ONLINE FIRST

ORIGINAL ARTICLE

A Missense Variant in LAMA3 Gene Causes Microcephaly and Epidermolysis Bullosa in a Pakistani Family

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ABSTRACT

OBJECTIVE: To identify disease causing mutation in a family with autosomal recessive primary microcephaly (MCPH).

METHODOLOGY: This cross sectional study was the continuation of an ongoing family based study initiated from 2016 at Department of Biochemistry, Quaid-e-azam University, Islamabad. The family was selected randomly and recruited from Sahiwal and has three members with MCPH. DNA was isolated from blood samples and genome wide scan was performed to map homozygous regions. Whole exome sequencing (WES) was performed to identify the plausible gene variant.

RESULTS: Whole genome data analysis identified multiple homozygous regions, but none of these contain known MCPH gene. Whole exome sequencing (WES) data identified six potentially pathogenic variants but only Laminin subunit alpha-3 (LAMA3) (c.5260C/T) variant segregates in the family and is also present within genomic region mapped on chromosome 18. The reevaluation of affected members of family revealed the presence of blisters on their hand and feet indicating the presence of epidermolysis bullosa along with microcephaly.

CONCLUSION: The casual finding of LAMA3 variant (c.5260C/T; p. Arg1754Trp) and absence of any other MCPH causing variant in affected members of this family expands the phenotypic spectrum of LAMA3 associated phenotype. Therefore, we can conclude that LAMA3 variant can probably cause recessive microcephaly and epidermolysis bullosa, but additional studies are needed to establish role of LAMA3 in microcephaly.

KEY WORDS: MCPH, LAMA3, Laminin-5, Genome scan, homozygous regions, whole exome sequencing.

INTRODUCTION

Microcephaly is a type of neurodevelopmental disorder which is frequently present in patients with intellectual disability (ID). Microcephaly is characterized by reduced cranial volume (below the 3 standard deviations at birth) and mild to severe ID but additional neurological deformities are absent in the patients[1]. The reduction in cranial volume of the newborns and children can be detected by measuring occipital–frontal circumferences (OFC), which is significantly reduced in the microcephalic cases. Detailed neurological imaging studies of microcephalic patients have shown the decrease in cerebral cortex which leads to a simplified gyral patterning without affecting the cerebral cortex thickness[2, 3]. To date twenty five genes have been identified that cause autosomal recessive primary microcephaly (MCPH)[3] and among these 12 genes (*MCPH1*[4], *WDR62*[5], *CDK5RAP2*[6], *ASPM*[7], *CENPJ*[6], *CEP135*[8], *CEP152*[9], *CDK6*[10], *SASS6*[11], *MFSD2A*[12], *KIF14*[13], and *NUP37*[14]) have been identified by studying Pakistani families. Previous studies on Pakistani and other populations have identified *ASPM* and *WDR62* genes as major players responsible for MCPH[1, 2].

The Laminin-5 protein (laminin-332) is a large glycoprotein mainly expressed in skin and comprises of three subunits α3 chain, β3 chain and γ2 chains which are encoded by *LAMA3*, *LAMB3*, *LAMC3* genes respectively[15]. This protein plays a vital role in adhesion of epidermal and dermal layer[16]. The *LAMA3* gene located on chromosome 18q11.2, consists of 75 exons and encode a protein with 3333 amino acids[17]. The mutation in *LAMA3* gene causes skin disease Herlitz type junctional epidermolysis bullosa (JEB) with recessive trait[18, 19] and Laryngo-onycho-cutaneous syndrome[20].

In this study we performed genetic analysis of a family segregating autosomal recessive primary microcephaly and identified a missense mutation in *LAMA3* gene.

METHODOLOGY

The cross sectional study was performed at Biochemistry Department of Quaid-I-Azam University, Islamabad from 2016-2018 after getting approval by the Bio-Ethical Committee (BEC-FBS-QAU-59/2016), of Quaid-i-Azam University (QAU). The randomly selected two generation family segregating microcephaly was recruited from a remote village of Sahiwal district from Punjab province. Written informed consent was obtained from all the subjects and their parents according to the instruction specified in Declaration of Helsinki. Detailed interview was conducted with elders of the family to collect information about pedigree, patient's behavior, disease status and degree of disease progression. The occipital–frontal circumferences (OFC) of all available family members were also measured.

Peripheral blood samples were collected in EDTA (Ethylene diamine tetra-acetic acid) tubes (BD, Franklin Lakes, NJ, USA) from available family members. Genomic DNA was extracted by standard organic phenol- chloroform method, quantified by using Colibri Microvolume Spectrometer (Titertek Berthold, Germany). After DNA quantification samples were diluted to 50ng/ul and used for genome wide genotyping and exome sequencing in a international collaborator laboratory at Center for Statistical Genetics, Columbia University, New York, USA. Infinium® HumanCoreExome BeadChip (Illumina, USA) was used for genome wide scan and the resulting genotype data was analyzed by homozygosity mapper (Seelow et al., 2009).

The whole exome sequencing (WES) of one affected individual was performed by using NimbleGen SeqCap EZ Human Exome Library v.2 (Roche Diagnostics, San Francisco, CA). The variants identified by WES were further filtered to enrich potentially pathogenic and rare variants. Selected potentially pathogenic variants were further tested for segregation in the available family members by using Sanger sequencing. Each candidate gene variant was amplified from DNA samples of available family members by using standard PCR protocol and the PCR product was purified by Gene JETTM PCR Purification Kit (Fermentas, London, UK). Sanger sequencing was performed with Big Dye Terminator cycling sequencing kit v. 3.1(Applied Biosystems, Foster City, USA) and the products were analyzed on ABI Genetic Analyzer (ABI, USA). The data was analyzed on Bioedit software version 7.0.9.0.

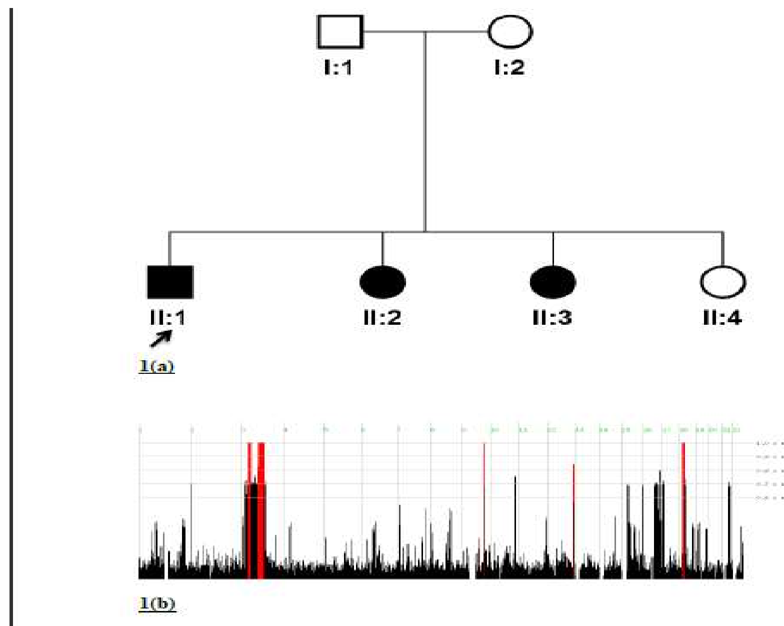
For prediction of pathogenic nature of identified variants public data base like Mutation Taster (http://www.mutationtaster.org/), SIFT (https://sift.bii.a-star.edu.sg), polyphen2 (http://genetics.bwh.harvard.edu/pph2), and PROVEAN (http://provean.jcvi.org/) were used.

The frequency of these variants was tested with 1000 Genomes Browser (http://www.1000genomes.org), genomAD (https://gnomad.broadinstitute.org), and dbSNP (http://www.ncbi.nlm.nih.gov/SNP/).

RESULT

The two generation family (Figure I(a)) resides in Sahiwal district, Punjab province comprising of three affected including one male (II-1) and two females (II-2, II-3) individuals. The ages of three patients range from 9 to 14 years but all presented reduced head circumferences and severe ID. Both parents were unable to explain the exact ancestral relationship however, their elders were certain about a convergence of ancestry three generations earlier, therefore we concluded an autosomal recessive inheritance of MCPH in this family.
Analysis of the genome wide genotyping data identified homozygous regions on chromosome 3, 9 and 18 (Figure I(b)). The two larger 25 Mb and 11.7 Mb homozygous by descent (HBD) regions were mapped on chromosome 3 and we anticipated that MCPH causing gene may be present within any one of these regions. Further analysis of candidate genes located within these genomic regions could not identify any known MCPH gene. Therefore, one affected individual underwent for WES to identify disease causing variant in this family.

Figure I(a): Family pedigree showing three MCPH patients in the 2nd generation. The blackarrow points the individual (II: I) which was utilized for WES (I(b)) HBD regions mapped on the respective chromosomes are shown by vertical red lines. The green row on X axis indicates the chromosome numbers while homozygosity score is presented on Y axis.



The exome data analysis detected 79,637 sequence variants in affected individual 2-1. These pathogenic variants were scrutinized with different prediction tools which resulted in the shortlisting of six rare detrimental coding variants in *LAMA3* (c.5260C/T), *PIK3C2B* (c.3038G/C), *DYRK3* (c.982C/T), *GADL1* (c.1097A/G), *RGMA* (c.886C/T) and *PVRL3* (c.1492G/C) gene (Table I).

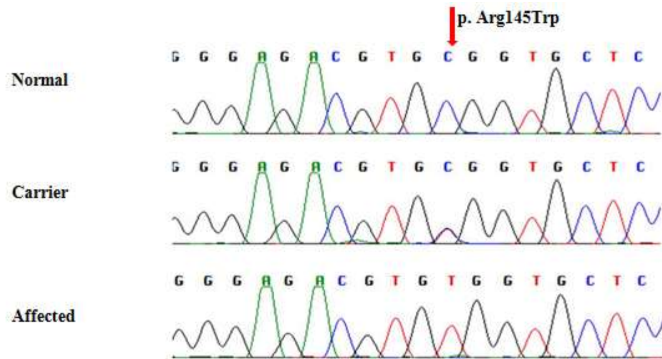
Table I: Variants identified by WES in the family with microcephaly

Gene	chr	Ref Seq Mutation	Protein change	gnomAD MAF south Asian Allele	gnomAD All MAF	1000g	MT	PP2	SIFT
LAMA3	**18**	**c.5260C/T**	**p.R1754W**	**0.000098**	**0.00004**	**-**	**DC**	**PD**	**Del**
GADL1	3	c.1097A/G	p. Q366R	0.00003	0.000003	-	DC	PD	Del
RGMA	15	c.886C/T	p. R296C	0.00006	0.00001	-	DC	PD	Del
DYRK3	1	c.982C/T	p. R328C	0.00019	0.00009	-	DC	B	Del
PIK3C2B	1	c.3122G/C	p. S1041T	0.000217	0.00169	-	P	PD	T
PVRL3	3	c.1492G/C	p.V498L	0.00016	0.00006	-	DC	B	T

MT= Mutation Tester, 1000g= 1000 genome, chr= chromosome, MAF= Minor allele frequency, PD= Probably Damaging, DC= Disease Causing, Del= Deleterious, SIFT= sorting intolerant from tolerant, PP2=polyphen2.

Sanger sequencing also confirmed the segregation of *LAMA3* variant (c.5260C/T; p. Arg1754Trp) in this family (Figure II(A)). Affected individual is homozygous for mutant allele whereas the parent is heterozygous for both alleles. This identified variant is also found within the homozygous genomic region mapped in the family by genome wide scan. The *LAMA3* variant (c.5260C/T; p. Arg1754Trp) is predicted as damaging by mutation taster. SIFT and PolyPhen2 also predicted this variant as deleterious. The variant is reported in the heterozygous form (6/121216) and (13/282308) in ExAC and gnomAD databases, respectively. Conservation analysis exhibited that the arginine amino acid residue at 1754 was almost in orthologue of higher vertebrates (Figure II (B)).

Figure II(A) Chromatogram shows the segregation of LAMA3 gene variant (c.5260C/T) in family (B) Multiple alignment in the orthologues of LAMA3 gene showing Arginine 1754 amino acid residue is conserved among some vertebrate species



(A)

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                     1754
H.sapiens       TNSFATGCVVNGGDVRCSCKAGYTGTQCER
P.troglodytes   TNSFATGCVVNGGDVRCSCKAGYTGTQCER
M.mulatta       TNRFATGCVVNGGDVRCSCKAGYTGIQCER
C.lupus         SNSFATGCVVTGGNVRCSCKPGYTGTQCER
M.musculus      TNSFATGCAVDGGAVRCACKPGYTGTQCER
R.norvegicus    TNSFATGCAVDGGAVRCACKPGYTGAQCER
B.taurus        SDSFATGCVVTGENVKCSCKPGYTGTRCER
G.gallus        TNRFATGCVANGGEIQCLCKEGYTGVRCER
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DISCUSSION

In present study, we found a *LAMA3* gene variant (c.5260C/T; p. Arg1754Trp) in all affected members of a family with autosomal recessive microcephaly. Laminin subunit alpha-3 (*LAMA3*) gene comprised of 75 exons that encodes α3 subunit for laminin-5 protein. Laminin-5 protein is highly complex glycoprotein that is extensively secreted in the skin and other epithelial structures where it serves as anchorage with the extracellular material and also thought to involve in cellular migration, tissue binding and organization in embryo[16,21,22]. *LAMA3* variant were found to be associated with skin disorder, autosomal recessive in nature, that results in the blister formation[19]. Mouse with targeted mutation in *LAMA3* genes exhibit severe epithelial abnormalities. Malformation of hemi desmosomes and skin blister formation was observed in *LAMA3* knock out mice[23].

At the time of initial visit to this family in 2016, we noticed the presence of reduced head circumference and severe ID. However, careful reevaluation of the patients after genetic testing also revealed the presence of blisters on the skin of hands and feet. This contrasts with previous findings where patients with *LAMA3* gene mutation presented Herlitz type junctional epidermolysis bullosa (JEB)[18, 19] and Laryngo-onycho-cutaneous syndrome[20,24]. The segregation analysis confirmed that *LAMA3* gene variant (p. Arg1754Trp) is homozygous in affected individuals of our family, whereas unaffected individuals were either heterozygous or homozygous for wild type allele. This variant is reported in public databases in the heterozygous state (Table 1) but affected members of our family were homozygous and thus coincides with the autosomal recessive inheritance of phenotype in our family.

Microcephaly is a heterogeneous disorder that is characterized by small cortical size and reduction in the cortical convolution patterns[1, 2]. This reduction was associated with number of genes malfunction that affects the specific pattern of cell division of neural progenitor cells and their migration to neo cortical region[1]. At the beginning of neurogenesis the neuro-epithelial cells gives rise to redial glial cells (RGCs), these cells are self-renewing in nature and serves as precursor for neural progenitors cells (NPCs) in the ventricular zone (VZ)[25]. With each division one of the daughter cells moves radially to the cortical plate, while one of the daughter cell remains in the VZ via its end feet attachment that spawn through neocortical wall to the pial and ventricular surface[26-29]. These cellular attachments not only provide the anchorage to RGCs and maintain the precursor number but also important for the migration of NPCs to the cortical plate[30, 31]. Nidogen, a laminin binding cell adhesion protein is highly expressed in the RGCs and therefore may be important in the regulation on their division and migration. This led us to presumption that laminin-5 (protein encoded by *LAMA3* gene) might play some role in the organization of RGCs and early neurogenesis[32]. It is probable that laminin-5 plays role in embryonic cellular adhesion, division and migration, and its expression in CNS prompts for additional studies to explore its role beyond recessive skin disorders.

CONCLUSION

This is the first report regarding the involvement of *LAMA3* variant (p. Arg1754Trp) in autosomal recessive microcephaly and epidermolysis bullosa. None of the known gene for the microcephaly in recessive trait was observed in the same affected members of family and only *LAMA3* variant appears as a possible factor responsible for the phenotype of our family. This casual finding of *LAMA3* variant in microcephaly patients is suggestive of its role in early cell division and migration of neural progenitor cells.

ETHICAL PERMISSION: Department of Biochemistry, Qauid-i-Azam University Islamabad, Pakistan, BEC letter Number: BEC-FBS-QAU-59, dated 4-5-2016.

ACKNOWLEDGEMENT: We are grateful to family who has participated in this study. We are also grateful to our collaborator Professor Suzanne M Leal at Center for Statistical Genetics, Columbia University, New York, USA for providing support for genome scan and whole exome sequencing.

CONFLICT OF INTEREST: There is no conflict of interest.
FUNDING: There was no funding agency.

AUTHOR CONTRIBUTION:
Mughal ZU: Conceived of the study. Collected samples, conducted the research, organized, analyzed, and interpreted the data, and wrote the initial and final drafts of the article.
Zai JA: collected, gathered and managed data and also provide logistic support.
Ansar M: Conceived and supervised the study. Data interpretation. Critical revision, suggestions, and draft finalization.
All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

REFERENCE

1. Rasool S, Baig JM, Moawia A, Ahmad I, Iqbal M, Waseem SS, et al. An update of pathogenic variants in ASPM, WDR62, CDK5RAP2, STIL, CENPJ, and CEP135 underlying autosomal recessive primary microcephaly in 32 consanguineous families from Pakistan. Molecular Genetics & Genomic Med. 2020; 8(9): e1408. doi:10.1002/mgg3.1408.
2. Mahmood S, Ahmad W, Hassan MJ. Autosomal recessive primary microcephaly (MCPH): clinical manifestations, genetic heterogeneity and mutation continuum. Orphanet J Rare Dis. 2011; 6(1): 1-5. doi:10.1186/1750-1172-6-39.
3. Zaqout S, Morris-Rosendahl D, Kaindl AM. Autosomal recessive primary microcephaly (MCPH): an update. Neuropediatrics. 2017; 48(03): 135-42. doi:10.1055/s-0037-1601448.
4. Jackson AP, Eastwood H, Bell SM, Adu J, Toomes C, Carr IM, et al. Identification of microcephalin, a protein implicated in determining the size of the human brain. Am J Human Genetics. 2002; 71(1): 136-42. doi:10.1086/341283.
5. Nicholas AK, Khurshid M, Désir J, Carvalho OP, Cox JJ, Thornton G, et al. WDR62 is associated with the spindle pole and is mutated in human microcephaly. Nature Genetics. 2010; 42(11): 1010-4. doi:10.1038/ng.682.
6. Bond J, Roberts E, Springell K, Lizarraga S, Scott S, Higgins J, et al. A centrosomal mechanism involving CDK5RAP2 and CENPJ controls brain size. Nature Genetics. 2005; 37(4): 353-5. doi:10.1038/ng1539.
7. Bond J, Roberts E, Mochida GH, Hampshire DJ, Scott S, Askham JM, et al. ASPM is a major determinant of cerebral cortical size. Nature Genetics. 2002; 32(2): 316-20. doi:10.1038/ng995.
8. Hussain MS, Baig SM, Neumann S, Nürnberg G, Farooq M, Ahmad I, et al. A truncating mutation of CEP135 causes primary microcephaly and disturbed centrosomal function. Am J Human Genetics. 2012; 90(5): 871-8. doi:10.1016/j.ajhg.2012.03.016.
9. Guernsey DL, Jiang H, Hussin J, Arnold M, Bouyakdan K, Perry S, et al. Mutations in centrosomal protein CEP152 in primary microcephaly families linked to MCPH4. Am J Human Genetics. 2010; 87(1): 40-51. doi:10.1016/j.ajhg.2010.06. 003.
10. Hussain MS, Baig SM, Neumann S, Peche VS, Szczepanski S, Nürnberg G, et al. CDK6 associates with the centrosome during mitosis and is mutated in a large Pakistani family with primary microcephaly. Human Molecular Genetics. 2013; 22(25): 5199-214. doi:10.1093/hmg/ddt374.
11. Khan MA, Rupp VM, Orpinell M, Hussain MS, Altmüller J, Steinmetz MO, et al. A missense mutation in the PISA domain of HsSAS-6 causes autosomal recessive primary microcephaly in a large consanguineous Pakistani family. Human Molecular Genetics. 2014; 23(22): 5940-9. doi:10.1093/hmg/ddu318.
12. Alakbarzade V, Hameed A, Quek DQ, Chioza BA, Baple EL, Cazenave-Gassiot A, et al. A partially inactivating mutation in the sodium-dependent lysophosphatidylcholine transporter MFSD2A causes a non-lethal microcephaly syndrome. Nature Genetics. 2015; 47(7): 814-7. doi:10.1038/ng.3313.
13. Moawia A, Shaheen R, Rasool S, Waseem SS, Ewida N, Budde B, et al. Mutations of KIF14 cause primary microcephaly by impairing cytokinesis. Annals Neurol. 2017; 82(4): 562-77. doi:10.1002/ana.25044.

14. Braun DA, Lovric S, Schapiro D, Schneider R, Marquez J, Asif M, et al. Mutations in multiple components of the nuclear pore complex cause nephrotic syndrome. J Clin Invest. 2018; 128(10): 4313-28. doi:10.1172/JCI98688.
15. Rousselle P, Scoazec JY. Laminin 332 in cancer: When the extracellular matrix turns signals from cell anchorage to cell movement. In: Seminars in cancer biology. 2020; 62: 149-165. Academic Press. doi:10.1016/j.semcancer.2019.09.026.
16. Lincoln V, Cogan J, Hou Y, Hirsch M, Hao M, Alexeev V, et al. Gentamicin induces LAMB3 nonsense mutation readthrough and restores functional laminin 332 in junctional epidermolysis bullosa. Proceedings of the National Academy of Sciences. 2018; 115(28): E6536-45. doi:10.1073/pnas.1803154115.
17. Ryan MC, Tizard R, VanDevanter DR, Carter WG. Cloning of the LamA3 gene encoding the alpha 3 chain of the adhesive ligand epiligrin. Expression in wound repair. J Biol Chem. 1994; 269(36): 22779-87. doi:10.1016/S0021-9258(17)31713-1.
18. Kivirikko S, McGrath JA, Baudoin C, Aberdam D, Ciatti S, Dunnill MG, et al. A homozygous nonsense mutation in the α3 chain gene of laminin 5 (LAMA3) in lethal (Herlitz) junctional epidermolysis bullosa. Human Molecular Genetics. 1995; 4(5): 959-62. doi:10.1093/hmg/4.5.959.
19. McGrath JA, Kivirikko S, Ciatti S, Moss C, Dunnill MG, Eady RA, et al. A homozygous nonsense mutation in the α3 chain gene of laminin 5 (LAMA3) in Herlitz junctional epidermolysis bullosa: prenatal exclusion in a fetus at risk. Genomics. 1995; 29(1): 282-4. doi:10.1006/geno.1995.1246.
20. McLean WI, Irvine AD, Hamill KJ, Whittock NV, Coleman-Campbell CM, Mellerio JE, et al. An unusual N-terminal deletion of the laminin α3a isoform leads to the chronic granulation tissue disorder laryngo-onycho-cutaneous syndrome. Human Molecular Genetics. 2003; 12(18): 2395-409. doi:10.1093/hmg/ddg234.
21. Rousselle P, Keene DR, Ruggiero F, Champliaud MF, Rest MV, Burgeson RE. Laminin 5 binds the NC-1 domain of type VII collagen. J Cell Biology. 1997; 138(3): 719-28. doi:10.1083/jcb.138.3.719.
22. Gatseva A, Sin YY, Brezzo G, Van Agtmael T. Basement membrane collagens and disease mechanisms. Essays Biochem. 2019; 63(3): 297-312. doi:10.1042/EBC20180071.
23. Ryan MC, Lee K, Miyashita Y, Carter WG. Targeted disruption of the LAMA3 gene in mice reveals abnormalities in survival and late stage differentiation of epithelial cells. J Cell Biol. 1999; 145(6): 1309-24. doi:10.1083/jcb.145.6.1309.
24. Ahmad F, Shah K, Umair M, Jan A, Khan S, Muhammad D, et al. Novel autosomal recessive LAMA3 and PLEC variants underlie junctional epidermolysis bullosa generalized intermediate and epidermolysis bullosa simplex with muscular dystrophy in two consanguineous families. Clin Exp Dermatol. 2018; 43(6): 752-5. doi:10.1111/ced.13610.
25. Fischer E, Morin X. Fate restrictions in embryonic neural progenitors. Curr Opinion Neurobiol. 2021; 66: 178-85. doi:10.1016/j.conb.2020.10.014.
26. Chenn A, McConnell SK. Cleavage orientation and the asymmetric inheritance of notchl immunoreactivity in mammalian neurogenesis. Cell. 1995; 82(4): 631-41. doi:10.1016/0092-8674(95)90035-7.
27. Govindan S, Jabaudon D. Coupling progenitor and neuronal diversity in the developing neocortex. FEBS letters. 2017; 591(24): 3960-77. doi:10.1002/1873-3468.12846.

28. Rakic P. Mode of cell migration to the superficial layers of fetal monkey neocortex. J Compar Neurology. 1972; 145(1): 61-83. doi:10.1002/cne.901450105.
29. Chou FS, Li R, Wang PS. Molecular components and polarity of radial glial cells during cerebral cortex development. Cellular Molecul Life Sci. 2018; 75(6): 1027-41. doi:10.1007/s00018-017-2680-0.
30. Zhang W, Yang SL, Yang M, Herrlinger S, Shao Q, Collar JL, et al. Modeling microcephaly with cerebral organoids reveals a WDR62–CEP170–KIF2A pathway promoting cilium disassembly in neural progenitors. Nature Comm. 2019; 10(1): 1-4. doi:10.1038/s41467-019-10497-2
31. Tang Y, Illes P. Regulation of adult neural progenitor cell functions by purinergic signaling. GLIA. 2017; 65(2): 213-30. doi:10.1002/glia.23056.
32. Li H, Chang YW, Mohan K, Su HW, Ricupero CL, Baridi A, et al. Activated Notch1 maintains the phenotype of radial glial cells and promotes their adhesion to laminin by upregulating nidogen. GLIA. 2008; 56(6): 646-58. doi:10.1002/glia.20643.
33. Buchman JJ, Tsai LH. Spindle regulation in neural precursors of flies and mammals. Nature Rev Neurosci. 2007; 8(2): 89-100. doi:10.1038/nrn2058.
34. Garcez PP, Diaz-Alonso J, Crespo-Enriquez I, Castro D, Bell D, Guillemot F. Cenpj/CPAP regulates progenitor divisions and neuronal migration in the cerebral cortex downstream of Ascl1. Nature Comm. 2015; 6(1): 1-4. doi:10.1038/ncomms7474.
35. Hardwick LJ, Ali FR, Azzarelli R, Philpott A. Cell cycle regulation of proliferation versus differentiation in the central nervous system. Cell Tissue Res. 2015; 359(1): 187-200. doi:10.1007/s00441-014-1895-8.