Fractal Geometry and Nonlinear Analysis in Medicine and Biology

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On the fractal analysis of gene sequences involved in atherosclerosis by binary image indicator matrix

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Abstract

In this paper, the gene sequences involved in Coronary Artery Disease (CAD) were analyzed by fractal analysis. In order to obtain a quantitative characterization of nucleotide patterns and to identify anomalies and singularities belonging to genes, the fractal dimension (FD) and lacunarity were studied as fractal parameters. To obtain accurateparametric values, the Binary Image Indicator Matrix (BIIM) was used. This method converts uni-dimensional sequences into 2- D images preserving the autocorrelation.

The results show like the fractal values of genes CAD are very similar to the random sequences. In particular, all FD values fall in the range 1.20 - 1.22 highlighting that the complexity/fractality is the same and suggesting that all genes equally contribute to the pathogenesis of atherosclerosis from statistical point a view. These results are in accordance with the biological studies that indicate CAD as a multi-factorial process, so that these values could be considered as characteristics for these genes.

Introduction

In this work, 60 genes involved in atherosclerosis process are studied in order to investigate a possible rule that relationship the gene sequences anomalies with possible complexity/fractal nature of the distribution of nucleotides. Thus, we are assuming that biological activity of bio-system could be correlate with fractal proprieties. Starting from this assumption, we could detect the physiological state from not physiological state by fractal values. In recent past, several studies were focused on the DNA complexity and on the complexity of some important diseases such as tumors, neurodegenerative and multi-factorial pathologies [1-4].

Some authors suggest that the fractality and complexity are involved in the degeneration of biological system that lead to origin of pathologies, for example the structural changes in the morphology of cells and tissues could cause the shift from a healthy to a pathological state, but this idea is still under investigation [5-9]. So that seems to be a link between fractal nature and biological function and the FD and lacunarity could be consider as parametric markers of these changes.

In order to identify the characteristics values of FD and lacunarity, a fractal analysis on gene sequences belonging to atherosclerosis was conducted and the results were compared with random sequences. The apparently random distribution is considered as sign of complexity of DNA and in recent papers the existence of a fractal organizationin some diseases was investigated [10,11]. The mean idea consider the biological activity correlated with fractal proprieties [12-15] and in particular, the normal and altered states could be identified by fractal values.

Atherosclerosis

The atherosclerosis is one of the most common chronic non-infectious unhealthy condition and can be considered as one of the main factor responsible for the development of coronary artery disease. Atherosclerosis is followed by the over-thickness of the arterial wall as the result of many factors, some of them include deposition of oxidized Low Density Lipoprotein (LDL) and cholesterol and shear stress of the wall induced by high blood pressure. Many diseases such as diabetes mellitus, or the unhealthy lifestyle and nutrition may speed up the process of atherosclerosis. The evolution of atherosclerosis and CAD is thought to have genetic basis. From physiological point of view, population-based epidemiological studies have confirmed that there isnot a single gene that can be considered as the main responsible for CAD pathogenesis. CAD is rather a multi-factorial disease so that multiple genes, located on various chromosomes, are proposed to havea role in its patho-physiology. Recently, many efforts have been made to identify these CAD-related genes and to design an adequate database for future research. Although today much is known about the location and structure of these genetic sequences, many aspects related to their biophysical properties still remain unknown [16-18].

BIIM method

The fractal values of CAD's genes have been obtained by BIIM method. This method allows the display of the nucleotide patterns in 2 dimensions with preservation of autocorrelation between symbols of sequence [19]. In this studies we have considered only 4 symbols, in particular Adenine (A), Cytosine (C), Thymine (T) and Guanine (G) that form the DNA sequences.

The BIIM method was usually used in the studies on alignment of

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proteins and sequences of DNA, to evaluate the degree of homology between biological sequences and for the analysis of DNA regions [20-23]. In this paper, the BIIM was used to convert uni- dimensional symbolic sequences (such as DNA sequences) in 2-D images and to compute the FD and lacunarity. This method allows to visualize a typical patterns of nucleotide that looks like a fractal arrangement such as demonstrated by Cattani in recent works and in the study of complexity of *Caenorhabditis elegans* [2,19,24-26].

Fractal

According to the most popular definition, we can consider a fractal as a geometric object that is characterized by the self-similarity, with its structure cyclically nested at different scales. In particular, the fractal is characterized from at least four properties: self-similarity, fine structure, irregularities and non-integer dimension. The fractal dimension is a parameter used to describe the degree of disorder within the object. In addition, this value is the measure of information contained in the sequence, so that the higher value corresponds to a higher information content [27,28].

Lacunarity

The lacunarity is a parameter which describes the gaps present within a structure or fractal object and in general the high lacunarity means a texture with many gaps (heterogeneous distribution), instead the low lacunarity corresponds to a texture with few gaps (homogeneous distribution) [29]. In the recent past, the analysis of lacunarity was applied on medical images and in particular in the studies of pathological and normal tissue [30,31].

FD and lacunarity correlation

As a statistical parameter to verify the relation between FD and lacunarity, the Pearson product moment correlation coefficient was used.

Materials and Methods

Materials

A representative number of genes sequences of Coronary Artery Disease Gene Database (CADGD)has been taken in account [32,33]. Indetail, we have selected 60 genes thatare shown in table 1. In particular, we have 12 different categories: Vascular smooth muscle cell abnormalities, rennin –angiotensin system, oxidation-reduction state, lipid and lipoprotein metabolism, endothelial integrity, immune and inflammation, gender difference, glucose metabolism, thrombosis, homocysteine metabolism, metalloproteinase - ECM and others generic genes involved in CAD.

The 60 genes are divided into 12 categories according to CAD database as follows:

Methods

The method consist in the computation of fractal dimension and lacunarity by BIIM. In particular,this method converts the un-dimensional sequences in 2-D imageswhereby is possible to compute the fractal parameters. Concerning the parameters, we should notice that all sequences have different lengths in bp and so that in order to make some reasonable comparisons, we havedivided the gene

Table 1. Genes.

Category	*Gene ID*	*Symbol Gene*	*Length bp*	*Category*	*Gene ID*	*Symbol Gene*	*Length bp*
Vasc. Smooth. Mus.	84159	ARID5B	195696	*Gender difference*	367	AR	186589
	339479	FAM5C	379964		1586	CYP17A1	7004
	3456	IFNB1	841		1588	CYP19A1	130543
	4045	LSAMP	643177		2099	ESR1	412780
	9927	MFN2	33336		2100	ESR2	111519
Renin-angiot.system	118	ADD1	86221	*Glucose metabolism*	3938	LCT	49337
	4878	NPPA	2076		11132	CAPN10	12395
	1636	ACE	21321		2645	GCK	45154
	185	AGTR1	45134		231	AKR1B1	16783
	133	ADM	2283		387082	SUM04	689
Ox-Red. state	84735	CNDP1	50571	*Thrombosis*	7450	VWF	175798
	5444	PON1	26217		5175	PECAM1	8083
	6648	SOD2	14207		2149	F2R	19729
	2730	GCLM	22424		2266	FGG	8618
	23564	DDAH2	3224		3690	ITGB3	58871
Lipid-LipoMetab.	5465	PPARA	93156	*Homocyst. Metab.*	875	CBS	23171
	350	APOH	17411		249	ALPL	69049
	1581	CYP7A1	9985		2524	FUT2	9965
	6720	SREBF1	25664		4524	MTHFR	20375
	126	ADH1C	16270		4507	MTAP	63337
Endothelial integrity	4205	MEF2A	150499	*Metalloprot. ECM*	7077	TIMP2	72415
	1573	CYP2J2	33445		7078	TIMP3	62228
	3082	HGF	68010		1471	CST3	4282
	5328	PLAU	6398		4314	MMP3	7816
	7035	TFPI	90264		4313	MMP2	27507
Imm. Inflamm.	4790	NFKB1	115975	*Others genes*	2247	FGF2	71529
	4048	LTA4H	42769		54658	UGT1A1	13028
	8600	TNFSF11	45279		6546	SLC8A1	400291
	10135	NAMPT	36909		10268	RAMP3	26484
	6868	ADAM17	66527		3791	KDR	47338

sequences in parts of 500 bp in length. The results shown in table 2 are the meanof values on 500 bp.

Fractal Dimension on correlation matrix

Fractal dimension iscomputed on the BIIM (correlation matrix) [13,19,20] as follows:

Let

$$\aleph_4 = \{A, C, G, T\}$$

be the finite alphabet of nucleotides and $x \in \aleph_4$ any member of the 4 symbols alphabet and let $D_1(N), D_2(N)$ be two *N*-length DNA sequences, the indicator functionis the map[34]:

$$u : D_1(N) \times D_2(N) \to \{0,1\} \quad (2.1)$$

such that the indicator (correlation) matrix:

$$u_{hk} = u(x_h, x_k) = \begin{cases} 1 & \text{if} \quad x_h = x_k \\ 0 & \text{if} \quad x_h \neq x_k \end{cases} \qquad (x_h \in D_1(N), x_k \in D_2(N)) \quad (2.2)$$

is a matrix of 0's and 1's showing the existence of correlation.

When the sequences are the same, the indicator function shows the existence of autocorrelation on the sequence. The indicator matrix can be used to obtain the binary image of DNA as a two dimensional dot-plot, which can be used to visualize some correlations between the two sequences [13]. It can be easily drawn by assigning a black dot to 1 and a white dot to 0 [19,20] (Figure 1).

G	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	...
C	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	...
A	**1**	**0**	**0**	**0**	**1**	**0**	**1**	**1**	**0**	**0**	...
A	**1**	**0**	**0**	**0**	**1**	**0**	**1**	**1**	**0**	**0**	...
T	**0**	**0**	**1**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	...
A	**1**	**0**	**0**	**0**	**1**	**0**	**1**	**1**	**0**	**0**	...
C	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	...
T	**0**	**0**	**1**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	...
G	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	...
A	**1**	**0**	**0**	**0**	**1**	**0**	**1**	**1**	**0**	**0**	...
$\boldsymbol{u_{hk}}$	**A**	**G**	**T**	**C**	**A**	**T**	**A**	**A**	**C**	**G**	...

Figure 1. Indicator Matrix for a given sequence of nucleotides.

So that the value of fractal dimension is given by

$$D = -\frac{1}{N}\sum_{n=2}^{N}\frac{\log p_x(n)}{\log n} \quad (2.3)$$

Lacunarity on correlation matrix

Let S_n be a given *N*-length DNA sequence and v^*_{hk} (*h*=1,...*N*; *k*=1,...4) be the 4 sequences of the Voss indicator map. On each indicator sequence, for the *h*-th nucleotide, we can consider a gliding box of *r*-length, so that

$$\mu_r(\ ) \quad \sum_{s\ k}^{k+r-} v \quad _{sh} \quad (2.4)$$

is the frequency of "1" within the box. The corresponding probability is

$$p_r(k) = \frac{1}{r}\sum_{s=k}^{k+r-1} v^*_{sh} \quad (2.5)$$

In two dimensions a natural generalization of the lacunarity will be computed as follows: let $u_{hk}(h,k=1,\ldots N)$ be the indicator matrix which gives rise to the binary image of DNA, the lacunarity is defined as a function of the square side, by the ratioof the second and first moment of the distribution.

$$\Lambda(r) = \frac{\sigma^2\left[p_r(h,k)\right]}{\left[\overline{p_r(h,k)}\right]^2} + 1 \quad (2.6)$$

Pearson product moment correlation coefficient

The Pearson product moment correlation coefficient is a statistical method used to describe the correlation between two or more data sets [35]. In particular, the coefficient value falls into the range [-1; 1], in particular (-1) for negative correlation, (+1) for positive correlation and (0) for no correlation.

Results

Fractal and Lacunarity values

In the following Table 2 the mean values with standard deviation of fractal dimension and lacunarity are given.

In table 2 are shown the higher (in yellow) and the lower (in red) values for gene and random sequences. For CAD's genes, the FDvalues fallinto the range1.20 to 1.22. The values of random sequences fall into the range 1.19 to 1.23 and this result suggest us that FD values for genes are very close to random.

For the lacunarity, the range of values between the higher (0.0065) and the lower (0.00019) is very small (referred to gene sequences). Similar result was obtained for the random sequences. In detail, the higher value is shown for the random sequence number 7 and the lower value belongs to the random sequence number 13. Also in this case, the difference of values is very low (0.00924).

Some notes on genes with extreme values are shown as follow:

PPARA (peroxisome proliferator- activated receptor alpha) encodes for PPARA protein involved in lipid metabolism. It is situated between 46,546,424 bp and 46,639,653 bp on chromosome 22. The main pathologies associated for PPARA are: hyperapobetalipoproteinemia and tularemia [36].

MEF2A (myocyte enhancer factor 2A) encodes for a protein involved in most important cellular processes such as muscle development, neuronal differentiation, cell growth control and apoptosis. This gene belongs to chromosome 15 and it is localized between 100,017,370 bp and 100,256,671 bp. The coronary artery disease and in particular the autosomal dominant 1 typeare associated to MEF2A gene dysfunction [36].

IFNB1 (interferon beta 1fibroblast) encodes to interferon beta protein. This protein has multiple activities as: antiviral, anticancer and antibacterial. This gene is positioned on chromosome 9 in particular between 21,077,104 bp and 21,077,943bp. A oral erosive lichen and cerebral primitive neuroectodermal tumor are associated with IFNB1 dysfunction[36].

ADD1 (adducing 1 alpha) encodes for a cytoskeleton protein

Table 2. Fractal dimension and lacunarity values of genes.

Symbol Gene	*Fractal Value*	*Lacunarity Value*	*Symbol Gene*	*Fractal Value*	*Lacunarity Value*
ARID5B	1,219 ± 0,002	0,00248 ± 0,001	AR	1,218 ± 0,002	0,002308 ± 0,003
FAM5C	1,216 ± 0,0004	0,00166 ± 0,001	CYP17A1	1,220 ± 0,0005	0,000166 ± 0,0001
IFNB1	1,220 ± 0,0001	0,00019± 0,0002	CYP19A1	1,220 ± 0,0003	0,001178 ± 0,001
LSAMP	1,219 ±0,0009	0,00337 ± 0,002	ESR1	1,218 ± 0,0006	0,00008 ± 0,0001
MFN2	1,216 ± 0,003	0,00018± 0,0002	ESR2	1,219 ± 0,001	0,00204 ± 0,002
ADD1	1,215± 0,008	0,00657± 0,006	LCT	1,220 ± 0,0002	0,00039 ± 0,0003
NPPA	1,220± 0,0006	0,00126 ± 0,001	CAPN10	1,216 ± 0,002	0,001188 ± 0,001
ACE	1,217± 0,004	0,00084 ± 0,0007	GCK	1,218 ± 0,001	0,00059 ± 0,0008
AGTR1	1,218± 0,001	0,00033± 0,0003	AKR1B1	1,218 ± 0,003	0,002308 ± 0,001
ADM	1,218± 0,001	0,00031± 0,0002	SUM04	1,220 ± 0,0003	0,000308 ± 0,0002
CNDP1	1,218 ± 0,003	0,00006 ± 0,00008	VWF	1,217± 0,004	0,005522 ± 0,004
PON1	1,219 ± 0,001	0,00033 ± 0,0003	PECAM1	1,220 ± 0,0002	0,001404 ± 0,001
SOD2	1,217 ± 0,003	0,00061 ± 0,0007	F2R	1,218 ± 0,002	0,00070 ± 0,0006
GCLM	1,215 ± 0,004	0,00008 ± 0,0001	FGG	1,216 ± 0,003	0,000167 ± 0,0002
DDAH2	1,218 ± 0,001	0,002351 ± 0,002	ITGB3	1,217 ± 0,005	0,00280 ± 0,003
PPARA	1,216± 0,007	0,001247 ± 0,001	CBS	1,217 ± 0,004	0,00067 ± 0,0007
APOH	1,220± 0,0005	0,00060± 0,0006	ALPL	1,218 ± 0,004	0,00519 ± 0,005
CYP7A1	1,218± 0,002	0,002124± 0,002	FUT2	1,217 ± 0,002	0,00023 ± 0,0002
SREBF1	1,217± 0,003	0,00150 ± 0,001	MTHFR	1,218 ± 0,002	0,000204 ± 0,0002
ADH1C	1,215± 0,002	0,00116± 0,001	MTAP	1,219 ± 0,002	0,001252 ± 0,001
MEF2A	1,214± 0,006	0,00006±0,00006	TIMP2	1,215 ± 0,006	0,000987 ±0,0008
CYP2J2	1,219± 0,0009	0,00063± 0,0008	TIMP3	1,220 ± 0,0005	0,000976 ± 0,001
HGF	1,217± 0,001	0,00026± 0,0002	CST3	1,217 ± 0,004	0,00082 ± 0,001
PLAU	1,217± 0,004	0,00057± 0,0007	MMP3	1,218 ± 0,002	0,00032 ± 0,0004
TFPI	1,217± 0,002	0,000132 ± 0,0001	MMP2	1,217 ± 0,002	0,00074 ± 0,0008
NFKB1	1,216 ± 0,005	0,001497 ± 0,001	FGF2	1,215 ± 0,006	0,002090 ± 0,001
LTA4H	1,219 ± 0,002	0,00063 ± 0,0004	UGT1A1	1,220 ± 0,0001	0,000705 ± 0,0009
TNFSF11	1,219 ± 0,0008	0,00036 ± 0,0004	SLC8A1	1,219 ± 0,001	0,000445 ± 0,0003
NAMPT	1,216 ± 0,003	0,00406 ± 0,005	RAMP3	1,217 ± 0,003	0,001580 ± 0,001
ADAM17	1,218 ± 0,0009	0,00071 ± 0,0009	KDR	1,218 ± 0,0005	0,000671 ± 0,0008
#Random 1	1,215 ± 0,002	0,00401± 0,0053	#Random 11	1,219± 0,0008	0,00101 ± 0,0008
#Random 2	1,214 ± 0,001	0,00064 ± 0,0006	#Random 12	1,214± 0,0008	0,000844 ± 0,0007
#Random 3	1.213 ± 0,001	0,00267 ± 0,003	#Random 13	1,216± 0,0002	0,00041 ± 0,0004
#Random 4	1,220 ± 0,0001	0,00065 ± 0,0006	#Random 14	1,197± 0,001	0,00717 ± 0,008
#Random 5	1,215 ± 0,002	0,00023 ± 0,0002	#Random 15	1,216 ± 0,0007	0,00105 ± 0,0009
#Random 6	1,210 ± 0,001	0,00072 ± 0,0007	#Random 16	1,213 ± 0,0006	0,00052 ± 0,0004
#Random 7	1,211 ± 0,001	0,00965± 0,009	#Random 17	1,217 ± 0,001	0,00235 ± 0,002
#Random 8	1,218 ± 0,0004	0,000235 ± 0,0003	#Random 18	1,235 ± 0,001	0,00363 ± 0,003
#Random 9	1,203 ± 0,006	0,00584 ± 0,0076	#Random 19	1,218 ± 0,001	0,00363 ± 0,003
#Random 10	1,217 ± 0,0003	0,00088 ± 0,001	#Random 20	1,219 ± 0,0004	0,00023 ± 0,0002

(adducin alpha) that acts binding to calmodulin. The gene is situated on chromosome 4 and in particular between 2,845,584 bp and 2,931,803 bp. The main disease associated to ADD1 is hypertension in particular the type salt- sensitive [36].

Statistical analysis

The Pearson product moment correlation coefficient is: -0,376 showing a negative correlation between FD and lacunarity.

Discussion

In recent past, the interest for efficient diagnosis and prognosis of tumors, inflammation of tissues, multi- factorial and degenerative disorders is increased.In order to better diagnosis many biological methods andcomputational tools have been used. In the last period these analytical methods were also supported by mathematical and statistical methods, because it is thought that behind the organization of cells, tissues and nucleotide distribution, seem to be some types of recurrences as fractals [37-40]. Concerning this conjecture, some authors have discussed the possibility to distinguish the physiological state from pathological state by fractal/ complexity values that biological systems assume when mutate their physiological conditions [41-44]. Thus, the idea that fractality is a valuable tool that allows to discriminate altered and normal states, it has been previously noticed in some diseases [45-47]. Therefore, the complexity and fractal nature seem to be involved in degeneration of biological system and the authors are searching by mathematical and statistical methods to better understanding the main features of bio- systems [48-50].

In this work, the multi-fractality, as an indicator of the degree of disorder/heterogeneity of the system, has been considered in the statistical study of atherosclerosis by parameters of FD and lacunarity (inversely related).

In order to find some singularities that enable us to characterized the nucleotide patterns within the gene sequences, the values of FD and lacunarity were achieved by BIIM. In the past, this method was applied

for the alignments of nucleotide sequences, analysis of different regions of DNA [14-17], while in this paperit was used to obtain fractal values.

In particular, the findings shown that all mean values tend to be very close both FD than lacunarity. The results lead to consider these values as characteristics for CAD's genes. To verify the correlation as well as the reliability of two parameters, the Pearson product moment correlation coefficient was applied. According to the negative correlation, if the value ofFDis high,it is expected that the value of lacunarity is low and vice-versa. The statistical hypothesis is that exists a negative correlation between FD and lacunarity. The analysis shows that between the two parameters there is a negative value (-0,376) and this means that two parameters can be assumed as reliable from statistical point of view.

The statistical study has shown that no genes seems to play a leading role in atherosclerosis and all genes considered seem to have the same fractal characteristics. This result, from a biological point a view, lead to assume that the genes have similar function in the origin of atherosclerosis confirming a multi-factorial nature of pathology.

Conclusion

In this paper, the FD and lacunarity values of CAD's genes and those of random sequences were calculated by BIIM. The analysis of fractal dimension and lacunarity showed values much similar between all sequences. In particular, the values of FD for all gene sequences fall in the range1.20 to 1.22, while the difference of values for lacunarity is only (0.00638). These results suggest that all the genes are involved equally in pathogenesis of CAD confirming the multi- factorial nature of pathology from biological point a view. Therefore, the values could be assumed as characteristics for CAD's genes and easily calculated by BIIM method. From statistical point a view, these results show that is also possible to conjecture a distribution of nucleotide very similar to a random distribution.

Conflict of interests

The author declare that there is no conflict of interests regarding the publication of this paper.

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