In a newborn screening by tandem mass spectrometry in Japan, SCHAD deficiency was not a target disorder, but was tentatively monitored using markers such as C4-hydroxyacylcarnitine (C4OH) (cut-off value; 0.23 nmol/ml) and a C4OH/acetylcarnitine (C2) ratio (cut-off value; 0.011). A Japanese girl was born with a birth weight of 1,230 g and a gestation age of 31 weeks. Acylcarnitine analysis in the first dried blood spot (DBS), which was made on day 49 after respirator therapy for respiratory distress, showed increased levels of C4OH (0.72 nmol/ml), C4OH/C2 (0.028), and glutarylcarnitine (C5DC) (0.28 nmol/ml, cut-off; 0.25). Because of the decreased level of C5DC (0.22 nmol/ml) in the second DBS on day 63, additional DBSs were not requested, although levels of C4OH (0.48 nmol/ml) and C4OH/C2 (0.026) were still increased. Because of several episodes of convulsion since 5 months of age and hypoglycemia (22 mg/dl) at 7 months of age, the patients had laboratory work-up, which indicated increased insulin levels during hypoglycemia. Serum acylcarnitines analysis revealed increased levels of C4OH, C4OH/C2, and C5DC, and urinary organic acid analysis showed increased excretion of 3-hydroxyglutaric acid. The patient did not experience hypoglycemia after diazoxide therapy. The diagnosis of SCHAD deficiency was confirmed by DNA analysis. The present case indicates that the markers mentioned above may be useful for the screening of SCHAD deficiency and should be tested further.