Exploring the Interplay of Sequence and Structural Features in Determining the Flexibility of AGC Kinase Protein Family: A Bioinformatics Approach

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Abstract

In this study, data mining approach was used to generate association rules for predicting average flexibility from the various derived sequence and structural features. 21 parameters were calculated and their variable importance was calculated for 115 sequences of AGC kinase family belonging to mouse and human using Classification and Regression Tree (CART). Beta turns were found to have maximum influence on average flexibility while the total beta strands were found to exert minimum impact on average flexibility. Understanding the variable importance will prove useful as a simple predictor of flexibility from an amino acid sequence. This will aid in better understanding of phenomenon underlying the average flexibility and thus, will pave a way for rational design of therapeutics.

Key words: AGC kinase; Protein flexibility; Data mining; Classification and Regression Tree (CART); Bioinformatics

Introduction

Every biological molecule is characterized and set apart from other biomolecules by a definite set of inherent intrinsic properties. Being the determinant of some vital functions like transport of metabolites (Anderson et al., 1990; Spurlino et al., 1991), catalysis (Bennett and Steitz, 1978; Remington et al., 1982) and regulation of protein activity (Perutz, 1970; Perutz, 1989) etc, average flexibility holds prime importance. Eukaryotic proteins demonstrate higher flexibility which influence conformational liability required in important biological processes like molecular recognition, interaction, assembly and modification. Moreover, protein flexibility is also known to influence stability and folding. There has been a sudden spur of interest in studies related to flexibility of proteins owing to discovery of role of some highly flexible proteins with implications in life threatening diseases like AIDS (HIV gp41) and scrapie (Chan et al., 1997). A comprehensive knowledge of fundamental nature of average flexibility will facilitate the unraveling of structure-function relationship and will also aid in development of novel therapeutics (Teague, 2003).

AGC protein kinase family, one among the eight ePK families defined in the Kinbase, includes many important enzymes such as cyclic nucleotide and calcium-phospholipid dependent kinases, ribosomal S6 phosphorolyzing kinases, G protein-coupled kinases, and few others. The AGC serine threonine kinases, known for phosphorylating sites surrounded by basic amino acids, are involved in many intra–cellular signaling pathways, critical cellular processes and control cell growth, differentiation and cell survival. Their crucial role in transmembrane signaling process hints on the importance of features of AGC kinases which may be responsible for membrane localization (Peterson and Schreiber, 1999). This group of protein kinases shares similarity within the catalytic domain and is characterized by similar mechanism of activation. Deregulation of AGC kinases is known to have implications in several diseases like Cancer, Diabetes, neurodegeneration, and thus, AGC kinases represent several attractive targets for small inhibitors of therapeutic significance (Breitenlechner, 2003).

Their stringent spatio-temporal regulation is attained through loop phosphorylation and repositioning of the key catalytic and substrate binding regions which indicates the importance of flexibility in these proteins (Kannan et al., 2007). There is preponderance of literature on flexibility of proteins but elucidating the effect of parameters influencing it is cumbersome. This study aims at exploring the importance of different parameters influencing the average flexibility of AGC kinase family using data mining approach.

Materials and Methods

Sequence Collection and Pre-Processing

Protein sequences of the enzymes belonging to AGC family of protein kinase super family in FASTA format were collected from the non redundant (NR) protein database of NCBI (http://www.ncbi.nlm.nih.gov). Partial sequences were excluded from the study and sequences were again put to manual filtering so as to minimize the redundancy. This approach resulted in 600 sequences from the total 1259 sequences of AGC family available in the database were obtained. Out of these, sequences belonging to Homo sapiens (59) and Mus musculus (56) were considered for this study.
| Parameter                  | Mean  | Standard Deviation | Skewness | Coefficient of variation | Variance | Kurtosis | Standard Error Mean |
|----------------------------|-------|--------------------|----------|--------------------------|----------|----------|---------------------|
| Accessible residues       | 5.8171| 0.42102            | 5.0288   | 0.072376                 | 0.17725  | 40.439   | 0.03926             |
| Buried Residues           | 5.7892| 0.72877            | -4.2973  | 0.12588                  | 0.5311   | 25.436   | 0.067958            |
| Amino acid composition    | 5.786 | 0.19749            | -0.034656| 0.034133                 | 0.039003 | -        | 0.15092             |
| Alpha helix               | 1.0192| 0.03128            | 4        | 1.4608                   | 0.030695 | 0.0009787| 9.3437             |
| Beta sheet                | 0.9709| 0.02598            | -0.20939 | 0.026761                 | 0.0006751| 1.077    | 0.002422            |
| Beta turn                 | 1.02  | 0.02791            | -0.11458 | 0.027365                 | 0.0007791| -        | 0.24003             |
| Coils                     | 1.0387| 0.0309             | 0.39441  | 0.029749                 | 0.0009548| -        | 0.40818             |
| Parallel Beta strands     | 1.0625| 0.05008            | 5        | 0.045298                 | 0.047139 | 0.0025085| -                  |
| Anti parallel beta strands| 0.9799| 0.03351            | -0.38504 | 0.034201                 | 0.0011231| -        | 0.003125            |
Table 1: Basic statistical features of parameters considered in the study.

| Parameter                  | Mean   | Standard Deviation | Minimum | Maximum | Median   | Range     | Q3       | Q1       |
|----------------------------|--------|--------------------|---------|---------|----------|-----------|----------|----------|
| Trans-membrane Tendency    | -0.5891| 0.27052            | 5.5183  | -0.45921| 0.07318  | 45.421    | 0.02     | 5226     |
| Total Beta strands         | 0.98868| 0.030955           | -0.56077| 0.031309| 0.000958 | 0.31456   | 0.002886 | 0.5      |
| Relative mutability        | 76.674 | 2.9206             | -0.085732| 0.038091| 8.53     | -0.19263  | 0.27235  |
| Refractivity               | 16.212 | 1.3109             | 0.12774 | 0.080856| 1.7184   | 0.27699   | 0.12224  |
| Recognition Factors        | 88.918 | 1.4693             | 0.43693 | 0.016525| 2.159    | -0.42356  | 0.13702  |
| Polarity                   | 19.936 | 1.9885             | 0.2598  | 0.099744| 3.954    | -        | 0.022502 | 0.18543  |
| Number of Codons           | 3.572  | 0.24312            | -2.0097 | 0.068063| 0.059107 | 11.473    | 0.022671 |
| Molecular weight           | 130.19 | 3.7174             | -0.33221| 0.028553| 13.819   | 1.203     | 0.34665  |
| Hydrophobicity             | -0.41118| 0.35214           | 2.7344  | -0.8564 | 0.124    | 15.724    | 0.032837 |
| Bulkiness                  | 14.261 | 1.1952             | -6.3417 | 0.083806| 1.4284   | 54.206    | 0.11145  |
| Average Area buried        | 124.92 | 7.8686             | -6.3319 | 0.062987| 61.915   | 55.828    | 0.73375  |
| Average Flexibility        | 0.44019| 0.0060555          | -0.11045| 0.013757| 3.6669e-005| 0.57539   | 0.000564 | 68       |
Figure 1: Frequency distribution chart for different parameters generated in CART 14 trees with different complexities and error values obtained using CART based on splitting criteria are reflected in table 2. Out of these trees, tree with 21 terminal nodes with minimum complexity and re-substitution relative error of 0.08501 and cross validated error of 0.72543 ± 0.12560 generated by Least Square splitting criteria was selected for generating decision rules. The topology of tree and error rate is represented in Figure 2. Splitters for the regression tree are shown in Figure 3. Decision rules obtained using CART are summarized in table 3(Supplement).
### Table 2: Details of trees generated in CART along with relative error and complexities

| Tree No. | Terminal Nodes | Cross-Validated Error       | Resubstitution Relative Error | Complexity |
|----------|---------------|-----------------------------|-------------------------------|------------|
| 1        | 21            | $0.72543 \pm 0.12560$       | $0.08501$                     | 0.00000    |
| 2        | 20            | $0.71808 \pm 0.12370$       | $0.08653$                     | 1.00000E-005 |
| 3        | 19            | $0.71000 \pm 0.11971$       | $0.08899$                     | 0.00002    |
| 4        | 15            | $0.67935 \pm 0.11594$       | $0.11571$                     | 0.00003    |
| 5        | 13            | $0.66759 \pm 0.11029$       | $0.14635$                     | 0.00007    |
| 6        | 11            | $0.66746 \pm 0.11162$       | $0.18358$                     | 0.00008    |
| 7        | 9             | $0.65670 \pm 0.11209$       | $0.22481$                     | 0.00009    |
| 8        | 8             | $0.57881 \pm 0.09948$       | $0.25020$                     | 0.00012    |
| 9        | 6             | $0.60897 \pm 0.08204$       | $0.35804$                     | 0.00023    |
| 10       | 5             | $0.66411 \pm 0.09268$       | $0.41964$                     | 0.00027    |
| 11       | 4             | $0.89325 \pm 0.08412$       | $0.52601$                     | 0.00045    |
| 12       | 3             | $0.92470 \pm 0.08126$       | $0.65254$                     | 0.00054    |
| 13       | 2             | $0.91504 \pm 0.07452$       | $0.78894$                     | 0.00058    |
| 14       | 1             | $1.00139 \pm 0.00159$       | $1.00000$                     | 0.00089    |
Figure 2: The tree sequence of lowest complexity which yielded 21 terminal nodes (A) with the cross validation error rate (B) and terminal node box plot (C).

Rules derived from CART can be interpreted in simple context of “If “and “Then” based statement and thus are self-explanatory.

For example: Rule 1 can be interpreted as:

**Rule 1:** IF “BULKINESS <= 14.2207” & “ALPHA-HELIX <= 1.01975” & “A.A COMPOSITION <= 5.55”, THEN “AVERAGE FLEXIBILITY=0.457”.

Figure 3: Details of splitter for the Decision tree

Rules derived from CART can be interpreted in simple context of “If “and “Then” based statement and thus are self-explanatory.

For example: Rule 1 can be interpreted as:

**Rule 1:** IF “BULKINESS <= 14.2207” & “ALPHA-HELIX <= 1.01975” & “A.A COMPOSITION <= 5.55”, THEN “AVERAGE FLEXIBILITY=0.457”.

**Rule 14:** IF “RECOGNITION FACTORS <= 89.4723” & “TRANSMEMBRANE TENDENCY <= -54225” & “ALPHA-HELIX > 1.01975” & “TOTAL BETA-STRAND > 0.95975 & <= 1.018” & “A.A Composition <= 6.0055” & “RELATIVE MUTABILITY <= 80.0835”, THEN “AVERAGE FLEXIBILITY = 0.436563”

**Variable importance**

Importance of different variables was calculated based on predefined scores in CART and summarized in Table 4.
| S. No. | VARIABLE                                | IMPORTANCE |
|-------|-----------------------------------------|------------|
| 1.    | BETA-TURN (CHOU & FASMAN)               | 100.00     |
| 2.    | % ACCESSIBLE RESIDUES                   | 93.57      |
| 3.    | ALPHA HELIX (CHOU & FASMAN)             | 86.18      |
| 4.    | TRANSMEMBRANE TENDENCY                  | 78.43      |
| 5.    | AMINOACID COMPOSITION                   | 71.15      |
| 6.    | BULKINESS                               | 55.69      |
| 7.    | COIL (DELEAGE & ROUX)                   | 50.69      |
| 8.    | PARALLEL BETA-STRAND                    | 50.03      |
| 9.    | RECOGNITION FACTORS                     | 49.06      |
| 10.   | MOLECULAR WEIGHT                        | 34.84      |
| 11.   | POLARITY (ZIMMERMAN)                    | 33.05      |
| 12.   | HYDROPHOBICITY (KYTE & DOOLITTLE)       | 32.08      |
| 13.   | AVERAGE AREA BURIED                     | 29.71      |
| 14.   | REFRACTIVITY                            | 29.16      |
| 15.   | BETA SHEET (CHOU & FASMAN)              | 27.81      |
| 16.   | NUMBER OF CODONS                        | 21.31      |
| 17.   | %BURIED RESIDUES                        | 17.72      |
| 18.   | RELATIVE MUTABILITY                     | 2.37       |
| 19.   | TOTAL BETA STRAND                       | 1.14       |
| 20.   | ANTI-PARALLEL BETA STRAND               | 0          |

**Table 4:** Variable importance of parameters influencing average flexibility.
Discussion

Dynamic nature of proteins, conferred by their structural flexibility, is associated with function. Average flexibility, an innate property of proteins is being recognized with implications in many important physiological processes recently (Wright and Dyson 1999; Bright et al. 2001; Dunker et al. 2001; Namba 2001). Recognition of several highly flexible proteins in some pathological conditions have led to the momentum in studies related to the flexibility of proteins. The huge gap in number of sequence and structures in PDB limits the utilization of 3-dimensional structure for deriving features affecting flexibility like B-factors. In unavailability of such data, sequence composition and secondary structure provides a rough estimation of structural properties. This warrants the need for an alternate and simplistic approach for determining the effect of various parameters on average flexibility in an easy to understand quantitative relationship. Data mining approaches based on decision tree based methods have been successfully exploited in elucidating importance of features affecting important biological processes (Banerjee et al., 2007). CART has been exploited in microarray studies (Boulesteix et al., 2003), ecological studies (De’ath & Fabricius, 2000), risk prediction (Gottschalk et al., 1998), diseases diagnosis (Hermanek & Holzmann., 1994) and social studies (Oge et al., 2004).

The dataset comprising of various derived features was used to elucidate decision rules by CART that can serve as rule of thumb for finding the effect of different parameters on average flexibility, which is virtually impossible to calculate in a lab simultaneously using conventional approaches. Among the secondary structure features, beta turn, parallel beta sheet, and total beta strands were found to influence the average flexibility in descending order. Among sequence features, % accessible residues, trans-membrane tendency, amino acid composition, bulkiness, recognition factors, molecular weight, polarity, hydrophobicity, average area buried, refractivity, no. of codons, % buried residues, and relative mutability were observed to affect the average flexibility in decreasing order(Table 4). Beta turns were found to have maximum impact while total beta strand, beta sheet and total beta strands were found to have minimum effect on average flexibility of the proteins considered in the study. As more and more studies are advocating the inclusion of protein flexibility in docking algorithms, it will be interesting to gain an insight on features influencing the flexibility of proteins. It is speculated that an extensive knowledge of protein flexibility and the various parameters contributing towards is important for rational drug design. Such an approach will lead to better understanding of underlying biological phenomena and aid in enzyme engineering processes.

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References

1. Anderson BF, Baker HM, Morris GE, Rumball SV, Baker EN (1990) Apolactoferrin structure demonstrates ligand-induced conformational change in transferrins. Nature 344: 784–787.
2. Banerjee AK, Arora N, Murty USN (2007) Stability of ITS2 Secondary Structure in Anopheles: What Lies Beneath? International Journal of Integrative Biology 3: 232-238.
3. Bennett WS Jr, Steitz TA (1978) Glucose-induced conformational change in yeast hexokinase. Proc Natl Acad Sci USA 75: 4848–4852.
4. Bhaskaran R, Ponnumswamy PK (1988) Positional flexibilities of amino acid residues in globular proteins. J Pept Prot Res 32: 242-255.
5. Boulesteix AL, Tutz G, Strimmer K (2003) A CART-based approach to discover emerging patterns in microarray data. Bioinformatics 19: 2465-2472.
6. Breiman L, Friedman JH, Olshen RA, Stone CJ (1984) Classification and regression trees. Chapman & Hall New York NY.
7. Breitenlechner C, Gabel M, Engh R, Bossemeyer D (2003) Structural Insights Into AGC Kinase Inhibition. Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics 14: 267-278.
8. Bright JN, Woolf TB, Hoh JH (2001) Predicting properties of intrinsically unstructured proteins. Prog Biophys Mol Biol 76: 131–173.
9. Chan DC, Fass D, Berger JM, Kim PS (1997) Core structure of gp41 from the HIV envelope glycoprotein. Cell 89: 263–273.
10. Chou PY, Fasman GD (1978) Prediction of the secondary structure of proteins from their amino acid sequence. Adv Enzymol Relat Areas Mol Biol 47: 45-148.
11. Dayhoff MO, Schwartz RM, Orcutt BC (1978) A model of evolutionary change in protein; in: M.O. Dayhoff (Ed.), Atlas of Protein Sequence and Structure, Nat. Biomed. Res Foundation Washington DC 5 Suppl 3: 345–352.
12. Death G, Fabricius KE (2000). Classification and regression trees: a powerful yet simple technique for ecological data analysis, Ecology 81: 3178–3192.
13. Deléage, Roux (1987) An algorithm for protein secondary structure prediction based on class prediction. Protein Engineering Design and Selection 1: 289-294.
14. Dunker AK, Lawson DJ, Brown CJ, Williams RM, Romero P, JS Oh, Oldfield CJ, Campen AM, Ratliff CM, Hippis KW, etal. (2001) Intrinsically disordered protein. J Mol Graph Model 19: 26–39.
15. Fraga S (1982) Theoretical prediction of protein. antigenic determinants from amino acid sequences. Can J Chem 60: 2606-2610.
16. Gottschalk KW, Colbert JJ, Feicht DL (1998) Tree mortality risk of oak due to gypsy moth. European Journal of Forest Pathology 28: 121-132.
17. Hermanek P, Guggenmos-Holzmann I (1994) Classification and regression trees (CART) for estimation of prognosis in patients with gastric carcinoma. J Cancer Res Clin Oncol 120: 309–313.
18. Joël Janin (1975) Amino acid properties and side-chain orientation in proteins: a cross correlation approach. J Theor Biol 50: 167-83.
19. Jones DD (1975) Amino acid properties and side-chain orientation in proteins: a cross correlation approach. J Theor Biol 50: 167-83.
20. Kannan N, Haste N, Taylor SS, Neuwald AF (2007) The hallmark of AGC kinase functional divergence is its C-terminal tail, a cis-acting regulatory module. Proc Natl Acad Sci USA 104: 1272-1277.
21. Kyte J, Doolittle RF (1982) A simple method for displaying the hydrophobic character of a protein. J Mol Biol 157: 105-132.
22. Lifson S, Sander C (1979) Antiparallel and parallel-strands differ in amino acid residue preferences. Nature 282: 109-111.
23. McCaldon P, Argo P (1988) Oligopeptide biases in protein sequences and their use in predicting protein coding regions in nucleotide sequences. Proteins: Structure Function and Genetics 4:99-122.

24. Namba K (2001) Roles of partially unfolded conformations in macromolecular self-assembly. Gene Cells 6:1-12.

25. Ozge C, Toros F, Bayramkaya E, Camdeviren H, Sasmaz T (2006) Which sociodemographic factors are important in smoking behaviour of high school students? The contribution of classification and regression tree methodology in a broad epidemiological survey. Postgraduate Medical School 82:532-541.

26. Parker PJ, Parkinson SJ (2001) AGC protein kinase phosphorylation and protein kinase C. Biochemical Society Transactions 29:860-863.

27. Perutz MF (1989) Mechanisms of cooperativity and allosteric regulation in proteins. Q Rev Biophys 22:139-237.

28. Perutz MF (1970) Stereochemistry of cooperative effects in haemoglobin. Nature 228:726-739.

29. Peterson RT, Schreiber SL (1999) Kinase phosphorylation: Keeping it all in the family. Curr Biol 9:R521-4

30. Remington S, Wiegand G, Huber R (1982) Crystallographic refinement and atomic models of two different forms of citrate synthase at 2.7 and 1.7 Å resolution. J Mol Biol 158:111-152.

31. Rose GD, Geselowitz AR, Lesser GJ, Lee RH, Zehhus MH (1985) Hydrophobicity of amino acid residues in globular proteins. Science 229:834-838.

32. Spurlino JC, Lu GY, Quirocho FA (1991) The 2.3-Å resolution structure of the maltose- or maltodextrin-binding protein, a primary receptor of bacterial active transport and chemotaxis. J Biol Chem 266:5202-5219.

33. Teague SJ (2003) Implications of protein flexibility for drug discovery. Nat Rev Drug Discov 2:527-41.

34. Wright PE, Dyson HJ, (1999) Intrinsically Unstructured Proteins: Re-assessing the Protein Structure-Function Paradigm. J Mol Biol 293:321-331.

35. Zhao G, London E (2006) An amino acid “transmembrane tendency” scale that approaches the theoretical limit to accuracy for prediction of transmembrane helices: Relationship to biological hydrophobicity. Protein Sci 15:189-201.

Accession numbers of the considered AGC kinase protein sequences are as follows:

O70291.1, POC605.1, P16054.1, P18654.2, P23298.1, P31750.1, P54265.1, P68181.2, P70268.3, P70336.1, Q3UU96.2, O70293.1, P05132.3, P18653.1, P20444.3, P28867.3, P49025.3, P63318.1, P68404.3, P70335.1, Q3U214.1, Q3UYYH7.1, Q7TPS0.2, Q7TSE6.1, Q7TSJ6.1, Q7TT50.1, Q8BSK8.1, Q8BW9.2, Q8BYR2.2, Q8C0P0.1, Q8C050.2, Q8K045.1, Q8VEB1.2, Q9ERE3.1, Q9QZS5.1, Q9R1L5.3, Q9WUA6.1, Q9WUT3.1, Q9WVC6.1, Q9WVL4.1, Q9Z0Z0.1, Q9Z1M4.1, Q9Z2A0.2, Q9Z2B9.1, Q8OUW5.2, Q91VJ4.1, Q99MK8.2, Q811L6.2, Q922R0.1, Q02111.1, Q02956.1, Q60592.1, Q60823.1, Q61410.1, Q62074.2, P41743.1, P43250.2, P51812.1, P51817.1, Q02156.1, Q16513.1, Q16512.1, Q15835.1, Q15418.2, Q15349.2, Q15208.1, Q13976.3, Q13464.1, Q13237.1, CAE55958.1, NP_443073.1, O00141.2, O15457.8, O15021.2, O15530.1, O60307.2, O75116.3, O75582.1, O75676.1, Q95835.1, P05129.3, P05771.4, P14619.1, P17252.3, P17612.2, P22612.3, P22694.2, P22433.2, P24256.1, P24273.2, P25098.2, P31749.2, P31751.2, P32298.3, P34947.1, P35626.2, Q09013.1, Q05655.1, Q05513.4, Q04759.3, Q96GX5.1, Q96BR1.1, Q9Y243.1, Q9Y25S2.2, Q9Y2H9.2, Q9Y2H1.3, Q9UK32.1, Q9UBS0.1, Q9NRM7.1, Q9HY8.1, Q8WTQ7.1, Q9PZ22.1, Q6PQ8.2, Q6DT37.1, Q5VT25.1.
| Node | Bulkiness | Polarity | Recognition factors | Trans membrane tendency | % Accessible residues | Alpha-helix | beta-sheet | Coll | Total beta-strand | Anti Parallel beta-strand | Paralleled beta-strand | A.A. composition | Relative mutability | Average flexibility |
|------|-----------|---------|---------------------|------------------------|----------------------|------------|-----------|------|------------------|-------------------------|-------------------------|----------------|------------------|------------------|
| 1    | <= 14.2207 |        |                     |                        |                      | <=         |           |      | <= 5.55          |                         |                         |                | 0.457            |                  |
| 2    | <= 14.2207 |        |                     |                        |                      | <=         |           |      | <= 0.977         | > 5.55 & <= 5.55625      |                         |                | 0.4494          |                  |
| 3    | <= 14.2207 | <= 90.611 |                   |                        |                      | <=         |           |      | > 0.977          | > 5.55625               |                         |                | 0.447667        |                  |
| 4    | <= 14.2207 | <= 90.611 |                   |                        |                      | <=         | <= 0.9825 | > 0.977 | > 5.55625       |                         |                         |                | 0.443143        |                  |
| 5    | <= 14.2207 | <= 90.611 |                   |                        |                      | <=         | > 0.9825  | > 0.977 | > 5.55625       |                         |                         |                | 0.441429        |                  |
| 6    | <= 14.2207 | > 90.611 |                   |                        |                      | <=         | > 0.977   | > 5.55  |                 |                         |                         |                | 0.4479          |                  |
| 7    | > 14.2207  | <= 19.9293 |                 |                        |                      | <=         |         1.01975 | <= 1.0425 |                     |                         |                         |                | 0.4336          |                  |
| 8    | > 14.2207  | <= 19.9293 |                 |                        |                      | <=         |         1.01975 | > 1.0425 |                     |                         |                         |                | 0.438722        |                  |
| 9    | > 14.2207  | > 19.9293 |                   |                        |                      | <=         |         1.01975 | <= 0.97275 |                     | <= 5.68875              |                         |                | 0.4419          |                  |
| 10   | > 14.2207  | > 19.9293 |                   |                        |                      | <=         |         1.01975 | <= 0.97275 |                     | > 5.68875               |                         |                | 0.444667        |                  |
Association rules obtained in CART

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|   | 0.4402 | 0.4407 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 | 0.4485 |
| 11 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 | <= 6.0055 |
| 12 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 | <= 89.4723 |
| 13 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 | > -0.54225 |
| 14 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 | 1.01975 |
| 15 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 |
| 16 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 |
| 17 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 |
| 18 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 |
| 19 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 |
| 20 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 |
| 21 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 | <= 6.0055 | > 1.018 |

Table 3: Association rules obtained in CART