Non-syndromic Autosomal Recessive Mental Retardation in Tunisian families: Exclusion of GRIK2 and TUSC3 genes

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Analyse génétique et moléculaire de Retard Mental Non Syndromique Autosomique Récessif chez des familles Tunisiennes: Exclusion de la liaison pour les gènes GRIK2 et TUSC3

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RÉSUMÉ

Prérequis : Le retard mental est l'handicap majeur le plus fréquent, avec une fréquence de 1- 3 % dans la population générale, il constitue un problème majeur de santé publique. Les récents progrès de la biologie moléculaire et de la cytogénétique ont permis d'identifier des nouveaux gènes de retard mental non syndromique autosomique récessif (RMNS-AR).
But : Tester une liaison génétique entre deux gènes GRIK2 (6q16.3), TUSC3 (8p22) et une forme autosomique récessive de retard mental non syndromique.
Méthodes : Quatre familles Tunisiennes dont au moins deux enfants malades sont inclues dans cette étude. Le génotypage est effectué avec des marqueurs microsatellites, les analyses statistiques sont réalisées par le logiciel Easy Linkage (Version 4:00 beta).
Résultats : Le génotypage et les analyses statistiques ont montré une exclusion de la liaison génétique pour les deux gènes GRIK2 et TUSC3.
Conclusion : Les résultats de cette étude confirment l'extrême hétérogénéité clinique et génétique de RMNS-AR.

Mots-clés

RMNS-AR; Liaison Génétique; Génotypage; Haplotypes; Hétérogénéité Génétique

SUMMARY

Background: Mental retardation is one of the most frequent major handicap, with a 1-3 % frequency in the general population, it appear a major problem of public health. The recent progress of molecular biology and cytogenetic allowed to identify new genes for non syndromic autosomal recessive mental retardation (NSAR-MR).
Aim: Genetic analysis of NSAR-MR: the GRIK2 gene (6q16.3-q21) and the TUSC3 gene (8p22).
Methods: Four Tunisian families with NSAR-MR were included in this study. Genotyping was made using polymorphic microsatellite markers and statistical analysis was validated using the Fast Link programme of the Easy linkage software (V4:00beta).
Results: Genotyping and linkage analysis excluded linkage of the GRIK2 gene and TUSC3 gene.
Conclusion: Our results confirm the extreme genetic heterogeneity of NSAR-MR.

Key-words

NSAR-MR, Linkage analysis, Genotyping, Haplotypes, Genetic heterogeneity.

Mental retardation (MR) is one of the major handicaps in general population. It is defined as a generalized disorder, characterized by significantly impaired cognitive functioning and deficits in two or more adaptative behaviors (such as social skills and communication), with onset before the age of 18 years [1]. Moderate to severe mental retardation (IQ‹50) was estimated to affect 0.4-0.5% of the population [2]. These statistics oscillate among the different epidemiological studies. MR is characterized by high heterogeneous etiology [3], as an important number of studies indicate that the MR may result from genetic impairment [4, 5, 6].

The research from the genetic of non syndromic mental retardation has progressed during last years. More than 20 genes associated with non syndromic X-linked mental retardation have been identified so far [7, 8], while the role of autosomal genes remains limited. To date, only six genes have been reported in NSAR-MR [9, 10]. These are PRSS12 (MRT1: OMIM 606709) [11, 12], CC2D1A (MRT3: OMIM 608443) [13, 14], CRBN (MRT2: OMIM 607417) [15, 16, 17], GRIK2 (MRT6: OMIM 611092) [18], TUSC3 (MRT7: OMIM 601093) [19] and TRAPPC9 (MRT13:OMIM 613192) [10].

GRIK2 gene encodes a Kainate receptor subunit involved in synaptic transmission [20]. TUSC3 encodes one subunit of the oligosaccharyltransferase (OTase) complex that catalyzes the transfer of an oligosaccharide chain on nascent proteins [21].

In the present study we report the genetic analysis of GRIK2 and TUSC3 in four Tunisian families with NSAR-MR.

MATERIALS AND METHODS

MR families

Four Tunisian families including at least two affected children were selected in a cohort for families with MR, and recruited at the department of congenital and hereditary disorders. Consanguinity was present in two families (MR-S and MR-D). A total of 9 patients (5 males and 4 females) and their parents were considered for linkage analysis. The pedigrees are shown in figures 1 and 2. All affected children were born from healthy parents with normal delivery. Clinical examination did not reveal facial dysmorphism, congenital malformations and neurological disturbance. Head circumferences, body heights and weights were normal. The degrees of mental retardation were ranged from mild to severe (Table 1). Biological investigation revealed normal karyotype and normal metabolic screening. Resonance magnetic imaging (MRI) of the brain for all patients did not reveal abnormalities.

Genotyping and haplotypes analysis

After obtaining informed consent from patients and parents for minors, DNA was extracted from peripheral blood of all 21 individuals. A genetic Linkage analysis was carried out for the 4 families using two intragenic polymorphic microsatellite markers for GRIK2 gene: D6S449 (AFM 296 ze5) and D6S1543 (AFMa 111zf5). For TUSC3, four extragenic microsatellites markers were selected covering the locus: D8S1827 (AFM107ya1), D8S1731 (AFMa311wd1) and D8S549 (AFM303zc1), D8S261 (AFM123xg5) available in Ensembl database (http://www.ensembl.org) (Tel-D8S1827-D8S1731-D8S549-D8S261-Cent) (Table 2). The genetic

Table 1 : Clinical Features of the 9 affected patients

Patient	Age at examination	Mental retardation	Height	Weight	OFC	SD
MR.A-III: 1	10 years	Mild	136 cm	20,5 Kg	51cm	- 1.15 SD
MR.A-III: 2	8 years	Mild	121 cm	23 Kg	51cm	- 1.30 SD
MR.B-II : 2	19 years	Moderate	164 cm	48,5 Kg	52 cm	- 2.38 SD
MR.B-II : 3	17 years	Moderate	175 cm	58 Kg	56 cm	- 0.37 SD
MR.B-II : 5	10 years	Moderate	136 cm	28 Kg	51,5cm	- 1.37 SD
MR.D-IV:1	12 years	Mild	156 cm	39 Kg	51,5 cm	- 1.35 SD
MR.D-IV:3	4 years	Severe	99 cm	16 Kg	53 cm	+ 1.75 SD
MR.S-IV :1	11 years	Moderate	129 cm	27 Kg	ND	-
MR.S-IV :2	6 years	Moderate	113 cm	22 Kg	51 cm	+ 0.15 SD

OFC: Measure of occipito-frontal circumference.

SD : Standard Deviations. ND: non determined.

Table 2 : Lod Score of GRIK2 gene linkage analysis in families MR-B, MRD and MR-A

Family	Marker	Lod Score at θ =						θ Maximum	Zmax
		0	0.1	0.2	0.3	0.4	0.5		
MR-B	D6S449	1.45	1.17	0.84	0.49	0.15	0.00	0	1.45
	D6S1543	1.20	0.97	0.65	0.34	0.095	0.00	0	1.20
MR-D	D6S449	0.72	0.54	0.36	0.19	0.055	0.00	0	0.72
	D6S1543	0.72	0.54	0.36	0.19	0.055	0.00	0	0.72
MR-A	D6S449	0.60	0.46	0.31	0.17	0.049	0.00	0	0.60
	D6S1543	0.60	0.46	0.31	0.17	0.049	0.00	0	0.60

distance between the flanking markers D8S1827 and D8S261 was 6.55cM according to the version of the Genethon map [22]. All PCR reactions were carried out in 50μl aliquot containing 150 ng of genomic DNA, one aliquot of PCR buffer, dNTPs mix (0.2mM each), 1.5 mM MgCl2, 1 μM of each forward and reverse primer, 0.5 unit of Taq DNA polymerase (Invitrogen, Foster City, CA, USA). The cycling conditions were: 1 cycle at 96°C for 5 min, 30 cycles at 96°C for 30s, specific annealing temperature for 30s, and 72°C for 30s, and one final cycle of extension at 72°C for 7 min. PCR reactions were carried out in a Perkin Elmer 2400 thermocycler . Amplified markers were electrophoresed on an ABI Prism 3130 DNA capillary sequencer (Applied Biosystems, Foster City, USA) and were analyzed with Gene Mapper software (Applied Biosystems).

Statistical and Linkage analysis

Informativeness of markers

A marker was considered as informative, if at least one of the two parents was heterozygous for the tested marker.

Linkage analysis

Two point parametric Lod scores between the disease loci and markers were calculated using the Fast Link programme of the easy Linkage Plus software package (v4.00 beta) [23].

RESULTS

Informativeness of the markers:

GRIK2 gene: Haplotypes analysis revealed only one non informative family (MR-S) for the two tested markers (D6S449 and D6S1543).

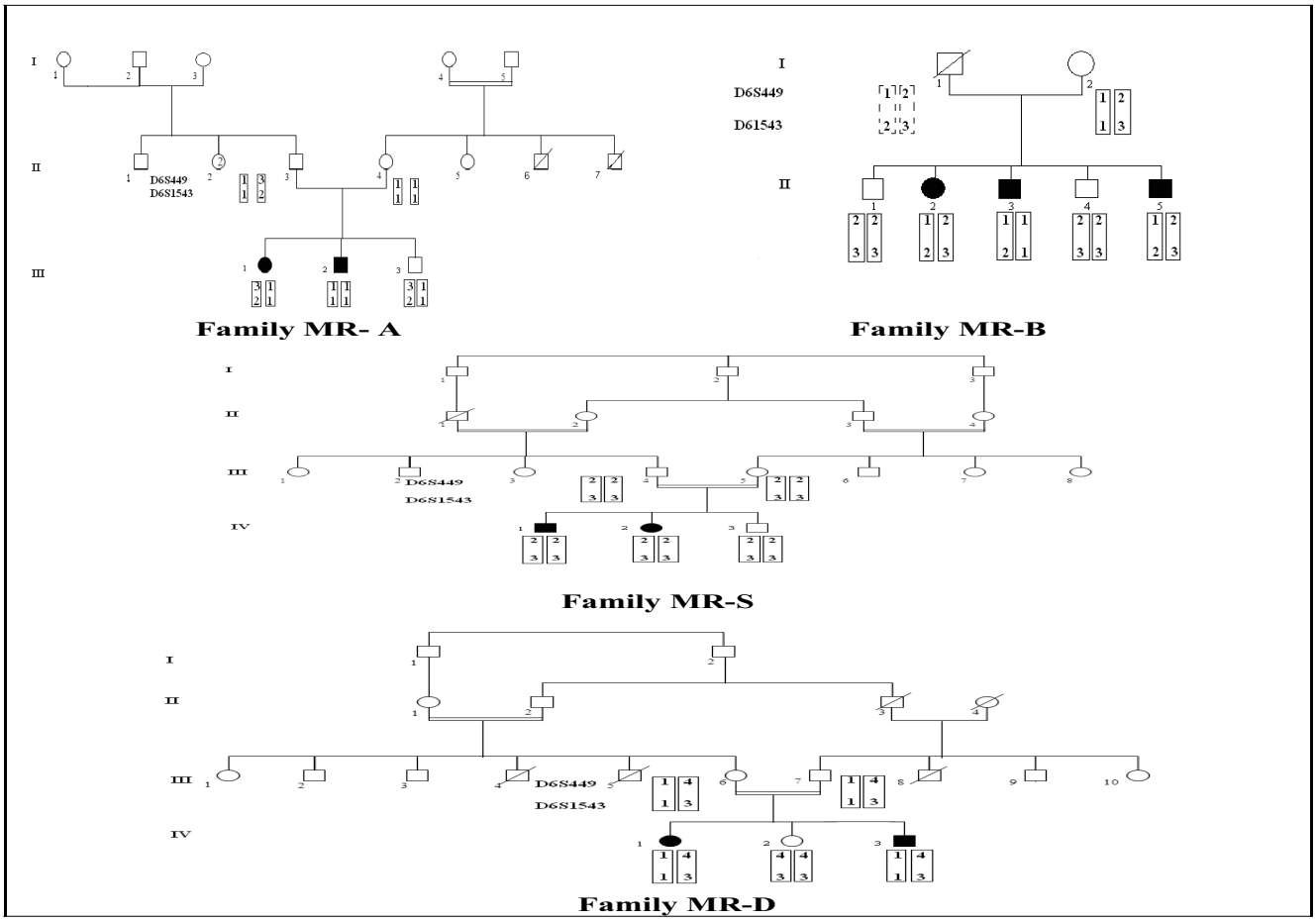
TUSC3 gene: All families were subtyped with two polymorphic markers (D8S549 and D8S1731), in order to increase informativeness, two other microsatellites markers (D8S1827 and D8S261) were added to families MR-A and MR-D. All the studied families were informative for all tested markers.

Haplotypes analysis

GRIK2 gene: For the three studied families, haplotypes analysis allowed us to exclude linkage to GRIK2 gene; indeed this analysis revealed that different haplotypes segregated with NSARMR in two families (MR-D and MR-B). For family MR-B, affected and unaffected individuals shared a common haplotype with unaffected individual (Fig 1).

TUSC3 gene: Haplotypes analysis for families MR-B and MR-S showed that different haplotypes segregated with NSAR-MR phenotype. Families MR-D and MR-A were genotyped in the

Figure 1 : Pedigrees and haplotypes analysis for GRIK2 gene. All black circles and squares indicate confirmed affected status.



first step with two polymorphic microsatellites markers D8S1731 and D8S549. Haplotypes analysis showed that affected individuals shared the same haplotypes, this giving evidence for linkage. Two additional microsatellites markers D8S1827 and D8S261 covering TUSC3 region were used; new haplotypes were shared by affected individuals but with absence of homozygosity (Fig. 2).

Lod scores analysis

Two point parametric Lod scores were calculated for three informative families MR-D, MR-A and MR-B between GRIK2 gene and the two tested markers. For TUSC3 Lod scores were calculated for all tested markers in all families. No significant values were obtained in all cases (Tables 3 - 4).

DISCUSSION

NSARMR is a clinically and genetically heterogeneous disease which is reported from different populations. To date only six genes has been identified [9, 10]. Motazacker et al. [21] confirmed linkage to the GRIK2 gene in a large Iranian family and identified a novel mutation within this gene, in the same way Garshasbi et al. [19] and Molinari et al. [21] concluded linkage to the TUSC3 gene in one Iranian and one European families and reported two different novel mutations. In the present study we reported the linkage analysis of GRIK2 and TUSC3 genes in Tunisian families. The pedigrees were consistent with an autosomal recessive inheritance pattern of the disease. The current strategy for identification of a new defect gene in NSAR-MR affected families remains positional cloning. For consanguineous families, the strategy of linkage analysis is usually based on homozygosity mapping method. The power of homozygosity mapping is to localize a disease gene, using a small number of patients. Affected individuals in a sibling should share a common homozygous haplotype transmitted by heterozygous parents [25]. In our study genotyping, haplotypes analysis and lod score calculation concluded the exclusion of the GRIK2 gene in the three informative families. For TUSC3 gene, haplotypes analysis for family MR-D could conclude to linkage but the absence of homozygozity on patients' haplotype in consanguineous family

Figure 2 : Pedigrees and haplotypes analysis for TUSC3 gene. Black circles and squares indicate confirmed affected status.

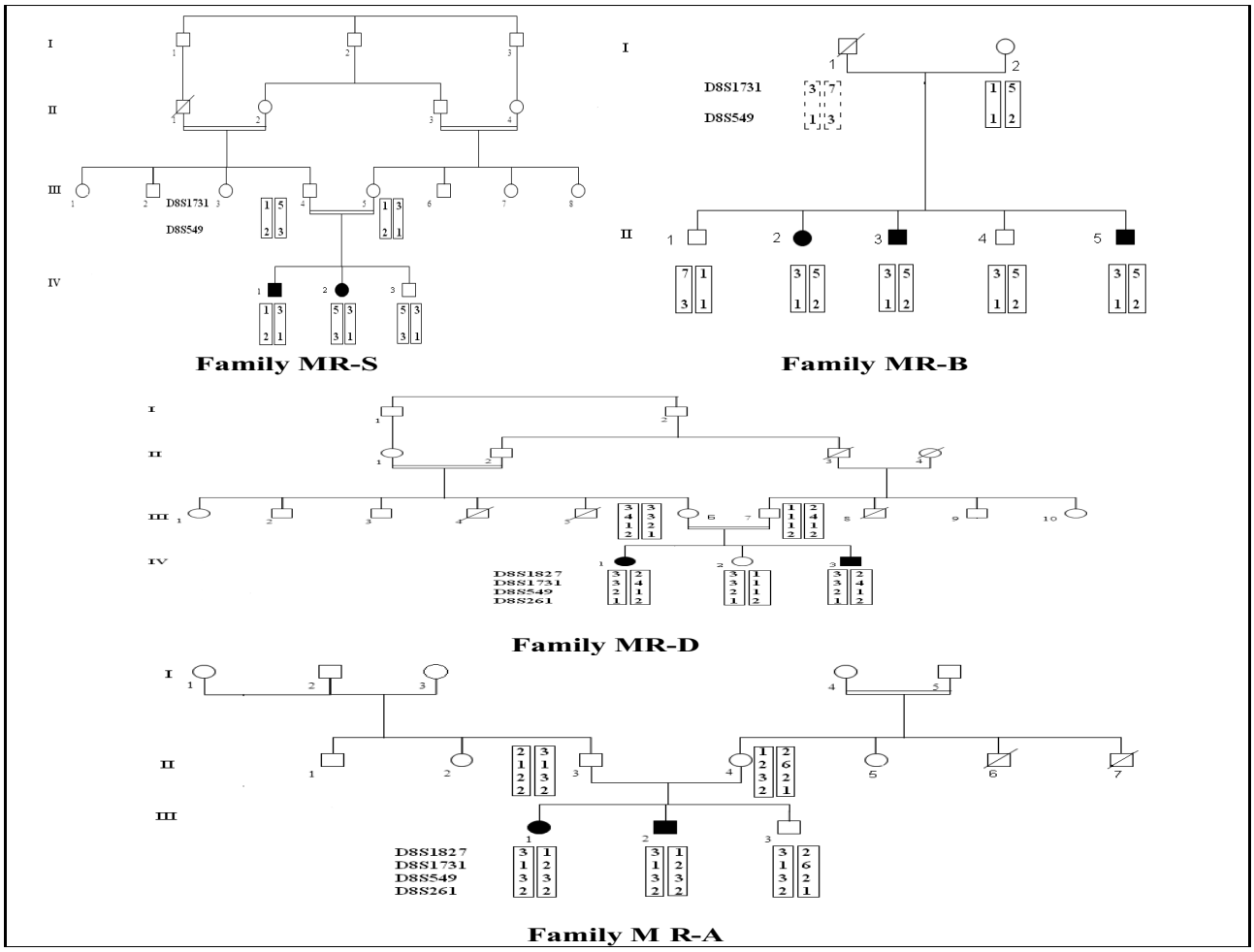


Table 3 : Lod Score of TUSC3 gene linkage analysis in families MR-D, MR-B, MR-S, MR-A.

Family	Marker	Lod Score at θ =						θ Maximum	Zmax
		0	0.1	0.2	0.3	0.4	0.5		
MR-D	D8S1827	0.72	0.54	0.36	0.19	0.055	0.00	0	0.72
	D8S1731	0.72	0.54	0.36	0.19	0.055	0.00	0	0.72
	D8S549	0.72	0.54	0.36	0.19	0.055	0.00	0	0.72
	D8S261	0.72	0.54	0.36	0.19	0.055	0.00	0	0.72
MR-B	D8S549	1.45	1.17	0.84	0.49	0.15	0.00	0	1.45
	D8S1731	1.45	1.17	0.84	0.49	0.15	0.00	0	1.45
MR-S	D8S549	1.2	0.78	0.63	0.34	0.098	0.00	0	1.20
	D8S1731	1.2	0.78	0.63	0.34	0.098	0.00	0	1.20
MR-A	D8S1827	0.72	0.54	0.36	0.19	0.055	0.00	0	0.72
	D8S1731	0.72	0.54	0.36	0.19	0.055	0.00	0	0.72
	D8S549	0.72	0.54	0.36	0.19	0.055	0.00	0	0.72
	D8S261	0.72	0.54	0.36	0.19	0.055	0.00	0	0.72

and reduced lod scores lead us to exclude the TUSC3 gene linkage. While for family MR-A linkage to TUSC3 may be a candidate gene which has to be confirmed by gene sequencing. These results are in accordance with previous papers revealing that NSAR-MR is very heterogeneous disease [9]. Genetic analysis in the same families with 3 genes (PRSS12, CRBN, CC2D1A) confirmed exclusion of linkage (personal data). It is noteworthy that other genes were implicated in our pathology, indeed Inlow and Restifio [24] estimated that the total number of genes defects causing autosomal recessive MR could run into the thousands. Linkage analysis in these families is often complemented by the genome wide scan; this approach has been proven to be successful in the identification of new genes causing NSAR-MR.

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