# A Novel Framework for Accurate Segmentation of Brain Tumor Using Multiple Kernel Fuzzy Clustering Algorithm

# Praylin Selva Blessy SELVARAJ ASSLEY<sup>1,\*</sup>, Helen Sulochana CHELLAKKON<sup>2</sup>

<sup>1</sup>Department of Electronics and Communication Engineering, Bethlahem Institute of Engineering, Karungal, Nagercoil, 629257 Tamilnadu, India

<sup>2</sup> Department of Electronics and Communication Engineering, St.Xavier's Catholic College of Engineering, Nagercoil, 6290003 Tamilnadu, India

E-mails: praylinstalin@gmail.com (\*); helentcr@gmail.com

\* Author to whom correspondence should be addressed; Tel.:91-9486271100

Received: March 25, 2020/Accepted: June 16, 2020/ Published online: June 30, 2020

## Abstract

Magnetic Resonance Imaging (MRI) is one of the prominent imaging techniques for assessment of brain tumor progression. Intensity inhomogeneity, partial volume effect (PVE) and diverse nature of tumor render a challenging task for automatic segmentation of brain tumors from MR images. Existing MRI brain tumor segmentation methods focus one or two of the above mentioned challenges. We aimed to present a framework for automatic brain tumor segmentation that effectively tackles all major challenges. In the proposed framework, first the intensity inhomogeneities in the MRI images are corrected using an Enhanced Homomorphic Unsharp Masking algorithm. Following intensity inhomogeneity correction, features are extracted. Finally, the extracted features are fused and clustered using Multiple Kernel FCM (MKFCM) clustering algorithm. The MKFCM clustering algorithm employed in the proposed framework overcomes the PVE and form more generalized clusters, thus proved to be effective for images with diverse tumor shape. To demonstrate the effectiveness of the proposed framework, it is compared with four other clustering algorithms using different validation measures.

Keywords: Magnetic Resonance Imaging (MRI); Brain tumor; Segmentation; Fuzzy; Clustering; Kernels

## Introduction

Abnormal and uncontrolled growth of cells in the brain results in brain tumors [1]. Various imaging modalities such as X-ray, Computed Tomography (CT), Magnetic Resonance Imaging (MRI), etc. can be used in brain imaging studies. MRI offers contrast and spatial resolution among various soft tissues in the brain [2, 3]. Therefore, it has been widely preferred than other imaging modalities. Brain tumor segmentation from MR images refers to the task of delineating brain tumors from other brain tissues in MR images. Accurate segmentation of brain tumors plays a vital role in nearly all MR imaging applications such as diagnosis, quantification of tissue volumes, treatment planning, and surgery.

Automatic segmentation of brain tumors from MR images is a difficult task due to the presence of intensity inhomogeneity and partial volume effect (PVE) in MR images and the diverse nature of the tumor. A lot of methods are available in the literature for MR image segmentation [4-6]. Among the existing techniques, fuzzy segmentation methods are concentrated in MRI as they could retain more details than hard segmentation methods [7]. Fuzzy c-means (FCM) clustering algorithm, a most popular fuzzy segmentation method, provides the flexibility of allowing pixels to occupy multiple clusters and thus tends to overcome PVE. In FCM, tasks of PVE and segmentation are interleaved iteratively to yield better results. However, the FCM algorithm fails to form generalized clusters, and it is sensitive to noise. To overcome these problems, many researchers have concentrated on modifying the objective function of FCM [8]. FCM algorithm is useful only for spherical shape clusters due to the presence of Euclidean distance norm in its objective function. Gustafson and Kessel proposed the GK algorithm, an adaptive distance norm to detect clusters of various shapes in one data set effectively. But the GK algorithm deforms the cluster shapes in the presence of noise. To rectify this, Gath and Geva developed an unsupervised optimal fuzzy clustering (UOFC) algorithm that performs well with different geometrical shapes, different densities, and the number of data points in each cluster. UOFC algorithm converges to a local optimum with the presence of exponential distance in its objective function, thus limiting its performance. In our previous works [9, 10], we discuss the usefulness of GK and UOFC clustering algorithms for MRI brain tumor segmentation. However, due to the above-mentioned limitations, these clustering algorithms may prove inadequate for MRI brain tumors of diverse shapes and sizes. Efficient segmentation of brain tumors of diverse nature could be reached withkernel-based clustering algorithms.

In recent years, kernel FCM (KFCM) addresses the problem of spherical clusters by mapping data with nonlinear relationships to appropriate feature space using kernel tricks [11]. Kernel selection plays an essentialrole in effective clustering. Unfortunately, the right combination of kernels limits its performance. To overcome this, Huang et al. [12] introduced the Multiple Kernel FCM clustering algorithm (MKFCM) by extending multiple kernels to fuzzy clustering. MKFCM incorporates multiple kernels and automatically adjusts kernel weights, thus immune to irrelevant features and kernels.

This paper presents a framework that effectively tackles all the challenges present in MRI brain tumor segmentation. In the proposed framework, the intensity inhomogeneity in MR image is corrected using Enhanced Homomorphic Unsharp Masking (EHUM) algorithm and features are extracted for efficient clustering. MKFCM clustering algorithm employed in the proposed framework fuses and also clusters the extracted features, thus eliminating the need for an additional process of feature fusion before clustering. This clustering algorithm overcomes the PVE and form more generalized clusters, thus proved to be effective for images with diverse tumor shape.

#### Material and Method

## Database

Brain tumor image dataset used in this work are obtained from the "Challenge on Multimodal Brain Tumor Segmentation BRATS2015" [13]. The dataset is composed of multi-contrast MRI scans of 30 glioma patients (with and without resection and both low-grade and high-grade) with proficient annotations for "active tumor" and "edema". In addition to the patient image, it consists of simulated images for 25 high-grade and 25 low-grade glioma subjects. The proposed framework is implemented in MATLAB 2010 Rb software.

#### Method

The overall flow diagram of the proposed framework is shown in Figure 1. In the pre-processing step, the intensity inhomogeneity of the input MR image is minimized using Enhanced Homomorphic Unsharp Masking (EHUM) algorithm. Intensity and texture features extracted from the pre-processed image are fused and clustered using the MKFCM clustering algorithm.

A Novel Framework for Accurate Segmentation of Brain Tumor Using Multiple Kernel Fuzzy Clustering Algorithm



Figure 1. Flow diagram of the proposed framework

# Intensity in Homogeneity

Intensity inhomogeneity arising from the radiofrequency coil in MR imaging obstructs the diagnostic process of a physician. Such inhomogeneities afford difficulties in processing MR images as they produce spatial variations in tissue statistics. To correct the intensity variations of the MRI, an Enhanced Homomorphic Unsharp Masking (EHUM) method is used as a pre-processing stepc[14]. The approach for intensity inhomogeneity correction is summarized below:

- 1. Find out the region of interest 'R' of input image 'P'
- 2. Find the log transform of input image 'P'
- 3. Use low pass filter to both input image and region of interest. Let it be  $P_{Llog}$  and  $R_{L}$
- 4. Compute pixel by pixel division of  $P_{Llog}$  and  $R_L$

5. Determine the difference between the images obtained in Step 2 and Step 4 and expressed in exponential form. This indicates the bias field.

6. Perform dynamic compression to obtain the bias-corrected image. The linear mapping of the restored image intensity Rx from the interval  $[P_1, P_2]$  ( $P_1$  and  $P_2$  representing the range of intensities of interest) to standard scale  $[S_1, S_2]$  ( $S_1$  and  $S_2$  are the minimum and maximum intensities) is expressed as:

$$Y = S_1 + \frac{Rx - P_1}{P_2 - P_1} (S_1 - S_2)$$
(1)

where Y indicates the image obtained from the intensity mapping.

#### Appl Med Inform 42(2) June/2020

7. The intensity mapped image is normalized to the original gray level dynamics using dynamic compression. The corrected image  $I_C$  is restored from the mapped image using the following dynamic compression expression.

$$I_{C} = \frac{Y - \min(Y)}{\max(Y) - \min(Y)} \max(Y)$$
<sup>(2)</sup>

#### Feature Extraction

After intensity standardization, features such as intensity and texture are extracted from the standardized image. In our work, to describe the visual content of interest, it is important to understand the structural variations in the brain, which has been associated with volumetric reductions in medial temporal lobes and skull regions of the brain. For understanding the structural changes, feature extraction plays an important role. Intensity and textures are some of the commonly used features. Here, thecorrected brain tumor MR image  $I_C$  is converted into feature vectors  $X = \{x_1, x_2, \ldots x_n\}$ . Intensity features relyon the probability density P (i,j) of occurrence of the intensity levels (i,j) with the total number of pixels N in the image. We have used the intensity features such as mean, variance, median, mode, and standard deviation. Mean and median are computed as,

$$Mean = \frac{1}{N} \sum_{i=1}^{N} P(i,j)$$
<sup>(3)</sup>

 $Median = \frac{N+1}{2} \text{th term of X}$ (4)

The texture features are extracted using Gray Level Co-occurrence Matrix (GLCM) [15]. A GLCM is a matrix in which the number of rows and columns is equal to the number of gray levels N, in the image.

The GLCM is a two-dimensional array thattakes into account the specific position of a pixel related to other pixels. TheseGLCM matrices are constructed at a distance of d=1, 2, 3, 4 and for the direction of data given. The GLCM features include correlation, energy, entropy, cluster shade, homogeneity, sum of square variance, etc. In our work, the following GLCM features are computed.

Entropy gives the measure of randomness. It is given as N = N

$$Entropy = -\sum_{i=1}^{N} \sum_{j=1}^{N} P(i,j) X log(P(i,j))$$
<sup>(5)</sup>

Correlation provides the linear dependency of grey levels of neighboring pixels. It is calculated as

$$[Correlation = \frac{\sum_{i=1}^{N} \sum_{j=1}^{N} \{iXj\} XP(i,j) - \{\mu_X X \mu_y\}}{\sigma_X X \sigma_y}$$
(6)

where  $\mu_x$ ,  $\mu_y$ ,  $\sigma_x$  and  $\sigma_y$  are the means and standard deviation of P<sub>x</sub> and P<sub>y</sub>.

Following equations define sum average and contrast

$$Sumaverage = \sum_{i=0}^{2N} iP_{x+y}(i)$$
<sup>(7)</sup>

$$Contrast = \sum_{n=0}^{N-1} n^2 \left\{ \sum_{i=0}^{N} \sum_{j=0}^{N} P(i,j) \right\}, |i-j| = n$$
(8)

#### Feature Fusion and Clustering

The MKFCM algorithm employed in the proposed framework performs both feature fusion and clustering, and is related to multiple kernel learning. The success of kernel methods relies in the formation of suitable kernel. However, selection of single kernel from a data set is insufficient in all

cases. Diverse features chosen for a data set corresponds to distinct kernels. Combination of such distinct kernels yield the way to multiple kernel learning. MKFCM extends FCM algorithm with multiple kernel learning setting and embeds feature weight computation into clustering algorithm [16]. This algorithm concurrently decides the best degrees of membership and optimal kernel weights for a combination of set of kernels. The integration of multiple kernels and adjusting kernel weights automatically provides MKFCM more immune to unreliable features or kernels.

Given a data set  $X = \{x_1, x_2, ..., x_n\}$  to be partitioned into C fuzzy clusters, a set of weight vectors  $w_j = w_1 + w_2 + \dots + w_L$  is associated withcluster  $v_i$  for feature selection. Application of kernel learning to the FCM algorithm aims at turning the original nonlinear data X to F by mapping  $\Psi_L$ .

The objective function of such MKFCM algorithm with a composite kernel is defined as

$$J_{MKFCM} = \sum_{i=1}^{c} \sum_{k=1}^{n} U_{ik}^{m} \|\Psi_{L}(x_{k}) - \Psi_{L}(v_{i})\|^{2}$$
(9)

The transformation function in Eq(9) is deduced using composite kernel function  $K_L$  as

$$K_L = \langle \Psi_L(x_k), \Psi_L(v_i) \rangle \tag{10}$$

A composite kernel function is defined as

$$K_L = w_1^b K_1 + w_2^b K_2 + \dots + w_j^b$$
(11)

where b > 1 is a coefficient similar to fuzzy coefficient,  $K_1, K_2, \dots, K_j$  denote the individual kernels, and the weights  $w_i, w_2, \dots, w_j$  in Equation (6) satisfy the constraint:

$$\sum_{j=1}^{L} w_j = 1 \tag{12}$$

As a result, different kernel functions can be defined separately for different features and can be combined as composite kernel  $K_L$  to improve the segmentation results. The incorporation of multiple kernels and adjusting kernel weights automatically make MKFCM more immune to unreliable features or kernels.By minimizing an objective function, the membership function is obtained as

$$U_{ik} = \frac{1}{\sum_{j=1}^{n} \left(\frac{D_{ik}^2}{D_{jk}^2}\right)^{\frac{1}{m-1}}}$$
(13)

The distance function  $D_{ik} \ is$ 

$$D_{ik}^{2} = \sum_{j=1}^{L} \alpha_{ikj} w_{j}^{2}$$
(14)

where the coefficient  $\alpha_{ikj}$  can be obtained as

$$\alpha_{ikj} = K_j(x_i, x_j) - 2\sum_{n=1}^N U_{ik}K_j(x_i, x_j)$$
(15)

## <u>Algorithm</u>

*Step 1*: Initialization. Set number of clusters c, fuzzification degree m, kernels, stop criterion  $\in$  , and max iteration  $I_{max}$ .

Initialize the membership matrix  $U_{ik}$ 

Step 2: Computation and updating parameters

for  $t = 1, 2, ..., I_{max., do}$ 

- a) Compute membership using Eq(13)
- b) Calculate coefficients  $\alpha_{ikj}$  using Eq(15)
- c) Update weights using Eq(12)
- d) Compute the distance by Eq(14)
- e) Update membership  $U_{ik}$  using Eq(13)
- f) Repeat steps (a-e)

Step 3: Check for the termination condition: Compute until  $||U^{(t)}-U^{(t-1)}|| \leq \varepsilon$ . Stop

## <u>Effectiveness Assessment</u>

To check the effectiveness of segmentation methods, various validation techniques are in practice. In this work, the proposed methodis validated using Jaccard coefficient (JC), Dice coefficient (DC), sensitivity and specificity measures. Four criteria: the True positive (TP), False positive (FP), True negative (TN) and False negative (FN) are used to obtain performance metrics. TP represents the number of samples where both the proposed method decision and the ground truth label confirms the presence of tumor; TN represents the number of samples where both the proposed method decision and the ground truth label confirms the absence of tumor; FN and FP is the number of samples where the decisions mismatch. A total error represents the sum of FP and FN.

Specificity = 
$$\frac{TN}{TN + FP}$$
 (16)

Sensitivity = 
$$\frac{TP}{TP + FN}$$
 (17)

$$DC = \frac{2|G \cap S|}{(|G| + |S|)}$$
(18)

$$JC = \frac{|G \cap S|}{|G \cup S|} \tag{19}$$

The misclassified pixels are computed as

% Misclassified pixels 
$$=\frac{(G \cap S)}{G} \times 100$$
 (20)

where **G** is the number of pixels in the ground truth for brain tumor pixels and **S** is the number of pixels in the segmented image for brain tumor pixels.

#### **Results and Discussion**

The proposed method sisteatically obtained the smallest percentage of pixels misclassification (Table 1).

Table 1. Percentage of pixels misclassified by method

Images	Percentage of pixels misclassified				
	FCM	GK	UOFC	KFCM	Proposed method
1	28	23	21	20	15
2	52	49	45	50	18
3	45	43	44	38	13
4	63	50	49	59	21
5	58	43	39	52	18

FCM = Fuzzy c-means; GK = an algorithm proposed by Gustafson and Kessel; UOFC = unsupervised optimal fuzzy clustering, KFCM = kernel FCM

Figure 2(a) shows three MRI sample images. The sample MR images are corrupted by intensity inhomogeneity and noise due to the imaging nature of the device. Figure 2(b) shows the intensity standardized image of the sample image in Figure 2(a). It can be seen that the intensities of the original image in Figure 2(a) is quite homogeneous in the intensity inhomogeneitycorrected image in Figure 2(b). That is after intensity inhomogeneity correction, pixels having the same intensity value are more likely to contain the same kind of tissue. The improvement of the image quality in terms of intensity inhomogeneity corrected images in Figure 2(b). Figure 2(c) represents the corresponding segmentation results of the proposed method.



Figure 2. (a) Sample image (b) Intensity standardized image (c)Segmentation results

The intensity inhomogeneity corrected brain tumor MR image is converted into feature vectors  $X = \{x_1, x_2, \ldots, x_n\}$  that belongs to non-spatial features associated with the spatial location in the image. Here, first the brain tumor MR image is divided into blocks with fixed number of size like  $n \times n$  (*n* may be 3, 5 etc). After that, the intensity and texture features are extracted block by block in each image.

In this work, various intensity features like mean, median and the GLCM texture features such as correlation, energy, sum-average, and contrast are extracted. Usually, the individual feature which has been extracted is not sufficient to identify the tumors with its correct shape, size and location. For accurate diagnoses, we integrate information from various features. By applying the features to the MKFCM clustering algorithm, all the features are fused and clustered without using a separate feature fusion algorithm. This reduces the complexity of the segmentation process. Initial parameters of the proposed algorithm is set as fuzzification degree m = 1.12, stop threshold  $\epsilon = 0.0001$ , parameter  $\alpha = 1$ , number of clusters C=2 for an average of 50 runs. Figure 2(c) shows examples of clustering results for MR images of three tumor patients in Figure 2(a).

In Figure 3 the proposed method is compared with some other clustering algorithms such as FCM, GK, UOFC and KFCM, which are used to validate crisp and well-separated clusters. Figure3(a) shows MRI patient data, Figure3(b)-(f) show the segmentation results obtained using FCM, GK, UOFC, KFCM and proposed algorithm, respectively. It can be observed that some classification error exists in FCM, as it is very sensitive to noise. GK and UOFC provide better segmentation results than FCM, but increase the computational complexity, as mentioned earlier. The qualitative

#### Praylin Selva Blessy SELVARAJ ASSLEY, Helen Sulochana CHELLAKKON

results show the improvement in accuracy for KFCM algorithm, but the problem in the selection of kernels reduces its performance. The result of the proposed algorithm is presented in Figure 3(f). Visually analyzing the results, it is evident that the proposed algorithm is promising when compared to other clustering algorithms. Here, one can view that the tumor is segmented well from other tissues. The main goal of segmentation is to extract worthy information by grouping similar information, the proposed approach successfully performs this task. This is quantitatively displayed in Table 1.The lower misclassification percentage provides better segmentation. From Table 1, it is to be noted that the proposed algorithm provides lower misclassified pixels compared to other clustering algorithms.



Figure 3. Results produced by various clustering algorithms for MRI brain tumor image. a)MRI slices b) FCM c) GK d) UOFC e) KFCM and f) Proposed method

Figure 4 shows the plot of different validation measures. Figure 4(a) shows the sensitivity chart for various clustering algorithms. Each clustering algorithm is represented in a separate color. Sensitivity shows how good the method is to identify the MR image having tumors. The proposed method produces 96% sensitivity results compared to other clustering algorithms. High sensitivity is obviously significant where the method is used to identify the tumors in the dataset. The proposed method produces a better result compared to the proposed method. All the specificity values for different clustering algorithms are displayed as bars in various colors in the Figure4(b). This shows that specificity is considerably decreased for the proposed method when compared to other algorithms. But, if the dataset increases, the numerical value obtained is low for the MKFCM method. At that instant, the proposed method produces the optimum value and diagnoses the tumors perfectly in the MR image.

Jaccard similarity index measures the similarity of clusters and Dice coefficient provides a quantitative measurement of the tissues. JC and DC values of the four clustering algorithms in delineating brain tumors from MR images are compared in Figure 5(a) and Figure 5(b), respectively. It shows that the MKFCM algorithm employed in the proposed framework is substantially more accurate than other algorithms.



Figure 4. Plot for (a) Sensitivity and (b)Specificity

While comparing the results of all the clustering algorithms, the proposed algorithm provides a better classification of pixels. The standard FCM algorithm cannot provide accurate results as it fails to form more general clusters. UOFC algorithm provides more generalized clusters, but increases the computational complexity. KFCM algorithm is an efficient clustering algorithm; however, in our case, due to the presence of multiple features, it does not provides sufficient results. The proposed method efficiently fuses the multiple features and clusters the tumor and normal tissues providing better clustering results.



Figure 5. Plot for (a) Jaccard coefficient and (b) Dice coefficient

To sum up, a robust segmentation framework has been presented for brain tumor delineation from MR images. The intensity inhomogeneity of the input MR image is corrected using Enhanced Homomorphic Unsharp Masking algorithm. The conventional FCM clustering algorithm is associated with the problem of spherical clusters which degrades its performance in brain tumor segmentation. The MKFCM clustering algorithm employed in the proposed framework uses kernel trick for clustering. Strength of MKFCM clustering algorithm includes immune to redundant, unreliable features and noise. This algorithm also performs feature fusion thus reducing the complexity of using separate feature fusion algorithm. The proposed framework is applied to different MR images in the dataset and compared with four other clustering algorithms.

## Conclusion

. Results have demonstrated that the proposed approach for brain tumor segmentation successfully overcomes the difficulties of intensity inhomogeneity, partial volume effects, and efficiently perform segmentation for brain tumors of diverse shape and size, hence provides more accurate segmentation than other algorithms. Future work is focused on extending the proposed approach to multiclass segmentation.

# List of abbreviations

MRI- Magnetic Resonance Imaging PVE - Partial Volume Effect FCM -Fuzzy C means KFCM- Kernel Fuzzy C means GK - Gustafson and Kessel UOFC-Unsupervised Optimal Fuzzy Clustering MKFCM-Multiple Kernel Fuzzy C means EHUM-Enhanced Homomorphic Unsharp Masking

# **Conflict of Interest**

The authors declare that they have no conflict of interest.

# References

- 1. Fisher JL, Schwartzbaum JA, Wrensch M, Wiemels JL. Epidemiology of brain tumor in the Neurologic Clinics 2007:25:867-890.
- 2. Alfred Horowitz L. MRI Physics for Radiologist: A visual approach in the Springer, Third ed. 1994.
- 3. Ahmed S, Iftekharuddin KM, Vossough A. Efficiency of Texture shape and Intensity Feature Fusion for Posterior-Fossa Tumor Segmentation in MRI in the IEEE Trans. Inf. Technol. Biomed 2011:15:206-213.
- 4. Praylin Selva Blessy SA, Helen Sulochana C. A comparative study on medical image segmentation methods. Applied Medica Informatics 2014:32:32-46.
- 5. Praylin Selva Blessy SA, Helen Sulochana C. A review on MRI brain tumor segmentation methods in theApplied Mechanics and Materials 2014:626:38-43.
- 6. Pham DL, Xu C, Prince JL. A survey of current methods in medical image segmentation. Annu. Rev. Biomed 2000:2:315-337.
- 7. Masulli F, Schenone A. A fuzzy clustering based segmentation system as support to diagnosis in medical imaging. Artif. Intell. Med. 1999:16:129-147.
- 8. Ji ZX, Sun QS, Xia DS. A framework with modified fast FCM for brain MR images segmentation. Pattern Recogn. 2011;44:999-1013.
- 9. Praylin Selva Blessy SA, Helen Sulochana C. Performance analysis of unsupervised optimal fuzzy clustering algorithm for MRI brain tumor segmentation. Tech. and Health Care 2015;23:23-35.
- 10. Praylin Selva Blessy SA, Helen Sulochana C. Comparison and evaluation of clustering methods for MRI brain tumor segmentation. Int. Journal of App. Eng. Research 2014;9:9345-9348.
- 11. Zhang DQ, Chen SC. Clustering incomplete data using kernel-based fuzzy c-means algorithm. Neural Process. Lett. 2003;18:155-162.
- 12. Hsin-Chien Huang, Yung-Yu Chuang, Chu-Song Chen. Multiple Kernel Fuzzy Clustering in theIEEE Trans.on Fuzzy Systems 2012:20: 120-134.
- 13. Challenge on Multimodal Brain Tumor Segmentation BRATS2015 [cited February 2020] Available from: www.smir.ch
- 14. Praylin Selva Blessy SA, Helen Sulochana C. Enhanced Homomorphic Unsharp Masking method for intensity inhomogeneity correction in brain MR images. Computer Methods in Biomechanics and Biomedical Engineering: Imaging & Visualization 2000;8:40-41.

- 15. Fritz Albregtsen, Statistical Texture Measures Computed from Gray Level Co-occurrence Matrice in the 3<sup>rd</sup> Image processing Laboratory Department of Informatics. University of Oslo. 2008.
- 16. Long Chen, San Antonia, Mingzhu Lu A. Multiple Kernel fuzzel C means algorithm for image segmentation in the IEEE Trans on Sym, Man and Cybernetics-Part B: Cybernetics 2011;41:1263-1274.