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Khan et al.
A novel deletion mutation in the TUSC3 gene in a consanguineous Pakistani family with autosomal recessive nonsyndromic intellectual disability

Muzammil Ahmad Khan1,2, Muhammad Arshad Rafiq2, Abdul Noor2, Nadir Ali1, Ghazanfar Ali1,3, John B Vincent2,4 and Muhammad Ansar1*

Abstract

Background: Intellectual disability (ID) is a serious disorder of the central nervous system with a prevalence of 1-3% in a general population. In the past decades, the research focus has been predominantly on X-linked ID (68 loci and 19 genes for non syndromic X linked ID) while for autosomal recessive nonsyndromic ID (NSID) only 30 loci and 6 genes have been reported to date.

Methods: Genome-wide homozygosity mapping with 500 K Nsp1 array (Affymetrix), CNV analysis, PCR based breakpoint mapping and DNA sequencing was performed to explore the genetic basis of autosomal recessive nonsyndromic ID in a large Pakistani family.

Results: Data analysis showed linkage at 8p23 locus with common homozygous region between SNPs rs6989820 and rs2237834, spanning a region of 12.494 Mb. The subsequent CNV analysis of the data revealed a homozygous deletion of 170.673 Kb which encompassed the TUSC3 gene.

Conclusion: We report a novel deletion mutation in TUSC3 gene which is the second gene after TRAPPC9 in which mutation has been identified in more than one family with autosomal recessive NSID. The study will aid in exploring the molecular pathway of cognition.

Background

Intellectual disability (ID), also frequently referred to as Mental Retardation or cognitive impairment (CI), is a condition where intelligence quotient is less than 70, there is deficiency in at least two adaptive skills like communication, reading, writing, self care etc and onset before 18 year of age [1]. Assuming a population mean IQ of 100, ID is subcategorized as mild (50-55 to 70), moderate (35-40 to 50-55), severe (20-25 to 35-40) and profound (below 20-25) [1,2].

Generally it is believed that ~25% of genetic ID patients are thought to have autosomal recessive mode of inheritance [3]. To date 30 loci including six known genes have been reported to be involved in autosomal recessive NS-ID (ARNS-ID) [4]. These include PRSS12 (Protease, Serine, 12 or Neurotrypsin; MIM# 606709) [5], CRBN (Cereblon; MIM# 609262) [6], CC2D1A (Coiled-coil and C2 domain containing protein 1A; MIM# 610055) [7], GRIK2 (Glutamate receptor, ionotropic, kainite 2; MIM#138244) [8], TUSC3 (Tumor suppressor candidate 3; MIM# 601385) [9,10], TRAPPC9 (Trafficking protein particle complex subunit 9; MIM# 611966) [11-13].

Chromosome 8 is considered an average chromosome with respect to size (146.364 Mb), number of genes (1198), repeat content and degree of segmental duplication [14]. But its p arm showed high degree of sequence variations, particularly within its distal-most ~15 mega-base region. This region is believed to be of prime importance in the human genome because of the high expression pattern of nervous system related genes, and has recently been touted as a “hub” for neuropsychiatric developmental disorders [15]. Many genomic imbalances on 8p locus, such as duplication of 8p23.1-8p22.2, are...
associated with learning disability [16]. Also one gene for microcephaly (MCPH1) and one gene for NS-ID, namely TUSC3, have been identified on 8p.

In this study we present the clinical and molecular analysis of a consanguineous Pakistani family with autosomal recessive NS-ID, and report a novel mutation containing deletion of the entire TUSC3 gene (except for the promoter and 1st exon) and its down stream region at the 8p23 locus.

Methods
A: Sampling and DNA extraction
The family was recruited from a rural part of Sindh province of Pakistan, after getting prior approval from Institutional Review Board (Quaid-I-Azam U IRB#1-Biomedical; IORG0002926; IRB00003532), and blood samples were taken from available affected and unaffected family members and DNA was extracted from whole blood by following the standard proteinase K/phenol/chloroform isolation method. Written informed and photography consents (Translated in local Urdu language) were obtained from the parents/guardians of patients participating in this study, which conforms to Helsinki Declaration and local legislation.

B: Clinical Assessments
Affected family members were evaluated with the help of standard questionnaire (translated and amended version of Wechsler Intelligence Scale in Urdu) for severity of disease and IQ assessments. Photographs of affected members were taken with written consent to study and publish their facial features. Two individuals from two different loops of the pedigree were selected for cranial CT scan (IV-10 and IV-14) to screen for abnormal brain anatomical features. For information on disease onset, parents were interviewed about the prenatal, perinatal and neonatal medical histories of all affected individuals were normal. Ophthalmological and otorhinolaryngological findings were also normal. Cranial computed tomography (CT) performed on the patients from two separate loops did not reveal any brain dysmorphology or neurologic symptoms (Data not shown). No facial dysmorphism was observed except for affected individual IV-10 who had strabismus (Figure 2). In individual IV-15 cognitive impairment was also accompanied by a form of muscular dystrophy, so this individual was not treated as affected for the purposes of the analysis which was later supported by the homozygosity and CNV data. The clinical information of the affected individuals (on the basis of questionnaire) is presented in Table 1.

Molecular studies
(i) Genome-wide homozygosity mapping and CNV analysis
Genome wide homozygosity mapping revealed homozygosity-by-descent (HBD or autohomozygosity) among four affected individuals (IV-11, IV-12, IV-13) on 8p23 (Figure 3) between SNPs rs6989820 and rs2237834, which delineates a critical region of 12.494 Mb (UCSC genome Browser, May 2004 [NCBI35/hg17]). In order to confirm segregation of the identified HBD region in the entire family STS markers D8S1781, D8S262, D8S518, D8S1140, D8S277, D8S351, D8S1469, D8S1721, D8S550, D8S552, D8S1106, D8S1790, D8S1731, D8S1145 and D8S298 were genotyped. Haplotypes were generated and are presented in Figure 1, which also indicate the segregation of a minimum critical region flanked by above mentioned SNPs. Linkage analysis yielded two-point and multipoint LOD scores above 3 and 5 respectively at several markers (Table 2). This region contained a total of 96 known genes {UCSC Genome Browser, May 2004 (NCBI35/hg17)} including...
MCPH1 and TUSC3, which have already been reported to be involved in microcephaly with ID. Initially MCPH1 gene was sequenced to detect pathogenic mutation in this family but the sequence analysis only revealed the presence of known SNPs in exon 1 (rs2305023), 6 (rs2442513) and 8 (rs930557 and rs2920676). Subsequent CNV analysis of microarray data showed homozygous deletion of 170.673 Kb region in all affected individuals. The deletion encompassed almost the entire TUSC3 gene (minus the promoter and first exon) and its downstream region.

(ii) Deletion breakpoint mapping

The CNV analysis of microarray data revealed that in all affected individuals no hybridization signals were generated for 22 SNPs on chromosome 8; from rs4258002 to rs352769 as borderline deleted SNPs. With the evidence of microarray data of four affected individuals, size of deletion fragment was mapped by PCR amplification.
Table 1 Summary of the clinical data of affected individuals

| Clinical Findings       | IV-12 | IV-14 | IV-13 | IV-6  | IV-10 | IV-11 |
|------------------------|-------|-------|-------|-------|-------|-------|
| Sex                    | Female| Female| Female| Male  | Male  | Male  |
| Age on assessment      | 15 years| 10 years| 13 years| 18 years| 10 years| 11 years|
| Developmental delay    | +     | +     | +     | +     | +     | +     |
| Head Circumference     | 52 cm | 51 cm | 51 cm | Not Available | 52 cm | 50 cm |
| Speech Development     | +     | +     | +     | +     | +     | +     |
| Dysmorphic feature     | -     | -     | -     | -     | -     | -     |
| Skeletal Problem       | -     | -     | -     | -     | -     | -     |
| Ophthalmological problem | -   | -     | -     | -     | -     | -     |
| Epilepsy               | -     | -     | -     | -     | -     | -     |
| Mental retardation     | Severe| Severe| Severe| Severe| Severe| Severe|
| Growth                 | Normal| Normal| Weak  | Normal| Normal| Normal|
| Schooling              | -     | -     | -     | -     | -     | -     |
| Learning Disability    | +     | +     | +     | +     | +     | +     |
| Muscular dystrophy     | -     | -     | -     | -     | -     | -     |
| Self biting            | -     | -     | -     | -     | -     | -     |

Each column indicates the data of an individual and row presents the category of clinical symptoms, (- indicates absence, + indicates presence).

Table 2 Two point and multipoint LOD score between identified HBD and chromosome 8 markers

| Markers | Genetic Position in cm (Rutgers map, build 36) | Physical Position in bp (GRCh37/hg19) | Two point LOD Score | Multipoint LOD Score |
|---------|-----------------------------------------------|----------------------------------------|---------------------|----------------------|
| D8S1781 | 6.8                                           | 3678400                                 | 2.2342              | 4.2286               |
| D8S262  | 7.13                                          | 3777275                                 | 1.0814              | 4.2143               |
| D8S18   | 9.88                                          | 4587958                                 | 0.5706              | 1.456                |
| rs6989820 |                                         | 5041417                                 | -3.8284             | -4.0196              |
| D8S1140 | 11.96                                         | 5617059                                 | 3.1114              | 2.9368               |
| D8S277  | 14.92                                         | 6616946                                 | 2.9565              | 5.0000               |
| D8S351  | 18.94                                         | 8877155                                 | 3.0286              | 5.1617               |
| D8S1469 | 19.38                                         | 9090104                                 | 1.523               | 5.1693               |
| D8S1721 | 19.7                                          | 10240822                                | 1.8241              | 5.1717               |
| D8S550  | 20.85                                         | 10981913                                | -Infinity           | -Infinity             |
| D8S552  | 24.76                                         | 12842458                                | 2.9823              | 5.1646               |
| D8S1106 | 24.76                                         | 12936149                                | 3.2113              | 5.1646               |
| D8S1790 | 26.35                                         | 13166462                                | 3.2843              | 5.1229               |
| D8S1731 | 27.95                                         | 15338590                                | 1.3825              | 4.5748               |
| rs2237834 |                                         | 17479493                                 | -3.8284             | -4.4686              |
| D8S1145 | 32.78                                         | 18452816                                | -Infinity           | -Infinity             |
| D8S298  | 40.11                                         | 21862145                                | -Infinity           | -Infinity             |

Figure 3 Graphical representation of the homozygous by decent (HBD) regions identified by homozygosity mapper. The red bar indicates the HBD identified on chromosome 8.
the presently identified *TUSC3* involving CNVs exist in heterozygous state.

**Discussion and Conclusion**

Autosomal recessive NSID accounts for ~25% of genetic ID cases and may be more common than X linked cases, however, the molecular basis of AR-NSID is relatively poorly known because of clinical and genetic heterogeneity, and the absence of distinguishing clinical criteria. In non-syndromic ID, cognitive deficit is the sole clinical feature among the patients. It is suggested that ID may be caused by the disruption of the biological and molecular processes in the nervous system such as neuronal differentiation and synaptic plasticity, synaptic vesicles cycling and gene expression, regulation profiling etc [22]. There are number of co-translational and post-translational modifications required for protein stability and proper protein folding into its 3D structure, which are essential for normal protein function. One such post-translational modification, N-glycosylation, has previously reported to be involved in non-syndromic X-linked ID at a *TUSC3* paralogous gene, *IAP* [9].

In the current study we analyzed a four generation Pakistani family with 6 affected individuals having severe psychomotor developmental delay, segregating autosomal recessive mode of inheritance. The major clinical presentations of all patients were normal except for the cognitive dysfunction. However comparison of the clinical data (Biometric and neurological data) of our family with the patients of earlier reports did not reveal any significant difference in the phenotypic expression [9,10]. *TUSC3* is the 5th gene in autosomal recessive NSID and the 2nd in which a 3rd mutation has been identified, after *TRAPPC9*. Our study will aid in diagnostic assessment of AR-NSID individuals.

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**Author details**

1. Department of Biochemistry, Quaid-i-Azam University Islamabad, Islamabad, Pakistan.
2. Molecular Neuropsychiatry & Development Lab, Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, ON, Canada.
3. Center of Excellence in Biotechnology Research, King Saud University, Riyadh, Kingdom of Saudi Arabia.
4. Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada.

**Authors’ contributions**

MAK did the DNA extraction, microarray data analysis, PCR based breakpoint mapping and sequencing of junction fragments, MAR and AN performed...
microarray data analysis, NA and GA recruited, sampled and done clinic work up while the project designing and funding was arranged by MA and JBV. All authors have read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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