mia-1 patients from Thailand and Singapore, respectively, using this multiplex-PCR assay, suggests that the $--^{FIL}$ and $--^{THAI}$ alleles are much less common than the $--^{SEA}$ allele in these populations (data not shown). These results are in agreement with the findings of Eng et al. *(4)*. With the exception of the $--^{HW}$ total deletion spanning >300 kb of the α-globin cluster that was described in a Chinese family *(3, 9)*, this multiplex-PCR assay detects all described Southeast Asian double-gene deletions in *cis* and the two most commonly observed single-gene deletions in a single reaction. This assay should simplify and substantially reduce the effort and cost of screening for α-thalassemia deletions in individuals of Southeast Asian origin.

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Serum Creatinine and Fat-free Mass (Lean Body Mass), *R. Swaminathan,[1*] Phillipa Major,[2] Harold Snieder,[2] and Timothy Spector[2]* ([1] Department of Chemical Pathology and [2] Twin Research Unit, The Guy's, King's College and St. Thomas' Medical and Dental School, St. Thomas' Campus, London, United Kingdom SE1 7EH; * author for correspondence: fax 44-20-7-928-4226, e-mail r.swaminathan@kcl.ac.uk)

Serum creatinine is widely used as an index of glomerular filtration rate (GFR) *(1)*. However, it is not a sensitive index because GFR may have to decrease by >50% before serum creatinine becomes "abnormal", and this may be at least partly attributable to the wide reference interval for serum creatinine (44–124 μmol/L). One of the factors influencing the creatinine concentration is said to be muscle mass *(1, 2)*. Thus, it can be argued that if the reference interval for serum creatinine can be adjusted for muscle mass, serum creatinine may be a better index of GFR. However, there is little information on the relationship between muscle mass and serum creatinine. We previously have shown that the contribution of lean body mass (LBM) to the variation in serum creatinine in healthy subjects is small *(3)*. In this report, we extend these observations to another group and used a more precise method to measure LBM.

We studied 664 female subjects (age range, 19–76 years) who attended the Twin Research Unit as part of a larger study. Of these, 346 were monozygotic and 318 were dizygotic twins. Body composition (total fat mass and LBM) was determined by dual energy x-ray absorptiometry using a QDR 2000 scanner (Hologic). The long-term imprecision with this scanner is 2%. Fasting blood samples were obtained from all subjects, and serum creatinine was measured by an enzymatic method on a dry chemistry analyzer (Vitros 950; Johnson & Johnson Diagnostic). The CV of the method is 3% at a concentration of 120 μmol/L.

The age, height, weight, total fat mass, LBM, and serum urea and creatinine values for the group are shown in Table 1. Simple correlation analysis with correction for the dependency between observation of twin pairs (using the Generalised Estimating Equation) showed that serum creatinine was correlated significantly with LBM ($r = 0.171$; $P < 0.0001$), percentage of body fat ($r = -0.10$; $P = 0.011$), and age ($r = 0.152$; $P < 0.0001$) but not with body mass index or total fat mass. When stepwise multiple regression analysis was done using serum creatinine as the dependent variable, and age, percentage of fat, and LBM as independent variables, the contributions of LBM, age, and percentage of fat to the variation of the serum creatinine were 2.9%, 2.1%, and 0.9%, respectively. To exclude the contribution of genetic influences, the analysis was done with one of the twins, and the contributions for LBM, age, and fat were 3.8%, 1.9%, and 2.1%, respectively.

Creatinine is produced nonenzymatically in the skeletal muscle, and the amount of creatinine production, and therefore the 24 h excretion of creatinine, is directly related to muscle mass *(4)*. This relationship has been used to predict muscle mass from creatinine excretion *(5, 6)*. In estimating GFR from serum creatinine, body weight is included in the formula *(7)* or nomogram *(8)* to allow for variation in creatinine excretion.

Table 1. Characteristics of the subjects.

	Mean ± SD	Range
Age, years	54.1 ± 8.7	19.0–70.3
Weight, kg	64.3 ± 11.0	49.0–139.0
Height, cm	161.9 ± 5.8	144–183
BMI,[a] kg/m^2	24.5 ± 4.2	18.4–51.2
Total fat mass, kg	24.9 ± 8.3	6.9–65.0
Lean body mass, kg	37.2 ± 4.6	23.0–53.7
Serum urea concentration, mmol/L	4.6 ± 1.1	2.0–9.2
Serum creatinine, μmol/L	73.0 ± 9.9	34–117

[a] BMI, body mass index.

When interpreting serum creatinine, it is believed that LBM should be taken into account *(1)*. However, as shown here and previously *(3)*, the contribution of LBM to variations in serum creatinine is small. The group of subjects studied were all females, and this may account for the low contribution. However, their LBM covered a wide range (23.0–53.7 kg), and thus it is likely that the findings here would be widely applicable.

These results suggest that although creatinine production increases with increasing LBM, there is a concomitant increase in the volume of distribution of creatinine, thereby reducing the relationship between serum creatinine and LBM. Total body water is related to LBM, and Shutte et al. *(9)* have shown that LBM and total plasma creatinine (plasma volume × creatinine concentration) are well correlated.

We conclude that the contribution of LBM to serum creatinine is small and that correction of serum creatinine according to LBM is unlikely to improve the usefulness of this measurement.

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Rapid Detection of the Two Most Common *CLN2* Mutations Causing Classical Late Infantile Neuronal Ceroid Lipofuscinosis, *Marek Bodzioch,[1,2] Charalampos Aslanidis,[2] Marek Kacinski,[1] Nanbert Zhong,[3] Krystyna E. Wisniewski,[4] and Gerd Schmitz[2*]* ([1] Department of Child Neurology, Polish-American Children's Hospital, Collegium Medicum, Jagiellonian University, Wielicka 265, 30-663 Krakow, Poland; [2] Institute for Clinical Chemistry and Laboratory Medicine, University of Regensburg, Franz-Josef-Strauss-Allee 11, D-93042 Regensburg, Germany; [3] Molecular Neurogenetic Diagnostic Laboratory, Specialty Clinic Laboratories and [4] Department of Pathological Neurobiology, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Rd., Staten Island, NY 10314; * author for correspondence: fax 49-941-944-6202, e-mail gerd.schmitz@klinik.uni-regensburg.de)

Neuronal ceroid lipofuscinoses (NCLs) are a group of genetically transmitted neurodegenerative disorders characterized clinically by intellectual and motor decline, visual loss, and myoclonic seizures, in most cases preceded by a variable period of apparently normal development. A common pathological feature of all NCLs is the intracellular accumulation of an autofluorescent material resembling ceroid or lipofuscin. Five genes (*CLN1*, *CLN2*, *CLN3*, *CLN5*, and *CLN8*) have been identified that are mutated in different forms of NCL: respectively, infantile NCL *(1)*; late infantile NCL *(2, 3)*; classical juvenile NCL *(4–6)*; Finnish variant late infantile NCL *(7)*; and the progressive epilepsy with mental retardation (EPMR, also called Northern epilepsy) *(8)*. Adult-onset NCL (*CLN4*) follows either an autosomal recessive (Kufs disease) or an autosomal dominant (Parry disease) pattern of inheritance and is likely to be linked to different, as yet unknown, gene loci.

The classical late infantile and juvenile forms, by far the commonest NCLs reported in different populations, are leading causes of neurodegeneration in childhood and adolescence. More than 30 mutations, scattered along the whole *CLN2* gene, have been reported in association with the classical late infantile NCL (cLINCL) phenotype *(9, 10)*. However, studies performed on large groups of cLINCL patients demonstrated that two mutations, 636C→T and T523-1G→C, are particularly common *(9, 11)*. They occur in ~60% of cLINCL chromosomes, and at least one of these mutations can be identified in >75% of patients *(12)*. We report here (*a*) successful development of a real-time multiplex fluorescence PCR with two dyes for the rapid detection of these two mutations and (*b*) genetic analysis of five new cLINCL families from South-Eastern Poland.

We obtained DNA samples for genotyping from five previously unreported cLINCL families with 5 cLINCL patients and 18 healthy relatives. Family members gave informed consent, and the study was approved by the appropriate ethics boards. The diagnosis of cLINCL was based on the typical clinical features and characteristic ultrastructural picture with curvilinear profiles. DNA was isolated from cultured skin fibroblasts using the QIAGEN Blood and Cell Culture DNA Mini reagent set (QIAGEN). Six DNA samples (C10516, C10557, C7153, C8878, C9542, and C11488) with known genotype *(12)* were obtained from Batten Disease Registry, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York. For mutation detection, we used the LightCycler (Roche Diagnostics) *(13, 14)*. The method uses two hybridization probes, labeled with a pair of fluorescent dyes, that bind adjacent to each other on a single-stranded DNA template. After excitation by a laser beam, fluorescein, a donor dye attached to the 3′ end of one probe, transfers energy to an acceptor dye, located on the 5′ end of the other probe, which in turn emits light detected by the instrument. The use of thin glass capillaries allows rapid heat transfer and short cycling times. Each pair of hybridization probes consists of a longer anchor probe and a shorter detection probe that overlaps