Unusual clustering of coefficients of variation in published articles from a medical biochemistry department in India

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ABSTRACT **A simple statistical method is described to test whether data are consistent with minimum statistical variability expected in a biological experiment. The method is applied to data presented in data tables in a subset of 84 articles among more than 200 published by 3 investigators in a small medical biochemistry department at a major university in India and to 29 "control" articles selected by key word PubMed searches. Major conclusions include: *1*) unusual clustering of coefficients of variation (CVs) was observed for data from the majority of articles analyzed that were published by the 3 investigators from 2000–2007; unusual clustering was not observed for data from any of their articles examined that were published between 1992 and 1999; and *2*) among a group of 29 control articles retrieved by PubMed key word, title, or title/abstract searches, unusually clustered CVs were observed in 3 articles. Two of these articles were coauthored by 1 of the 3 investigators, and 1 was from the same university but a different department. We are unable to offer a statistical or biological explanation for the unusual clustering observed.—Hudes, M. L., McCann, J. C., Ames, B. N. Unusual clustering of coefficients of variation in published articles from a medical biochemistry department in India. *FASEB J.* 23, 689–703 (2009)**

Key Words: lipoic acid · oxidative stress · antioxidants · aging · data variability · data manipulation

In 2004 and early 2005, as part of the peer review process, our group was asked to review several articles submitted for publication by Chinnakannu Panneerselvam, an investigator in a small medical biochemistry department at a major university in India. In these articles, the coefficients of variation [CVs; CV=100×(SD/mean)], calculated from data presented in data tables, appeared to be similar regardless of the treatment group or variable being measured. Subsequently, we observed a similar effect in a published article from the same investigator (1).

In late 2005, after visual inspection of a number of published articles from C. Panneerselvam and the other 2 investigators (Palaninathan Varalakshmi, the department chair, and Dhanapal Sakthisekaran) in the same department with publications listed in PubMed, we decided to conduct a more systematic analysis on a sample of their articles. Here, we describe the method of sampling and analysis, present results for a subset of 84 of more than 200 articles published by the 3 investigators and 29 "control" articles selected by PubMed key word, title, or title/abstract searches. Expected effects of the violation of assumptions utilized in deriving the method of analysis are discussed. Simulation trials are presented in an appendix.

MATERIALS AND METHODS

Selection of articles for analysis

All searches were conducted using PubMed (http://www.ncbi.nlm.nih.gov). The majority of the more than 200 publications of the 3 investigators appeared after the year 2000, but articles are listed that extend from 2008 back over periods of 29 yr (P. Varalakshmi), 27 yr (C. Panneerselvam), or 19 yr (D. Sakthisekaran). This project was initiated in late 2005. The period from 2000 to 2005 was initially chosen for analysis. Subsequently, the analysis was expanded to earlier and later periods. The period 1992–1999 was arbitrarily chosen as the earlier period; articles appearing in PubMed in 2006–2007 represented the later period. Methods used to select articles for analysis are indicated below.

Selection of articles published by the 3 investigators from 1992 through 2006

To select these articles in a nonbiased fashion for each of the 3 investigators, the first 2 articles with data tables listed for each year were selected, unless the first 2 articles had the same first author, in which case the first article and the next listed with a different first author were selected. Using this procedure, a total of 28, 14, and 17 articles were selected, respectively, for P. Varalakshmi, C. Panneerselvam, and D. Sakthisekaran.

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Selection of articles published in 2007

All articles dated 2007 with data tables that were retrieved in a PubMed search conducted in February 2008 were sampled, a total of 25 (16, P. Varalakshmi; 8, C. Panneeerselvam; and 1, D. Sakthisekaran). These articles included several e-pub publications that subsequently have appeared in print dated 2008.

Selection of control articles

Three sets of control articles were selected using PubMed key word searches conducted in December 2005 (control set 1) and February 2006 (control sets 2 and 3).

Control set 1 With PubMed limits set for title/abstract, articles were selected using the key word entry "aging oxidative stress rats antioxidants." Key words were selected to extract articles that included measurements made from *in vivo* experiments using rats that measured endpoints similar to those in many articles from the 3 investigators. All articles retrieved by this search that had data tables, group sizes (n), and a measure of variability (SD or SE) were selected, a total of 10.

Control set 2 With PubMed limits set for title, articles were selected using the key word entry "on oxidative stress rats." Key words were selected to retrieve *in vivo* experiments using rats that were more specifically targeted at measuring the effects of an agent on oxidative stress markers than were studies used for control set 1. A total of 594 references were retrieved. Starting with the first reference on the list, we selected the first 15 articles that presented results in at least 1 data table and included biochemical measurements of oxidative stress. In the articles selected, tables of nonbiochemical measurements such as body weight and red blood cell count were excluded. When a table contained both biochemical and nonbiochemical measurements (*e.g.*, Hung, ref. 2), the table was included.

Control set 3 One article of C. Panneerselvam (1) that suggested unusual clustering of CVs measured a single endpoint (mitochondrial respiration) in an *in vivo* rat experiment using a common methodology (polarographic measurement of oxygen consumption using a standard Clark-type oxygen electrode). A PubMed search was conducted to identify other laboratories that had measured the same endpoint in *in vivo* rat experiments using the same methodology. The first 4 articles identified that included data tables were chosen.

Statistical procedures

The rationale for this analysis was to use the CV as a measure of variability, to define boundaries within which CVs are likely to fall, and then to compute an approximate probability value ("P value") to describe how well results from a particular article correspond to what would be expected statistically. The analysis was designed to be conservative, that is, to disfavor the tentative conclusion of unusual clustering of CVs.

Construction of boundaries within which 50% of CVs are likely to fall

We make the assumption that the observed data are normally distributed with finite mean μ and finite variance σ^2. The population CV (expressed below as a proportion), CV_{pop}, would then be $CV_{pop} = \sigma/\mu$. This is estimated by the sample CV, $CV_{sample} = s/\bar{x}$, where $\bar{x}$ and s are the sample mean and sample SD. For a sample of size n from a normal distribution, $T = \sqrt{n}/CV_{sample}$ has a noncentral Student t distribution with $n - 1$ degrees of freedom and noncentrality parameter ncp = $\sqrt{n}/CV_{pop}$ (3).

The above can be used to construct an interval within which CV_{sample} will fall 50% of the time. Let t'_p denote the percentile of the noncentral t distribution (with degrees of freedom $n - 1$ and noncentrality parameter $\sqrt{n}/CV_{pop}$) which cuts proportion p to the left and proportion $1 - p$ to the right. We then have Eqs. 1–3:

$$P(t'_{0.25} < T < t'_{0.75}) = 0.50 \quad (1)$$

$$P\left[\frac{1}{t'_{0.25}} > \frac{CV_{sample}}{\sqrt{n}} > \frac{1}{t'_{0.75}}\right] = 0.50 \quad (2)$$

$$P\left[\frac{\sqrt{n}}{t'_{0.75}} < CV_{sample} < \frac{\sqrt{n}}{t'_{0.25}}\right] = 0.50 \quad (3)$$

The median CV from each journal article was used to estimate CV_{pop}.

The construction of these intervals makes the following assumptions:

a) All measurements are on the same variable, such as a particular enzyme activity;

b) All measurements are from the same treatment group, such as the control group or a group treated with lipoic acid;

c) The median CV is an adequate estimate of CV_{pop}.

d) Each variable being examined has an approximate normal distribution.

The violation of assumptions *a* and *b*, which occurs for all articles analyzed, would make it more likely that the sample CV would be outside the constructed 50% boundaries. On the other hand, utilization of the median to estimate CV_{pop}, assumption *c*, may slightly increase the number of sample CVs within the 50% intervals. We expect that the violation of assumptions *a* and *b* will have a much greater effect than the use of the median in assumption *c*. Simulation trials were performed to evaluate the violation of the normality assumption (See Appendix).

Calculation of an approximate "P value"

The binomial distribution was used to compute the likelihood of obtaining k or more (out of a total of n) CVs inside the constructed 50% limits. This is analogous to computing the probability of k or more heads in n tosses of a fair coin. These "P values" are not P values in the usual sense; that is, they do not relate in the usual way to null and alternative hypotheses, test statistics, and such. They are probability numbers computed from a binomial model assuming independence. The assumption of independence, however, is not true of the experiments analyzed here because multiple measurements were taken from the same animals. Such dependence would tend to result in a smaller "P value" than that calculated under the assumption of independence. Conversely, if assumptions *a* and *b* above are violated (*e.g.*, measurements are made on more than one variable on subjects from more than one population), as is the case in all the experiments we analyzed, this will result in fewer observations inside the constructed 50% limits, resulting in a computed "P value" much larger than if these assumptions are not violated. Because the computed "P values" are rough approximations, we have adopted a somewhat conservative value of $P < 0.01$ as the criterion for unusual clustering.

RESULTS

Using the selection procedures indicated in Materials and Methods, a subset of 84 articles of more than 200 published by 3 investigators in the medical biochemistry department at the University of Madras in Chennai, India,

TABLE 1. *Palaninathan Varalakshmi: variability of CVs in articles published 1992–2007*

Reference	Journal	Total means	Group size (n)	Median CV (%)	50% CV limits	CVs falling within the 50% limits	"P value"[a]
Veena *et al.,* 2008 (E-pub 2007) (25)	*Eur. J. Pharmacol.*	44	6	11.7	8.6–13.5	35	5.3×10^{-5}
Amudha *et al.,* 2007 (26)	*Mol. Cell. Biochem.*	80	6	10.2	7.4–11.7	66	1.6×10^{-9}
Amudha *et al.,* 2007 (27)	*Int. Immunopharmacol.*	20	6	12.1	8.8–14.0	11	0.41
Amudha *et al.,* 2007 (28)	*Eur. J. Pharmacol.*	68	6	13.0	9.5–15.0	28	0.94
Josephine *et al.,* 2007 (4)	*Arch. Toxicol.*	80	6	11.0	8.0–12.6	75	2.1×10^{-17}
Josephine *et al.,* 2007 (29)	*Mol. Nutr. Food. Res.*	60	6	13.2	9.6–15.2	37	0.046
Josephine *et al.,* 2007 (30)	*Biol. Pharm. Bull.*	32	6	12.8	9.4–14.8	22	0.025
Josephine *et al.,* 2007 (31)	*Basic Clin. Pharmacol. Toxicol.*	40	6	10.8	7.9–12.5	28	0.0083
Mythili *et al.,* 2007 (32)	*Life Sci.*	40	6	10.8	7.9–12.5	30	0.0011
Rasool and Varalakshmi, 2007 (33)	*Fundam. Clin. Pharmacol.*	168	6	8.3	6.1–9.6	122	2.0×10^{-9}
Sakthivel *et al.,* 2007 (34)	*Clin. Chim. Acta*	1	4	8.7	5.5–10.2	19	>0.99
		1	6		6.3–10.0		
		17	55		8.1–9.2		
		17	70		8.1–9.1		
		17	90		8.2–10.0		
		17	113		8.3–10.2		
Sudhahar *et al.,* 2007 (35)	*Vascul. Pharmacol.*	60	6	9.53	7.0–11.0	52	2.6×10^{-9}
Sudhahar *et al.,* 2007 (36)	*Mol. Cell. Biochem.*	78	6	9.3	6.8–10.7	69	6.9×10^{-13}
Veena *et al.,* 2007 (37)	*Clin. Exp. Nephrol.*	7	6	12.2	8.9–14.1	5	0.23
Veena *et al.,* 2007 (38)	*Hum. Exp. Toxicol.*	36	6	10.7	7.9–12.3	28	5×10^{-4}
Veena *et al.,* 2007 (39)	*J. Pharm. Pharmacol.*	52	6	10.1	7.4–11.7	33	0.035
Deepa and Varalakshmi, 2006 (40)	*Int. J. Cardiol.*	114	6	7.44	5.44–8.57	86	2.4×10^{-8}
Sudharsan *et al.,* 2006 (41)	*Mol. Cell. Biochem.*	72	6	12.74	9.30–14.72	56	1.2×10^{-6}
Sudharsan *et al.,* 2005 (42)	*J. Pharm. Pharmacol.*	126	6	9.91	7.24–11.43	65	0.40
Sudhahar *et al.,* 2006 (E-pub 2005)	*Life Sci.*	108	6	10.15	7.41–11.71	93	3.2×10^{-15}
Prahalathan *et al.,* 2004 (44)	*Mol. Cell. Biochem.*	76	6	7.46	5.45–8.60	39	0.45
Mythili *et al.,* 2004 (45)	*Chem. Biol. Interact.*	60	6	9.92	7.25–11.44	50	8.1×10^{-8}
Deepa and Varalakshmi, 2003 (5)	*Mol. Cell. Biochem.*	108	6	9.50	6.94–10.96	93	3.2×10^{-15}
Varalakshmi *et al.,* 2003 (46)	*Mol. Cell. Biochem.*	52	6	7.45	5.45–8.59	25	0.66
Vidya *et al.,* 2002 (47)	*Phytother. Res.*	102	6	6.30	4.61–7.26	50	0.62
Sivaprasad *et al.,* 2002 (48)	*Arch. Toxicol.*	80	6	8.95	6.54–10.32	62	4.1×10^{-7}
Lenin *et al.,* 2001 (49)	*Prostaglandins Leukot. Essent. Fatty Acids*	80	6	11.21	8.19–12.94	43	0.29
Sunitha *et al.,* 2001 (24)	*Fitoterapia*	85	6	8.12	5.94–9.37	65	5.2×10^{-7}
Vidya and Varalakshmi, 2000 (50)	*Fitoterapia*	102	6	6.33	4.63–7.29	44	0.93
Nagaraj *et al.,* 2000 (23)	*J. Appl. Toxicol.*	60	6	10.10	7.38–11.65	14	>0.99
Geetha and Varalakshmi, 1999 (51)	*Mol. Cell. Biochem.*	156	6	7.24	5.29–8.35	67	0.97
Anuradha and Varalakshmi, 1999 (52)	*J. Appl. Toxicol.*	216	6	1.96[b]	1.43–2.26	52	>0.99
Latha *et al.,* 1998 (53)	*Gen. Pharmacol.*	76	6	8.87	6.48–10.23	25	>0.99
Geetha *et al.,* 1998 (54)	*Pharmacol. Res.*	78	6	4.89	3.57–5.63	15	>0.99
Sandhya and Varalakshmi, 1997 (55)	*J. Appl. Toxicol.*	56	6	10.93	7.99–12.62	18	>0.99
Sumathi *et al.,* 1996 (56)	*Jpn. J. Med. Sci. Biol.*	60	6	10.6	7.8–12.3	37	0.046
Saravanan *et al.,* 1996 (57)	*Br. J. Urol.*	28	6	11.4	8.3–13.2	10	0.96
Malini *et al.,* 1995 (58)	*Jpn. J. Med. Sci. Biol.*	40	6	11.7	8.5–13.5	10	>0.99
Saravanan *et al.,* 1995 (59)	*Pharmacol. Res.*	96	6	8.9	6.5–10.3	50	0.38
Jayanthi *et al.,* 1994 (60)	*Pharmacol. Res.*	36	6	10.9	7.9–12.5	11	>0.99
Sumathi *et al.,* 1993 (61)	*Pharmacol. Res.*	55	6	5.9	4.3–6.8	20	>0.99
Subha and Varalakshmi, 1993 (62)	*Pharmacol. Res.*	96	6	7.5	5.5–8.7	30	>0.99
Selvam *et al.,* 1992 (63)	*Pharmacol. Res.*	112	6	8.3	6.0–9.5	51	0.85
Subha *et al.,* 1992 (64)	*Biochem. Int.*	60	6	9.0	6.6–10.4	28	0.74

Articles in PubMed (as of April 2008) that list P. Varalakshmi as coauthor include 134 articles from the Department of Medical Biochemistry at the University of Madras published 1990–2008. Two articles before 1990 are listed in PubMed, one published in 1983 (from Ohio State University) and the other, of which we were unable to obtain a copy, in 1979. We do not know if the individual listed on the 1979 and 1983 articles is the same P. Varalakshmi as is listed on the articles from the University of Madras. Articles published prior to 1992 were not analyzed. All articles that appeared in PubMed in 2007 were analyzed. Articles analyzed from 1992 to 2006 were those sampled using the procedure described in Materials and Methods. [a] The "P value" is an approximated probability value calculated under certain assumptions (see Materials and Methods). We consider $P < 0.01$ to suggest unusual clustering. [b] This is such a small number, possibly SES instead of SDS were presented.

TABLE 2. *Chinnakannu Panneerselvam: variability of CVs in articles published 2000–2007*

Reference	Journal	Total means	Group size (*n*)	Median CV (%)	50% CV limits	CVs falling within the 50% limits	"*P* value"[a]
Murali *et al.*, 2008 (9) (E-pub 2007)	*Int. J. Dev. Neurosci.*	60	6	9.4	6.9–10.9	60	8.7×10^{-19}
Thangasamy *et al.*, 2008 (65) (E-pub 2007)	*Clin. Chim. Acta*	112[b]	6	14.5	10.6–16.8	89	1.2×10^{-10}
Kadirvel *et al.*, 2007 (66)	*Hum. Exp. Toxicol.*	15	6	13.0	9.5–15.0	8	0.5
Murali and Panneerselvam, 2007 (67)	*J. Gerontol. A Biol. Sci. Med. Sci.*	120	6	8.6	6.3–9.9	99	1.3×10^{-13}
Savitha and Panneerselvam, 2007 (68)	*Mech. Ageing Dev.*	18	6	10.6	7.7–12.2	16	6.5×10^{-4}
Savitha *et al.*, 2007 (69)	*Eur. J. Pharmacol.*	18	6	11.4	8.3–13.1	18	3.8×10^{-6}
Tamilselvan *et al.*, 2007 (70)	*Free Radic. Biol. Med.*	24	6	13.6	9.9–15.7	16	0.076
Tamilselvan *et al.*, 2007 (71)	*Rejuvenation Res.*	44	6	11.6	8.5–13.4	31	0.0048
Balu *et al.*, 2006 (72)	*Brain Res. Bull.*	16	6	9.72	7.1–11.2	16	1.5×10^{-5}
Sethumadhavan and Chinnakannu, 2006 (8)[c]	*J. Gerontol. A Biol. Sci. Med. Sci.*	240	6	11.02	8.0–12.7	236	7.8×10^{-65}
Kumaran *et al.*, 2005 (1)	*Mol. Cell. Biochem.*	60	6	9.42	6.88–10.86	59	5.3×10^{-17}
Sangeetha *et al.*, 2005 (14)	*Exp. Gerontol.*	72	6	10.10	7.38–11.66	45	0.022
Arivazhagan and Panneerselvam, 2004 (73)	*Ann. N. Y. Acad Sci*	60	6	8.70	6.36–10.04	51	1.5×10^{-8}
Haripriya *et al.*, 2004 (74)	*Biogerontology*	72	6	8.93	6.52–10.29	63	1.3×10^{-10}
Arivazhagan *et al.*, 2003 (75)	*J. Gerontol. A Biol. Sci. Med. Sci.*	90	6	9.43	6.89–10.88	76	8.9×10^{-12}
Juliet *et al.*, 2003 (76)	*J. Gerontol. A Biol. Sci. Med. Sci.*	120	6	6.61	4.83–7.62	37	>0.99
Arivazhagan and Panneerselvam, 2002 (77)	*Exp. Gerontol.*	90	6	7.90	5.78–9.11	68	6.3×10^{-7}
Arockia Rani and Panneerselvam, 2001 (78)[d]	*Exp. Gerontol.*	240	6	9.11	6.66–10.51	79	>0.99
Ramanathan *et al.*, 2002 (79)	*Hum. Exp. Toxicol.*	150	6	10.60	7.75–12.24	115	1.9×10^{-11}
Arivazhagan *et al.*, 2001 (80)	*Exp. Gerontol.*	102[e]	6	8.76	6.40–10.10	61	0.03
Arivazhagan and Panneerselvam, 2000 (81)	*Pharmacol. Res.*	54	6	9.10	6.65–10.50	45	3.6×10^{-7}
Arivazhagan and Panneerselvam, 2000 (82)	*Pharmacol. Res.*	120	6	7.87	5.75–9.07	84	7.0×10^{-6}

Articles in PubMed (as of April 2008) that list Chinnakannu Panneerselvam as a coauthor include: 1981–1983, 2 articles published from the Department of Biochemistry at the University of Madras; 1987–1993, 11 articles published from the Department of Biochemistry at Cornell University; 2000–2008, 49 articles published from the Medical Biochemistry Department at Madras University; and 2006, 3 articles under the name Panneerselvam Chinnakannu, also from the Medical Biochemistry Department at Madras University. There are no listings in PubMed for C. Panneerselvam or P. Chinnakannu for 1994–1999. There were no data tables in the articles published from Cornell University from 1992 to 1993; we did not analyze articles published prior to 1992. [a]The "*P* value" is an approximated probability value calculated under certain assumptions (see Materials and Methods). We consider $P < 0.01$ to suggest unusual clustering. [b]SE was presented in this article; it was converted to SD for this analysis. [c]We have assumed that Panneerselvam Chinnakannu is the same individual who usually publishes as Chinnakannu Panneerselvam. [d]Note that the first author on this article is listed as P. Juliet Arockia Rani. In the 2003 article, the first author is listed as Packiasamy A. R. Juliet. These are most likely the same person, but we have listed the 2 names following PubMed. [e]Standard error was presented in the article; it was converted to SD for this analysis.

and 29 control articles from the general published literature were sampled for analysis. Results of the analysis of these articles are presented here. Data used to calculate the 50% limits and "*P* values" and results of the calculations are in **Tables 1–4**. The great majority of articles analyzed presented means and SDs in data tables. When SEs were presented instead, SEs were converted to SDs by multiplying the SE by the square root of *n*, the number of observations used to calculate the mean.

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Studies in articles coauthored by each of the 3 investigators have similar experimental designs. Typically, rats are divided into groups consisting of 6 rats each and subjected to various treatments, and then results are presented in data tables that compare group means and SDs (or occasionally SEs) for various outcome measures. Outcome measures varied but were predominantly biochemical measurements, frequently enzymatic and nonenzymatic measures of oxidative stress or other enzyme activities. The same outcome measures were frequently made in multiple tissues. Below are results of the analysis of articles sampled from publications of each of the 3 investigators.

Palaninathan Varalakshmi

P. Varalakshmi is the chair of the Medical Biochemistry Department on the Chennai campus of the University of

TABLE 3. *Dhanapal Sakthisekaran: variability of CVs in articles published 1991–2007*

Reference	Journal	Total means	Group size (n)	Median CV (%)	50% CV limits	CVs falling within the 50% limits	"P value"[a]
Thirunavukkarasu *et al.,* 2008 (83)	*Mol. Cell. Biochem.*	64	6	25.3 (SE)	18.4–29.6	25	0.97
Chodon *et al.,* 2007 (84)	*Toxicol. In Vitro*			No data tables			—
Chodon *et al.,* 2007 (85)	*Mol. Cell. Biochem.*			No data tables			—
Senthilnation *et al.,* 2006 (86)	*Cancer Sci.*	35	6	8.8	6.4–10.1	19	0.37
Padmavathi *et al.,* 2006 (87)	*Comp. Biochem. Physiol.*	75	6	8.5	6.2–9.9	60	7.9×10^{-8}
Rajkapoor *et al.,* 2006 (E-pub 2005) (88)	*J. Ethnopharmacol.*	33[b]	6	6.40	4.68–7.38	14	0.85
Thirunavukkarasu *et al.,* 2005 (89)	*Mol. Cell. Biochem.*	72	6	9.88	7.22–11.40	53	3.8×10^{-5}
Banu *et al.,* 2004 (90)	*Hum. Exp. Toxicol.*	56	6	9.75	7.12–11.24	48	2.3×10^{-8}
Selvendiran *et al.,* 2004 (91)	*Clin. Chim. Acta*	90	6	10.26	7.49–11.83	47	0.38
Selvendiran *et al.,* 2003 (92)	*Fitoterapia*	70	6	8.13	5.94–9.37	28	0.96
Thirunavukkarasu and Sakthisekaran, 2003 (93)	*Biomed. Pharmacother.*	60	6	10.44	7.63–12.05	49	3.8×10^{-7}
Palanivel and Sakthisekaran, 2002 (94)	*Ann. N. Y. Acad. Sci.*	48	6	9.09	6.64–10.48	41	3.1×10^{-7}
Thirunavukkarasu *et al.,* 2002 (95)	*Cell Biochem. Funct.*	144	6	8.78	6.42–10.13	91	9.7×10^{-4}
Ebrahim *et al.,* 2001 (10)	*Drug Chem. Toxicol.*	25	6	8.82	6.44–10.17	20	0.0020
Thirunavukkarasu *et al.,* 2001 (11)	*Cell Biochem. Funct.*	32 48	6 5	9.26	6.76–10.68 6.41–10.76	77	7.1×10^{-20}
Jagadeeswaran *et al.,* 2000 (96)	*Fitoterapia*	28	6	12.50	9.13–14.44	13	0.71
Babu *et al.,* 1999 (97)	*Ren. Fail.*	60	6	5.4	4.0–6.2	22	0.99
Ebrahim *et al.,* 1996 (98)	*J. Appl. Toxicol.*	78	6	14.3	10.5–16.6	41	0.37
Babu *et al.,* 1995 (99)	*Mol. Cell. Biochem.*	21	6	2.5	1.8–2.9	4	>0.99
Ebrahim *et al.,* 1995 (100)	*Mol. Cell. Biochem.*	36	6	6.6	4.8–7.6	10	>0.99

Articles in PubMed (as of April, 2008) that list D. Sakthisekaran as coauthor include 45 articles published 1989–1991 from the Department of Biochemistry at University of Madras, and 1995–2008 from the Medical Biochemistry Department. All articles appearing in PubMed in 2007 were analyzed. Articles published 1995–2006 were those sampled as described in Materials and Methods. Articles prior to 1992 were not analyzed. [a]The "P value" is an approximated probability value calculated under certain assumptions (see Materials and Methods). We consider $P < 0.01$ to suggest unusual clustering. [b]SE was presented in the article; it was converted to SD for this analysis.

Madras and has been a coauthor on 136 articles (cited in PubMed as of April 2008) published from 1979 to 2008. From 1990 to the present, Dr. Varalakshmi has been associated with the Medical Biochemistry Department.

Table 1 includes constructed 50% CV limits and "P values" for all 16 articles that appeared in PubMed in 2007, 14 articles sampled from the 88 that appeared between January 2000 and December 2006, and 14 articles sampled from the 27 that appeared between 1992 and 1999 (articles published prior to 1992 were not included).

As shown in Table 1, values of $P < 0.01$ were observed for 16 (57%) of the 28 articles analyzed that were published after January 2001 but for none of the 16 articles analyzed that were published prior to 2001. As indicated in Materials and Methods, we consider that values of $P < 0.01$ suggest unusual clustering of CVs. Note that most values of $P < 0.01$ are far below 0.01, ranging up to 2.1×10^{-17} (4). CVs for one of these unusually clustered articles, Deepa and Varalakshmi, (5) are illustrated in **Fig. 1*A***. As shown, 93 of 108 CVs fell within the constructed 50% limits, with a value of $P = 3.2 \times 10^{-15}$.

Chinnakannu Panneerselvam

C. Panneerselvam has been a coauthor on 62 articles (cited in PubMed as of April 2008), published from 1981 to 2008. From 2000 to April 2008, Dr. Panneerselvam has published 49 articles from the Medical Biochemistry Department at the University of Madras. From 1987 to 1993, he was a coauthor on 11 articles from a laboratory at Cornell University, and from 1981 to 1983, he coauthored 2 articles from the Department of Biochemistry at the University of Madras. In 2006, 3

TABLE 4. *Control articles*

Reference	Total means	Group size (n)	Median CV (%)	50% CV limits	CVs falling within the 50% limits	"P value"[a]
Control set 1[b]						
Albano *et al.,* 2002 (101)	14[d]	6	24.1	17.49–28.06	4	0.97
Augustiniak *et al.,* 2005 (22)	210	6	6.89	5.03–7.94	113	0.15[c]
Balu *et al.,* 2005 (12)	112	6	9.45	6.90–10.90	99	7.5×10^{-18}
Luczaj *et al.,* 2004 (17)	174	6	6.49	4.75–7.48	92	0.25[c]
Mosoni *et al.,* 2004 (102)	48[d]	6	19.30	14.06–22.39	12	0.99
Payne *et al.,* 2003 (103)	32[d]	10	10.87	8.79–12.25	0	1.0
Sangeetha *et al.,* 2005 (14)	72[e]	6	10.10	7.38–11.66	45	0.022
Savitha *et al.,* 2005 (13)	84	6	10.82	7.90–12.48	82	1.9×10^{-22}
Shimizu *et al.,* 2004 (104)	28[d,f]	6	29.27	21.21–34.32	10	>0.99
	50[d]	7		22.01–34.03		
	9[d,g]	12		24.10–33.09		
Suh *et al.,* 2005 (105)	12	4	21.36	13.52–25.30	5	0.81
Control set 2[h]						
Coskun *et al.,* 2005 (106)	12	10	28.58	22.94–32.58	8	0.19
Faine *et al.,* 2006 (107)[i]	32	6	18.55	13.51–21.51	13	0.89
Gupta and Flora, 2006 (16)[j,k]	112[k]	5	11.30	7.82–13.15	36	>0.99
Hung, 2005 (2)	82[k]	8	26.81	20.74–30.87	28	>0.99
Kanter *et al.,* 2005 (108)[l]	15	10	9.90	8.01–11.16	3	>0.99
Klepac *et al.,* 2005 (109)	20	7	10.73	8.13–12.30	11	0.41
Lei *et al.,* 2005 (110)[m]	24	4	9.37	5.95–10.99	7	0.99
Liu *et al.,* 2006 (111)[i]	15[k]	9	28.04	22.14–32.13	7	0.70
Mastrocola *et al.,* 2005 (112)	10	6	13.77	10.01–15.92	4	0.83
Montilla *et al.,* 2005 (113)[i]	24[k]	6	27.26	19.78–31.89	19	>0.99
	56[k]	5		18.75–32.19		
Montilla *et al.,* 2005 (114)	104[k]	5	29.81	20.48–35.32	45	0.93
Muthuvel *et al.,* 2006 (15)[i]	18[k]	6	15.28	11.15–17.68	17	7.2×10^{-5}
Naik *et al.,* 2005 (115)	8[k]	6	6.08	4.45–7.00	0	1.0
Ozturk *et al.,* 2006 (116)	12	10	12.72	10.28–14.35	5	0.81
Pustovrh *et al.,* 2005 (117)	12[k]	8	28.72	22.19–33.13	5	0.81

Articles selected in a PubMed search intended to extract articles that included measurements from *in vivo* experiments using rats that measured end points similar to those in a number of the articles from the 3 Medical Biochemistry Department investigators. [a]The "P value" is an approximated probability value calculated under certain assumptions (see Materials and Methods). We consider $P < 0.01$ to suggest unusual clustering. [b]Articles listed were retrieved by a search of PubMed using the key words "aging oxidative stress rats antioxidants," with limits set for title/abstract (*i.e.*, with the search confined to articles in which key words appear in the title or abstract). All articles that had data tables and that specified group sizes (n) and a measure of variability (SD or SE) were selected. [c]"P value" estimated. [d]SEs were listed in the article; they were converted to SDs for this analysis. [e]The reported values for 6 of the SDs in this data set were 0, yielding values of CV = 0, which were included in the analysis. The reported zero values are most likely due to the fact that SDs were reported to only 2 significant figures and corresponding mean values were <0.1. [f]Two $n = 6$ sample means were reported as 0, and these were excluded from the total means. [g]One $n = 12$ data entry was 0 and was excluded from the total means. [h]Articles selected in a PubMed search intended to specifically target articles that included measurement of effects on oxidative stress markers. Articles listed are the first 16 retrieved by a search of PubMed using the key words "on oxidative stress rats" with limits set for title (*i.e.*, with the search confined to articles in which key words appear in the title). [i]Table 1 excluded. [j]Tables 1 and 2 excluded. [k]SE was listed in the article; it was converted to SD for this analysis. [l]Tables 2 and 3 excluded. [m]Tables 1–3 and 5 excluded.

additional articles from the Medical Biochemistry Department were published under the name Panneerselvam Chinnakannu (6–8), whom we assume to be the same individual as Chinnakannu Panneeerselvam.

Table 2 includes results for all 8 articles that appeared in PubMed in 2007 and 14 articles sampled from the 42 that appeared between January 2000 and December 2006. There are no listings in PubMed for C. Panneerselvam from 1994 to 1999, no data tables in the one article from Cornell University published in 1993, and no articles listed for 1992. We did not include articles published prior to 1992.

As shown in Table 2, values of $P < 0.01$ were observed for 16 (73%) of the 22 articles analyzed, all of which were published during or after the year 2000. All but 1 of these 16 "P values" are far less than 0.01, ranging up to 7.8×10^{-65} (8). CVs for 2 unusually clustered articles are in Fig. 1*B*, Murali *et al.* (9) and Fig. 3A, Kumaran *et al.* (1). As shown in Fig. 1*B*, in Murali *et al.* (9), all 80 CVs fell within the constructed 50% limits, resulting in a "P value" of 7.1×10^{-20}. For Kumaran *et al.* (1), shown in Fig. 3*A*, 59 of 60 CVs fell within the 50% limits, resulting in a "P value" of 5.3×10^{-17}. Kumaran *et al.* (1) involved measurement of a single endpoint (mitochondrial respiration) using a commonly employed methodology and is discussed below in comparison with 4 control articles.

Dhanapal Sakthisekaran

Articles in PubMed (as of April 2008) that list D. Sakthisekaran as coauthor include 42 published be-

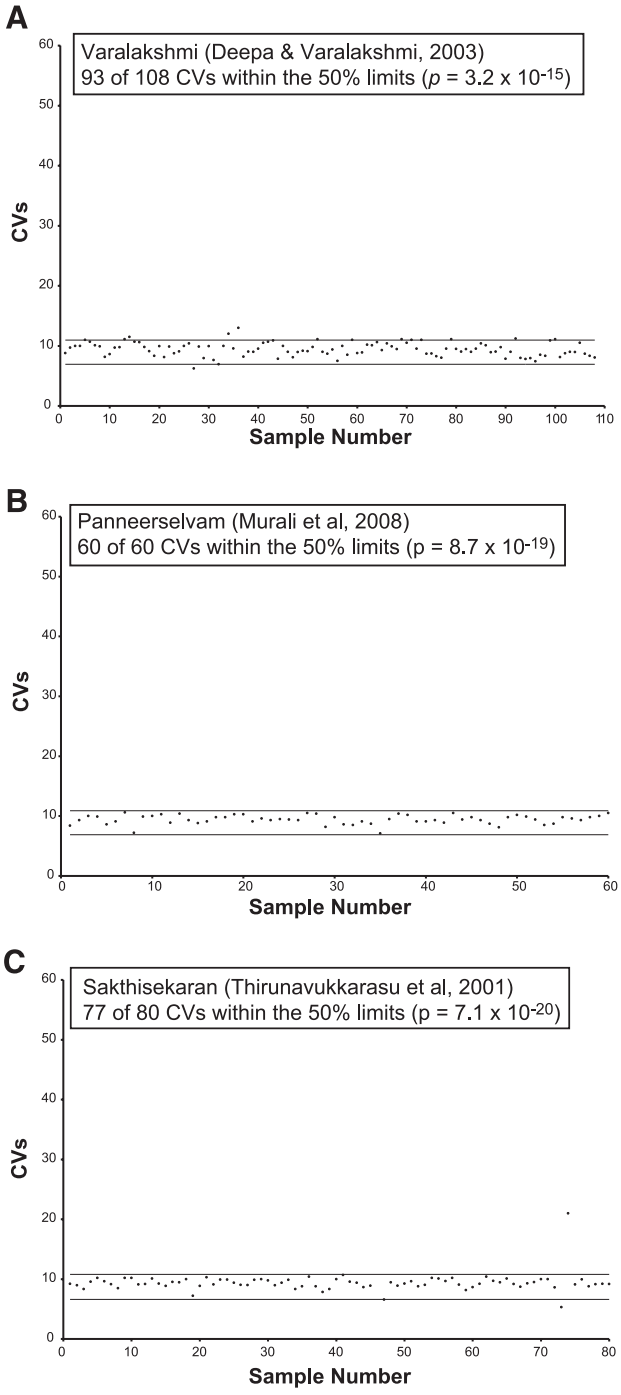

Figure 1. Illustrative results for P. Varalakshmi, C. Panneerselvam, and D. Sakthisekaran. CVs are plotted on the *y* axis for each mean and SD (or SE), which are consecutively numbered on the *x* axis. Constructed 50% limits are illustrated by horizontal lines. "*P* values" were calculated as described in Materials and Methods. Quantitative values used to construct 50% limits and "*P* values" are specified in the accompanying tables. Outcome measures are as listed below. *A*) Varalakshmi (Deepa and Varalakshmi, ref. 5): lipid peroxidation (MDA), antioxidant enzymes (SOD, catalase, GSH-Px), and antioxidants (GSH, vitamin C, α-tocopherol) measured in heart, liver, and kidney in rats ± an atherogenic diet ± treatment with low molecular weight heparin. *B*) Panneerselvam (Murali *et al.*, ref. 9): lipofuscin, Na^+K^+ ATPase, Mg^{2+} ATPase, Ca^{2+} ATPase, and intracellular calcium, each measured in 3 brain regions in young or old rats ± treatment with glutathione monoester. *C*) Sakthisekaran (Thirunavukkarasu *et al.*, ref 11): TCA cycle enzymes (ICDH, SDH, MDH, α-KGDH) were measured in hepatoma and surrounding tissue, liver, and kidney in rats ± selenium supplementation, ± hepatoma induction by N-nitrosodiethylamine-phenobarbital. Each figure displays CVs (plotted on the *y* axis) for each mean and SD (or SE) appearing in data tables in a particular article. The CVs appear consecutively from left to right, grouped according to table (*i.e.*, all CVs corresponding to Table 1 in a particular article appear before all CVs corresponding to Table 2, and so on). Within each table grouping, CVs are grouped according to the specific end point measured. GSH, glutathione; GSH-Px, glutathione peroxidase; ICDH, isocitrate dehydrogenase; α-KGDH, α-ketoglutarate dehydrogenase; MDA, malondialdehyde; MDH, malate dehydrogenase; SDH, succinate dehydrogenase; SOD, superoxide dismutase; TCA, tricarboxylic acid.

tween 1995 and 2008 from the Medical Biochemistry Department, and 3 articles published from 1989 to 1991 from the Department of Biochemistry at the University of Madras. We analyzed all articles appearing in PubMed in 2007 (only 1 of 3 published contained data tables); articles sampled as described in Materials and Methods from those published from 2000 to 2006 (a total of 13 of 36 published); and all 4 articles published from 1995 to 1999. Articles published previous to 1992 were not included.

As shown in Table 3, values of $P < 0.01$ were observed for 8 (62%) of the 13 articles analyzed that were published after January 2001 but for none of the 5 articles analyzed that were published prior to 2001. The "*P* values" for all but 1 (10) of the 8 articles with unusual clustering of CVs were far below 0.01, ranging up to 7.1×10^{-20} (11). CVs for one of these unusually clustered articles, Thirunavukkarasu *et al.* (11), are shown in Fig. 1*C*. As shown, 77 of 80 CVs calculated from the data tables in Thirunavukkarasu *et al.* (11) fell within the constructed 50% limits, resulting in a "*P* value" of 7.1×10^{-20}.

Control articles: results obtained by other laboratories

We conducted 3 PubMed searches (see Materials and Methods) to identify articles from other laboratories that conducted experiments similar to those conducted by the Madras investigators. The constructed 50% limits and "*P* values" for the 29 articles sampled are listed in **Tables** 4 and **5,** and example scatter plots of CVs calculated from data in 7 control articles are shown in **Figs. 2** and **3**.

Control sets 1 and 2

As indicated in Materials and Methods, these articles were selected without regard to authorship or research institution. Three of the 10 articles retrieved as control set 1 were from the laboratory of C. Panneeerselvam. The remaining 7 articles were from 6 different laboratories in various parts of the world. As shown in Table

TABLE 5. *Control set 3: four articles that measure oxygen consumption by mitochondria isolated from rat tissues*

Reference	Journal	Total means	Group size (n)	Median CV (%)	50% CV limits	CVs falling within the 50% limits	"P value"[a]
Chen *et al.,* 2006 (18)	*J. Pharmacol. Exp. Ther.*	14	5	19.09	13.18–22.34	11	>0.99
		14	11		15.61–21.51		
		14	12		15.79–21.42		
Kerner *et al.,* 2001 (21)	*Am. J. Physiol. Endocrinol. Metab*	12	7	12.38	9.38–14.19	10	0.85
		12	8		9.63–14.11		
Leichtweis *et al.,* 1996 (19)	*Acta Physiol. Scand.*	8	5	24.74	17.04–29.13	17	0.99
		8	6		17.98–28.86		
		8	7		18.65–28.63		
		20	8		19.17–28.27		
		4	9		19.57–28.27		
Patel *et al.,* 2007 (20)	*Clin. Biochem.*	2	8	20.50	15.91–23.47	31	0.98
		6	9		16.25–23.34		
		18	10		16.52–23.22		
		4	11		16.75–23.12		
		38	12		16.95–23.02		
		8	13		17.12–22.94		
		4	14		17.27–22.86		

[a]The "P value" is an approximated probability value calculated under certain assumptions (see Materials and Methods). We consider $P < 0.01$ to suggest unusual clustering.

4, the only articles in control set 1 for which unusual clustering of CVs around the median was suggested ($P<0.01$) were 2 of the 3 Panneerselvam articles (12, 13). Calculated "P values" for these articles were, respectively, 7.5×10^{-18} and 1.9×10^{-22}. The calculated "P value" for the third Panneerselvam article (14) was 0.022, which was suggestive. "P values" for the other 7 articles ranged from 0.15 to >0.99.

The 15 articles retrieved as control set 2 were from 14 different laboratories in various parts of the world. As shown in Table 4, "P values" for all but 1 of these 15 articles ranged from 0.19 to 1.0. The one article with a value of $P < 0.01$, Muthuvel *et al.* (15), was the only article from Madras University. It was from the same campus of Madras University as the other 3 Madras investigators, but from a different department. The "P value" for Muthuvel *et al.* (15) is 7.2×10^{-5}.

A number of the control articles in Table 4 have large "P values", often >0.99. Scatter plots of CVs from 2 such articles (2, 16) are presented in Fig. 2*A, B*. As indicated in Materials and Methods, these very large "P values" most likely reflect the fact that assumptions *a* and *b*, used to construct the 50% intervals (see Materials and Methods), are consistently violated because publications always present data for more than 1 variable and more than 1 treatment group. Thus, a 50% interval constructed assuming only 1 variable and 1 treatment group might be expected to have relatively few sample CVs within the constructed interval, leading to a high "P value".

Figure 2*C* illustrates another expected characteristic of CVs for measurements of diverse variables. That is, CVs might be expected to have different values and also to vary differently for different kinds of measurements. This appears to be the case for CVs calculated from data tables in Luczaj *et al.* (17). CVs plotted, from left to right in groups of 12, are for measurements of a series of 8 different endpoints.

Control set 3

One of the unusually clustered articles analyzed from C. Panneerselvam (1) involved measurement of one simple endpoint using a standard procedure. CVs calculated from data in data tables in this article are shown in Fig. 3*A*. The endpoint measured was mitochondrial respiration in skeletal muscle mitochondria isolated from adult or aged rats ± pretreatment with L-carnitine and DL-alpha-lipoic acid. All data presented in the article were results of polarographic measurements of oxygen consumption using a standard Clark-type oxygen electrode, with measurements taken using the various substrates indicated in the caption to Fig. 3*A*.

As indicated in Materials and Methods, we searched PubMed to find examples of articles from other laboratories that had also measured mitochondrial respiration using a Clark-type oxygen electrode in mitochondria isolated as part of experiments conducted *in vivo*. The distribution of CVs calculated from data in the first 4 articles (18–21) identified that contained data tables is shown in Fig. 3*B–E* (see Table 5 for quantitative values used to construct 50% limits and "P values"). As shown, "P values" varied from 0.85 to >0.99, in contrast to the "P value" of 5.3×10^{-17} calculated for the Panneerselvam article (1).

DISCUSSION

In this study, we used the CV as a measure of variability, constructed boundaries within which CVs from any particular study would be expected to fall ~50% of the

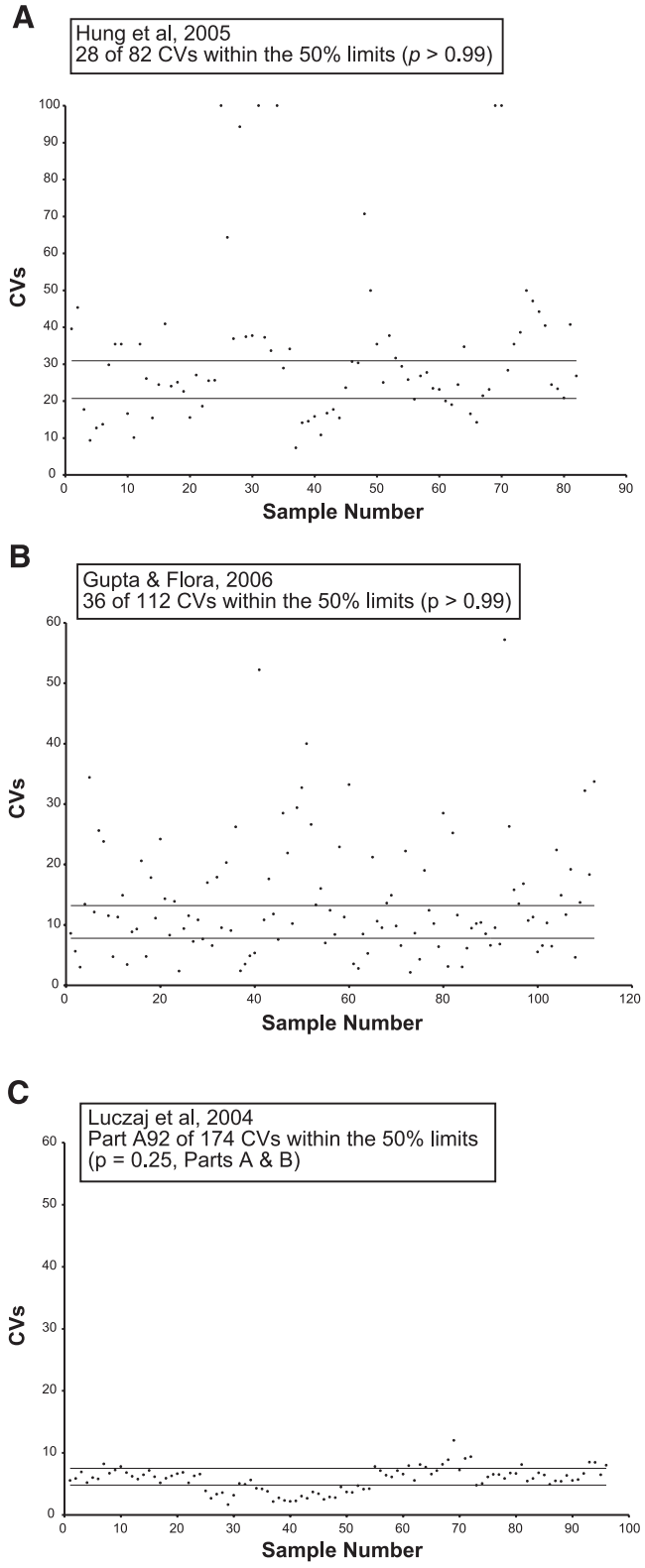

Figure 2. Illustrative results for control articles. CVs are plotted on the *y* axis for each mean and SD (or SE), which are consecutively numbered on the *x* axis. Constructed 50% limits are illustrated by horizontal lines. "*P* values" were calculated as described in Materials and Methods. Quantitative values used to construct 50% limits and "*P* values" are specified in the accompanying tables. Outcome measures are as listed below. *A*) Hung (2): gastric biochemical parameters (acid back-diffusion, GSH, MDA, mucus, hemoglobin, ulcer area were measured in rats with or without streptozotocin-induced diabetes ± supplementation with a betel quid chewing diet or treatment with lysozyme chloride ± indomethacin. Five data points exceeded 100, the upper limit of the *y* axis; they are plotted as 100. The actual values are 283, 121, 154, 141, and 141. *B*) Gupta and Flora (16): hematological variables and oxidative stress markers (ALAD, ZPP, GSH, GSSG, TBARS, SOD, catalase) were measured in blood, liver, kidney, and brain of rats treated with various amounts of *Centella asiatica* extracts ± arsenic supplementation. *C*) Luczaj *et al.* (17): antioxidant enzymes (SOD, GSH-Px, GSSG-R) and antioxidants (GSH, vitamin C, vitamin E, vitamin A, β-carotene) were measured in blood serum of rats of several ages ± supplemention with ethanol and/or green tea. ALAD, δ-aminolevulinic acid dehydratase; GSSG-R, oxidized glutathione; TBARS, thiobarbituric acid reactive substance; ZPP, zinc protoporphyrin.

time, and used an approximate probability number (termed the "*P* value") to describe how well results from a particular article corresponded to what was expected statistically. We applied this procedure to a sample of 84 articles of more than 200 published by 3 investigators in a small medical biochemistry department at a major university in India and to 29 control articles selected by key word PubMed searches. As presented in detail here, for articles coauthored by the 3 investigators, very small "*P* values", most less than 10^{-6}, were observed for the majority of articles sampled that were published after January 2000 but for none of those published before 2000. Specifically, all articles analyzed with data showing this unusual clustering effect appeared after January 2001 for two of the investigators (P. Varalakshmi and D. Sakthisekaran) and after January 2000 for the third investigator (C. Panneerselvam).

As shown in Table 4, 3 articles from 1 of the 3 investigators and 1 from the same university but a different department were retrieved among the 29 control articles sampled. "*P* values" for these articles were 7.5×10^{-18} (12), 1.9×10^{-22} (13), 0.022 (14), and 7.2×10^{-5} (15). In contrast, the smallest "*P* value" observed for the other 26 control articles, all from other universities, was 0.15 (22), but most "*P* values" were quite high, many >0.90. Visually, differences between scatter plots of CVs for sample articles from the 3 investigators with unusual clustering and representative control articles are striking (Figs. 1–3).

It is important to consider whether there are statistical or biological factors that could have caused the unusual clustering effect in articles from the 3 investigators but not in control articles. As detailed in the body of the present study, our conclusions rely on probability values ("*P* values") that compare the proportion of CVs in a particular article that fall within defined boundaries (50% limits) to what would be expected statistically. Both the "*P* values" and the 50% limits were calculated under certain assumptions. As indicated in Materials and Methods, 3 of the 4 assumptions used in constructing the 50% limits were purposely designed to be conservative (*i.e.*, to disfavor the

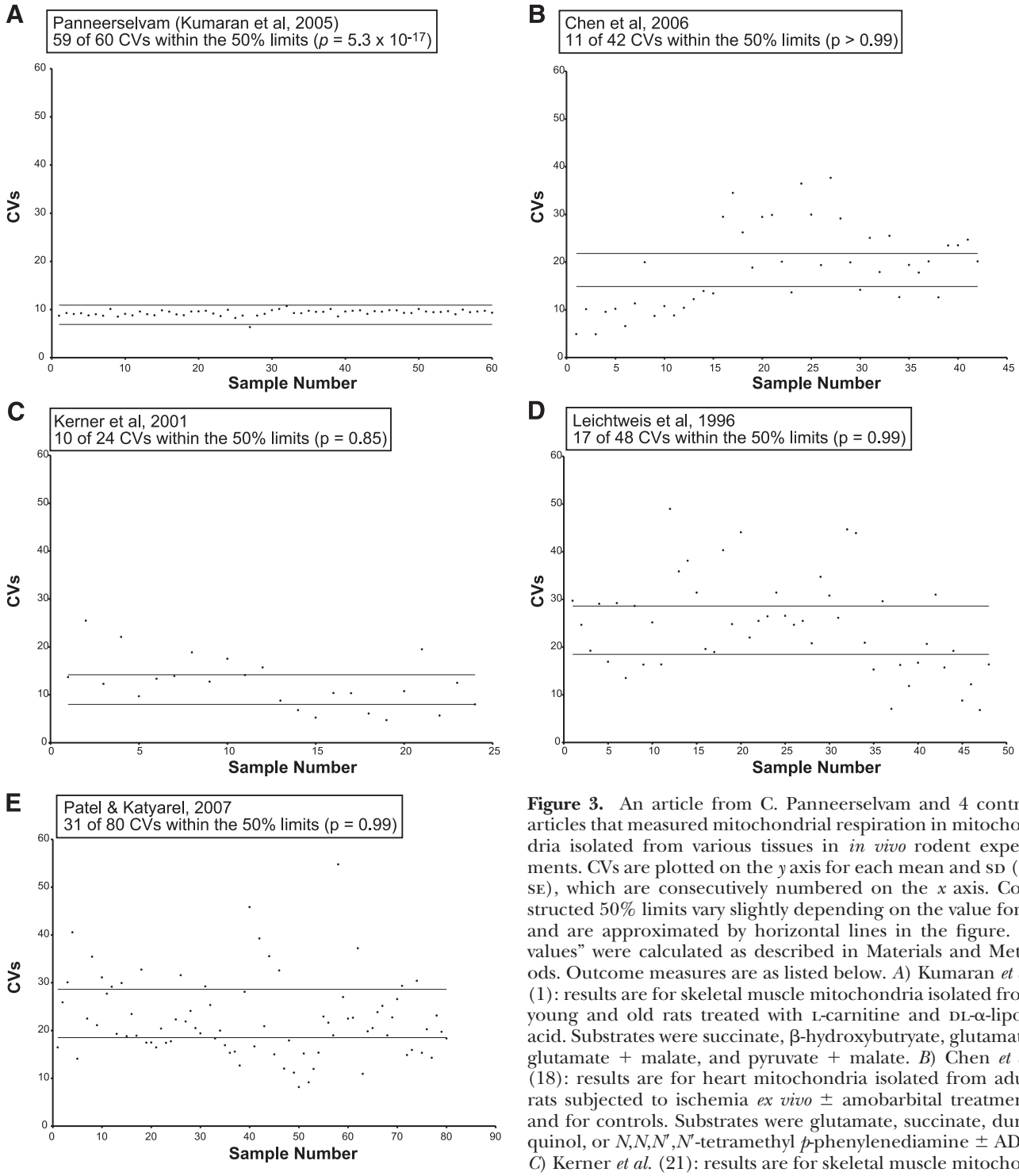

Figure 3. An article from C. Panneerselvam and 4 control articles that measured mitochondrial respiration in mitochondria isolated from various tissues in *in vivo* rodent experiments. CVs are plotted on the *y* axis for each mean and SD (or SE), which are consecutively numbered on the *x* axis. Constructed 50% limits vary slightly depending on the value for *n* and are approximated by horizontal lines in the figure. "*P* values" were calculated as described in Materials and Methods. Outcome measures are as listed below. *A*) Kumaran *et al.* (1): results are for skeletal muscle mitochondria isolated from young and old rats treated with L-carnitine and DL-α-lipoic acid. Substrates were succinate, β-hydroxybutryate, glutamate, glutamate + malate, and pyruvate + malate. *B*) Chen *et al.* (18): results are for heart mitochondria isolated from adult rats subjected to ischemia *ex vivo* ± amobarbital treatment, and for controls. Substrates were glutamate, succinate, duroquinol, or *N,N,N′,N′*-tetramethyl *p*-phenylenediamine ± ADP. *C*) Kerner *et al.* (21): results are for skeletal muscle mitochondria isolated from adult or aged rats. Substrates were glutamate, pyruvate + malate, palmitoyl-CoA, and octanoyl-CoA, ± ADP. *D*) Leichtweis *et al.* (19): results are for heart mitochondria isolated from adult rats ± cardiac hypertrophy followed by ischemia. Substrates were pyruvate + malate or succinate, ± ADP. *E*) Patel and Katyarel (20): results are for liver and brain mitrochondria isolated from adult rats ± treatment with dehydroepiandrosterone. Substrates were glutamate succinate, pyruvate + malate, and ascorbate + *N,N,N′,N′*-tetramethyl *p*-phenylenediamine, ± ADP. CoA, coenzyme A.

conclusion of unusual clustering of CVs). The assumptions that all measurements are on the same variable (*e.g.*, a particular enzyme activity) and are from a single treatment group (*e.g.*, a control group) are clearly strongly conservative, because a number of variables and treatment groups characterize all articles analyzed. Similarly, as addressed in simulations in the Appendix, the assumption that each variable being examined has an approximate normal distribution is also likely to be conservative.

We believe it is reasonable to expect that the conservative effects of these 3 assumptions strongly predominate

over what are expected to be relatively weak opposing effects of the fourth assumption (*i.e.*, use of the median to approximate the population mean) and the assumption of independence used to calculate the approximate probability number we have termed the "*P* value". We have been unable to think of any realistic condition under which a selective effect of either of these assumptions could be responsible for the very small "*P* values" observed for many articles published by the 3 investigators without also affecting their other articles and the control articles. Simulation trials support these conclusions (see Appendix).

We have not observed any other obvious distinguishing characteristics of articles with, as opposed to without, unusual clustering of CVs. For example, there is no association of a particular coauthor's name, or of a technician's name in the acknowledgments, with articles that are unusually clustered. In addition, specific endpoints do not appear to obviously correlate with unusual clustering. For example, 2 articles (23, 24) from P. Varalakshmi's group have the same authorship and measure almost all of the same endpoints either in the liver (24) or the kidney (23). The experimental protocols are very similar except for differences in treatment doses of cadmium and lupeol and different durations of treatment. The only apparent difference is that Nagaraj *et al.* (23) was submitted for publication in December 1998 and Sunitha *et al.* (24) over 2 yr later, in April 2000. As shown in Table 3, only the later article (24) exhibited unusual clustering of CVs ($P=5.2\times10^{-7}$).

There are many factors that affect experimental variability, including treatment conditions, analytical methods, technician experience, and such, and precise comparisons between results obtained in various laboratories were not possible. Some of the control articles from other laboratories measured a more diverse array of endpoints than some articles from the 3 investigators, which would be expected to decrease clustering of CVs (*i.e.*, increase the spread of CVs about the median, leading to the high "*P* values" observed). However, even when data are obtained on a single, easily measurable variable, as was the case for several variables examined in simulations in the Appendix, the probability of a CV falling inside the constructed 50% limits appears to be ≤ 0.5 for all of the real variables examined. These variables include some biochemical measurements that were in some of the articles analyzed here (see Appendix). In addition, as shown in Fig. 3 and Table 5, direct comparison of one of the highly clustered articles (1) that measured a single endpoint using a standard methodology to 4 control articles that measured the same end point using the same methodology indicates a striking disparity between results obtained in the Panneerselvam laboratory compared with the 4 control laboratories.

At this point, we are unable to offer a biological or statistical explanation for the unusual clustering of CVs observed. In lieu of such an explanation, we conclude that the analysis presented here strongly suggests that the data presented in 61% of the 66 articles we analyzed that were published after January 2000 exhibit significantly less statistical variability than the minimum expected in a biological experiment.

APPENDIX

Simulation trials were performed to evaluate the violation of the normality assumption. The following simulations were performed to examine whether the constructed 50% intervals are valid for normally distributed data and to examine how CVs fall relative to these intervals when the distribution is not normal.

Simulations assuming normality

To illustrate that the constructed 50% intervals are valid for normally distributed data, we performed 10 sets of simulations, each set drawing 1,000 samples of 6 observations from a normal distribution. For each of the 1000 samples, we computed $CV = s/\bar{x}$. Using the population mean μ and SD σ, we constructed a 50% interval and determined how many of these CVs would fall within this interval. Results for the 10 sets were 50.2, 51.2, 47.8, 51.5, 53.3, 50.4, 49.8, 49.0, 52.9, and 47.9. Overall, the percentage inside was 50.4%. This result suggests that, for data that are normally distributed, the simulated results are in accordance with the theory.

Simulation using the uniform distribution

We also simulated results for the uniform distribution, which is known to have lighter tails than a normal distribution. Results for the 10 sets were 61.2, 61.4, 61.7, 58.6, 59.8, 63.7, 60.6, 60.8, 59.8, and 62.2. Overall, the percentage inside was 61.0%.

Simulations using real data sets

Seldom are real data normally distributed. Typically, the distributions for real data tend to have heavier tails than the normal distribution. This would imply heavier tails for the distribution of the sample CVs. We would then expect more than 50% of the sample CVs to fall outside the 50% intervals that were constructed assuming normality (*i.e.*, we would expect fewer than 50% of the CVs to fall within these intervals.

Data from the National Heart, Lung, and Blood Institute Growth and Health Study

Data were used with permission (P. Crawford, University of California, Berkeley, CA, USA; personal communication). Data from this multicenter study have been the basis for many publications; two examples (118, 119) are cited. The data used were from a population of ~3000 9- to 10-yr-old girls. For each of 8 variables (*e.g.*, height), the population CV was used to construct 50% limits. Then, 10 separate sets were selected, each comprised of 1000 samples, and each sample consisted of 6 girls. For each sample, a sample CV was calculated. This was done for 8 different variables. Average results (percentage of CVs within the 50% limits) of simulations for each of the 8 variables were body weight (41.4%), height (48.6%), body mass index (41.2%), hours of TV watched per week (49.8%), percentage of calories from fat

(45.7%), kcal intake (41.7%), vitamin A intake (13.9%), and vitamin C intake (40.3%). We note that for height, well known to be approximately normally distributed for a group of similar age and sex, the percentage of CVs in the 50% limits is close to 50%. Vitamin A is known to be highly variably distributed in the population (heavy tails).

The above data were obtained from measurements made at several centers. To minimize interlaboratory variability, we ran simulations on 3 additional variables (total cholesterol, HDL, and LDL) from this data set using only measurements made at the Berkeley Center. Average results (percentage of CVs within the 50% limits) were total cholesterol (47.0%), HDL (42.4%), and LDL (42.7%). (Simulations on these variables that use data from all centers resulted in slightly smaller percentages inside the 50% limits: 45.7%, 40.4%, 42.1%, respectively, consistent with the expected greater variability.)

National Health and Nutrition Examination Survey (NHANES) data (2003–2004 data release)

Simulations were performed to evaluate the effect of violation of the normality assumption using a large data set with biochemical endpoints. Unweighted data were used. For each of 6 variables (*e.g.*, cholesterol), the population CV was used to construct 50% limits. Then, 10 separate sets were selected, each comprised of 1000 samples, where each sample consisted of 6 subjects. For each sample, a sample CV was calculated. This was done for 6 different variables. Depending on the variable, the actual data involved 6500–7800 individuals (men and women of all ages) sampled in the NHANES survey. Because each variable was measured by a single NHANES laboratory (http:// www.cdc.gov/nchs/about/major/nhanes/nhanes2003–2004/lab_methods_03_04.htm), interlaboratory variability is not a component of the variance for any particular measurement. Average results of simulations for each of the 6 variables were (percentage within the constructed 50% limits): total cholesterol (48.8%), HDL (47.8%), LDL (47.1%), alanine aminotransferase (7.1%), aspartate aminotransferase (4.5%), and γ-glutamyl transferase (7.3%). The cholesterol, HDL, and LDL measures were not far from normally distributed, whereas all 3 enzyme activities were very skewed. FJ

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