Journal of
Proteomics & Bioinformatics

Rauf and Mir, J Proteomics Bioinform 2012, 5:10
DOI: 10.4172/jpb.1000245

Rapid Communication Open Access

# Phylogenetic Analysis of ASPM, a Major Contributor Gene of Microcephaly

**Sobiah Rauf and Asif Mir***

*Bioinformatics & Biotechnology, DES, International Islamic University, Islamabad, Pakistan*

**Abstract**

ASPM gene at MCPH5 locus is considered a major causative gene of autosomal recessive primary microcephaly (MCPH), which is a rare neurodevelopemental disorder that affects head and brain size. Mutations in this gene have contribution of more than 50% in causing MCPH for which seven loci (MCPH1 to MCPH7) have been discovered so far. The current study includes bioinformatics analysis of ASPM gene at MCPH5 locus. Bioinformatics analysis includes syntenic relationship of ASPM and its phylogenetic studies with reference to various selected orthologs. These studies have revealed information about conservation of genes among different ortholog species and their evolutionary relationship.

## Introduction

Autosomal Recessive Primary Microcephaly (MCPH) is a rare neurodevelopmental disorder in which individuals are born with small head size. Head circumference of MCPH patients reduces at least 3 standard deviations below the mean for a given age, sex, race and gestation [1-3]. It is a genetically heterogeneous disorder for which seven loci (MCPH1-MCPH7) with the corresponding genes (MCPH1, WDR62, CDK5RAP2, CEP152, ASPM, CENPJ, and STIL) have been discovered so far from different world populations and have been mapped [4,5]. Studies in Drosophila revealed the influence of mutations in MCPH genes on asymmetrical cell division leading to a reduced central nervous system neuronal growth during embryogenesis [4-7]. Individuals affected with MCPH have less weight and volume of brain and a reduced cerebral cortex. Although size reduces but the gyral pattern remains well preserved and there is no major affect on cortical architecture [8,9]. Beside a reduced cerebral cortex and mild-to-moderate mental retardation primary microcephaly patients display no other developmental or neurological deficits [5,10]. Sloping foreheads and reduced intelligence are common criteria for the diagnosis of microcephaly [4,10].

According to Mahmood et al. [4], ASPM (at MCPH5 locus) and WDR62 (at MCPH2 locus) are the two most common genes for primary microcephaly found mutated in more than 55-60% of the affected families. The ASPM (Abnormal Spindle-like Microcephaly Associated) gene consisting of 3477 amino acids contains 28 exons [11-13]. The loci for MCPH5 on chromosome 1q31.3 have already been discovered in 2000 by Jamieson et al. [14]. ASPM is an important and specific regulator gene which regulates the brain size [15]; Progenitors are important for the expansion of cerebral cortex size. ASPM being a part of mitotic spindle may control progenitor's proliferative symmetry [16]. During the neurogenic cycle, ASPM is preferentially expressed in the neuroepithelium of the lateral ventricles. This is the fact which supports its role in human neurogenesis [17].

In MCPH patients, mutated ASPM gene causes a mitotic defect which is specific to the brain that affects its size [18]. In model organism mice, ASPM gene studies were carried out. These studies have shown that in the regions of active neurogenesis, expression of ASPM is maximum and it is down regulated on the completion of neurogenesis. This also indicates the involvement of ASPM in neuron production [19]. According to Riparbelli et al. [20], ASPM is required in microtubule organization of the mitotic spindle poles and the central spindle in meiosis and mitosis in Drosophila [20]. Considering the above mentioned importance of ASPM gene, it can be hypothesized that during neurogenesis, it is involved in the organization of microtubules at the spindle pole during mitosis and during cytokinesis, it is involved at the central spindle [21].

## Materials and Methods

### Bioinformatics analysis

After linkage establishment to the known locus (MCPH5) in family, the findings were analyzed through following bioinformatics methods.

**Sequence retrieval:** The gene lies within MCPH5 locus at chromosome 1q31.3 having 28 functional exons and a genomic size of 62567 base pairs. The amino acid sequence of ASPM is 3477 residues long and was obtained from ensemble database (Ensemble Protein ID: ENSP00000356379; MIM #: 605481) [22].

**Genome syntenic relationship:** Synteny analysis was performed using Ensembl syntenyview in ensemble database [23] and the visual analysis of conserved regions was carried out using a web-based genome synteny viewer GSV [24].

**Phylogenetic tree reconstruction:** In the current study, MEGA5 [25] was used for phylogenetic tree reconstruction. We used neighbor joining (NJ) method and constructed tree for human ASPM gene of microcephaly. Sixteen ortholog species of Human have been considered in the current study as shown in Figure 1.

## Results

### Genome synteny analysis

In order to find out the genomic elements that are functionally conserved, we find out a set of conserved genomic features (genes or other genetic loci) in the same relative ordering on a set of homologous chromosomes (of human and its four orthologs). We studied

***Corresponding author:** Asif Mir, Bioinformatics & Biotechnology, DES, International Islamic University, Islamabad, Pakistan, E-mail: asif.mir@iiu.edu.pk

**Received** August 22, 2012; **Accepted** September 01, 2012; **Published** September 03, 2012

**Citation:** Rauf S, Mir A (2012) Phylogenetic Analysis of ASPM, a Major Contributor Gene of Microcephaly. J Proteomics Bioinform 5: 252-255. doi:10.4172/jpb.1000245

conservation of human 15 genes (both upstream and downstream of ASPM) with genes of its orthologs as shown in Table 1 (data collected from ensembl syntenyview in ensemble database). Human ASPM gene lies between 1:197053258 bp 197115824 bp, fifteen upstream genes of Human are between 192605275 bp to 197036397 bp while downstream genes are between 197127572 bp to 200843306 bp. Four orthologs which have been considered for this study are chimpanzee (*Pan troglodytes*), mouse *(Mus musculus),* dog *(Canis familiaris)* and chicken *(Gallus gallus).* Conserverd regions were also generated using genome synteny viewer GSV web server which produces graphical representations facilitating the quick visualization of conserved regions in the form of colored blocks with the ruler indicating positions of these conserverd regions (Figure 2: a, b, c, d). Our analysis showed that majority of the portion is conserved among two orthologs (chimpanzee, Chicken) in relevance to human then with some deletions in mouse and dog. Synteny analysis showed that there exists only one deletion in chimpanzee, three in chicken and six in mouse (in relevance to human) according to our synteny location map (Table 1). Maximum deletions (i.e. 7) exist in case of dog ortholog with respect to human. ASPM gene remained conserved in all four orthologs in relevance to human indicating importance of this gene. Changes which lead towards the evolution of these organisms are given in Table 2.

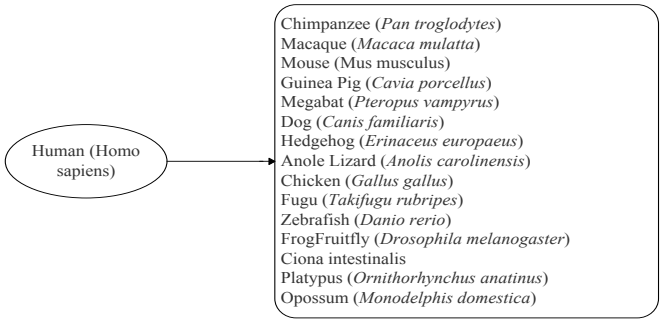


**Figure 1:** Sixteen Ortholog Species of Human Selected for Phylogenetic Analysis.

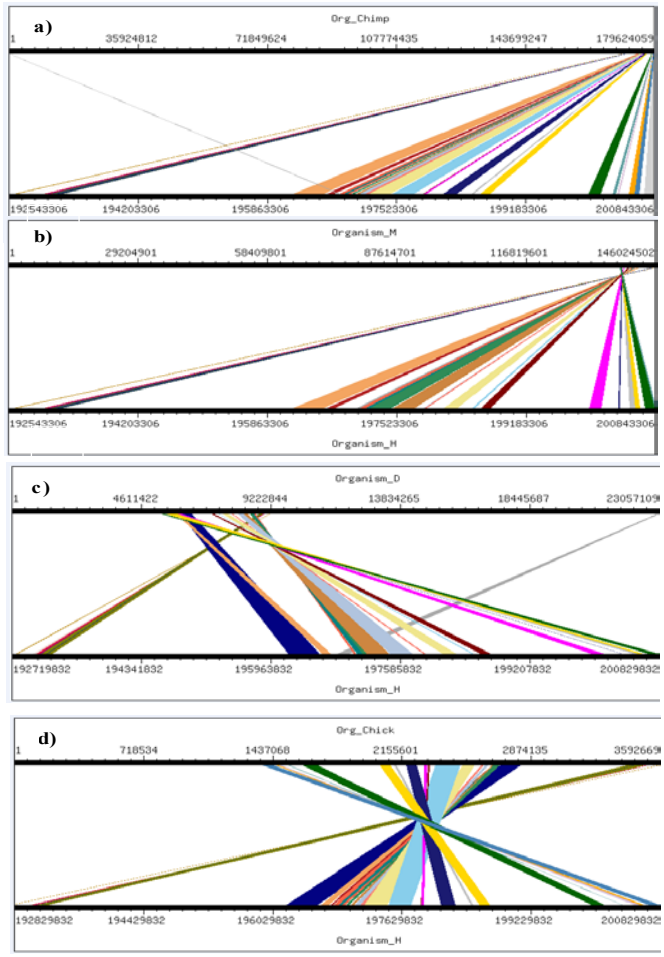


**Figure 2:** Results of GSV **a)** Conserved regions between Org_Chimp (Chimpanzee) and Organism_H (Human); **b)** Conserved regions between Organism_M (Mouse) and Organism_H (Human); **c)** Conserved regions between Organism_D (Dog) and Organism_H (Human); **d)** Conserved regions between Org_Chick (Chicken) and Organism_H (Human).

## Phylogenetic analysis

Neighbor joining method was used to construct the phylogenetic tree of Human ASPM gene using software MEGA5 as shown in Figure 3(a). Bootstrap analysis was also carried out which is an accurate way to control and check stability of results. In current study, bootstrap test uses 500 replicates and assigns each branch a value ranging from 0 to 100 which gives an idea that how much a sequence is evolutionary closer to each other and also validates each branch. The tree shows evolutionary relationship among human and its orthologs selected in the current study as shown in Figure 1. According to this tree, *Ciona intestinalis* is outgroup. Human is making cluster with Chimpanzee with 97 as a bootstrap value. Macaque and ancestor of Human/Chimpanzee have same ancestor from which they evolved. Macaque is evolving with a bootstrap value of 100 and is close to Human/Chimpanzee cluster. Anole Lizard, Fruitfly, Frog and Mouse have been deleted from the tree as they were not according to the time of divergence. The tree was

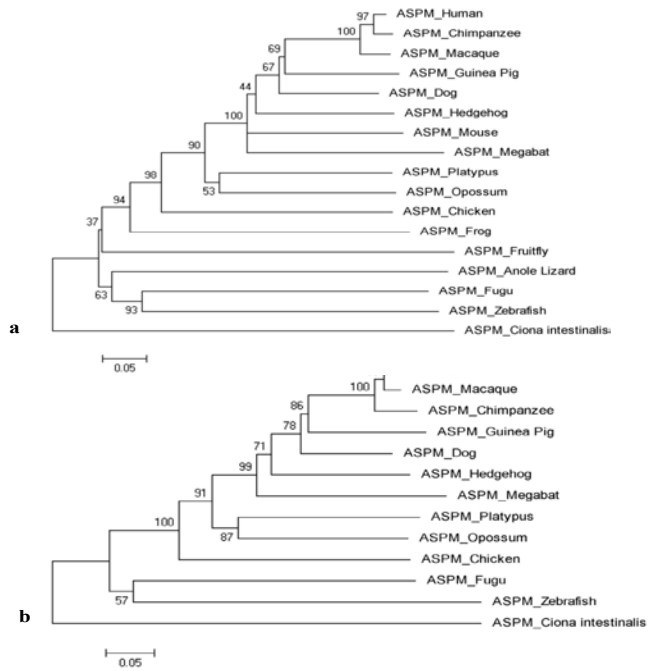


**Figure 3:** Neighbor Joining (NJ) Tree for Human ASPM using MEGA5; **a)** Original Tree, **b)** Reconstructed Tree. Numbers on branches represent Bootstrap Values (based on 500 replications).

| | Chimpanzee [Gene (Location)] | Mouse [Gene (Location)] | Human [Gene (Location)] | Dog [Gene (Location)] | Chicken [Gene (Location)] |
|---|---|---|---|---|---|
| Upstream Genes | RGS13 (1:171271723-171296304) | Rgs13 (1:145985797-146024502) | RGS13 (1:192605275-192629390) | No homologues | No homologues |
| | RGS2 (1:171446984-171450224) | Rgs2 (1:145846468-145851291) | RGS2 (1:192778169-192781403) | RGS2 (38:9200721-9205445) | Q7ZZS5_CHICK (8:3589865-3592669) |
| | UCHL5 1:171541538-171694397 | Uchl5 1:145624408-145654596 | UCHL5 1:192984889-193029237 | UCHL5 38:9004422-9043753 | UCHL5 (8:3533310-3546012) |
| | TROVE2 (1:171694878-171719735) | Trove2 (1:145597920-145624198) | TROVE2 (1:193028552-193060907) | TROVE2 (38:8981256-8993877) | TROVE2 (8:3522339-3528954) |
| | GLRX2 (1:171731504-171740886) | Glrx2 (1:145586159-145596806) | GLRX2 (1:193065598-193075244) | GLRX2 (38:8967853-8971492) | GLRX2 (8:3511466-3516178) |
| | CDC73 (1:171757336-171885799) | Cdc73 (1:145445927-145550023) | CDC73 (1:193091147-193223031) | A2SXS7_CANFA (38:8825461-8937816) | CDC73_CHICK (8:3416992-3512154) |
| | B3GALT2 (1:171813559-171821452) | B3GALT2 (1:145487794-145497536) | B3GALT2 (1:193148175-193155784) | B3GALT2 (38:8873830-8875098) | B3GALT2 (8:3457928-3459196) |
| | KCNT2 (1:174928575-175322202) | KCNT2 (1:142142793-142508640) | KCNT2 (1:196194909-196577541) | KCNT2 (38:6006542-6381048) | KCNT2 (8:2711236-2818986) |
| | CFH (1:175366304-175461537) | CFHR3 (1:141471762-141524888) | CFH (1:196621008-196716634) | CFH (38:5878742-5955949) | ENSGALG00000002431 (8:2667080-2699650) |
| | No homologues | No homologues | CFHR3 (1:196743925-196764536) | No homologues | ENSGALG00000002431 (8:2667080-2699650) |
| | CFHR1 (:14458-26908) | No homologues | CFHR1 (1:196788875-196801319) | ENSCAFG00000013809 (7:23027426-23057109) | ENSGALG00000002431 (8:2667080-2699650) |
| | CFHR2 (1:175526799-175647439) | No homologues | CFHR2 (1:196788898-196928356) | ENSCAFG00000013809 (7:23027426-23057109) | ENSGALG00000002431 (8:2667080-2699650) |
| Upstream Genes | CFHR4 (1:175597111-175627003) | No homologues | CFHR4 (1:196857144-196887843) | No homologues | ENSGALG00000002431 (8:2667080-2699650) |
| | CFHR5 (1:175667259-175699392) | No homologues | CFHR5 (1:196946667-196978804) | No homologues | ENSGALG00000002431 (8:2667080-2699650) |
| | F13B (1:175728895-175756997) | F13B (1:141398284-141420333) | F13B (1:197008321-197036397) | F13B (7:8630400-8653643) | F13B (8:2658697-2665269) |
| ASPM | **ASPM (1:175788897-175841858)** | **ASPM (1:141351350-141390667)** | **ASPM (1:197053258-197115824)** | **ASPM_CANFA (7:8549449-8616618)** | **ASPM (8:2624989-2640191)** |
| Downstream Genes | ZBTB41 (1:175854378-175897808) | Zbtb41 (1:141318960-141349582) | ZBTB41 (1:197127572-197169672) | ZBTB41 (7:8500413-8539342) | ZBTB41 (8:2600301-2612540) |
| | CRB1 (1:175966292-176182998) | CRB1 (1:141093633-141273677) | CRB1 (1:197170592-197447585) | CRB1 (7:8233979-8376206) | CRB1 (8:2498842-2561808) |
| | DENND1B (1:176259102-176479095) | DENND1B (1:140860013-141072620) | DENND1B (1:197473878-197744826) | DENND1B (7:8061958-8217662) | DENND1B (8:2344861-2480256) |
| | LOC736288 (1:176609633-176614485) | 2310009B15Rik (1:140748556-140753431) | C1orf53 (1:197871777-197876497) | No homologues | C1orf53 (8:2307510-2310552) |
| | LHX9 (1:176624964-176636400) | LHX9 (1:140721763-140745153) | LHX9 (1:197881618-197904608) | LHX9 (7:7838543-7852551) | E1BSF2_CHICK (8:2285101-2299119) |
| | NEK7 (1:176863350-177027704) | NEK7 (1:140381291-140516273) | NEK7 (1:198126093-198291550) | NEK7 (7:7517158-7669901) | NEK7 (8:2184533-2239018) |
| | ATP6V1G3 (1:177243743-177261536) | ATP6V1G3 (1:140170315-140186037) | ATP6V1G3 (1:198492352-198510075) | ATP6V1G3 (7:7324804-7345445) | ATP6V1G3 (8:2115841-2127214) |
| | Q6QIM2_PANTR (1:177359410-177478533) | PTPRC (1:139959438-140071882) | PTPRC (1:198607801-198726545) | PTPRC (7:7112906-7176214) | PTPRC (8:2034560-2092242) |
| | A2T752_PANTR (1:178759166-178910858) | NR5A2 (1:138740161-138857004) | NR5A2 (1:199996730-200146552) | NR5A2 (7:5883612-5998115) | F1NVB5_CHICK (8:1599597-1682676) |
| Downstream Genes | C1orf98 (1:179079446-179110894) | No homologues | C1orf98 (1:200311672-200343482) | No homologues | No homologues |
| | ZNF281 (1:179145766-179149507) | Zfp281 (1:138521478-138526630) | ZNF281 (1:200375827-200379184) | ZNF281 (7:5673236-5675937) | ZNF281 (8:1521894-1523615) |
| | KIF14 (1:179298886-179362964) | KIF14 (1:138364535-138428088) | KIF14 (1:200520628-200589862) | KIF14 (7:5507000-5556803) | KIF14 (8:1469715-1481893) |
| | DDX59 (1:179386276-179417105) | DDX59 (1:138311848-138336735) | DDX59 (1:200593024-200639126) | DDX59 (7:5458800-5835853) | DDX59 (8:1452258-1459617) |
| | CAMSAP1L1 (1:179488265-179610562) | CAMSAP2 (1:138164700-138242681) | CAMSAP2 (1:200708686-200829832) | CAMSAP2 (7:5277092-5367783) | CAMSAP2 (8:1377489-1435829) |
| | GPR25 (1:179622833-179624059) | GPR25 (1:138155491-138157450) | GPR25 (1:200842083-200843306) | No homologues | No homologues |

**Table 1:** ASPM with 15 Genes (Upstream and Downstream) in Human and its Four Orthologs.

| Organism | No. of Deletions | Genes |
|---|---|---|
| Chimpanzee | 1 | CFHR3 |
| Mouse | 6 | CFHR3, CFHR1, CFHR2, CFHR4, CFHR5, C1orf98 |
| Dog | 7 | RGS13, CFHR3, CFHR4, CFHR5, C1orf53, C1orf98, GPR25 |
| Chicken | 3 | RGS13, C1orf98, GPR25 |

**Table 2:** Number of deletions in four orthologs (Chimpanzee, Mouse, Dog, and Chicken) with respect to human ASPM and other genes (upstream and downstream of ASPM).

reconstructed after deleting these four orthologs and is shown in Figure 3(b). The reconstructed tree is reconciling the species divergence time. In this tree, Human is making cluster with Macaque instead of Chimpanzee with 50 as a bootstrap value. Chimpanzee is evolving with a bootstrap value of 100 and is close to Human/Macaque cluster. Zebrafish/Fugu, Opossum/Platypus are making cluster with 57 and 87 as bootstrap values, respectively. Bootstrap values changed when we reconstructed tree after deleting species. Evolutionary time for the tree is 0.05.

## Conclusion

ASPM has a major contribution in causing autosomal recessive primary microcephaly which is most commonly found in consanguineous populations. The syntenic relationship for ASPM gene has determined conservation of genomic elements among four human orthologs i.e. Chimpanzee, Mouse, Dog and Chicken (with respect to 15 upstream and downstream genes of Human ASPM i.e. 192605275 bp to 200843306 bp. Phylogenetic analysis of ASPM with respect to seventeen orthologs has revealed its evolutionary relationship among different ortholog species. As per our findings through MEGA5, Human ASPM gene with respect to orthologs is making cluster with Macaque and is closely related to Chimpanzee according to NJ tree Figure 3(b). ASPM mutations leading to the phenotypical characteristics of MCPH5 are now known but due to the high frequency of MCPH5 in primary microcephaly cases among consanguineous families (especially in Asians including Pakistani population), many more are expected to be revealed in the upcoming years. This autosomal recessive disorder can be reduced by genetic counseling, using better genotyping, neuroimaging approach and neuro-physiological testing. Clinical management, for example through carrier detection or prenatal diagnosis in families affected with MCPH could also be useful in this aspect.

### References

1. Passemard S, Kaindl AM, Titomanlio L, Gerard B, Gressens P, Verloes A (1993) Primary Autosomal Recessive Microcephaly: University of Washington, Seattle.
2. Thornton GK, Woods CG (2009) Primary microcephaly: do all roads lead to Rome? Trends Genet 25: 501-510.
3. Roberts E, Hampshire DJ, Pattison L, Springell K, Jafri H, et al. (2002) Autosomal recessive primary microcephaly: an analysis of locus heterogeneity and phenotypic variation. J Med Genet 39: 718-721.
4. Mahmood S, Ahmad W, Hassan MJ (2011) Autosomal recessive primary microcephaly (MCPH): clinical manifestations, genetic heterogeneity and mutation continuum. Orphanet J Rare Dis 6: 39.
5. Jackson AP, Eastwood H, Bell SM, Adu J, Toomes C, et al. (2002) Identification of microcephalin, a protein implicated in determining the size of the human brain. Am J Hum Genet 71: 136-142.
6. Wollnik B (2010) A common mechanism for microcephaly. Nat Genet 42: 923-924.
7. Cox J, Jackson AP, Bond J, Woods CG (2006) What primary microcephaly can tell us about brain growth. Trends Mol Med 12: 358-366.
8. McCreary BD, Rossiter JP, Robertson DM (1996) Recessive (true) microcephaly: a case report with neuropathological observations. J Intellect Disabil Res 40: 66-70.
9. Mochida GH, Walsh CA (2001) Molecular genetics of human microcephaly. Curr Opin Neurol 14: 151-156.
10. Teebi AS, Al-Awadi SA, White AG (1987) Autosomal recessive nonsyndromal microcephaly with normal intelligence. Am J Med Genet 26: 355-359.
11. Saunders RD, Avides MC, Howard T, Gonzalez C, Glover DM (1997) The Drosophila gene abnormal spindle encodes a novel microtubule-associated protein that associates with the polar regions of the mitotic spindle. J Cell Biol 137: 881-890.
12. Ponting CP (2006) A novel domain suggests a ciliary function for ASPM, a brain size determining gene. Bioinformatics 22: 1031-1035.
13. Kouprina N, Pavlicek A, Collins NK, Nakano M, Noskov VN, et al. (2005) The microcephaly ASPM gene is expressed in proliferating tissues and encodes for a mitotic spindle protein. Hum Mol Genet 14: 2155-2165.
14. Jamieson CR, Fryns JP, Jacobs J, Matthijs G, Abramowicz MJ (2000) Primary Autosomal Recessive Microcephaly: MCPH5 Maps to 1q25-q32. Am J Hum Genet 67: 1575-1577.
15. Mekel-Bobrov N, Gilbert SL, Evans PD, Vallender EJ, Anderson JR, et al. (2005) Ongoing adaptive evolution of ASPM, a brain size determinant in Homo sapiens. Science 309: 1720-1722.
16. Chenn A, Walsh CA (2003) Increased neuronal production, enlarged forebrain and cytoarchitectural distortions in beta-catenin overexpressing transgenic mice. Cereb Cortex 13:599-606.
17. Bond J, Woods CG (2006) Cytoskeletal genes regulating brain size. Curr Opin Cell Biol 18: 95-101.
18. Cox J, Jackson AP, Bond J, Woods CG (2006) What primary microcephaly can tell us about brain growth. Trends Mol Med 12: 358-366.
19. Bond J, Roberts E, Mochida GH, Hampshire DJ, Scott S, et al. (2002) ASPM is a major determinant of cerebral cortical size. Nat Genet 32: 316-320.
20. Riparbelli MG, Callaini G, Glover DM, Avides Mdo C (2002) A requirement for the Abnormal Spindle protein to organise microtubules of the central spindle for cytokinesis in Drosophila. J Cell Sci 115: 913-922.
21. Gul A, Tariq M, Khan MN, Hassan MJ, Ali G, et al. (2007) Novel Protein-Truncating Mutations in the ASPM Gene in Families with Autosomal Recessive Primary Microcephaly. J Neurogenet 21: 153-163.
22. Kong X, Matise TC (2005) MAP-O-MAT: internet-based linkage mapping. Bioinformatics 21: 557-559.
23. Clamp M, Andrews D, Barker D, Bevan P, Cameron G (2003) Ensembl 2002: accommodating comparative genomics. Nucleic Acids Res 31: 38-42.
24. Revanna KV, Chiu CC, Bierschank E, Dong Q (2011) GSV: a web-based genome synteny viewer for customized data. BMC Bioinformatics 12: 316.
25. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol 28: 2731-2739.