Menkes; Dopamine; Norepinephrine; Dopamine- β -hydroxylase; DHPG; DOPAC; Diagnosis

Introduction

Menkes disease (also called “kinky hair disease”) is an X-linked recessive neurodevelopmental disorder caused by defects in a gene that encodes a copper-transporting ATPase (ATP7A). Affected infants typically appear healthy at birth and show normal neurodevelopment for 2–3 months. Subsequently there is loss of milestones (e.g., smiling, visual tracking, head control) and death in late infancy or childhood.

Treatment with copper histidine injection can markedly improve clinical outcome and even normalize neuronal development in Menkes disease patients; however, to succeed, therapy must commence within the first weeks of life [1]. The usual biochemical pathologic markers, serum copper and ceruloplasmin, are unreliable for diagnosis in the neonatal period, because levels even in healthy newborns are low and overlap with those in patients with Menkes disease. Molecular assays are available to identify mutations at the ATP7A locus, but the testing involves multiple steps and may require several weeks.

A promising neonatal diagnostic test for Menkes disease is based on patterns of levels of catechols in plasma. Human plasma normally contains six catechols (Fig. 1)—the catecholamines dopamine (DA), norepinephrine (NE), and epinephrine (EPI), the catecholamine precursor *l*-3,4-dihydroxyphenylalanine (DOPA), the DA metabolite dihydroxyphenylacetic acid (DOPAC), and the NE metabolite, dihydroxyphenylglycol (DHPG). Dopamine- β -hydroxylase (DBH), the enzyme that catalyzes conversion of DA to NE, requires ionized copper as a co-factor. Menkes disease patients have relatively high concentrations of DOPA, DA, and DOPAC and relatively low concentrations of NE and DHPG [1–3]. Whether affected newborns have low levels of epinephrine (EPI) has been unknown. Since EPI is formed from NE, distal to the DBH step, one would presume that Menkes disease patients would have low plasma EPI levels.

The relative diagnostic efficiencies of levels of catechols, alone or in combination, in newborns at genetic risk for Menkes disease have not been compared; assessing diagnostic sensitivity and specificity was the main purpose of the present study.

Methods

Subjects

We studied 44 male infants less than 30 days old who were at genetic risk (potentially as high as 50%) for having Menkes disease, due to a family history of the disease. The study was approved by the Institutional Review Boards of the National Institute of Child Health and Human Development or the National Institute of Neurological Disorders and Stroke (NINDS). Written informed parental consent was obtained. Twenty-one of the subjects were included in a previous study [1].

Confirmation or exclusion of Menkes disease was determined through physical examination, ATP7A mutation analysis, or follow-up communication with referring physicians and subjects' parents.

Plasma Catechol Assays

Whole blood (2–4 ml) obtained by venipuncture was collected in heparinized tubes and placed on ice. The plasma was separated by refrigerated centrifugation, transferred to plastic specimen tubes, and frozen in dry ice. Specimens were sent in dry ice by courier to the CLIA-certified Clinical Neurochemistry Laboratory of the Clinical Neurocardiology Section in intramural NINDS. Plasma catechol levels were measured by high performance liquid

chromatography with electrochemical detection after batch alumina extraction, as described previously [4].

Statistical Analysis

Mean values for plasma levels of catechols or for plasma catechol ratios in affected and unaffected subjects were compared by independent-means *t* tests. Individual values in scatter plots were assessed by linear regression. Receiver operating characteristic (ROC) curves were constructed, showing values for test sensitivity as a function of one minus specificity, for each of the catecholamines and for ratios of DA:NE and DOPAC:DHPG.

Results

Among all newborns at genetic risk for Menkes disease who were evaluated by catechols assays at the NIH between January, 1999 and March, 2008, the group that proved to have the disease had higher mean plasma levels of DOPA, DA, and DOPAC and lower levels of NE and DHPG than did the unaffected group (Table 1). The groups did not differ in mean plasma EPI.

Although mean DA and DOPAC levels were increased and mean NE and DHPG levels decreased in Menkes disease patients compared to unaffected newborns, for each of these catechols there was some overlap in the distributions of individual values for the two groups, as indicated by the vertical and horizontal dashed lines in Fig. 2. One patient in particular had high plasma NE levels.

As indicated by the diagonal solid lines in Fig. 2, individual data for plasma NE vs. DA and for plasma DHPG versus DOPAC levels in the affected and unaffected groups could be separated completely. The affected and unaffected groups therefore did not overlap at all in individual values for plasma DA:NE or DOPAC:DHPG ratios (Fig. 3), ROC curves showed a sensitivity of 1.0 at 1-specificity of 0.0, and positive and negative predictive values for these ratios were unity (Fig. 4). DA:NE and DOPAC:DHPG ratios separated the affected from the unaffected groups, with *P* values of 2×10^{-8} and 2×10^{-4} .

Across individuals, plasma DHPG levels were positively correlated with those of NE ($r = 0.67$, $P < 0.0001$) and DOPAC with DA ($r = 0.56$, $P < 0.0001$). Plasma levels of DOPA were positively correlated with those of DOPAC ($r = 0.58$, $P < 0.0001$) and DA ($r = 0.56$, $P < 0.0001$) but were unrelated to those of NE ($r = -0.05$) or DHPG ($r = -0.11$).

The affected and unaffected groups had similar mean plasma levels of EPI (Table 1). The affected group had a higher EPI:NE ratio (0.58 ± 0.21) than did the unaffected group (0.10 ± 0.03 , $P = 0.013$).

The affected group had a lower mean DOPA:DA ratio the (39 ± 19) than did the unaffected group (211 ± 22 , $P = 0.003$)

Discussion

Previous reports noted abnormal catechol patterns in Menkes disease [1, 2]; however, those studies did not evaluate the relative efficiencies of catechol levels, considered alone or as ratios, in the neonatal diagnosis of Menkes disease. In this study, although mean plasma levels of DA and DOPAC were higher and of NE and DHPG lower in affected than unaffected subjects, distributions of individual values for all four catechols overlapped slightly. Therefore, no single catechol offers perfect sensitivity for detecting Menkes disease. Menkes disease patients also had higher plasma DOPA levels and EPI:NE ratios

than did unaffected at-risk newborns, findings that decreased DBH activity alone cannot explain.

Among newborns at genetic risk for Menkes disease, all who were affected had a DOPAC:DHPG ratio more than 5 and a DA:NE ratio more than 0.2, findings not seen in any unaffected at-risk newborns. That is, individual values for both DA:NE and for DOPAC:DHPG completely separated the two groups (Fig. 3). These distinctions would be expected from the dependence of NE formation and therefore of DHPG formation on DBH activity, so that decreased DBH activity builds up concentrations of the precursor, DA, and its metabolite, DOPAC, and decreases concentrations of the product, NE, and its metabolite, DHPG. Since DA:NE and DOPAC:DHPG ratios both completely separated the affected from the unaffected groups, with P values of 2×10^{-8} and 2×10^{-4} , the likelihood of incorrect group assignment based on combined measures of both ratios was essentially zero ($P = 4 \times 10^{-12}$). Plasma DHPG levels were positively correlated with NE levels, and DOPAC levels were positively correlated with DA levels, as expected for precursor-product relationships.

Unexpectedly, considering that L-aromatic-amino-acid decarboxylase, the enzyme that catalyzes the conversion of DOPA to DA, is not copper-dependent, affected newborns had higher plasma DOPA levels than did unaffected newborns. Buildup of DA in sympathetic nerves due to decreased DBH activity would be expected if anything to decrease tyrosine hydroxylase activity by feedback inhibition [5–7]. Origins of DOPA in the circulation are complex but include tyrosine hydroxylation in sympathetic nerves [8, 9]. Tyrosine hydroxylase activity may be increased in infants with Menkes disease [2], because of increased sympathetic nervous outflows as a compensation for decreased production and release of NE. Plasma levels of the three catechols distal to the tyrosine hydroxylase step and proximal to the DBH enzymatic step—DOPA, DOPAC, and DA—were not only increased but also strongly positively inter-correlated across subjects, consistent with increased catecholamine biosynthesis.

Other unexpected—and seemingly paradoxical—findings were that mean plasma levels of EPI did not differentiate the affected from the unaffected groups, and the affected group had a significantly higher mean EPI:NE ratio. We did not predict these findings, because EPI is formed from NE by the enzymatic action of phenylethanolamine-*N*-methyltransferase (PNMT), not DBH, and PNMT is not copper-dependent. At this point we can only speculate about bases for elevated EPI:NE ratios in neonates with Menkes disease. The sympathetic noradrenergic system is immature at birth, whereas chromaffin cells are already abundant during fetal development. Perhaps sufficient adrenomedullary development occurs under the influence of maternal factors to allow synthesis and storage of EPI. DBH deficiency is associated with increased rates of skeletal muscle sympathetic nerve traffic [10]. Increased sympathetic nerve traffic to the adrenal medulla, coupled with normal intrauterine EPI synthesis, might help explain normal plasma EPI levels in the first month of post-natal life.

The advent of liquid chromatography with tandem mass spectroscopy (LC/MS/MS) offers the potential for high throughput assays of catechols [11–14]. We propose that development of a LC/MS/MS assay method to measure DA:NE or DOPAC:DHPG ratios from filter paper blood spots may enable mass newborn screening and early institution of treatment for this otherwise invariably lethal pediatric disease.

Acknowledgments

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Abbreviations

DA	Dopamine
DBH	Dopamine- β -hydroxylase
DHPG	Dihydroxyphenylglycol
DOPA	<i>L</i> -3,4-Dihydroxyphenylalanine
DOPAC	Dihydroxyphenylacetic acid
NE	Norepinephrine

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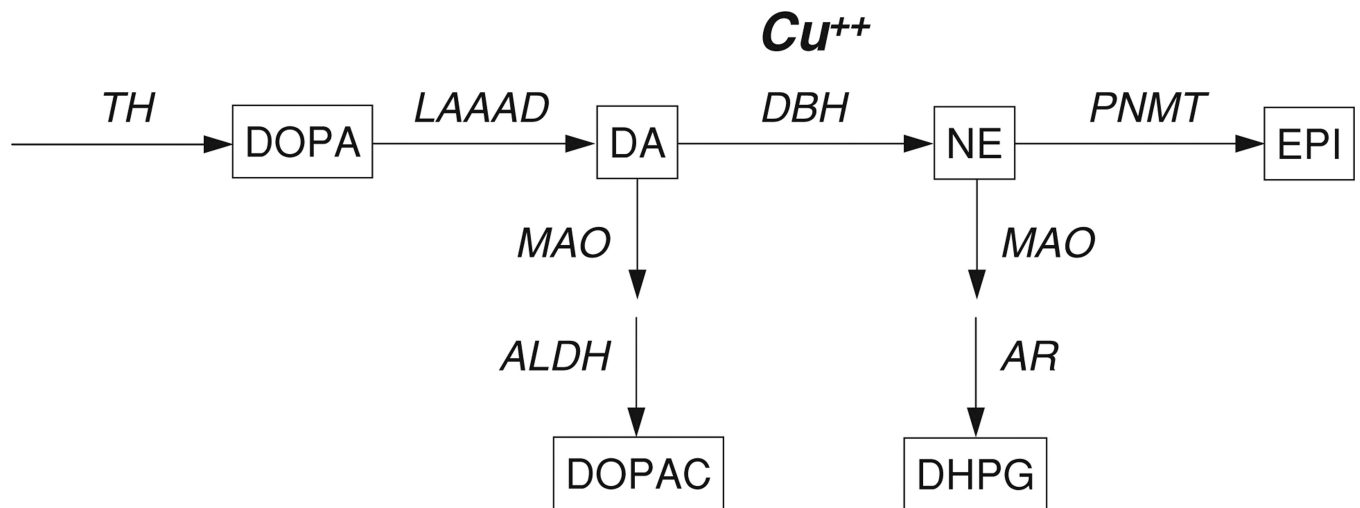


Fig. 1. Catecholamine biosynthetic cascade, showing the six endogenous catechols found normally in human plasma and the associated synthetic and degradative enzymes. TH, tyrosine hydroxylase; DOPA, *l*-3,4-dihydroxyphenylalanine; DA, dopamine; DBH, dopamine-beta-hydroxylase; NE, norepinephrine; PNMT, phenylethanolamine-*N*-methyltransferase; EPI, epinephrine; MAO, monoamine oxidase; ALDH, aldehyde dehydrogenase; AR, aldose/alcohol reductase; DOPAC, dihydroxyphenylacetic acid; DHPG, dihydroxyphenylglycol

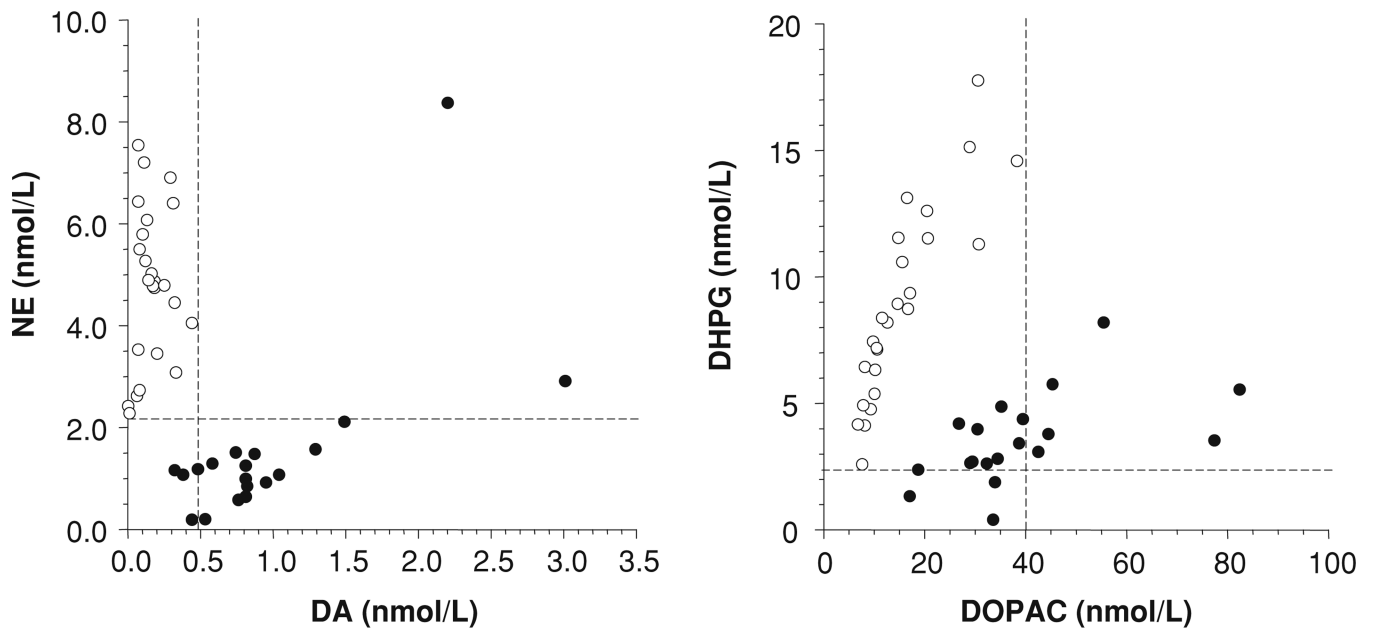


Fig. 2. Individual values for plasma levels of (*left*) norepinephrine (NE) and dopamine (DA) and (*right*) for dihydroxyphenylglycol (DHPG) and dihydroxyphenylacetic acid (DOPA) in newborns at genetic risk for Menkes disease. *White circles* show results for unaffected subjects and *black circles* results for affected subjects. Note some overlaps between the affected and unaffected groups, indicated by the *dashed lines*

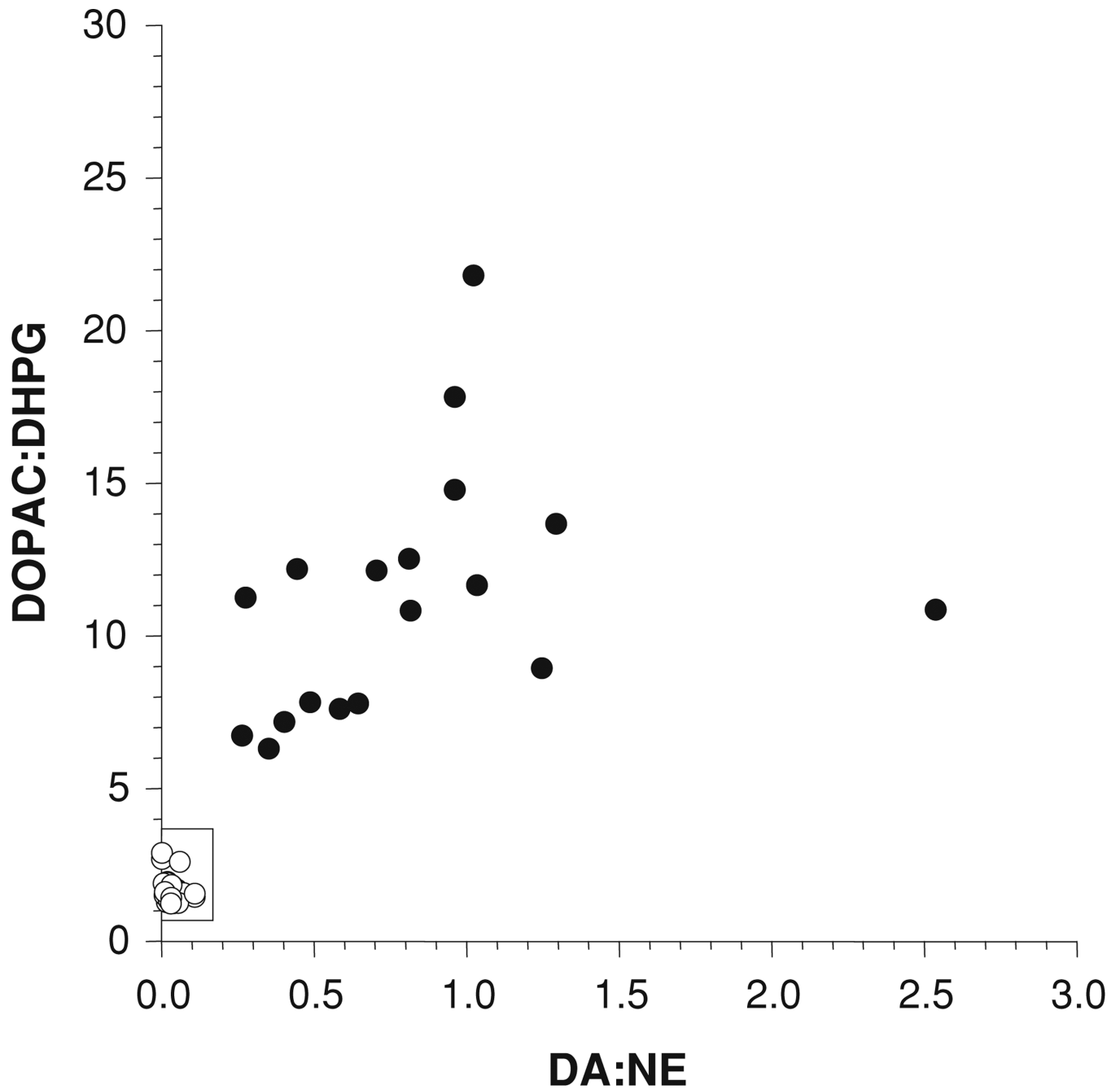


Fig. 3. Individual values for plasma DOPAC:DHPG as a function of DA:NE in newborns at risk for Menkes disease. *White circles* show results for unaffected subjects and *black circles* results for affected subjects. Note both high DOPAC:DHPG and high DA:NE ratios in all patients with Menkes disease

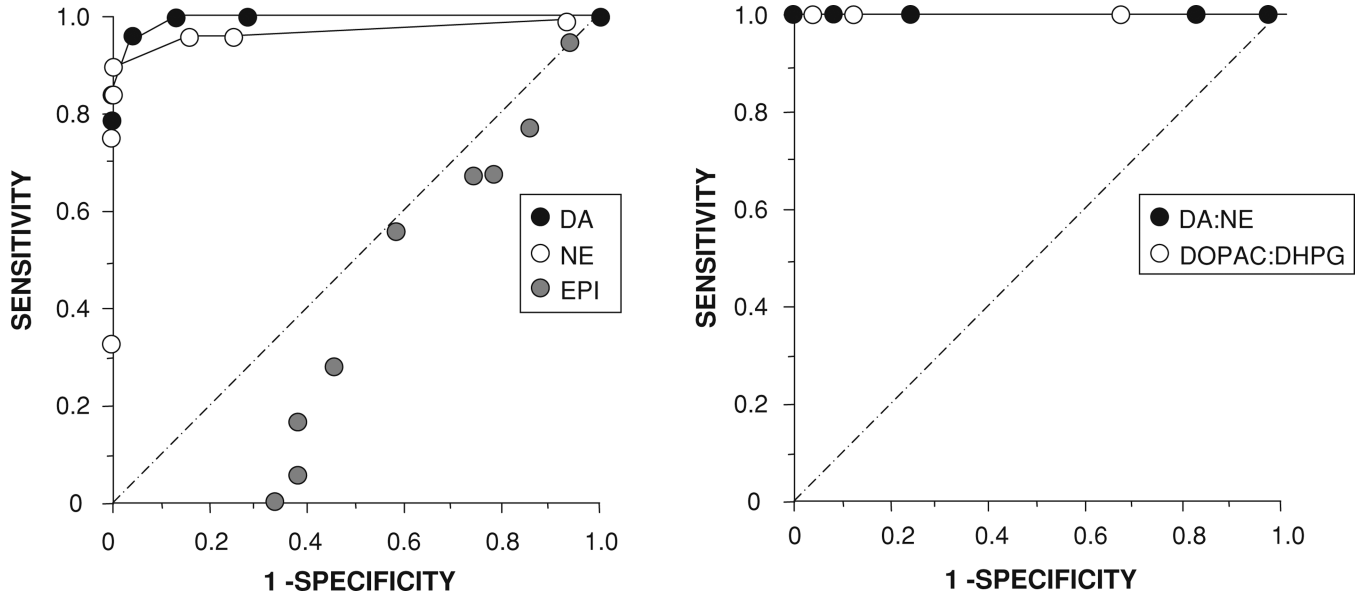


Fig. 4. Receiver operating characteristic curves for (*left*) the catecholamines dopamine (DA), norepinephrine (NE), and epinephrine (EPI) and (*right*) DA:NE and dihydroxyphenylacetic acid: dihydroxyphenylglycol (DOPAC:DHPG) ratios in newborns at risk for Menkes disease. Note high sensitivity and specificity of DA and NE and low sensitivity and specificity of EPI in distinguishing affected from unaffected newborns and sensitivities of 1.0 at 1-specificities of zero for DA:NE and DOPAC:DHPG ratios

Table 1Mean (\pm SD) values for plasma catechols in newborns at genetic risk for Menkes disease

Analyte	Affected	Unaffected	<i>P</i>
DOPA (nmol/l)	29.2 \pm 8.1	22.7 \pm 1.2	0.0015
DA (nmol/l)	0.97 \pm 0.15	0.16 \pm 0.02	<10 ⁻⁶
NE (nmol/l)	1.55 \pm 0.41	4.98 \pm 0.35	<10 ⁻⁶
EPI (nmol/l)	0.54 \pm 0.14	0.45 \pm 0.13	0.66
DOPAC (nmol/l)	39.3 \pm 3.9	15.5 \pm 1.7	<10 ⁻⁶
DHPG (nmol/l)	3.57 \pm 0.40	8.91 \pm 0.77	<10 ⁻⁵
DA:NE	0.90 \pm 0.14	0.04 \pm 0.01	<10 ⁻⁷
DOPAC:DHPG	14.9 \pm 3.75	1.74 \pm 0.09	<10 ⁻³
EPI:NE	0.58 \pm 0.21	0.10 \pm 0.03	0.013