Syndromic Choroideremia: Sublocalization of Phenotypes Associated with Martin-Probst Deafness Mental Retardation Syndrome

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PURPOSE. To identify the mutation leading to syndromic choroideremia (CHM) in two families and to define fundus autofluorescence (FAF) in CHM carriers.

METHODS. The ophthalmic and clinical phenotype was investigated including FAF, neuropediatric, otorhinolaryngologic, cardiologic, and nephrologic examinations of three male patients (age, 11–46 years) and three female carriers (age, 11–46 years) from two families. Genomic DNA amplification (PCR) of the REP1 gene as well as adjacent loci was used to determine the molecular basis of the phenotype.

RESULTS. Analysis of genomic DNA revealed large deletions that asymmetrically flank REP1 in both families, ranging from a minimum size of 6.3 and 8.5 mega base pairs (Mbp) to a maximum size of 9.7 and 14.1 Mbp, respectively. In addition to CHM, patients from these families exhibited mild syndromic features, including mental and motor retardation and low-frequency hearing loss. FAF showed a distinctive pattern characterized by small areas of reduced and increased autofluorescence in all female carriers.

CONCLUSIONS. Both CHM families are the first to be described with large deletions that manifest with a mild syndromic phenotype. The location of the deletions indicates that they may allow sublocalization of the syndromic features to the most proximal region of X-linked distal spinal muscular atrophy (DSMAX) and Martin-Probst deafness mental retardation syndrome (MPDMRS). The FAF pattern is specific to CHM carriers and thus will help to identify and differentiate between carriers of other X-linked recessive carrier states such as in X-linked proximal region of X-linked distal spinal muscular atrophy. The latter accounts for approximately 25% of all REP1 mutations published so far. No correlation has been found between the size of the deletion and the severity of CHM. Strikingly, nonsense mutations in REP1 have not been identified.

Complex phenotypes were found in a minor fraction of patients with CHM who showed larger deletions varying from 5 to 12 mega base pairs (Mbp). Deletions of this size can cause syndromic CHM in the sense of a contiguous gene syndrome. Such large deletions are associated with a severe clinical phenotype including choroideremia, severe mental retardation, agenesis of the corpus callosum, cleft lip and palate, and sensorineural deafness.

To this group of patients with syndromic CHM with complex phenotypes, we add two families with two novel, large interstitial deletions of at least 6.3 to 8.5 Mbp manifesting with CHM, motor retardation, moderate mental retardation, and hearing loss. In contrast to the previously described contiguous gene deletion syndromes, the syndromic features in these two families are rather mild. This is the first report on large deletions manifesting with such a mild syndromic phenotype. In addition, we describe a characteristic fundus autofluorescence (FAF) pattern in CHM carriers.

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METHODS

Patients and Clinical Investigation

Two unrelated families (three affected males, three carrier females) were included in the study. The pedigrees are illustrated in Figure 1.

Informed consent was obtained before examination and blood draws. The study adhered to the tenets of the Declaration of Helsinki. Members of family G (I-2, II-1, II-2 and II-3) and of family H (II-5, III-2, III-3 and III-4) underwent a complete ophthalmic examination including Goldmann perimetry and Panel D15 color vision test. FAF was recorded with a standard confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph [HRA]; Heidelberg Engineering, Heidelberg, Germany). Full-field electroretinograms (ERG, maximum flash intensity, 2 cd s/m²; Nicolet, Madison, WI) and multifocal electroretinograms (mfERG; VERIS 4.8, Electro-Diagnostic Imaging, Redwood City, CA) were performed with binocular stimulation, according to ISCEV (International Society for Clinical Electrophysiology of Vision) standards and guidelines.

The diagnosis of choroideremia was based on clinical and functional findings and was confirmed by genetic analysis, as described later in the following section. If indicated, patients underwent an interdisciplinary work-up including neuropediatric, otorhinolaryngologic, cardiologic, and nephrologic examinations.

DNA Preparation and Analysis

Genomic DNA was isolated from 3 mL venous blood by standard techniques (Chemagic Magnetic Separation Module I; Chemagen, Bässweiler, Germany). Primers were designed with the Primer 3 public domain software (Primer 3: http://frodo.wi.mit.edu/ developed by Steve Rozen and Helen Skaletsky, Whitehead Institute and Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA) and synthesized (Microsynth, Balgach, Switzerland). The sequences are listed in Table 1.

PCR conditions for 50 to 100 ng of genomic DNA template were as follows: Activation of the polymerase (HotFire; Solis BioDyne, Tartu, Estonia) for 15 minutes at 95°C was followed by denaturing at 95°C.

The sequences of forward and reverse primers for PCR are given from 5′ to 3′. Capital letters represent exon sequences.
annealing at 60°C, and extending at 72°C in 35 cycles, each step lasting 1 minute. Final extension at 72°C was continued for 10 minutes. Amplified DNA fragments were visualized after gel electrophoresis through 1% agarose in the presence of ethidium bromide. For each primer combination, a nontemplate (water) control sample was included. Based on the map view of the National Center for Biotechnology Information (NCBI) human genome build 36.2 (available at http://www.ncbi.nlm.gov/mapview/map_search.cgi?taxid=9606), a list of loci was established. Not every locus between LOC401602 and NAP1L3 was tested because of sequence duplications between X- and Y-chromosome.

RESULTS

Ophthalmic Observations in Patients with Choroideremia

Family G. The 15-year-old patient (II-1) (Fig. 1) reported having nyctalopia since early childhood. His best corrected visual acuity was 20/50 (right eye) and 20/30 (left eye) in standard room illumination. He had mild subcapsular posterior cataracts. Fundus examination showed peripheral to midperipheral loss of choriocapillaris, peripapillary sclerosis of the choroidal vasculature, normal retinal vessels, and normal optic nerve heads (Figs. 2A, 2B). Goldmann perimetry revealed arcuate scotomas extending from the periphery to the center. The Panel D-15 test showed several errors along the protan and deutan axes in accordance with a known protanomaly. The scotopic ERG amplitudes and the single-flash cone response were not detectable, and a discrete Fourier transform of the flicker response did not reveal significant cone responses. MFERG amplitudes were reduced to one fifth of the norm. FAF could not be recorded from the patient due to fixation problems.

Examination results for the patient’s two brothers (II-2 and II-3) were normal including those of functional tests.

Family H. The 21-year-old patient (III-2) (Fig. 1) reported adaptation difficulties. His best corrected visual acuities were 20/20 (right eye) and 20/25 (left eye) in standard room illumination. He had normal anterior segments. Fundus examination showed peripheral to midperipheral loss of choriocapillaris, peripapillary sclerosis of the choroidal vasculature, normal retinal vessels, and normal optic nerve heads with a peripapillary chorioretinal atrophy, the macula was relatively hyperpigmented (Figs. 2C, 2D). Goldmann perimetry revealed an arcuate scotoma (I/4) from 50° to 45° in the right eye. The saturated Panel D-15 test was without errors in both eyes. There was one error during unsaturated testing of the right eye and four errors of the left eye. The scotopic ERG signals were

FIGURE 2. Fundus (left two columns) and FAF (right two columns) of the patients with choroideremia. (A, B) II-1 (family G), (C–F) III-2 (family H), and (G–J) III-3 (family H). Fundus showed peripheral to midperipheral loss of choriocapillaris, normal retinal vessels, and optic nerve heads, peripapillary sclerosis (black arrows: some of the sclerosed vessels) of the choroid in II-1, and relative hyperpigmentation of the macula (✩) in III-2 and III-3. FAF was decreased (darker) in areas of chorioretinal atrophy (E, F, I, J).
not detectable, and photopic responses were reduced to one fourth of the norm. MfERG amplitudes were reduced without local preferences, yet in the normal range. FAF was almost intact in the macula but severely reduced in the midperiphery to periphery (Figs. 2E, 2F).

The 18-year-old sibling patient (III-3) had adaptation problems and mild nyctalopia. His best corrected visual acuities were 20/20 (both eyes) in standard room illumination. He had normal anterior segments. Fundus examination showed peripheral to midperipheral loss of choriocapillaris, normal retinal vessels, and normal optic nerve heads, the macula was relatively hyperpigmented (Figs. 2G, 2H). Goldmann perimetry results were normal. The saturated Panel D-15 test showed four errors in the right eye, six errors during unsaturated testing of the right eye, and five errors in the left eye, respectively. The scotopic ERG was reduced, and peak times of the photopic responses were prolonged. MfERG amplitudes were normal, yet showed a pathologic perifoveolar peak time increase. FAF was almost intact in the macular area but was severely reduced in the midperiphery to periphery (Figs. 2I, 2J).

**General Clinical Data of the Patients with Choroideremia**

**Family G.** Delayed motor and mental development became obvious during patient II-1’s first years of life. At the age of 4 years, a microhematuria episode was noted. Metabolic diseases were ruled out by serologic (thyroid stimulating hormone, triiodothyronine T3, thyroxine T4, circulating T3 and T4, electrolytes, creatinine, glucose, glutamic pyruvic transaminase, C-reactive protein, and total protein), blood (differential blood count), and urinary (uric acid, carbonic acid, fatty acids, and monosaccharides) assays. Cranial magnetic resonance imaging at age 7 was normal. Consequently, the diagnosis was global developmental delay of unknown origin. During the patient’s visit to our department, he displayed gait problems. His mother reported that he had learned to walk in his 24th month of life and was attending a special school for learning-impaired children. The patient underwent neuropsychiatric, nephrologic, and otorhinolaryngologic examinations. There was no hint of muscle disease. The gait problem and the mild mental retardation were attributed to general delayed development. A beginning of auditory hair cell damage affecting the low-frequency hearing was found in audiometry. The nephrologic examination was unremarkable except for a pelvic dilatation without functional impact.

**Family H.** Patient II-2 had had problems with motor coordination since infancy. He had learned to walk in his 18th month of life and showed delayed speech development with ongoing dyslalia. He had attended a special-needs school to graduate from a minimum-requirement school. In a neuropsychiatric examination he showed reduced facial expression, unilateral postural destabilization, dysdiadochokinesis, and delayed finger tapping.

His brother, patient II-3, displayed fine motor-skill problems during the third to fourth years of life. He had learned to walk in the 15th month of life. He graduated from a minimum-requirement school. A neuropsychiatric examination was unremarkable. Both brothers were not available for otorhinolaryngologic testing.

**DNA Analysis**

**Family G.** DNA amplification of all REP1 coding exons (1–15) from the proband (II-1) and his mother (I-2) revealed fragments of expected sizes only in the mother, indicative of a deletion of the entire coding region of REP1 (data not shown). In searching for the break points, we amplified DNA markers within various distances to REP1 (Fig. 3, Table 2).

Of the proximal markers CXorf43, FAM121A, and POF1B, only CXorf43 was amplified in the patient (Fig. 3).
The distal markers \textit{DACH2}, \textit{KLHL4}, \textit{CPXCR1}, and \textit{NAP1L3} did not amplify, but PCR fragments specific to the \textit{DIAPH2} locus were present (Fig. 5). From these data, we concluded that the proband carried a minimal-sized deletion of \(~8.5\) Mbp (Fig. 4). Based on the presence of the markers \textit{CXorf43} and \textit{DIAPH2}, the maximum size of the deletion was estimated as \(14.1\) Mbp (Fig. 4).
**Family H.** DNA amplification of *REP1* exon 10 from the proband (III-2) and his mother (II-5) revealed a fragment of expected size in the mother, but not in the proband, suggesting a deletion of *REP1* (data not shown). As described for family G, we used various flanking loci (Figs. 3, 4; Table 2) to identify the extent of the deletion. With the marker Cxorf43, a fragment of expected size was amplified from both DNA samples, mother and affected son. Marker EAM121A did not yield a fragment from DNA of the affected son. Markers DACH2, KLHL4, CPXCR1, USP12P2, and PABPC5, which are distal to *REPI*, were absent. From this, we conclude that the deletion had a minimum size of approximately 6.3 Mbp. Since amplification of marker LOC401602 was successful in both samples, the maximum deletion size was determined to be 9.7 Mbp (Figs. 3, 4). DNA analysis from patient III-3 revealed a pattern identical to that found for proband III-2. Sibling carrier III-4 showed the same DNA amplification pattern as the mother II-5 (data not shown).

**Ophthalmic Observations in the Carriers**

The age of the three carriers at the time of diagnosis was 44 (family G, I-2), 46 (family H, II-5), and 10 (family H, III-4) years. They did not have any ophthalmic or other medical symptoms. Carrier II-5 showed signs of bronchial asthma and allergies against pollen and animal fur. Visual acuities, anterior segments, visual fields, and color vision tests were normal. Scotopic and photopic full-field ERG and mFERG amplitudes were normal; however, the central mFERG amplitude was slightly diminished in individual I-2, and scotopic full-field ERG amplitudes of individual III-4 were at the lower normal limit. The fundi showed mottling of the RPE, discrete peripheral RPE clumping and small areas of chorioretinal atrophy (Figs. 5A, 5B, 5E, 5F, 5I, 5J).

In all three carriers, FAF displayed speckles, small areas of reduced or increased autofluorescence, and an otherwise normal autofluorescence (Figs. 5C, 5D, 5G, 5H, 5K, 5L). In the youngest carrier III-4, the speckled pattern was rather subtle (Figs. 5K, 5L).

**DISCUSSION**

In two families with syndromic CHM, we detected two novel large deletions of at least 8.5 and 6.3 Mbp to at most 14.1 and 9.7 Mbp, respectively. Patients in both families exhibited a mild but complex phenotype. Furthermore, a unique FAF pattern was found in CHM carriers.

Figure 5. Fundus (left two columns) and FAF (right two columns) of the choroideremia carriers. The fundi showed mottling of the retinal pigment epithelium, discrete peripheral RPE clumping, and small areas of chorioretinal atrophy (some marked with arrows in A, B, more subtle in the other fundi). There was a normal background autofluorescence with a characteristic speckled pattern of areas with reduced and increased autofluorescence. In the youngest carrier (II-4) these changes were very subtle. (A-D) Carrier I-2 (family G); (E-H) II-5 (family H); and (I-L) III-4 (family H).
FAF Pattern in CHM Carriers

FAF is used to monitor the natural autofluorescence of lipofuscin that accumulates in RPE cells after ingestion of rod outer segments and can be recorded in vivo by a confocal scanning laser ophthalmoscope. Some retinal dystrophies show distinctive changes in FAF, which can be displayed as a radial pattern in XLRD, or a ring-shaped pattern of increased FAF, indicative of autosomal RP, X-linked cone-rod dystrophy, autosomal dominant cone-rod dystrophy, or macular dystrophies such as Stargardt disease. To date, FAF data on choroideremia carriers are very limited. The characteristic flecked autofluorescence pattern observed in our carriers seems to be specific to choroideremia carriers and has not been described in any other retinal dystrophy so far. A normal background autofluorescence is flecked by some spots of decreased and others of increased autofluorescence. Spots of decreased autofluorescence are likely to correspond to small patches of photoreceptor loss associated with RPE atrophy. Spots of increased autofluorescence are possibly due to increased lipofuscin accumulation in RPE cells due to the high turnover rate of photoreceptor outer segments or to impaired phagocytosis. An age-dependent intensifying of the autofluorescence pattern seems possible, since the youngest carrier (family II, III-4) showed only very subtle changes compared with the adult carriers.

These three new cases of altered autofluorescence in choroideremia carriers confirm the preliminary results of Wegscheider et al. who reported similar findings in five independent carriers (Wegscheider E, et al. IOVS 2005;46:ARVO E-Abstract 4088) and Renner et al., who described one carrier.

According to these data and our findings, FAF promises to be a powerful means of identifying CHM carriers and of differentiating between X-linked retinitis pigmentosa carriers who show a radial FAF pattern.

Deletion Mapping: Understanding the Syndromic Aspect Displayed by the Two Families

The large deletions involving X-chromosome q21.1-q21.35 in our two families encompass several mapped syndromes, either in their entirety or only parts of them. REPI (MIM 300390) is completely lacking, which correlates with the molecular and clinical findings in the families described herein, as well as with previously reported deletions of the REPI gene leading to syndromic and nonsyndromic choroideremia. The region carrying the premature ovarian failure syndrome (POF2B; MIM 300064) is also entirely deleted, but as all affected members in our families were males, the syndrome is not expected to become manifest. It is unlikely that the female carriers of the deletion will be affected by this syndrome, but it remains to be seen, although heterozygotes have not been described to manifest this syndrome.

The X-linked distal spinal muscular atrophy DSMAX syndrome (MIM 300489) is only partially affected by the deletions described herein, although the distal end may be completely included in the deletion (Fig. 4). This syndrome has not yet been mapped to a particular gene. Rather, its region extends over 21 Mbp. It is an X-linked recessive progressive disorder causing atrophy of the upper and lower limbs. In addition to foot deformities, gait instability has been reported. Even though patient II-1 of family G had no diagnosed muscle disease, it is conceivable that his gait problems constitute part of this syndrome, as may the delayed motor coordination of patients III-2 and III-3 from family H. As no cognitive or sensory impairment has been described to part of this syndrome, the mental retardation diagnosed in the patients we have described appears unrelated to DSMAX. The fact that female carriers in our two families did not show signs of clinical conditions is consistent with reports that female carriers appear to be clinically normal.

It seems more likely that these aspects are components of the Martin-Probst deafness-mental retardation syndrome (MPDMS; MIM 300519). The entire chromosomal region assigned to this syndrome encompasses about 68 Mbp, of which only approximately 13% were deleted in our two families. A multitude of disorders such as congenital sensorineural hearing loss, mental retardation, short stature, congenital umbilical hernia, facial dysmorphism, abnormal teeth, positioned nipples, and abnormal dermatoglyphics all belong to this syndrome. Progressive pancytopenia in adulthood has also been observed. The mental retardation of the male patients described in this study could be a component of MPDMS syndrome. In addition, the early stages of auditory hair cell damage for low frequencies diagnosed in patient II-1 from family G may also be accounted for by the MPDMS syndrome. We can exclude the possibility that this hair cell damage is related to the deafness mixed with perilymphatic gusher syndrome, because marker CXorf43 is present in the patients and hence the deletion may not extend to POI3F4 (Fig. 4). Taken together, the partial overlap of the deletions described herein with previously reported syndromes may allow a sublocalization of particular phenotypes.

Two further syndromes, coronary heart disease, susceptibility to 3 (CHDS3; MIM 300464) and diaphanous homolog 2 (DIAPH2; MIM 300108) map only to the distal end of the potentially deleted region in family G. None of the characteristic phenotypical aspects were noted in our patients, suggesting that the deletion may not extend this far distally. Fine mapping of the deletion by using additional molecular markers may support our interpretation. We were not able to conduct fine-mapping because we encountered difficulties with sequence specificity due to partial sequence duplications of this X-chromosomal region on the Y-chromosome.

Large deletions have also been reported earlier to be associated not only with choroideremia but also with cleft lip and palate as well as mental retardation and deafness. While three of five syndromic deletions showed only partial overlap with deletions of our patients, two of the five (patient NP and XL62) covered the entire region deleted in both families reported herein. Of note, the phenotypes displayed in our families are considerably less severe. This lack of severity may in part be explained by the distal extension of the published deletions beyond the breakpoints seen in our patients. Similarly, the deafness phenotype reported by Cremers et al. and the here reported hearing loss of low frequencies are most likely of different origin, since the previously published deletions do not extend this far distally.

An alternative explanation could be breakage, repair, and rejoining of previously unrelated, nonconsecutive DNA sequences. Detailed knowledge of DNA sequences adjacent to the deletion breakpoints may shed light on this possibility. The individual genetic background may also contribute to the variable phenotypes.

Conclusions

In this study we found an FAF pattern that is unique to CHM carriers and thus will be a powerful tool for identifying CHM carriers alongside funduscopy and to distinguish between carriers of other X-linked recessive mutations such as in retinitis pigmentosa.

Further, we report two comparably large novel deletions in the range of minimally 6 Mbp to maximally 14 Mbp surrounding the REPI gene, which is associated with choroideremia. To date few large deletions of similar size and localization have
been characterized by severe syndromic features in addition to choroideremia. Our families are the first described as carrying large deletions yet showing rather mild clinical manifestations. Although our data do not narrow down the MDPMR syndrome locus, they potentially sublocalize the mild hearing defect as well as mental retardation and motor problems to the most proximal region of the locus for this complex syndrome.

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