RARE EXONIC VARIATIONS IN YOUNG ISCHEMIC STROKE: Preliminary results of case-control study

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INTRODUCTION

- Stroke is a complex, mutifactorial polygenic disease resulting from the interplay of genetics and environmental factors.
- The incidence rate of ischemic stroke over the world at young age is rising,10-30% are young adults in the age group between 18-45 years.
- Twin and family studies support a role for genetic factors in stroke risk.
- High peneterance with large effects of rare exonic variants was observed in many complex early onset disease. Young onset ischemic stroke may be elucidated by occurrence of rare exonic variations.
- In recent years, collecting data specifying that genetic variations plays an important risk contributor to the pathogenesis of ischemic stroke.
- This is the first study from India up to best of our knowledge to specifically addresses the genetic causality of young onset ischemic stroke with rare exonic variations through Whole exome sequencing.

OBJECTIVE

- To identify rare exonic variations among young onset ischemic stroke through whole exome sequencing.

MATERIALS AND METHODS

- This study is preliminary findings of an approved project by ***Indian Council Of Medical Research (ICMR),*** government of India.
- Study was approved by the Local Institutional Ethics Committee
- In this study Young (18-40ys) patients classified by TOAST were screened from neurological OPD/WARD and recruited after obtaining informed consent.
- The clinical evaluation was done under senior Neurologist and baseline data were collected in the standardized data collection form if they meet inclusion and exclusion criteria.
- 5ml blood samples were collected in an EDTA vials.
- DNA isolation from whole blood was done by phenol chloroform method.
- Out of 100 sample size, 35 samples were sequenced using Whole Exome Sequencing on illumina Hi Seq X TEN.
- *SENTIEON* tools were used for variant calling.
- The identified variants were annotated using *VARIMAT*.

Inclusion criteria for cases:

- Patients age 18-40 years old.
- With or without family history.
- Sudden onset of focal neurologic deficit or impairment of consciousness.
- Ischemic stroke ruled out by NCCT head within 10 days from the event or MRI
- No evidence of trauma, brain tumor/metastasis
- Willingness to provide written informed consent by patient or Legally Authorized Representative (LAR)

Exclusion criteria for cases:

- Unwillingness to provide written informed consent (by self or next of kin).
- Hemorrhagic stroke confirmed by CT or MRI.
- Concurrent major renal or hepatic disease.
- Stroke due to Pregnancy.

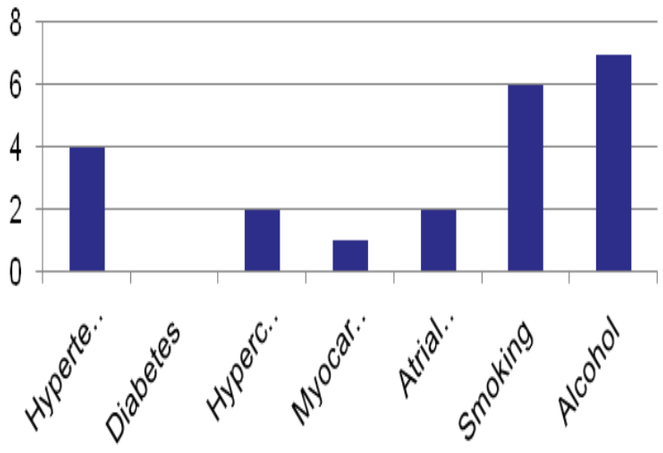
Inclusion criteria for controls:

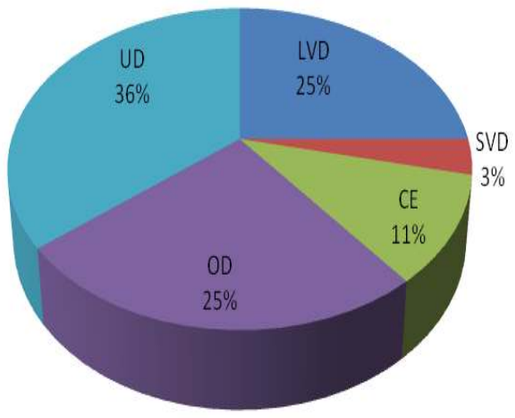
- Age will be more than 2 S.D of patient's age or more than 50 years.
- Unrelated ethnicity matched controls from mainly large population of India as described in IGV (Indian genome variation consortium study by CSIR-IGIB).
- Unaffected individuals within the family (Parents or Siblings of the probands).
- Willingness to provide written informed Consent by Legal Authorized Representative (LAR)

Exclusion criteria for controls:

- Unwillingness to provide written informed consent (by self or next of kin)

RISK FACTOR AND TOAST OF 28 PROBANDS

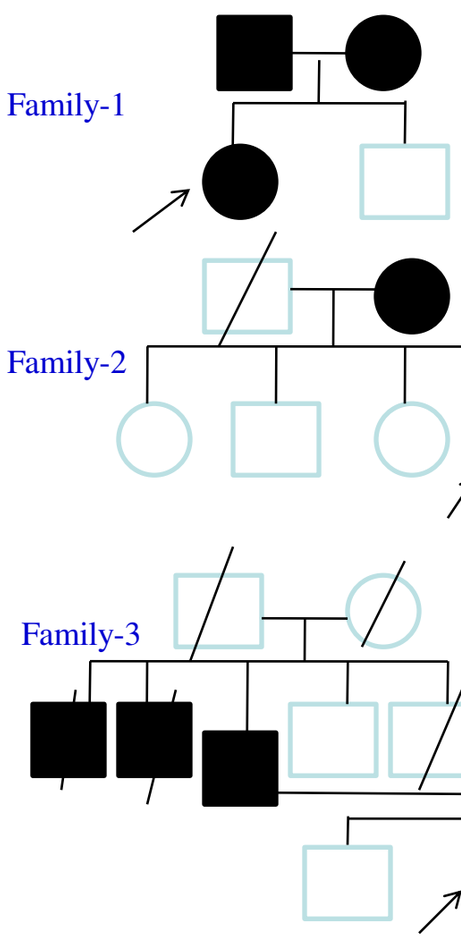




WHOLE EXOME ANALYSIS OF 28 PROBANDS

S. No.	VARIABLES	No. of VARIATIONS
1.	Total variants called 35 samples after Whole Exome Sequencing	30,63,107
2.	Pass and low DP (Depth), exonic and splicing, protein damaging variations (Nonsynonymous, frameshift deletion, frameshift insertion, stopgain, stoploss)	44,331
3.	Gene based filtering (Gene pannel-352 ischemic stroke gene)	655
4.	Autosomal Recessive Model- Homozygous(1000 G- 0.1%, Exac – 0.1%)	No
5.	Autosomal Dominant Model- Heterozygous(1000 G - 0.01%, Exac – 0.01%)	92
6.	Computational methods filter -SIFT/Polyphen2/Mutation taster (variant responsible for disease causing)	76
7.	SIFT + Polyphen2 + Mutation taster(variant responsible for disease causing)	25
8.	Novel Variants	15
9.	Variants reported in Exac with MAF ≤ 0.01%	7
10.	CLNVR Reported or HGMD (Pathogenic and Pathogenic/likely pathogenic)	3

RESULTS AND DISCUSSION



- In the whole exome analysis, panel of 352 genes were piled up from publicly available database.
- To find out the relevant number of variations we used dominant model (heterozygous), MAF ≥ 0.01%, 25 variants were identified as diseasing causing by three computational tools SIFT, POLYPHEN2 and MUTATION TASTER.
- Three genes rs397516456 in ***TNNT2*** gene located on chromosome 1q32.1, rs34889882 in ***HBB*** gene located on chromosome 11p15.4 and frameshift deletion variant in ***NOTCH3*** gene located on chromosome 19p13.12 were found to be pathogenic.

CONCLUSION

- Our preliminary analysis identified three reported pathogenic and 15 novel high penetrating with large effect rare variants among young onset Ischemic stroke.
- Precise conclusion can be derived after the completion of final sample size.

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