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Relationship between Genotype and Arcuate Fasciculus Morphology in Six Young Children with Global Developmental Delay: Preliminary DTI Study

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Abstract

Purpose—To investigate whether different genetic mutations observed in children with global developmental delay (GD) are associated with unique patterns of the arcuate fasciculus dysmorphology.

Materials and Methods—Six children with GD (age: 36.8 ± 14.1 months, 5 boys) having mutations in MID1, CDK4, SFRP1, EN2, RXRG-GLRB, or MECP2, and five children with typical development (TD, age: 38.5 ± 20.5 months, 4 boys) underwent a 3T MRI including diffusion weighted imaging (DWI). Five language pathway segments in the left hemisphere, “C₁: Broca's to Wernicke's area”, “C₂: Broca's to premotor area”, “C₃: premotor to Wernicke's area”, “C₄: Wernicke's to inferior parietal area”, and “C₅: premotor to inferior parietal area” were objectively identified using the DWI “maximum a posteriori probability” (MAP) classifier.

Results—Affinity propagation clustering analysis found that three arcuate pathway segments, C_{1,2,4}, of MID1, CDK4, EN2 and MECP2 had a similar pattern of volume ratio while those of SFRP1 and RXRG-GLRB had a heterogeneous pattern of volume ratio (net similarity = -0.01). Using receiver operating characteristic curve analysis, the fiber ratios of C_{1,2,4} showed a high probability to discriminate between GD and TD, yielding an accuracy of 0.91, 0.91, 1.00, respectively. The fiber volumes of C₁ and C₄ showed a strong correlation with expressive language ($R^2=0.6019$, p-value=0.033) and receptive language ($R^2=0.6379$, p-value=0.028), respectively.

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The authors declare no conflicts of interest.

Conclusion—The findings of the present study provide preliminary evidence to suggest that different segments of the arcuate fasciculus are formed under the regulation of different genes which, when mutated, may result in developmental delay.

Keywords

Diffusion weighted imaging; tractography; language pathway; arcuate fasciculus; axon guidance; genetics

INTRODUCTION

Global developmental delay (GD) is caused by a broad spectrum of etiologies that result in the impairment of multiple developmental domains such as language, motor, social interaction and activities of daily living (1). Its prevalence is estimated to be 1-3 % of children aged less than 5 years (1). A poor understanding of the underlying anatomic substrates and genetic causes of GD may explain the lack of specificity in diagnosing and treating GD. Similarly, standard clinical neuroimaging tools are of limited value in evaluating children with GD except to rule out a lesional/structural etiology (1). Advanced neuroimaging with diffusion weighted imaging (DWI) and whole exome sequencing techniques have the potential to improve our understanding of the anatomic substrates and underlying genetic causes of GD.

Recently, we reported that two major association brain tracts, arcuate fasciculus (AF) and inferior longitudinal fasciculus (ILF), were grossly abnormal anatomically in a significant proportion of children with GD (2, 3). In a subsequent tract based morphometric study, we showed that both diffusion and geometric properties of AF are abnormal in a subset of children with GD (4). In Angelman syndrome, a severe syndromic form of GD, we found abnormalities in multiple major cortical association tracts using DWI tractography (5) and tract based spatial statistics (6). Graph theoretical analysis based on DWI connectome was further applied to investigate significantly reduced connectivity strength in language network of the children with GD (7). White matter abnormalities in GD and other related neurodevelopmental disorders have also been reported by other groups (8-10).

Parallel to the demonstration of white matter abnormalities in GD using DWI, identification of causal mutations have enhanced our understanding of genetic/neurologic mechanisms of GD. A census of underlying causal mutations of GD implicated 282 genes (11). This was in the era before nextgen sequencing became a reality in genetic research and in the clinic. Whole exome sequencing studies have added to the repertoire of mutations causing GD (12, 13) even though some of these variants need to be validated with additional studies. Most mutations have yet to be identified as the total number of mutations associated with GD has been estimated to be over one thousand (14). With such a large number of potential mutations, evaluating the structural and functional consequences of mutations becomes even more important. From this perspective, performing simultaneous genetic and imaging studies provides a unique window of opportunity to infer the consequences of identified mutations. From a neuroanatomic perspective, the mutations may cause grey or white matter abnormalities or both. For example, several rare mutations of axon guidance pathways that

are known to cause primary white matter abnormality have been described (15). Indeed, in a recent study utilizing DWI and whole exome sequencing, we showed that mutations in two genes (EN2 and MID1) involving axon guidance pathways were associated with poorly developed AF in children with GD (16). In the same study, we also identified mutations in other genes whose relationship to white matter abnormalities is less obvious (16). Thus, the mutations may not only cause primary white matter abnormalities (if present in axon guidance pathway genes) or secondary white matter abnormalities. Characterizing the precise white matter abnormalities may provide further insights into the neuroanatomical substrates of GD.

However, in our previous study (16), we used a relatively simplistic classification scheme in which patients with GD were classified into those with and without identifiable AF on DWI tractography. Subsequent to that study, the present study re-analyzed these data using novel diffusion weighted imaging (DWI) analytical techniques such as high-resolution tracking of individual branches of AF called “DWI-based maximum *a posteriori* probability (DWI-MAP) classifier (17)” to automatically detect separate language pathway branches connecting four distinct language areas such as Broca’s area for speech, Wernicke’s area for comprehension, inferior parietal area for reading, and premotor area for fluency (8, 18, 19).

The present study applied the DWI-MAP classifier to determine whether abnormal patterns of language pathway branches in children with GD are associated with different genetic mutations found in these children. This study hypothesized that various gene mutations would be associated with distinct patterns of the arcuate fasciculus dysmorphology and ultimately correspond to atypical language function phenotypes.

MATERIALS and METHODS

Subjects

Six children with GD (age: 36.8 ± 14.1 months, 5 boys) having mutations in the genes, MID1, CDK4, SFRP1, EN2, RXRG-GLRB, or MECP2, were investigated. These mutations were identified in our previous whole exome sequencing study (16) on children who were clinically diagnosed with global developmental delay as defined by impaired global cognition (FSIQ < 70) and impairment in at least two domains of adaptive behavioral functioning (standard score < 70; gross/fine motor, speech/language, daily living skills, and socialization skills). Five children with typical development (TD, age: 38.5 ± 20.5 months, 4 boys) were also investigated and served as healthy controls. All participants were right-handed. Patients with known genetic etiology (such as fragile X syndrome, Rett Syndrome etc) or known environmental insults (such as perinatal hypoxia, fetal alcohol syndrome, etc.) were excluded from the study. All studies were performed in accordance with the policies of the Wayne State University Institutional Review Board with a written informed consent obtained from the parents or guardians.

Neuropsychological Assessment

All participants in the GD group completed comprehensive neurocognitive and behavioral assessments, including assessment of global, verbal and nonverbal intellectual functions,

using the age-appropriate Wechsler scale (WPPSI-4, WISC-IV). Receptive and expressive language functions were assessed using the age-appropriate version of the Comprehensive Evaluation of Language Fundamentals (CELF-P2, CELF-4). The Vineland Adaptive Behavior Scales – 2nd Edition (VABS-2) were used to quantify overall adaptive behavioral functioning as well as the Communication, Daily living, Socialization, and Motor skills domains. Finally, behavioral problems were assessed using the Behavioral Assessment System for Children – 2nd Edition (BASC-2). All of the above have excellent psychometric properties and are widely used with clinical populations and in research studies (20). Table 1 summarizes the obtained assessments for the present study.

All of the typically developing children also underwent cognitive assessments to ensure that they had intellectual function within normal limits (FSIQ>84), and also social historical interview to rule out any current and/or historical developmental, medical, or psychiatric conditions. Caregivers of this group also completed the BASC-2. Inclusion criteria for this group were measured global intellectual functioning (FSIQ>85) within normal limits; English speaking; right handed; regular school attendance; absence of current or historical medical and/or psychiatric diagnoses; absence of current or historical use of psychoactive medications, absence of any at-risk or clinical elevations on the BASC-2.

Genetic data analysis

The detailed method of identification of genetic mutations was previously published (16). We describe the procedure briefly here. Genomic DNA was extracted from whole blood using a DNA extraction kit (DNeasy blood and tissue kit; Qiagen, Valencia, CA). 5 micrograms of genomic DNA was used to extract the exome sequences by using the Agilent Sure Select V5, 51 MB exome capture kit. The exome sequencing was performed (with a minimum guaranteed coverage of 50) on the Illumina HiSeq 2000 sequencer by outsourcing the sequencing to a third party facility (Otogenetics, Atlanta, GA). Bioinformatics analysis was performed using standard exome sequencing alignment and variant calling procedures using DNAnexus bioinformatics service provider. An additional filtering was performed in which variants in only conserved genes that are also known to cause abnormal brain morphology in mouse (as defined in MGI mammalian phenotype browser database) were identified. Once variants were called, downstream statistical association of the variants was performed by in-house workflows developed to perform an integrated analysis of clinical, imaging and genetic data. All the candidate mutations were validated by Sanger sequencing.

MRI data acquisition

All participants underwent a 3T diffusion weighted MRI with eight channel head coil (GE-Signa scanner, GE Healthcare, Milwaukee, WI) at TR = 12,500ms, TE = 88.7ms, FOV = 24cm, 128×128 acquisition matrix (nominal resolution = 1.89mm), contiguous 3mm thickness in order to cover entire axial slices of whole brain using 55 isotropic gradient directions with $b = 1000 \text{ s/mm}^2$, one $b = 0$ acquisition, and number of excitations=1. The present study utilized the NIH TORTOISE DIFF_PREP software package to correct motion and eddy current distortion in the diffusion weighted MRI data (<https://science.nichd.nih.gov/confluence/display/nihpd/TORTOISE>).

Whole brain tractography

For each subject, whole brain tractography using independent component analysis with ball and stick model (ICA+BSM)(21) was performed to isolate up to 3 fiber bundles crossing at every voxel. Compared with standalone BSM (22) which guesses initial orientations of multiple tensor orientations randomly, ICA+BSM adapts an ICA approach to isolates independently attenuated diffusion profiles (up to three) from "neighboring voxels". The orientations of the resulting profiles were used as initial guesses of multiple stick-tensor orientations and then optimized via the framework of standalone BSM. The present study utilized conventional deterministic tractography algorithm implemented in DSI studio (www.dsi-studio.labsolver.org) to reconstruct whole brain tractography. Briefly, at every voxel of fractional anisotropy > 0.20, the first eigenvectors of the optimized stick-tensor components having the fractional ratio > 0.10 were considered as the reconstructed fiber orientations and then utilized for the streamline tractography: all orientations seeding, subvoxel seeds/voxel = 10, step size = 0.2 voxel, width, smoothing = 0, turning angle threshold = 60°, Runge-Kutta tracking, trilinear interpolation, and length constraint = 80 mm - 150 mm.

DWI-MAP classification

To identify five language pathway segments in the left hemisphere, "C₁: Broca's area to premotor area", "C₂: Broca's area to Wernicke's area", "C₃: premotor to inferior parietal area", "C₄: inferior parietal area-Wernicke's area" and "C₅: premotor area to Wernicke's area", the whole brain streamline tractography was sorted using a DWI-MAP classifier (17). Briefly, this classifier consists of the stereotactic MNI probability maps of five language pathways, $P(x,y,z|C_{i=1,2,3,4,5})$ obtained from semantic language fMRI and ICA+BSM tractography of healthy children. Five maximum a posteriori (MAP) probabilities of a given streamline tract are sequentially estimated from $P(x,y,z|C_{i=1,2,3,4,5})$ in order to make a classification of the streamline tract into five classes of interest, $C_{i=1,2,3,4,5}$. First, the maps of $P(x,y,z|C_{i=1,2,3,4,5,6})$ is spatially registered to individual subject's space via spatial deformation obtained between the subject's b0 image and MNI b0 template using SPM DARTTEL package (www.fil.ion.ucl.ac.uk/spm). Secondly, the resulting maps were used to approximate the conditional probability maps of a given voxel (x,y,z) , $P(x,y,z|C_i)$ for individual language pathways. Thirdly, we evaluated the a posteriori probability, $P(\text{fiber}_j|C_{i=1,2,\dots,5})$ that a given fiber streamline of whole brain tractography, $\text{fiber}_j(x,y,z)$ belongs to $C_{i=1,2,\dots,5}$, under an equal prior of $C_{i=1,2,3,4,5}$. The argument of i having the most probable a posteriori probability $P(\text{fiber}_j|C_i)$ determines the membership of the streamline tract, fiber_j . Details of the DWI-MAP classifier are available in our previous study (16).

Statistical analysis

The fiber volumes of the DWI-MAP derived language pathway segments were assessed by summing the voxels belonging to the streamlines of each segment and then normalizing to total white matter volume of left hemisphere in order to minimize the confounding effect of brain size across subjects. Two covariates (age and gender) were finally regressed out from the normalized fiber volume (i.e., fiber ratio of individual arcuate pathway segments, C_{1-5}).

To quantify the degree of ratio change in individual language pathway segments of each child with GD, z-score analysis was applied using the following equation: $z\text{-score} = (v - m_{TD}) / \text{std}_{TD}$ where v is the fiber ratio of the individual child with GD. m_{TD} and std_{TD} represent the average and standard deviation of fiber ratio in TD group, respectively.

Also, the present study utilized an affinity propagation clustering (APC) (23) to investigate exemplar genes existing in 6 different mutations. Three major language segments of the individual subject, $C_{1,2,4}$ were selected as two dimensional feature vectors, $[C_2, C_4]$ and $[C_1, C_2]$, respectively. These pair-wise feature vectors were then clustered to maximize the similarity objective function (i.e., negative net similarity). The APC method performs an iterative search to determine the optimal clusters maximizing an objective function, called “net similarity” that quantifies the degree of cluster compactness and between-cluster distance in the feature space. The advantage of this method is to automatically determine the number of clusters and exemplars (i.e., central genes of different clusters) existing in a given data pool. In the present study, the cluster centers of the GD group were compared with those of TD group in order to assess whether different gene mutations were indeed associated with differently developed patterns of individual language pathway segments.

Receiver operating characteristic (ROC) curve analysis was applied to assess the accuracy of individual ratios to differentiate GD group from the TD group. The accuracy of each fiber ratio for the differentiation of GD from TD was evaluated using the ROC-defined cut-off value to maximize sensitivity and specificity. Finally, Pearson’s correlation analysis was performed between the values of individual fiber ratios and specific language measures in order to investigate the correlation of anatomical versus functional phenotype in the language domain.

RESULTS

Five language pathway segments, C_{1-5} of six GD children having different mutations: MID1, CDK4, SFRP1, EN2, RXRG-GLRB, and MECP2 are visually reduced compared with healthy controls (Fig. 1 and 2). The magnitude of visually observed difference was quantified using z-score, but no statistical significance testing was performed due to small sample size. Compared with the TD group, the children with GD had decreased fiber ratios in “ C_1 : Broca’s area-premotor area”, “ C_4 : inferior parietal-Wernicke’s area”, and “ C_2 : Broca’s area-Wernicke’s area” where z-score was -2.7 ± 0.8 , -2.5 ± 0.7 and -1.6 ± 0.5 for C_1 , C_4 and C_2 , respectively.

Figure 3 shows the cluster plots of two pair-wise ratios maximizing the similarity in six mutations. The color of each dot indicates the gene belonging to an identical cluster whose exemplar is marked by an asterisk. Decreased fiber ratios in “ C_1 : Broca’s area-premotor area”, “ C_2 : Broca’s area-Wernicke’s area” and “ C_4 : inferior parietal-Wernicke’s area” were apparent in four mutations, MID1, CDK4, EN2 and MECP2 where CDK4 was an exemplar of this cluster, while two mutations, SFRP1 and RXRG-GLRB had their own exemplars, suggesting their high dissimilarities of $C_{1,2,4}$ from other mutations. Interestingly, SFRP1 had the fiber ratios of $C_{2,4}$ within normal range, which was classified into TD cluster having an exemplar of TD₃. In the fiber ratios of $C_{1,2}$ five mutations were classified into a cluster

having an exemplar of EN2 as all five controls were classified into a cluster having an exemplar of TD₁.

The ROC analysis found that the decreased fiber ratios of C₁₋₅ show higher probability to differentiate GD samples from TD samples (Figure 4). C₄ provided an excellent classification (i.e., area under curve (AUC) $\approx$ 1). The other three ratios (C_{1,2,3}) also performed a good classification (i.e., AUC > 0.8). The optimal cut-off values of fiber ratios, C_{1,2,3,4,5} were achieved at 0.10, 0.10, 0.02, 0.15 and 0.05, yielding a high accuracy of 0.91, 0.91, 0.82, 1.00 and 0.82, respectively (Table 2).

Figure 5 presents the neurological correlation analysis between DWI-MAP derived fiber ratio and specific language scores. It was found that a high correlation between the fiber ratio of the frontal language pathway segment (“C₁: Broca’s area-premotor area”) and expressive language score (Spearman’s $\rho=0.359$, $R^2=0.6019$ /one-tailed p-value=0.033). In contrast, the fiber ratio of the temporal language pathway segment (“C₄: inferior parietal-Wernicke’s area”) showed a high correlation with receptive language (Spearman’s $\rho=0.412$, $R^2=0.6379$ /one-tailed p-value=0.028). These correlations were much greater than those of global IQ demonstrating high specificity of DWI-MAP classification to detect specific patterns of language impairment in GD children. More interestingly, we found that the fronto-temporal pathway segment (“C₂: Broca’s area-Wernicke’s area”) was specifically correlated with performance and verbal IQs (Spearman’s $\rho=0.478$, $R^2=0.6724$ /one-tailed p-value=0.023 and Spearman’s $\rho=0.467$, $R^2=0.6619$ /one-tailed p-value=0.024, respectively).

DISCUSSION

The present study, although preliminary, suggests that the pattern of language network abnormality (i.e., the degree of decrease in DWI-streamline volume of specific segments of the arcuate fasciculus) may differ with specific mutations in children with GD. In three specific language pathway segments, C_{1,2,4}, MID1, CDK4, EN2 and MECP2 had a similar pattern of volume ratio while SFRP1 and RXRG-GLRB had a different pattern of volume ratio. The ROC analysis found that the fiber volume of C_{1,2,4} may be an effective imaging marker to achieve a high accuracy for the classification of GD. Indeed, the fiber volume of C₁ and C₄ showed strong correlation with expressive language score and receptive language score, respectively, indicating that different alterations of C_{1,2,4} may aid in understanding the biology of language impairment in GD and may assist in the establishment of detailed and specific genotype-phenotype relationships of GD. Interestingly, the same pattern of volume ratio can be seen with mutations directly regulating axon guidance pathways (EN2, MID1) or those with broader neuronal effects (MECP2, CDK4). This suggests that converging genetic/neurologic mechanisms may operate to produce similar patterns in DWI-MAP analysis. This analysis found a strong correlation of individual language pathway branches, with genetic abnormalities and specific language impairments. This finding, albeit in a small number of subjects, suggests that the integration of exome sequencing and DWI-MAP may be a novel and potentially powerful approach to link objective imaging metrics of semantic/phonologic/orthographic/fluency language pathways to different genetic variants linked to GD.

AF is the most recently developed white matter tract evolutionarily and is likely to be more prone to become dysfunctional with mutations of genes involved in white matter development (24). Thus, a detailed mapping of abnormalities in AF and its branches is likely to be particularly important in refining genotype-phenotype relationships in GD. Recent studies reported that the frontal aslant tract connecting Broca's area to pre-supplemental motor area (i.e., C₁) is a key pathway to affect verbal fluency and grammar(8, 25) suggesting that fine mapping of individual AF branches will facilitate the association of these tracts to specific clinical phenotypes.

Some interesting genetic mechanisms explain the plausibility of the observed genotype-phenotype relationships in this study. For example, MID1 and EN2 are transcription factors that are known to regulate axon guidance pathways (26, 27). Certain mutations in MID1 are known to cause variable developmental abnormalities including callosal dysgenesis (28). MID1 downregulates PP2AC and loss of function of MID1 results in accumulation of PP2AC causing abnormal axonal development (29). Similarly, EN2 regulates the expression of Ephrin A5, an axon guidance molecule. Abnormal ephrin signaling is implicated in misrouting of retino-tectal (30) and thalamo-cortical pathways (31). EN2 is also known to cause autism (32), suggesting its importance to language pathways. Thus, the present study localizes connectivity abnormalities to the specific branches of language pathways.

However, the present study has two main limitations for clinical translation. First, the current DWI-MAP classifier was limited in that it could only detect the portion of the language system mainly associated with direct/indirect AF segments in the left hemisphere. Other language areas such as the basal temporal area were not included (33). Second, the small sample size limits for a power analysis to present reliable statistics ensuring the correlation between genotype and DWI-MAP phenotype. GD is genetically highly heterogeneous with almost all causal mutations being very rare in the population. This makes it difficult to find multiple patients with the same type of gene mutation. Thus, the present study is a preliminary work to investigate if five different types of genetic mutations may share common patterns of dysmorphology in different branches of language pathways. Using larger cohorts, future studies should be designed to acquire comprehensive, evidence-based, correlative data of DWI-MAP classification, genotype and neuropsychological assessment in order to investigate neurological origins of language impairment in specific domains such as expression, comprehension, and reasoning.

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Figure 1.

Five DWI-MAP language pathway segments of five TD children ("C₁: Broca's area to premotor area", "C₂: Broca's area to Wernicke's area", "C₃: premotor to inferior parietal area", "C₄: Wernicke's area to inferior parietal area in left hemisphere, and "C₅: premotor area to Wernicke's area").

Figure 2.

Five DWI-MAP language pathway segments of six GD children having different mutations: MID1, CDK4, SFRP1, EN2, RXRG-GLRB, and MECP2 ("C₁: Broca's area to premotor area", "C₂: Broca's area to Wernicke's area", "C₃: premotor to inferior parietal area", "C₄: Wernicke's area to inferior parietal area in left hemisphere, and "C₅: premotor area to Wernicke's area"). For each GD child, the z-scores were assessed in five different pathway segments obtained from DWI-MAP analysis. Each image was enclosed by different color where red, orange, yellow and blue indicate different scales of z-score = -3, -2, -1 and 0, respectively.

Figure 3.

Affinity propagation clustering plots of two pair-wise ratios, $[C_2, C_4]$ and $[C_1, C_2]$ maximizing the similarity in six mutations (top row) and entire samples (bottom row). The color of each dot indicates the gene belonging to an identical cluster whose exemplar was marked by an asterisk.

Figure 4.

ROC curves indicating the performance of a binary classification (GD vs. TD) obtained from individual fiber ratios, “C₁: Broca’s area to premotor area”, “C₂: Broca’s area to Wernicke’s area”, “C₃: premotor to inferior parietal area”, “C₄: Wernicke’s area to inferior parietal area”, and “C₅: premotor area to Wernicke’s area”. AUC stands for an area under the curve which corresponds to the probability of correct classification. C₄ provided an excellent test (i.e., AUC $\approx$ 1), whereas C₁, C₂ and C₃ performed a good test (i.e., AUC > 0.8).

Figure 5.

Neuropsychological correlates with functional language pathway segments obtained from DWI-MAP classifier (“C₁: Broca’s area to premotor area”, “C₂: Broca’s area to Wernicke’s area”, “C₃: premotor to inferior parietal area”, “C₄: Wernicke’s area to inferior parietal area”, and “C₅: premotor area to Wernicke’s area”). Radar plots show correlation coefficients (R) between verbal (or performance) IQ and fiber ratios of DWI-MAP language pathway segments. The value of R was assessed in each of the five different branches (blue line) obtained from DWI-MAP analysis. No correlation (R= 0) is indicated by a black line. Note that the frontal pathway (C₁), temporal pathway (C₄), and fronto-temporal pathway (C₂) are specifically correlated with expressive language score, receptive language score, and verbal (or performance) IQ, respectively.

Table 1

GD patient demographics and neuropsychological assessments.

Mutations	Age (months)	Gender	Global IQ	Verbal IQ	Performance IQ	Receptive language	Expressive language
CDK4	26	M	70	N.A	N.A	72	64
MID1	101	F	64	45	82	75	70
MECP2	69	F	50	45	45	45	45
SFRP1	64	F	68	68	72	70	70
EN2	130	F	88	76	100	82	72
RXRGG-LRB	78	M	62	N.A	N.A	N.A	N.A

*
N.A: Not available

Table 2

Accuracy of fiber ratio of C₁₋₅ to differentiate children with GD having different mutations from healthy children.

Language pathway	Cut-off value	Sensitivity	Specificity	Accuracy
C ₁	0.0951	0.8333	1.0000	0.9091
C ₂	0.0996	0.8333	1.0000	0.9091
C ₃	0.0191	0.8333	0.8000	0.8182
C ₄	0.1460	1.0000	1.0000	1.0000
C ₅	0.0493	0.6667	1.0000	0.8182