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# Genetic linkage studies of a North Carolina macular dystrophy family

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ABSTRACT

*Background and objective:* North Carolina macular dystrophy (NCMD) is a very rare autosomal dominant hereditary disease. Up to date there are three types of NCMD described and consequently named macular dystrophy, retinal: MCDR1, MCDR2 and MCDR3. The aim of this study was to perform linkage and copy number variation analysis for the family affected by NCMD followed by the selected candidate gene sequencing.

*Materials and methods:* This study concerned a 3-generation, non-consanguineous Latvian family with NCMD. Genome-wide scan, copy number variation and non-parametric linkage analysis was performed. Analysis resolved the locus of interest to the 5p15.33 region. Two of the genes, iroquois homeobox 2 (*IRX2*) and iroquois homeobox 4 (*IRX4*), were selected and sanger sequencing was performed.

*Results:* Linkage analysis indicated a region on chromosome 5 for the analyzed family, corresponding to a genetic locus previously described for MCDR3 (5p15-p13). Chromosomal aberrations were not identified in the affected family members. An upstream intron variant (NM_001278634: c.-139G > A (rs6876836)) in *IRX4* gene segregated with NCMD phenotype in the analyzed family.

*Conclusions:* It is unlikely to be the causative mutation of NCMD due to its high minor allele frequency 0.3532. Therefore, the role of *IRX2* and *IRX4* genes in the pathogenesis of NCMD has not been proved. Considerable variability in visual acuity between individuals of the same age group in all the families examined was noted. No overlap between NCMD grade and family generation was seen in the family described in the present study.



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## 1. Introduction

North Carolina macular dystrophy (NCMD) is an autosomal dominant dystrophy that was first reported as hereditary macular degeneration and aminoaciduria by Lefler et al. [1]. Extensive genealogical investigations in the first reported NCMD family and other families in the United States established a common ancestry to two Irish brothers who settled in North Carolina in the 1830s [2], although at the time the macular dystrophy was termed dominant foveal dystrophy [3] or central areolar pigment epithelial dystrophy [4,5].

The disease grade 1 is characterized by symmetric, tiny drusen-like yellow deposits that are found in foveal region. It can have a little impact on the vision, but also it may be found without any visual impairment at all [5,6]. Grade 2 has yellow flecks and intermediately decreased visual acuity. The third grade's clinical characteristics are colobomatous macular chorioretinal trophy that might be accompanied by pigment aggregations. Third grades visual impairment is severe [5]. Also peripheral retinal drusen may be present in all grades [6]. Progression of disease is believed to be possible only during the childhood (should be uncommon after the age of 12 years) [7]. Grading does not correlate with the successive progression of NCMD [5]. It is possible that even with a severe macular lesion relatively good visual acuity is retained [5]. If progression occurs, a subretinal neovascular membrane may develop and lead to fibrosis and noticeable loss of vision. This means that the patient may be largely asymptomatic until this happens [8].

Up to date there are three types of NCMD described and consequently named as macular dystrophy, retinal: MCDR1, MCDR2 and MCDR3. None of these types have been attributed to any particular gene; there are only linkage data available for the selected loci. Historically MCDR1 was analyzed more intensively and in the large kindred of 2000 individuals. The genetic locus 6q16 has been described and it most probably will contain MCDR1 causative gene [9]. MCDR2 type is caused by a mutation in *PROM1* gene on chromosome 4p15 [10]. In 2010 Rosenberg described a family in Scandinavia and performed linkage analysis for the ten members of family [5]. The results overlap with the previously reported 5p13-p15 region for MCDR3 containing more than 55 genes [11].

The aim of this study was to perform linkage analysis for the family affected by NCMD followed by the selected candidate gene sequencing, and to have a clinical comparison between NCMD persons from the present study and previously reported cases.

## 2. Materials and methods

The study complies with the principles of the Declaration of Helsinki and was approved by the Central Medical Ethics Committee in Latvia. All subjects participating in this study were provided with information regarding the objectives of this study and issues regarding their possible participation in the study. All subjects or legal guardians of underage subjects included in this study have submitted a written informed consent form.

### 2.1. *Subjects*

This study concerns a three-generation, non-consanguineous Latvian family consisting of six family members, who agreed to participate in the study and donate blood samples (see the pedigree chart in Fig. 1). Other family members refused participation. One member (NC01, proband 1, born in 1994) of this family was independently referred to the Vision Center of the Children's Clinical University Hospital in Riga for a diagnostic examination. The findings initiated further examination of additional family members. Eight of them were found to have dystrophic changes in the macula congruent with the clinical characteristics of North Carolina macular dystrophy (NCMD). Subjects involved in this study underwent a standard examination, including a review of their visual symptoms, assessment of ocular alignment and motility and visual acuity, assessment of refraction, slit lamp examination, color vision screening, intraocular pressure measurement, color fundus photography. In addition, some of the patients underwent visual field assessment and optical coherence

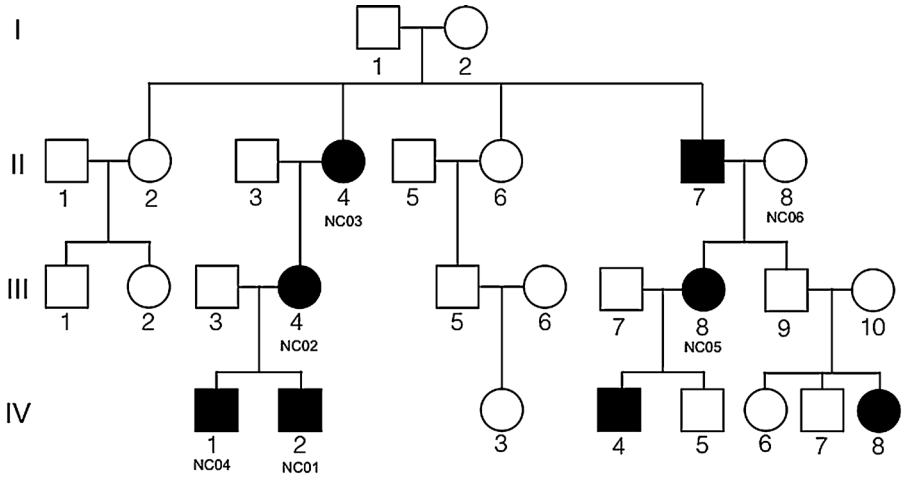


**Fig. 1 – Pedigree chart of family in Latvia affected by North Carolina macular dystrophy (NCMD). Black colored symbols represent NCMD affected individuals and NCMD unaffected family members are represented by open symbols.**