Research Article

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Candidate variants in *MLC1* gene causing Megalencephalic Leukodystrophy using in silico prediction methods

Abstract

Megalencephalic Leukodystrophy with sub cortical cysts is a type of Demyelinating Leukodystrophy caused by mutations in *MLC1* gene. Various mutations in *MLC1* gene have been reported worldwide but high throughput technologies aimed to discover novel variants underlying this disorder are scarce and there is a lot yet to be discovered. In silico analysis of SNPs in a gene known to cause a disease is a well effective and economic method of analyzing known variants deposited in public databases. This article aimed to analyze all SNPs in *MLC1* gene in order to be used in screening programs for patients with Megalencephalic Leukodystrophy. The SNPs in *MLC1* gene were retrieved from NCBI db SNP. Variants in VCF format were analyzed using Variant Effect Predictor (VEP) of the Ensemble database. The deleterious coding ns SNPs were detected by the web program SIFT, PolyPhen and Mutation Taster in addition to allele frequency and conservation score. The 3-D model of the human *MLC1* protein was predicted using the CPH models 2.0 server. The resulting modeled structure with positions of mutations was viewed using Chimera 1.8 software. In *MLC1* gene, 4 are nonsense, 18 are indels (frame shift mutations) and 10 potentially disrupt splicing. Six variants in *MLC1* gene were predicted to be pathogenic using the same tools. The results of our study will facilitate future studies aimed to analyze the genetics of Leukodystrophy patients from different families from different populations

Keywords: leukodystrophy, *MLC1*, SNPs, in silico

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Introduction

Leukodystrophies are group of inherited disorders caused primarily by defective myelination of the central nervous system with or without peripheral nervous system involvement.[1] There are over 30 of Leukodystrophy disorders have been described with various age of onset and clinical presentation but they all share white matter signals in brain MRI.[2] Individual Leukodystrophy types are rare-although they vary depending on the population- but collectively they are not uncommon.[3] Megalencephalic Leukodystrophy with sub cortical cysts is a type of Leukodystrophy caused by mutations in *MLC1* gene and less commonly in *HEPACAM* Gene.[4] The disease presents with early head enlargement, motor dysfunction and occasionally epilepsy. All kinds of mutations in *MLC1* gene were described.[4] However, high throughput technologies aimed to discover novel variants underlying these disorders are scarce, and there is a lot to be discovered. In silico analysis of SNPs in a gene known to cause a disease is a well effective and economic way of at least screen known variants deposited in public databases. And this approach has indeed proved valuable in many situations.[5–8] Since Leukodystrophy disorders are rarely studied especially in developing countries, approaches like in silico analysis, patients can be screened for known and predicted pathogenic variants first and if none found proceed to advanced technologies like whole genome or whole exome sequencing. This article aimed to analyze all SNPs in *MLC1* gene in order to be used in screening programs for patients with Megalencephalic Leukodystrophy.

Materials and methods

The SNPs and their related protein sequences for *MLC1* gene were retrieved from NCBI db SNP. Frame shift, nonsense and splicing variants were obtained from the NCBI database. The deleterious coding ns SNPs were detected by the web program SIFT and PolyPhen. Variants of both genes in VCF format were analyzed using Variant Effect Predictor (VEP) of the Ensemble database http://www.ensembl.org/Tools/VEP. Predicted pathogenic variants were filtered as follows: SIFT score <0.05, PolyPhen score >0.85 and Allele frequency <0.05. Pathogenicity of variants was verified using Mutation Taster and amino acid conservation from Alamut visual http://www.interactive-biosoftware.com/doc/alamut-visual/2.9/. Structural analysis was performed in order to evaluate and compare the stability of native and mutant structures. The 3-D model of the human *MLC1* protein was predicted using the CPH models 2.0 server.[9] The resulting modeled structure with positions of mutations was viewed using Chimera 1.8 software.[10]

Results

The *MLC1* gene contains 1471 SNPs, majority of which are intronic (44%) and upstream variants (22%). Majority of coding variants were missense (62%) and synonymous (30%), (Figure 1). In *MLC1* gene, 4 are nonsense, 18 are indels (frame shift mutations) and 10 potentially disrupt splicing, (Table 1). In *MLC1* gene, 6 missense variants were

predicted to be pathogenic using in silico tools, (Table 2). The result of *MLC1* protein modeling revealed globular proteins with multiple helices and beta sheets (Figure 2). The position of amino acid variants mentioned above is shown in (Figure 2).

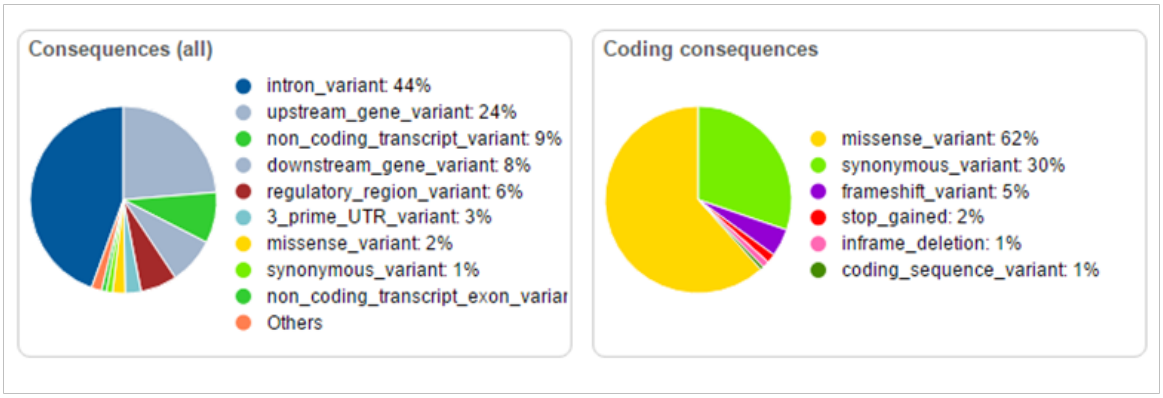

Figure 1 Percentages of the consequences of variants in *MLC1* gene (all) and (coding).

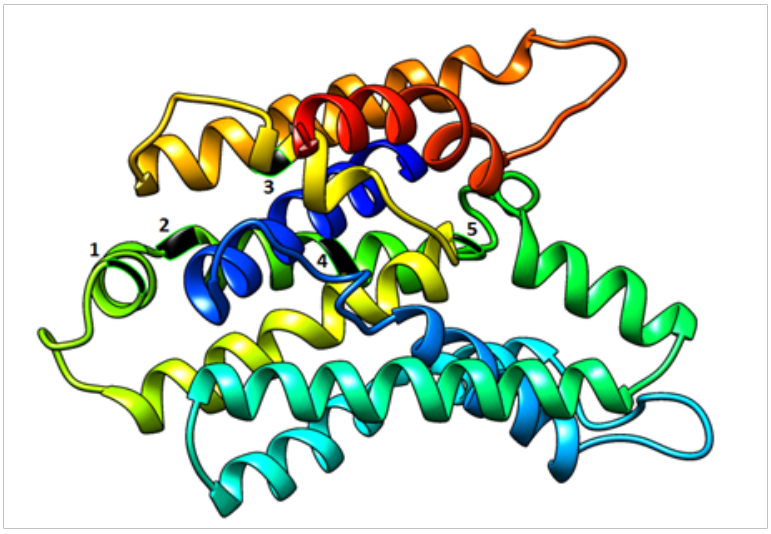

Figure 2 *MLC1* protein modeled with CPH modeling server with number indicating position of missense amino acid variants (1=N218K, 2=A208V, 3=V260L, 4=V200F, 5=R193W) (Note that one variant R20G is not in the figure because the first 55 amino acids could not be modeled).

Table 1 SNPs causing nonsense, frame shift and splicing impairment mutations in *MLC1* gene

MLC1		
Nonsense	**Frame shift**	**Splicing**
rs992764755	rs4513390	rs992020383
rs992830566	rs4569573	rs992021756
rs992920871	rs4569574	rs992057554
rs993014432	rs4569575	rs992070962
	rs4600768	rs992340649
	rs4838816	rs992349844
	rs4838817	rs992369866
	rs4838819	rs992430530

Table continued....

Nonsense	**Frame shift**	**Splicing**
	rs4838879	rs992566519
	rs4838880	rs992712599
	rs4838882	
	rs4838883	
	rs4990416	
	rs5771140	
	rs5771141	
	rs5771142	
	rs5771143	
	rs5771144	

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Table 2 Missense variants in *MLC1* gene predicted to be pathogenic with SIFT (and its score) Poly Phen (and its score), Mutation Taster, allele frequency and conversation

SNP	AA*	SIFT (score)	Poly phen (score)	Mutation taster	AF**	CS
rs143061714	V260L	Deleterious (0)	Probably damaging (-0.987)	Disease causing	0.0006	High
rs568289086	A208V	Deleterious (0)	Probably damaging (-0.994)	Disease causing	0.0002	High
rs41302601	N218K	Deleterious (0)	Probably damaging (-0.997)	Disease causing	0.0022	High
rs78644350	V200F	Deleterious (0)	Probably damaging (-0.997)	Disease causing	0.0002	High
rs555304253	R193W	Deleterious (0)	Probably damaging (-0.997)	Disease causing	0.0002	High
rs533294413	R20G	Deleterious (0)	Probably damaging (-0.997)	Disease causing	0.0004	High

*AA, Amino acid change; **AF, Allele frequency

Discussion

In silico analysis of SNPs in disease causing genes deposited in public databases and whose clinical significance is unknown is a valuable and economic method for preliminary screening studies especially for neglected diseases like Leukodystrophy. This study aimed to which variants in *MLC1* gene are likely to be pathogenic using in silico prediction methods. The most common mutation we found were indel variants causing frame shift mutations. Frame shifting mutations are known to disrupt protein synthesis and very rarely don't impair protein function.[11] Frame shifting mutations were indeed found in many studies underlying these forms of Leukodystrophy.[12–15] Splicing impairing variants and non-sense variants were also found in our study and from pathogenesis point of view they are even stronger as culprits than either frame shift or missense damaging variants especially for disease which loss of function is their known mechanism[16]. Nonsense variants were found also in other studies[17–19] and so are splicing defects.[15,20] Missense variants causing *MLC1*were previously reported in many studies.[21] Judging from the in silico prediction methods which include conservation, allele frequency and tolerance of amino acid changes, our variants reported in our study are very likely to be pathogenic, but they are all novel and their association with these types of leukodystrophies await to be confirmed. These variants were deposited in NCBI public database with unknown clinical significance because all individuals sampled for genotyping were in heterozygous state and these diseases are recessive. The results of our study will facilitate future studies aimed to analyze the genetics of Leukodystrophy patients from different families from different populations. By implementing in silico predicted pathogenic variants in disease panels, this will perhaps lower the need to do exome sequencing and hence the cost of the study. The results of in silico predictive studies will also accelerate variants annotation keeping in pace with advancing international genome and exam studies.

Acknowledgments

None.

Conflict of interest

The authors declare that they have no conflict of interest.

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