Author's response to reviews

**Title:** Variation in the circle of Willis in a Sri Lankan Population

**Authors:**

- K Ranil D. De Silva (ranilds@sltnet.lk)
- Rukmal Silva (ranil@sjp.ac.lk)
- Lal S Gunasekera (l_gunasekera@yahoo.com)
- Dhammika Amaratunga (damaratung@yahoo.com)
- Rohan W Jayasekera (rohanwj@hotmail.com)

**Version:** 8  **Date:** 24 February 2010

**Author's response to reviews:** see over
Author’s comments in blue

Reviewer's report
Title: Variation in the circle of Willis in a Sri Lankan Population
Version: 6 Date: 26 October 2009
Reviewer: Ljiljana Vasovic

Reviewer's report:
Minor essential revisions

1. Is the question posed by the authors well defined? Yes
2. Are the methods appropriate and well described? Yes
3. Are the data sound? Yes
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes
5. Are the discussion and conclusions well balanced and adequately supported by the data? Yes
6. Are limitations of the work clearly stated? Yes
7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Not in general
8. Do the title and abstract accurately convey what has been found? Yes
9. Is the writing acceptable? Not in general

Comments
I recommend minor essential revisions because there was not an equality in citation of references (according to the volumen, number of corresponding notebooks, number of pages as well as marking of paper as the shortcut or full name).

The reviewer is correct, Thank you, revised the references in pages 15-18

In the Table 1 it is necessary to change the year (see also heading Results) and country for Eftekhar et al. and name of the country for El Khamlichi et al.

The reviewer is correct, Sorry, Thank you, revised in page 19, Row 1, Table 1 as follows;

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Country</td>
<td>USA (1)</td>
<td>Iran</td>
<td>USA (2)</td>
<td>France</td>
<td>Morocco</td>
<td>Sri Lanka</td>
</tr>
</tbody>
</table>

Page 12 has new sentence that needs revision. Hypoplasia instead of Hyoplasia

The reviewer is correct, Sorry, Thank you, revised in page 12 last para 1st line, as follows;

"However, the methods and definition of hypoplasia differ among anatomical studies in"
Author’s comments in blue

Reviewer’s report
Title: Variation in the circle of Willis in a Sri Lankan Population
Version: 6 Date: 22 October 2009
Reviewer: Peter B Canham

Reviewer’s report:
General comment: significant revisions have been made to the manuscript that provide a much better scientific basis for the conclusions. The key addition is the statistical analysis.

There are a number of suggested further changes – mostly related to clarity, and others as minor errors. The inclusion of two photographed circles is a good concept, but not well done. The photos are of poor quality.

2 New photos are included
Figure 1 (Type 1) – “Typical CW”
Figure 2 (Type 6) – Bilateral hypoplastic PcoA

The discussion of postulates such as neck movement and hemodynamics adds little to the value (mainly because the causes of death are not part of the data presented, or analysed).

We have addressed this comment on page 12 by deleting the whole sentence:
amplitude of neck movements,[18]  hemodynamic factors,[23,24] postnatal development,[25]  and genetic factors. [26] have been considered

A brief discussion of the criteria for obtaining diameters from the several studies might provide a valid explanation of why some of the various studies have different results. The authors might remark (if it is true) that to the best of their knowledge all of the several studies appear to have the same methods of measurement, and definitions of hypoplastic, normal size, etc.

We have addressed this comment on page 9, para 1 lines 3-7 lines in the Methods section, by adding the following new sentence:
“To the best of our knowledge only six studies [2,3,16-19] and present study has investigated the variations of CW as a whole and has classified all vessels with diameter under 1 mm as hypoplastic, but the nomenclature and the methodology adapted to measure the external diameter of the vessels were not identical”.

i) Abstract, line 2 uses the word “efficient” to describe collateral circulations. (also used in the text). Strong preference would be the word “effective”. Efficient implies some sort of energy saving feature. While energy related to
hemodynamics may be involved it is unlikely that the authors wish to imply that it is a causative factor in ‘circle’ design.

The abstract in pages 3 and the text in pages 5 has been changed as the reviewer indicates as follows:

page 3, line 2;
“patients with efficient effective collateral circulations have a lower risk of transient”

Page, 5th sentence
“efficient effective collateral circulations have a lower risk of transient ischemic attack and”

ii) Abstract line 6: grammar: “as a whole HAVE has not been studied.

The abstract in line 6, pages 3 has been changed as the reviewer indicates.
“variations in the anatomy of the CW as a whole have not been studied in the Indian”

iii) Abstract (methods) “ . . . occurrence of variations in the CW” (this addition is consistent with similar language elsewhere)

The abstract in: line 5, pages 3 has been changed as the reviewer indicates by;
“the frequency of occurrence prevalence of the different variations with previous autopsy”

Last line , pages 6 has been changed as the reviewer indicates by;
“the frequency of occurrence prevalence of the different variations”

iv) Racial and ethnic population groups are challenging to define. In the abstract, but not in the text, the inference is that the Japanese study was not included as “Asian” whereas the Iranian data and Sri Lankan data are Asian. If one is dividing up the world we might use America (north and south), Europe, Australia, Africa and Asia, but that isn’t useful for racial or ethnic boundaries. In this reviewer's mind the main Asian population groups would be Japan, China, Korea etc. STRONG suggestion to define racial groupings, and preferentially using a common standard (if there is one). This reviewer doesn’t know the proper approach.

We have addressed this comment on page 9, last para in the Methods section by adding the following;

In order to study the relationship of the anatomical variations of the CW between the studies in Caucasian dominant (USA [2,17] and France [18]), African (Morocco) [16] and Asian ( Iran, [19] and present study from Sri Lanka)

v) Abstract conclusions are confusing: “marked variations in the CW . . . . (p<0.5 among Caucasian dominant . . .) Possibly this is a typographical error, but such a high p range is not significant -- maybe p<0.05?? The sentence reads awkwardly, as well.
We have addressed this comment on page 4, under conclusion in the abstract by adding;

**Conclusion:** The present study reveals that there are marked variations in the CW among intra and inter ethnic groups (Caucasian, African and Asian: Iran and Sri Lanka dominant populations), and warrants further studies keeping the methods of measurements, data assessment, and the definitions of hypoplasia the same.

vi) Background (page 5 top). The sentence is long, and the referencing seems a bit out of place: suggesting: “Based on anatomical [1-4] and radiological studies, [5-8] it has been shown that more than 50% of healthy control subjects have anatomical variations in the circle of Willis (CW). Comparisons based on radiological studies [5-8] in living patients and anatomical autopsy studies [1-4] are not possible as in-vivo data from angiography record luminal . . .”

The reviewer is correct, Thank you, revised in page 5 para 1 by adding the following;

“Based on anatomical [1-4] and radiological studies, [5-8] it has been shown that more than 50% of healthy control subjects have anatomical variations in the circle of Willis (CW). Comparisons based on radiological studies [5-8] in living patients and anatomical autopsy studies [1-4] are not possible as in-vivo data from angiography record luminal diameters of vessels distended by normal arterial blood pressure, whereas the cadaveric studies report on external diameters of collapsed vessels with zero luminal pressures”

vii) Word choice and grammar: page 5, line 8: “as a collateral anastomotic network; patients with effective collateral circulations . . .”

**Pages 5, line 8, has been changed as the reviewer indicates. Thank you, by;**

“cerebral haemodynamics as a collateral anastomotic channel network and patients with”

viii) The sentence beginning “Autopsy studies have . . .” is very confusing to this reviewer.

**Pages 5, last 6 lines, has been changed as the reviewer indicates;**

“Autopsy studies have shown that more Fetal configuration [where the blood supply to the occipital lobe is mainly via the internal carotid arteries due to a hypoplastic precommunicating (P1) segment of the posterior cerebral artery (PCA)] were found in autopsy brains with infarcts than in brains without.[11,12]

Studies have shown that there also exists a correlation between cerebral aneurysms and certain variations of the CW.[13-15]"
ix) Methods: line 8: suggesting 2 sentences: Blood was carefully washed out from the CW with isotonic saline. Line diagrams of all 225 circles were obtained, including photographic records in some cases. 

**Pages 7, para 1, last line, has been changed as the reviewer indicates by adding:**

“Blood was carefully washed out from the CW with isotonic saline. Line diagrams of all 225 circles were obtained, including photographic records in some cases.”

x) Methods page 7,last paragraph , line 1: internal internal

Sorry, has been changed as the reviewer indicates, by deleting. Thank you.

“left internal internal carotid arteries (ICA)”

xi) Method (top of page 8) unclear as to the random “section” or whether several diameters were obtained for each vessel segment, and then averaged. How many readings per segment. (issue raised by another reviewer) This is a strongly requested clarification.

The reviewer is correct, we have addressed this comment on page 7 last line and page 8 first 4 lines. Thank you.

“Transverse sections were then cut from each of the segments obtained as stated above in a plane that was perpendicular to the vessel on a microtome (Shandon M1R, UK) at 40 µm, a random “section” was then obtained from the water bath, three measurements of the external diameter were performed on each section by the first Author under a stereomicroscope equipped with a micrometer-calibrator (Leica, WILD M3B, Stereomicroscope).”

xii) Page 9 top: “ . . . variations of the CW and their classification into a few clearly arranged groups is hardly possible. We classified variations of CW using 28 Types . . . .”

The reviewer is correct, we have addressed this comment on page 9, Para 1, as follows*. Thank you.

“There are many anatomical variations of the CW, their classification into a few clearly arranged groups is hardly possible, we classified variations of CW to using 22 Types as defined by Ozaki et al., 1977,[3] Lazorthes et al.,1979, [18] and Eftekharp et al., 1985[19]. To the best of our knowledge only six studies [2,3,16-19] and the present study have investigated variations of CW as a whole and have classified all vessels with diameter under 1 mm as hypoplastic, but the selection of cases, nomenclature and the methodology adapted to measure the external diameter of the vessels were not identical”;

xiii) Perhaps here, page 9, the authors could clarify their use of the “Asian” labelling.

We have addressed this comment on page 9, 2nd para first sentence as follows;
In order to study the relationship of the anatomical variations of the CW between the studies in Caucasian dominant (USA [2,17] and France [18]), African (Morocco) [16] and Asian (Iran, [19] and present study from Sri Lanka) we performed a global chi-squared test to compare across all 6 studies, and then a series of pairwise chi-squared tests to test for differences between each pair of studies.

xiv) RESULTS: line 3 “present study is are
We have addressed this comment on page 10, 4th line.

“Variations in the CW in the present study are shown in table I.”

xv) Table 1: there seems to be a few minor errors in the table, perhaps one more serious error, needing explanation: The Sri Lankan study is claimed to have 225 autopsies, while this reviewer adds up the numbers in the Sri L. Column and gets a total of 216. Are there undecided autopsies, that are omitted?

Riggs and Rupp column (#10) 33 is stated as 0.1% (why not 3.3%?)
Sorry, has been changed as the reviewer indicates Table 1 in type 10. Thank you

<table>
<thead>
<tr>
<th></th>
<th>Bilateral hypoplastic P1s</th>
<th>33(3.3)</th>
<th>1(1)</th>
<th>16(3.8)</th>
<th>6(3)</th>
<th>0(0)</th>
<th>1(0.44%)</th>
</tr>
</thead>
</table>

The Ozaki study is said to have 134, but the column only adds up to 108. Is row “23” an undecided row?

For some of the columns there are “0” entries, and others are left blank. What are the criteria?
We have addressed this valuable point indicated by the reviewer by filling all empty spaces please refer Table 1

Line 18 in the table has 58 entries as 3.8%, why not 5.8%.
Sorry, has been changed as the reviewer indicates Table 1 in type 18. Thank you

<table>
<thead>
<tr>
<th></th>
<th>A1 and bilateral hypoplastic PcoAs</th>
<th>58(5.8)</th>
<th>0(0)</th>
<th>21(5.0)</th>
<th>6(3)</th>
<th>0(0)</th>
<th>4(1.7%)</th>
</tr>
</thead>
</table>

There are many entries in the Sri Lankan study, and the Japanese study that have zero or “1” entry in a category. This reviewer wonders if this large number of insignificant categories has any impact on the conclusions. A MUST FIX CATEGORY IS THE DOUBLE CHECKING ON THE COLUMN ENTRIES FOR THE ENTIRE TABLE, AND THE VERIFICATION THAT SIMILAR CONCLUSIONS COULD BE MADE AFTER THE STATISTICS ARE RERUN. There needs to be clarification of the difference between a blank entry, and a zero entry.
The reviewer is correct, we have addressed this by deleting the Japanese study and doing the Table 1 again.

The Results now read as follows;
Page 10 para 1;
“Results

We report 15 types of variations of CW out of 22 types previously described by Lazorthes et al., 1979, [18] and Eftekhari et al., 1985.[19] and one additional type: hypoplastic A1 and contralateral PcoA 5(2.2%), categorized under “others” in Table I. Variations in the CW in the present study are shown in table I

The most common variations are as follows:

Type 1-“Typical CW”: 32(14.2%); Type 3 – Hypoplastic AcoA: 32(14.2%); Type 4 - Unilateral hypoplastic PcoA: 26(11.5%); Type 5 - Unilateral hypoplastic PcoA and AcoA: 15(6.6%); Type 6 - Bilateral hypoplastic PcoAs: 52(23.1%); Type 7 - Bilateral hypoplastic PcoAs and hypoplastic AcoA: 37(16.4%).”

xvi) Discussion, para 2: the first sentence is confusing regarding the numbers: e.g. “in Chandigarh, India [4]” what percentage? What are the sizes of the Indian subcontinent studies, and is the claim that these must be ethnic/cultural differences since there is racial similarity??

xvii) If the Indian sub population studies appear as very similar racially, are the authors confident that the variations among the data could not be due to differences in data assessment?

XVI and XVII: The reviewer is correct, we have addressed this comment Thank you

We have addressed this comment on page 11 last para by;
“Table I reveals that there is a marked variation in CW among ethnic and racial populations. There exist several postulates as to the underlying reasons for the anatomical variation of the CW among which are, selection of cases: brains obtained from those who have died of causes unrelated to the brain in [16,19] and the present study, brains obtained from pathological or infarcted brains in [2] and in unselected
cases in [17]. Gender: male and female cases were studied in [16] and the present study, male only in [19]. Other studies [2,17,18] had not mentioned the sex distribution of their cases.”

AND

We have addressed this comment on page 12, first para..

“The definition of hypoplasia was consistent in these studies [2, 16-19] but the diameter of component vessels of the CW has not been performed in all the studies [2,18]. Prevalence of the “typical” configuration in the present study is 14.2% in 225 brains examined as compared to studies reported in India: 26.8% in Maharashtra, India [21] 53.2% in South India, [22] and 45.20% in Chandigarh-Northwest India, [4] in 175, 357 and 1000 apparently normal brains examined respectively. The definition of hypoplasia was consistent in these studies [4, 21, 22] but the diameter of component vessels of the CW has not been performed in all the samples, and has not investigated the variations of CW as a whole. It is believed that Sri Lankans have a common origin from India, further studies are needed to ascertain reasons for the wide range in the prevalence of “typical” configuration between studies in India and Sri Lanka.”

xviii) The discussion on pages 11 and 12 is rather peripheral – since there were no matching data between cause of death in the Sri Lankan study and the anatomical configurations of the CWs. This reviewer suggests that the discussion SHOULD focus mainly on the circle variations rather than the speculations of stroke incidence at various hospitals. We have addressed this comment on page 12 by deleting the following;

“Studies have also shown that there are variations in the incidence of cerebrovascular diseases in different ethnic or racial groups. Hospital based prospective studies conducted at the National Hospital of Sri Lanka, in 1974 and in 1989 revealed that the incidence of stroke in young adults (aged 15–45
years) was 10.4% [27] and 33.6% [28] respectively. Hospital-based studies from India in 2001 stated that young stroke (aged 15–45 years) accounted for (15%–30%) of all strokes. [29] The average age of patients in the developing countries with stroke is 15 years younger than in developed countries. [30] The reasons for these differences are not well-understood and the etiology of the majority of strokes in young adults in Sri Lanka is unexplained. [27,28,31,32] In a post mortem study in the below 40 year age group, 92% of patients with cerebral infarction has been due to occlusive arterial disease of large vessels due to nonatherosclerotic vasculopathy. [33] It has been reported that in Asians the incidence of intracranial atherosclerosis in the anterior circulation stroke is much higher compared to Caucasians. [27] Prevalence of posterior circulation stroke among Asians [28] also has been reported to be much higher as compared to the West. [29–31] Further studies are needed to ascertain the role of variations in the configurations of the of the CW in the pathogenesis of cerebrovascular diseases in different ethnic or racial groups in the Indian subcontinent. “

REFERENCES: it is noted that some of the titles of papers include capitalized nouns, where most do not (the more common convention) e.g. #10,21,22. Sorry, has been changed as the reviewer indicates. Thank you.


xx) The two new figures – photographs of circles, have very unclear lettering (fig. 1 or 2) and from this reviewer’s printout were of quite poor resolution. No scales were presented, and no features noted regarding either circle. While one of the other reviewers did suggest including photographs of example circles, it would be assumed that high quality, informative photos were to be expected.

We have addressed this comment

**New photos are included**

Figure 1 (Type 1) – “Typical CW”

Figure 2 (Type 6) – Bilateral hypoplastic PcoA
Author’s comments in blue

Reviewer’s report
Title: Variation in the circle of Willis in a Sri Lankan Population
Version: 6 Date: 19 October 2009
Reviewer: Hisashi Tanaka

Reviewers report:
The authors measured the size of brain vessels of Sri Lankan people and compared the results with previous similar measurements for other races.

Major compulsory revisions
I have concern about the comparison, because some of the previous autopsy studies might use threshold of “normal” vessel size which was different from that in the present study. The authors should remove the data from the previous studies using different threshold of “normal” vessel size.

We have addressed this comment on page 9, 1st and 2nd para in the Methods section by adding the following;

“There are many anatomical variations of the CW, their classification into a few clearly arranged groups is hardly possible, we classified variations of CW using 22 Types as defined by Lazorthes et al., 1979, [18] and Eftekhari et al., 1985[19]. To the best of our knowledge only six studies [2,3,16-19] and the present study have investigated variations of CW as a whole and have classified all vessels with diameter under 1 mm as hypoplastic, but the selection of cases, nomenclature and the methodology adapted to measure the external diameter of the vessels were not identical.”

“In order to study the relationship of the anatomical variations of the CW between the studies in Caucasian dominant (USA [2,17] and France [18]), African (Morocco) [16] and Asian (Iran, [19] and present study from Sri Lanka)…..”

In addition to it, I’m unsure that Pearson Brown ranked correlation is an appropriate statistical strategy. In order to perform Pearson Brown ranked correlation, I assume variations of the Circle of Willis to be ranked. However, it is difficult to determine which is highly ranked, for example, type 5 (unilareral hypoplastic PcoA and AcoA) or type6 (bilateral hypoplastic PcoAs) in Table1. Chi-square test, suggested by referee 2, is more appropriate.
We have addressed this very important comment on page 9, last para in the Methods section by adding the following:

“In order to study the relationship of the anatomical variations of the CW between the studies in Caucasian dominant (USA [2,17] and France [18]), African (Morocco) [16] and Asian (Iran, [19] and present study from Sri Lanka) we performed a global chi-squared test to compare across all 6 studies, and then a series of pairwise chi-squared tests to test for differences between each pair of studies. Thereafter we performed a correspondence analysis [23], which is an effective way to display and compare configuration profiles with two-way categorical data. This analysis allows us to see which studies are most similar to one another and, for those studies that differ, which configurations are primarily responsible for the difference.”

These are the comments by the Biostatistician;
“I first did the requested chi-squared analysis, initially a global chi-squared test to compare across all 6 countries, and then a series of pairwise chi-squared tests to test for differences between each pair of countries. All these come up as being highly significant in part due to the large number of degrees of freedom; all the p-values are essentially zero.

Global chi-squared test: 0.0000

Pairwise chi-squared tests:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>NA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[2]</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[3]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[4]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[5]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>[6]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

In other words, each country is significantly different from each of the other

Then the biostatistician did a correspondence analysis. With two-way categorical data, correspondence analysis (Greenacre, 2007) is an effective way to display and compare configuration profiles. Correspondence analysis would allow us to see which countries are most similar to one another and, for those countries that differ, which configurations are primarily responsible for the difference. Fig 1 is a correspondence analysis plot of the data in Table 1. The
6 countries are represented by filled circles and the 23 configurations are represented by
unfilled circles.

Countries with similar profiles of configurations would lie close together. It can be seen that Sri
Lanka, Asia and France all have somewhat similar profiles. USA2 is clearly distinct from the rest
driven by differences in configuration numbers 11, 22, 17, 19, 1 and 4; USA1 and Morocco also
separate out slightly.

As a check, I also did a simple cluster analysis and the results, particularly the similarity of Sri
Lanka, Asia and France with the others separating out, come up there as well.

Minor essential Revisions
page 7 line 11-12 right and left internal internal carotid arteries. Please remove
one of the “internal”
Sorry, has been changed as the reviewer indicates, by deleting. Thank you.
“left internal internal carotid arteries (ICA”

Discretionary Revisions
Some people have multiple anterior communicating arteries. Each of the arteries
may be less than 1mm in diameter, however, as a whole, they suffice as effective
collateral. If the authors encountered such case, please describe how the case to
be classified.
We have addressed this comment on page7,last para, line 5,6 lines by adding the
following
“...and anterior communicating artery (AcoA) (with its variations if present) at its middle point..”

Level of interest: An article whose findings are important to those with closely
related research interests
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the
statistics.
Declaration of competing interests: