

Research Article

Stroke Risk Assessment for the Community by Automatic Retinal Image Analysis Using Fundus Photograph

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ABSTRACT

Background: Primary prevention of stroke is vital for saving lives and disabilities, and retina characteristics have been investigated as potential tools for stroke risk assessment. This study reports the development of a statistical model for stroke risk assessment using manually digitized retinal image characteristics obtained from a case-control study. We further report the results of a fully automatic version of the analysis (ARIA-stroke) on the study. The model was then validated using a separate dataset to show that it can be applied in a primary care setting.

Methods: We have carried out a case-control study with 244 subjects (122 strokes and 122 controls). About 66% of each group was diabetes patients. A manual digitization process was used to measure retinal characteristics including central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE), arteriole-venule ratio (AVR), bifurcation coefficients, bifurcation angles, and bifurcation asymmetries, arteriole-venous nicking, tortuosity, hemorrhage, exudates, and arteriole occlusions. Logistic models were developed to evaluate both the clinical and retinal characteristics. A fully automatic approach for the analysis of the retinal images was developed and the method was validated using a separate data set with 412 subjects (138 normal controls, 198 hypertensions and 76 stroke cases)

Results: The manual analysis shows that retinal characteristics are valuable in stroke risk assessment with AUC of 0.78 (95% C.I. 0.72-0.84) for retinal characteristics alone versus AUC of 0.66 (95% C.I. 0.59-0.73) for clinical variables alone. The combined model with both clinical and retinal characteristics has an AUC of 0.84 (95% C.I. 0.78-0.89) outperformed model using clinical or retinal variables alone. For the automatic ARIA-stroke model, the average probability of stroke for the control group was 0.141 (95% CI: 0.126-0.156), and the case group was 0.847 (95% CI: 0.839-0.855). When we looked at the patient subgroups with and without diabetes, the average probability of stroke for the control without diabetes was 0.054 (95% CI: 0.046-0.063), control with diabetes was 0.185 (95% CI: 0.170-0.199), stroke without diabetes was 0.853 (95% CI: 0.841-0.866), stroke with diabetes was 0.843 (95% CI: 0.833-0.854). The sensitivity and specificity was 100% in the case-control study using a probability cutoff of 0.5. We have also estimated the retinal

parameters that are potentially useful for interpretation of the results. The observed data have significantly high correlations with the estimated values showing high goodness-of-fits. The validation study using a separate data set with normal controls, hypertension controls, and stroke cases have confirmed the results with a cutoff probability of 0.5, the sensitivity is 94.7% and specificity is 100%.

Conclusion: This study demonstrated that retinal images contain valuable information for stroke risk assessment in addition to conventional clinical variables. A fast and fully automatic method can be used to estimate risk of stroke based on fundus photographs alone. We have also shown that a number of retinal characteristics may provide insights on clinical interpretation of the risk estimate and this method may be used in community setting or population screening.

Keywords: Stroke risk prediction; Disease screening; Cardiovascular health; Biostatistics methods; Image processing

Background

Stroke is a disease with high mortality and debilitating even for survivors. It generates great financial burden on survivors' families and the health care system worldwide. Krishnamurthi et al. reported that the global burden of ischemic and hemorrhagic stroke increased significantly between 1990 and 2010 in terms of the absolute number of cases, number of deaths, and disability-adjusted life years (DALY) lost.[1] They found that the global burden of strokes increased in low-income and middle-income countries as opposed to high-income countries. This has become an important global health issue.

Various interventions for stroke prevention are available and some have shown to be effective, but the challenge is on the ability to provide a more specific and accurate classification. From an individual-based prevention perspective, there are various ways to assess the risk of stroke. They include ultrasound, computed tomography angiography (CTA), and magnetic resonance angiography (MRA). Ultrasound can assess stenosis and blood velocity of vessels in relative superficial surfaces and is widely used to evaluate the carotid stenosis. More than 70% stenosis is indication for carotid endarterectomy. However, stroke caused by carotid stenosis accounts for only 4% of all stroke cases.[2] CTA and MRA can detect abnormality of larger cerebral vessels, but these techniques are costly, inconvenience and invasive. From a population-based prevention perspective, we can substantially reduce the burden of stroke if we reduce blood pressure, promote physical activity, increase smoking cessation, and a healthy diet.[3]

However, tools to estimate stroke risk for an individual are not well developed. Feigin et al. suggested the use of Stroke Riskometer App in addition to other tools such as Framingham and QSTROKE stroke risk prevention algorithms. The mobile app-based approach is promising and may increase general awareness of the importance of stroke risk reduction, but the accuracy remains to be proven.[4]

Cerebral vascular change is one of the major pathology causes of stroke. Retina vessel circulation shares similar morphology, function, and pathologic changes with cerebral vascular system. Since retina is the only place throughout the body where a small part of the vascular system can be observed directly, cerebral vascular changes can be explored through retinal image to determine the risk of strokes. Previous studies have shown that a number of retinal characteristics were significantly associated with strokes.[5-9] However, none of them demonstrated they were adequate for stroke risk estimation. In this paper, we extracted the retinal parameters from color fundus images and identified risk factors associated with stroke cases; we further explored the use of retinal characteristics in a multivariate model for stroke risk assessment. Furthermore, we employed a novel method to automate the analysis of the retinal image for stroke risk assessment and to estimate the retinal parameters using data from a case-control study. We then validated the methodology using a separate data set.

METHODS

In the initial case-control study, 122 stroke cases were entered from an Acute Stroke Unit in collaboration with the diabetic retinopathy screening program in Hong Kong. The patients were diagnosed with either ischemic stroke or hemorrhagic stroke and had adequate sitting balance to carry out the retinal photography. There were 81 stroke cases with diabetes and 41 stroke cases without diabetes. Patients who were age 80 years or older were not included, since this age group is likely to have optical opacity and other complication that was not suitable for capturing color retina photo and may introduce bias of other sources. Patients with eye disease that had influence on the retinal vessel structures or spot characteristics and those with stroke subtypes of cardioembolic stroke, and subarachnoid hemorrhage were excluded. Patients suspected to suffer from cerebral diseases and those with disease that influence vessel morphology were also excluded. 122 control subjects matched with age and diabetic status were selected. Controls subjects without stroke were recruited from Eye Outpatient Clinics or diabetic retinopathy screening program. Only patients with routine eye checkup, recovered central serous chorioretinopathy, mild quiet age related maculopathy confirmed by fluorescein and indocyanine green angiography were included as controls. The mean length of follow-up period from the date of taking the retinal image was 4.3 years. All the controls were aged from 50 to 80 years old and have no retinal disease or with only mild diseases without influencing vessel structure in color retina images, such as mild dry age-related maculopathy, central serous chorioretinopathy, post-cataract extraction, retinal pigment epithelial detachment. Written informed consent was obtained, and the project was done according to the guidelines of the Declaration of Helsinki and approved by the Joint CUHK-NTEC Clinical Research Ethics Committee.

Clinical risk factors

Stroke risk factors including age, gender, hypertension, diabetes, hyperlipidemia, smoking status, histories of ischemic heart disease and atrial fibrillation were recorded in the study. Hypertension was defined as systolic blood pressure greater than 140 mm Hg, diastolic blood pressure above 90 mm Hg, or use of antihypertensive medication during the previous 2

weeks. Diabetes mellitus was defined as a fasting blood glucose concentration above 7.0 mmol/L, a non-fasting value of more than 11.1 mmol/L, or a history of treatment for diabetes. Hyperlipidemia was defined as history of administration of lipid lowering drug. Smokers included ex-smokers or current smokers.

Retinal characteristics

Retinal vessel measurements: The formula developed by Knudtson et al. was used to summarize the retinal vessel measurements into the central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE).[10] Six largest arterioles within the circle, 0.5 to 1 disc diameter apart from the edge of optic disc were selected as a measurement of the diameter by drawing a line perpendicular to the edge of the vessel walls. The six diameters were summarized into one parameter as CRAE using Knudtson's formula to represent the arteriole diameter of the retina. Similarly, CRVE was used to summarize the venule diameters. Arteriole-venule ratio (AVR) was calculated as the ratio of CRAE to CRVE. In order to make the parameters compatible, all retina images were resized and adjusted into JPG format with 1365*1024 pixels.

Arteriole-venous nicking and arteriole occlusion: The sign of arteriole-venous nicking was marked as the narrowing of venule at the crossing point of arteriole. The arteriole occlusions were presented as thread-like arterioles when the blood inside the arterioles was stopped by emboli.[7]

Hemorrhages and exudates: Status of hemorrhage and exudates were recorded as either present or absent. Hemorrhage and exudates were key determinants for the severity of diabetic retinopathy as they were found to be associated with stroke in other studies.[5-7]

Tortuosity: Tortuosity was assessed by visual grading of one fovea-centred and one disc-centred fundus image from each image.[11] The grading levels for retinal arterial tortuosity were either predominantly straight arteries or mild to severe tortuosity with at least one inflection of at least one major artery.

Bifurcation coefficients (BC): Bifurcation coefficient (BC) or "area ratio" is the ratio of the sum of the cross-sectional areas of the daughter vessels of a bifurcation to that of the parent stem. Niall Patton et al.[12] have shown that the bifurcation coefficients (BC) of different bifurcation of vessels in the same retina image did not correlate to the eccentricity to the edge of optic disc. In this study, three largest branching points were selected and lines perpendicular to the vessel walls were drawn by the image software. We marked the diameter of trunk, the smaller branch and the larger branch as D_0, D_1, D_2. The BC for a specific bifurcation was calculated as:

$$BC=(D_1^2+D_2^2)/D_0^2$$

The calculation was the same in arterioles and venules. The means of the bifurcation coefficient of arterioles (BCA) and venules (BCV) were used.

Asymmetry of branches and bifurcation angles: Asymmetry index (AI) is the ratio of diameters of two daughter branches.[13,14] The AI was calculated as: $AI=D_1/D_2$, where D_1 and D_2 was smaller and larger branch respectively. The mean of the 3 sets of AI of arterioles (Aasymmetry) and venules (Vasymmetry) were used.

The angle between two daughter branches of the same branches studied in the BC was measured. The centerline of two branches was drawn, and the angle was calculated to represent the branching angle. The mean of the bifurcation angles of arterioles (Aangle), and mean of bifurcation angles of venules (Vangle) from the three sets of vessels in one retinal image were used for the analysis.

Retinal photography and image analysis procedure

A Canon non-mydriatic retinal camera CR-1, with a 45 degree angle view was used in the diabetic retinopathy screening program. Topcon Retinal Camera TRC-50IX with a 50 degree angle was used in the eye outpatient clinics to capture the color retinal images. After 5 min of dark adaptation, photographs of the retina were taken from one randomly selected eye. All continuous parameters were measured and quantified by using ImageJ in pixel units. Retinal images were adjusted to the same resolution of 1365*1024 pixels. The length and angle measurement tools were used to measure the length and angle of vessels.

Statistical analysis

Manual analysis method for stroke risk assessment: For the analysis of clinical and retinal characteristics measured manually, we used two sample independent t-tests to compare continuous data and chi-square tests for categorical data. The p-values<0.05 was considered as statistical significant. Odd ratios (OR) and the corresponding 95% confidence interval (95% CI) were obtained by simple logistic regressions. Stepwise logistic regression was employed to select the best model. The classification accuracy, and area under the curve (AUC) of the receiver operating characteristic (ROC) were measured. All data was analyzed using software SAS 9.3.

Automatic analysis method for stroke risk assessment: The fully automatic retinal image analysis method for stroke risk assessment (ARIA-stroke) was developed using R and Matlab computer software. The detailed methods of the automatic retinal imaging analysis method have been reported in Zee.[15] The methods include the use of fractal analysis, high order spectra analysis, and statistical texture analysis. Each of the methods targeted to specific characteristics on the retinal image, and the combination of all three approaches was used to accomplish the overall estimation of stroke risk. The methods for detection of neovascularisation and exudates for diabetic retinopathy are also applied in stroke risk assessment.[16,17]

Validation data

In order to validate the method we developed from the original case-control study for stroke risk assessment, we employed a completely separate data set to test the ARIA-stroke algorithm. The validation data include 138 normal elderly subjects as control recruited from the community in Hong Kong without stroke history and 198 well managed hypertension patients from the General Outpatient Clinic of Kwong Wah Hospital of Hong Kong. We have also recruited 76 stroke cases under rehabilitation program in the Shenzhen Traditional Chinese Medicine Hospital. These patients have been discharged from

the hospital within a short period, an average of 3.6 months (95% C.I. 2.8-4.5 months).

We have also developed the method of estimation for each of the retinal parameters of interest. These parameters have been reported in previous literature either directly or indirectly related to stroke. In order to verify the accuracy of the method, the observed values were plotted against the estimated values for all retinal parameters to examine their goodness-of-fit and linear relationship. The processing speed for the analysis of an individual retinal image takes about one minute.

RESULTS

Main study based on manual analysis

Descriptive demographics variables: A total 244 patients were recruited in the main study, 122 of them with stroke and the 122 without stroke. The statistics of demographic data was summarized in Table 1. There were 81 subjects with diabetes and 41 subjects without diabetes for both the case and control groups. Since the status of diabetes and age were matched, there was no significant difference in demographics between the two groups with respect to these two variables. Among the stroke patients, ten of them suffered from hemorrhagic stroke, and others were ischemic stroke.

Univariate analysis of retina characteristics: The summary of retina characteristics is given in Table 2. From the results of univariate analysis, both the CRAE and CRVE were significantly smaller in the stroke group with odds ratio (OR) of 0.52 (95% CI: 0.38-0.70) and 0.73 (95% CI: 0.55-0.96) per standard deviation (SD) unit changes respectively. The OR for AVR was 0.58 (95% CI: 0.43-0.78). BCV was also significantly different between the two groups (OR 0.74, 95% CI: 0.56-0.97). The retinal characteristics measured by dichotomous variable and their frequencies were also summarized and compared in Table 2. The occurrence of vessel tortuosity (OR 3.75, 95% CI: 1.94-7.22), hemorrhages (OR 3.45, 95% CI: 1.64-7.25), and arteriole-venous nicking (OR 3.08, 95% CI: 1.45-6.53) significantly increase the risk of stroke. Exudates contributed comparatively lower risk to stroke (OR 2.30, 95% CI: 1.07-4.96).

Stroke risk estimates: Stepwise logistic regression analysis was used to determine the best set of retinal characteristics and clinical features associated synergistically with stroke. The results of the logistic regression analyses are presented

Table 1: Demographic and clinical data for the initial study.

	All subjects	Controls (n=122)	Stroke (n=122)	p-value
Age in years (Mean ± Standard deviation)	65 ± 8.2	66 ± 8.1	65 ± 8.3	0.276
Male	155	81	74	0.304
Hypertension*	174	80	94	0.048
Diabetes	162	81	81	1.000
Smoker	68	29	39	0.164
Cardiac complication	24	10	14	0.401
Atrial fibrillation*	12	1	11	0.003
Hyperlipidemia	190	93	97	0.994

*p-value<0.05

Table 2: Summary and comparison of retinal characteristics between case and control.

		Control			Stroke		
Variables	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**OR (95% CI)†**
CRAE*	112	14.353	3.1924	108	11.4757	1.5369	0.52 (0.38, 0.70)
CRVE*	114	21.164	3.6397	108	18.1623	2.0542	0.73 (0.55, 0.96)
AVR*	112	0.6777	0.0921	107	0.6331	0.0796	0.58 (0.43, 0.78)
BCA	112	1.590	0.3323	107	1.639	0.4813	1.13 (0.86, 1.48)
BCV*	115	1.304	0.2366	108	1.239	0.2029	0.74 (0.56, 0.97)
Aangle	114	70.32	12.51	108	72.59	11.44	0.87 (0.66, 1.13)
Vangle	112	72.52	11.86	107	70.85	11.56	1.21 (0.93, 1.58)
Aasymmetry	112	0.8344	0.0803	107	0.8270	0.1008	0.92 (0.71, 1.20)
Vasymmetry	115	0.7755	0.0907	108	0.7572	0.0901	0.82 (0.62, 1.06)
		Control			**Stroke**		
Variables	**n**	**Count**	**%**	**n**	**Count**	**%**	**OR (95% CI)**
Arteriole-venule Nicking*	122	11	9.02%	122	28	22.95%	3.08 (1.45, 6.53)
Tortuosity*	122	15	12.30%	122	42	34.43%	3.75 (1.94, 7.22)
Hemorrhage*	122	11	9.02%	122	31	25.41%	3.45 (1.64, 7.25)
Exudates*	122	11	9.02%	122	23	18.85%	2.30 (1.07, 4.96)
Arteriole occlusion	122	2	1.64%	122	7	5.74%	3.68 (0.75, 18.1)

*p-value<0.05

†OR: Odd ratio. CI: Confidence Interval. The ORs of continues variables are in per standard deviation (SD) unit change

in Table 3. The logistic model using demographic and clinical variables give a 59% correct classification. Using manually measured retinal parameters alone has an accuracy of 72.2%. The combination of significant clinical risk factors and manually measured retinal characteristics further improved the classification result to 80.4%. It indicates that interactions between clinical and retinal characteristics contain useful information for the classification of stroke.

Main Study based on ARIA-Stroke

In the fully automatic ARIA-stroke model we used a cutoff probability of 0.5 or higher for stroke classification and obtained 100% accuracy (Table 3). A box plot of individual probabilities for the cases and controls stratified by diabetes status is shown in Figure 1. The average probability of stroke for the control group was 0.141 (95% CI: 0.126-0.156), and the case group was 0.847 (95% CI: 0.839-0.855). The average probability of stroke for the control without diabetes was 0.054 (95% CI: 0.046-0.063), control with diabetes was 0.185 (95% CI: 0.170-0.199), stroke without diabetes was 0.853 (95% CI: 0.841-0.866), stroke with diabetes was 0.843 (95% CI: 0.833-0.854).

We have also developed the method for estimating the retinal parameters using the ARIA-stroke methodology. The observed values were plotted against the estimated values for each of the retinal parameters to evaluate if the model generated accurate estimates of the retinal parameters (Figure 2). We confirmed that all the retinal parameter estimates have high level of goodness-of-fit and with clear linear relationships between observed and estimated values. With the support of these results we would be able to provide automatic estimates of retinal characteristics from any fundus photo.

Validation study

The validation study for ARIA-stroke was done on a completely separate data set. The sensitivity and specificity for the validation were 94.7% and 100% respectively using a 0.5 probability as cutoff. The box plot for the probability of stroke

Table 3: Analysis results for stroke risk classification.

Model	Variables included in logistic regression model	Accuracy (%)	AUC† (95% CI)
1	Main demographic and clinical variables	59.0%	0.66 (0.59, 0.73)
2	Manual measure of retinal characteristics	72.2%	0.78 (0.72, 0.84)
3	Manual measure of retinal characteristics and clinical variables	80.4%	0.84 (0.78, 0.89)
4	Automatic retinal image analysis for stroke	100%	

†AUC: Area under ROC Curve

Model 1: Age, gender, hypertension, diabetes, atrial fibrillation, smoking status, hyperlipidemia, and family history of ischemic heart disease

Model 2: CRAE, Vasymmetry, tortuosity, arteriole-venous nicking, and hemorrhage

Model 3: Hypertension, diabetes, atrial fibrillation, CRAE, Vasymmetry, tortuosity, arteriole-venous nicking, hemorrhage with interactions of Vasymmetry-CRAE, CRAE-hemorrhage, Vasymmetry-tortuosity

Model 4: ARIA-stroke

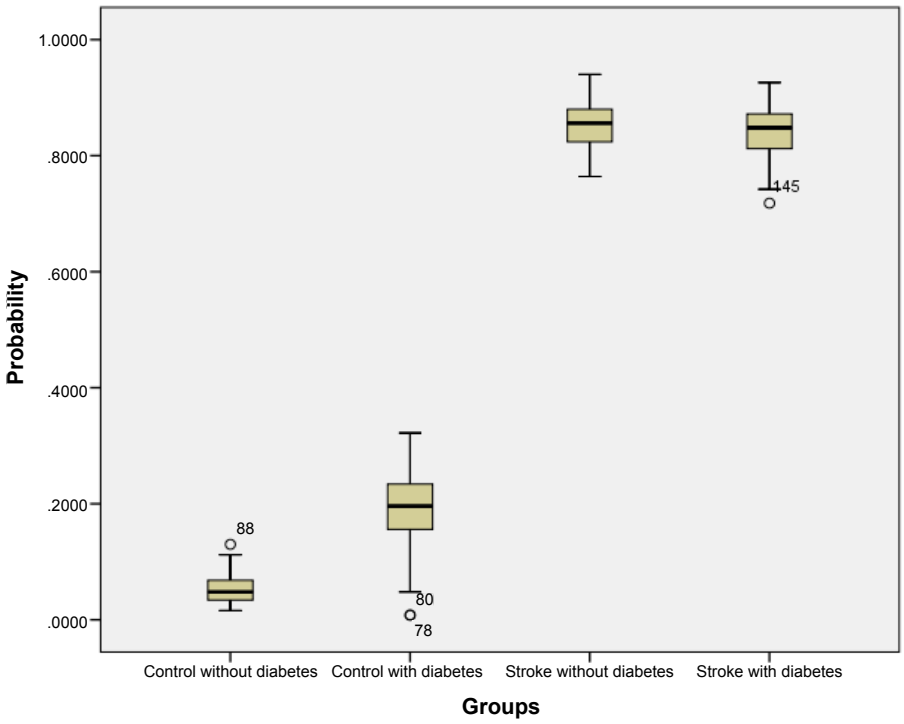

Figure 1: Probability of stroke for the case-control study data by group and diabetes status.

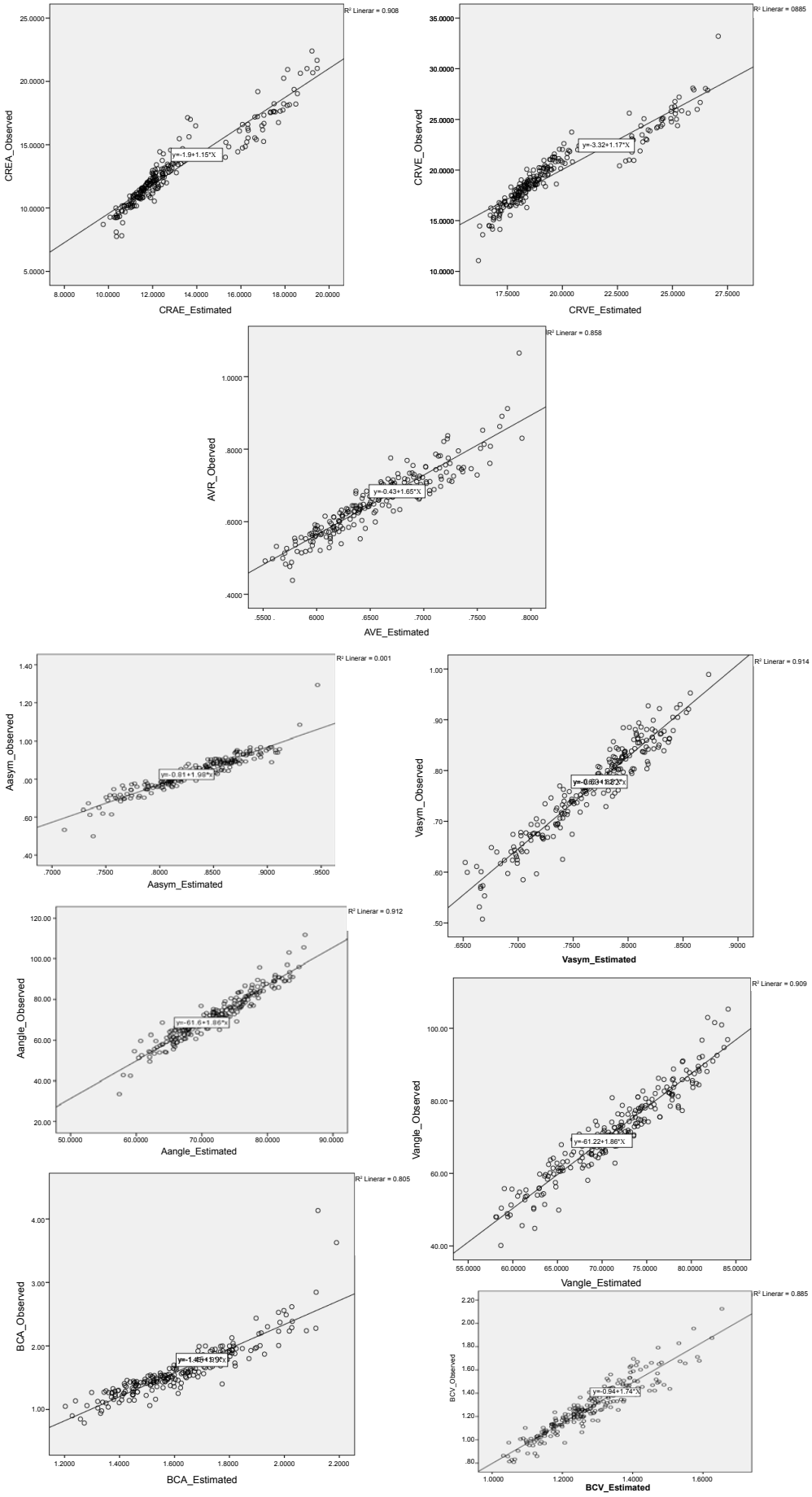

Figure 2: Observed versus estimated values for retinal parameters.

estimated using ARIA-stroke method for the three groups is shown in Figure 3. The normal control group has a mean probability of 0.159 (95% CI: 0.148-0.170) and the hypertension group has a mean probability of 0.274 (95% CI: 0.269-0.280). The stroke cases are under rehabilitation program during their recovery period, the mean probability is 0.570 (95% CI: 0.556-0.584).

We have also examined the retinal parameters for the validation data. The box plots for the retinal parameters are shown in Figure 4. The means and the 95% confidence intervals for selected retinal parameters are shown in Table 4. A number of retinal variables for stroke are significantly different from the normal controls, including CRAE CRVE, venule asymmetry, arteriole and venule angles and bifurcation coefficients. The binary retinal parameters estimates are shown in Table 5. Stroke patients have high proportion of tortuosity as compared to healthy normal control and hypertension groups. (0% vs. 5.6% vs. 78.9%), hemorrhage (0% vs. 10.6% vs. 22.4%) and exudates (0.7% vs. 1.5% vs. 52.6%). The arteriole occlusion has a significantly higher risk of stroke as compared to both control and hypertension patients (0% vs. 0% vs. 21.1%).

DISCUSSION

Previous studies have shown that a number of retinal characteristics were significantly associated with strokes. The combination of retinal characteristics and other clinical features was able to classify the case and control cases with about 80% accuracy once the interaction of the retinal parameters were being considered as opposed to 59% using clinical variables alone. Since ARIA-stroke is tailored to take full advantages of complex interaction, the classification result was significantly improved to 100% in the training data even without clinical variables, and a sensitivity of 94.7% in the validation study using stroke patients already in the rehabilitation stage. With a sample of 336 subjects without stroke in the validation, there is no false positive case and the specificity rate remains 100%. The probability plots have shown that healthy normal subjects have an average probability of less than 0.2, and for stroke patients in the acute care stroke unit the average probability was around 0.8. We have also demonstrated that both the diabetes subgroup in the training data and well managed hypertension patients in a general outpatient clinic have significantly higher probabilities as compared to normal controls but the absolute probabilities were still much lower than the 0.5 cutoff. More research is needed to identify the mild and moderate risk groups of subjects in the community and ideally a long-term follow up study on patients with hypertension and gradually developing stroke incidence should be done. However, a cohort study will take an extensive period of time to eventually confirm its sensitivity. In order to gain more insights at the moment, we have selected 27 hypertension patients with stroke history from the general outpatient clinic with their hypertension well under control for a few years. Their average probability of stroke was 0.37 (95% CI: 0.331-0.406) which is significantly higher than the other hypertension patients without a stroke history with an average probability of 0.27 (95% CI: 0.269-0.280).

In this study we have also identified a number of retinal characteristics that are potentially useful to explain differences among groups. Specific retinal parameters are being observed in Figure 4. According to a study from Rotterdam,[18] smaller diameters of arterioles were not associated with stroke, whereas the widest venule diameters (4th quantile) were associated with an increased risk of stroke. Similar finding was demonstrated in the Cardiovascular Health Study[19] with an OR around 2.2 (95% CI: 1.1-4.3). In our study, the arterioles and venules diameters for stroke and hypertension are significantly smaller than that of the normal control. Patients with stroke have significantly higher probability on venule asymmetry as compared to hypertension and normal. A significantly smaller arterial bifurcation angle and a significantly larger venule bifurcation angle were found in the stroke group as compared to the normal control. Vessel tortuosity was found to be the main risk factor associated with hypertension and stroke as the wall of tortuous vessel may affect the blood flow turbulent which might lead to the damage of the endothelium of the wall and atherosclerosis formation. The existence of hemorrhage is an indicator of long-term damage of systemic vessels by hyperglycemia which may

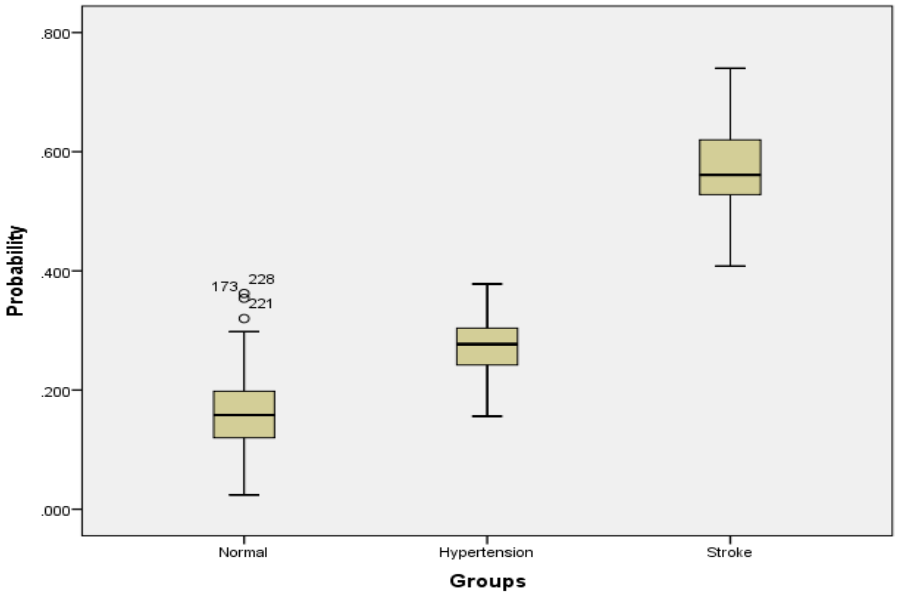

Figure 3: Probability of stroke using automatic retinal image analysis (ARIA) for the validation data set.

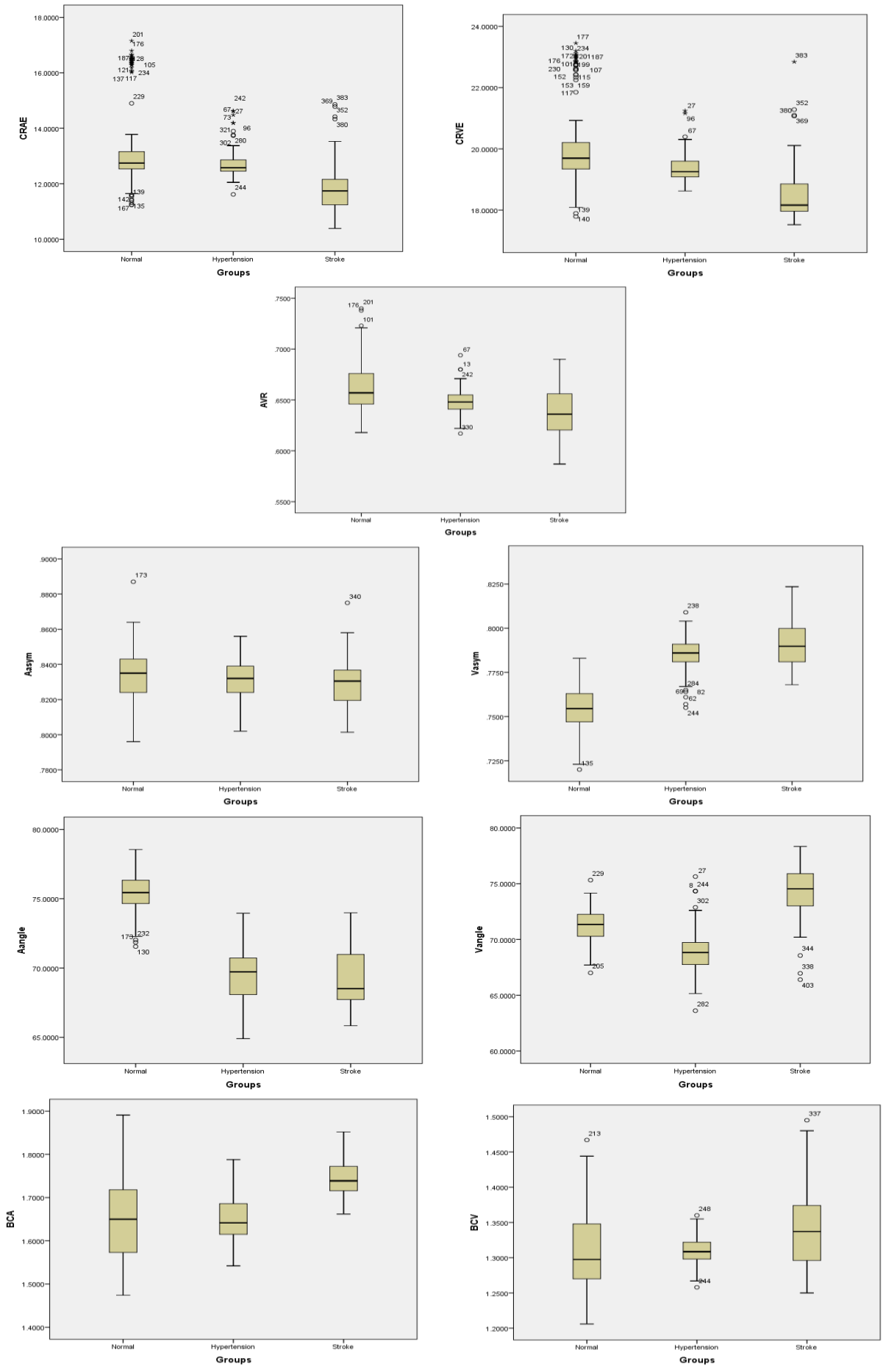

Figure 4: Retinal parameters estimates for the validation data.

Table 4: Continuous retinal parameters estimated by ARIA for the validation data.

		N	Mean	Std. Deviation	95% Confidence Interval	
					Lower Bound	Upper Bound
CRAE*	Normal	138	13.33	1.579	13.068	13.599
	Hypertension	198	12.56	0.406	12.498	12.612
	Stroke	76	11.84	0.957	11.620	12.057
CRVE*	Normal	138	20.08	1.382	19.850	20.315
	Hypertension	198	19.35	0.422	19.292	19.411
	Stroke	76	18.52	0.948	18.299	18.733
AVR	Normal	138	0.66	0.025	0.659	0.668
	Hypertension	198	0.65	0.012	0.648	0.651
	Stroke	76	0.64	0.025	0.632	0.645
Aasym	Normal	138	0.83	0.014	0.832	0.836
	Hypertension	198	0.83	0.010	0.826	0.829
	Stroke	76	0.83	0.016	0.827	0.834
Vasym*	Normal	138	0.76	0.012	0.757	0.761
	Hypertension	198	0.788	0.009	0.787	0.789
	Stroke	76	0.791	0.012	0.788	0.794
Aangle*	Normal	138	74.03	1.217	73.822	74.232
	Hypertension	198	68.67	1.646	68.435	68.897
	Stroke	76	69.31	2.152	68.816	69.799
Vangle*	Normal	138	70.13	1.506	69.877	70.384
	Hypertension	198	68.61	1.589	68.392	68.837
	Stroke	76	74.22	2.403	73.667	74.766
BCA*	Normal	138	1.70	0.076	1.685	1.710
	Hypertension	198	1.71	0.046	1.703	1.716
	Stroke	76	1.74	0.041	1.734	1.752
BCV*	Normal	138	1.32	0.048	1.312	1.328
	Hypertension	198	1.31	0.018	1.307	1.312
	Stroke	76	1.34	0.055	1.330	1.355

Table 5: Binary retinal parameters estimated by ARIA for the validation data.

Variables	**Healthy** % (count/n)	**Hypertension** % (count/n)	**Ischemic Stroke** % (count/n)	p-value
Arteriole-venous Nicking*	0.7% (1/138)	67.7% (134/198)	0% (0/76)	< 0.001
Tortuosity*	0% (0/138)	5.6% (11/198)	78.9% (60/76)	< 0.001
Hemorrhage*	0% (0/138)	10.6% (21/198)	22.4% (17/76)	< 0.001
Exudates*	0.7% (1/138)	1.5% (3/198)	52.6% (40/76)	< 0.001
Arteriole occlusion*	0% (0/138)	0% (0/198)	21.1% (16/76)	< 0.001

explain the higher risk of stroke. Some epidemiological studies reported arteriole-venous nicking associated with the incidence stroke or prevalence stroke after adjustment of common stroke risk factors such as hypertension, diabetes, and smoking,[5,20-22] however these were not observed in our study.

In conclusion, our results demonstrated that retinal image contains large amount of information for stroke risk estimation and they can be used at a cross-sectional time point. Based on our study, we found that subjects with a probability of less than 0.5 can be considered low risk group as both the control groups in the case-control study and the healthy normal subjects in the validation study have stroke probability less than this value. We would suggest that a probability of 0.7 or above as high risk as majority of acute care stroke patients (i.e., 60%) in the case-control study have a probability value of 0.7 or above. The range of 0.5-0.7 is considered moderate risk. We have shown that the retinal parameters may enhance our understanding and provide explanation on the development of the disease. In particular, CRAE CRVE, AVR, venule asymmetry, arteriole and venule bifurcation angles, arteriole and venule bifurcation coefficients have been shown to be significantly different between stroke and normal control. Tortuosity, hemorrhage, exudates and arteriole occlusion are also significantly higher in the stroke patients group. With the ARIA-stroke we would be able to standardize the retinal image assessment in an automatic fashion for the management of patients with hypertension and diabetes, and be able to use this tool in a community primary healthcare setting or for large population screening.

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REFERENCES

1. Krishnamurthi RV, Feigin VL, Forouzanfar MH, Mensah GA, Connor M, et al. Global and regional burden of first-ever ischaemic and haemorrhagic stroke during 1990-2010: Findings from the Global Burden of Disease Study 2010. Lancet Glob Health 2013; 1: e259-281.
2. Goldstein LB, Bushnell CD, Adams RJ, Apple LJ, Braun LT, et al., Guidelines for the primary prevention of stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 42: 517-584.
3. O'Donnell MJ, Xavier D, Liu L, Zhang H, Chin SL, et al. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. Lancet 2010; 376: 112-123.
4. Feigin VL, Krishnamurthi R, Bhattacharjee R, Priya P, Alice T, et al. RIBURST study collaboration writing group. "New strategy to reduce the global burden of stroke". Stroke 2015; 46: 1740-1747.
5. Wong TY, Klein R, Couper DJ, Cooper LS, Shahar E et al. Retinal microvascular abnormalities and incident stroke: The Atherosclerosis Risk in Communities Study. Lancet 2001; 358: 1134-1140.
6. Wong TY, Klein R, Sharrett AR, Couper DJ, Klein BE, et al. Cerebral white matter lesions, retinopathy, and incident clinical stroke. JAMA 2002; 288: 67-74.
7. Doubal FN, Hokke PE, Wardlaw JM. Retinal microvascular abnormalities and stroke: a systematic review. J Neurol Neurosurg Psychiatry 2009; 80: 158-65.
8. Cheung N, Rogers S, Couper DJ, Klein R, Sharrett AR, et al. Is diabetic retinopathy an independent risk factor for ischemic stroke? Stroke 2007; 38: 398-401.
9. Hirai FE, Moss SE, Knudtson MD, Klein BE, Klein R. Retinopathy and survival in a population without diabetes: The Beaver Dam Eye Study. Am J Epidemiol 2007; 166: 724-730.
10. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, et al. Revised formulas for summarizing retinal vessel diameters. Curr Eye Res 2003; 27: 143-149.
11. Witt N, Wong TY, Hughes AD, Chaturvedi N, Klein BE, et al. Abnormalities of retinal microvascular structure and risk of mortality from ischemic heart disease and stroke. Hypertension 2006; 47: 975-981.
12. Patton N, Aslam T, Macgillivray T, Dhillon B, Constable I. Asymmetry of retinal arteriolar branch widths at junctions affects ability of formulae to predict trunk arteriolar widths. Invest Ophthalmol Vis Sci 2006; 47: 1329-1333.
13. Murray CD. The physiological principle of minimum work applied to the angle of branching of arteries. J Gen Physiol 1926; 9: 835-841.
14. Zamir M. Shear forces and blood vessel radii in the cardiovascular system. J Gen Physiol 1977; 69: 449-61.
15. Zee B, Lee J, Li Q. Method and device for retinal image analysis. Patent No. US8787638 B2 2014.
16. Lee J, Zee B, Li Q. Detection of neovascularization based on fractal and texture analysis with interaction effects in diabetic retinopathy. PLoS One 2013; 8: e75699.
17. Lee J, Zee B, Li Q. Segmentation and texture analysis with multimodel inference for the automatic detection of exudates in early diabetic retinopathy. J Biomedical Science and Engineering 2013; 6: 298-307
18. Ikram MK, de Jong FJ, Bos MJ, Vingerling JR, Hofman A, et al. Retinal vessel diameters and risk of stroke: The Rotterdam study. Neurology 2006; 66: 1339-1343.
19. Wong TY, Kamineni A, Klein R, Sharrett AR, Klein BE, et al. Quantitative retinal venular caliber and risk of cardiovascular disease in older persons: The cardiovascular health study. Arch Intern Med 2006; 166: 2388-2394.
20. Mitchell P, Wang JJ, Wong TY, Smith W, Klein R, et al. Retinal microvascular signs and risk of stroke and stroke mortality. Neurology 2005; 65: 1005-1009.
21. Cooper LS, Wong TY, Klein R, Sharrett AR, Bryan RN, et al. Retinal microvascular abnormalities and MRI-defined

subclinical cerebral infarction: The atherosclerosis risk in communities study. Stroke 2006; 37: 82-86.

22. Longstreth W Jr, Larsen EK, Klein R, Wong TY, Sharrett AR, et al. Associations between findings on cranial magnetic resonance imaging and retinal photography in the elderly: The cardiovascular health study. Am J Epidemiol 2007; 165: 78-84.

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