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Genetic Heterogeneity of Syndromic X-Linked Recessive Microphthalmia-Anophthalmia: Is Lenz Microphthalmia a Single Disorder?

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Nonsyndromic congenital microphthalmia or anophthalmia is a heterogeneous malformation with autosomal dominant, autosomal recessive, and X-linked modes of inheritance. Lenz microphthalmia syndrome comprises microphthalmia with mental retardation, malformed ears, skeletal anomalies, and is inherited in an X-linked recessive pattern. Prior studies have shown linkage of both isolated (or nonsyndromic) anophthalmos (ANOP1, [MIM 301590]) and Lenz syndrome [MIM 309800] to Xq27–q28. Nonsyndromic colobomatous microphthalmia [MIM 300345] has been linked to Xp11.4–Xq11.1. We describe a five-generation African-American family with microphthalmia or anophthalmia, mental retardation, and urogenital anomalies, in an X-linked recessive inheritance pattern, consistent with Lenz syndrome. Initial linkage analysis with microsatellite markers excluded the region in Xq27–q28 previously reported as a candidate region for ANOP1 [MIM 301590]. An X-chromosome scan revealed linkage to a 10-cM region between markers DXS228 and DXS992 in Xp11.4–p21.2. Multipoint analysis gave a maximum LOD score of 2.46 at marker DXS993. These data show that X-linked recessive syndromic microphthalmia exhibits genetic heterogeneity. In addition, it suggests that Lenz microphthalmia syndrome, previously thought to be a single disorder, may represent an amalgam of two distinct disorders.

KEY WORDS: microphthalmia; X-linked; syndromic; linkage analysis

INTRODUCTION

Lenz microphthalmia syndrome (microphthalmia with associated anomalies or MAA [MIM 309800]) is a type of syndromic microphthalmia (XLSM or X-linked syndromic microphthalmia) and was credited to Lenz for his description of a family in 1955 [Lenz, 1955]. The features in that family included mental retardation, microphthalmia, high palate, anteverted ears, dental abnormalities, congenital heart defect, skeletal defects of the fingers and clavicles, unilateral renal aplasia, and cryptorchidism. The eye findings in the family were variable and ranged from anophthalmia to severe microphthalmia to mild microphthalmia with retained vision, horizontal nystagmus and iris coloboma. Subsequent to the report by Lenz [1955], 15 additional case reports [Hoefnagel et al., 1963; Hermann and Opitz, 1969; Goldberg and McKusik, 1971; Ogunye et al., 1975; Baraitser et al., 1982; Glanz et al., 1983; Pallota, 1983; Brunquell et al., 1984; Traboulsi et al., 1988; Graham et al., 1991; Antoniades et al., 1993; Ozkinay et al., 1997; Temtamy et al., 2000; Forrester et al., 2001; Lehman et al., 2001] of X-linked microphthalmia have been published. These reports show that anophthalmia and microphthalmia are part of a spectrum of ophthalmic malformations. Furthermore, they also show that there is a wide range of associated extra-ocular malformations in Lenz microphthalmia that up to now were assumed to be caused by allelic heterogeneity. It has also been assumed that microphthalmia without associated skeletal, dental or urogenital anomalies (Clinical Anophthalmos; ANOP1 [MIM301590]) represented a disorder distinct from Lenz microphthalmia.

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Given that the formation of the globe is a complex multistep embryologic process (requiring the interaction of multiple transcription factors and their target genes), we hypothesize that separate genetic defects disrupting steps along this developmental pathway could produce phenotypes with similar and overlapping associated anomalies and that the level of residual gene function may explain this broad phenotypic spectrum.

We identified an African-American family with six affected males exhibiting variable features of microphthalmia or anophthalmia, microcephaly, mental retardation, renal aplasia, cryptorchidism, and hypospadias. The pedigree of this family is shown in Figure 1. Of the six affected males, three are currently living and two were available for analysis. We performed linkage analysis on this family to test the hypothesis that Lenz syndrome is a single disorder distinct from nonsyndromic microphthalmia.

CLINICAL REPORT

The proband (Fig. 2) was a full term African-American male born to a 22-year-old gravida 1 para 0 mother and her nonconsanguineous partner. His birth weight was 1.9 kg (< 3rd centile), length 40 cm (< 3rd centile), and occipitofrontal circumference (OFC) was 29.5 cm (< 3rd centile). Apgar scores were 6 at 1 min and 8 at 5 min. The patient was noted to be small for gestational age and have microcephaly. His ears were posteriorly rotated with a right preauricular pit, he had mild micrognathia, and his palpebral fissures were short. His eyelids were tightly closed, and attempts to visualize the globes were unsuccessful. His hands were clenched with extra flexion creases located proximally at each finger, his testes were partially descended, and a Grade I hypospadias with chordee was present. His anus was normally positioned and patent. An ophthalmologic examination under sedation revealed a 5 mm and 14 mm globe, respectively, in the right and left orbits. Visual Evoked Response testing suggested that the left, but not the right globe could have light perception. A cranial MRI showed a hypoplastic corpus callosum, cingulate gyrus, and optic chiasm (Figs. 3,4). Peripheral blood chromosome analysis revealed a normal 46,XY karyotype (resolution 650 bands).

He is growing well currently with his height near the 50th centile and weight between the 5th-10th centile. His microcephaly persists with his OFC at the 50th centile for a 5-month-old when measured at a chronological age of 14 months. From 7 months of age he

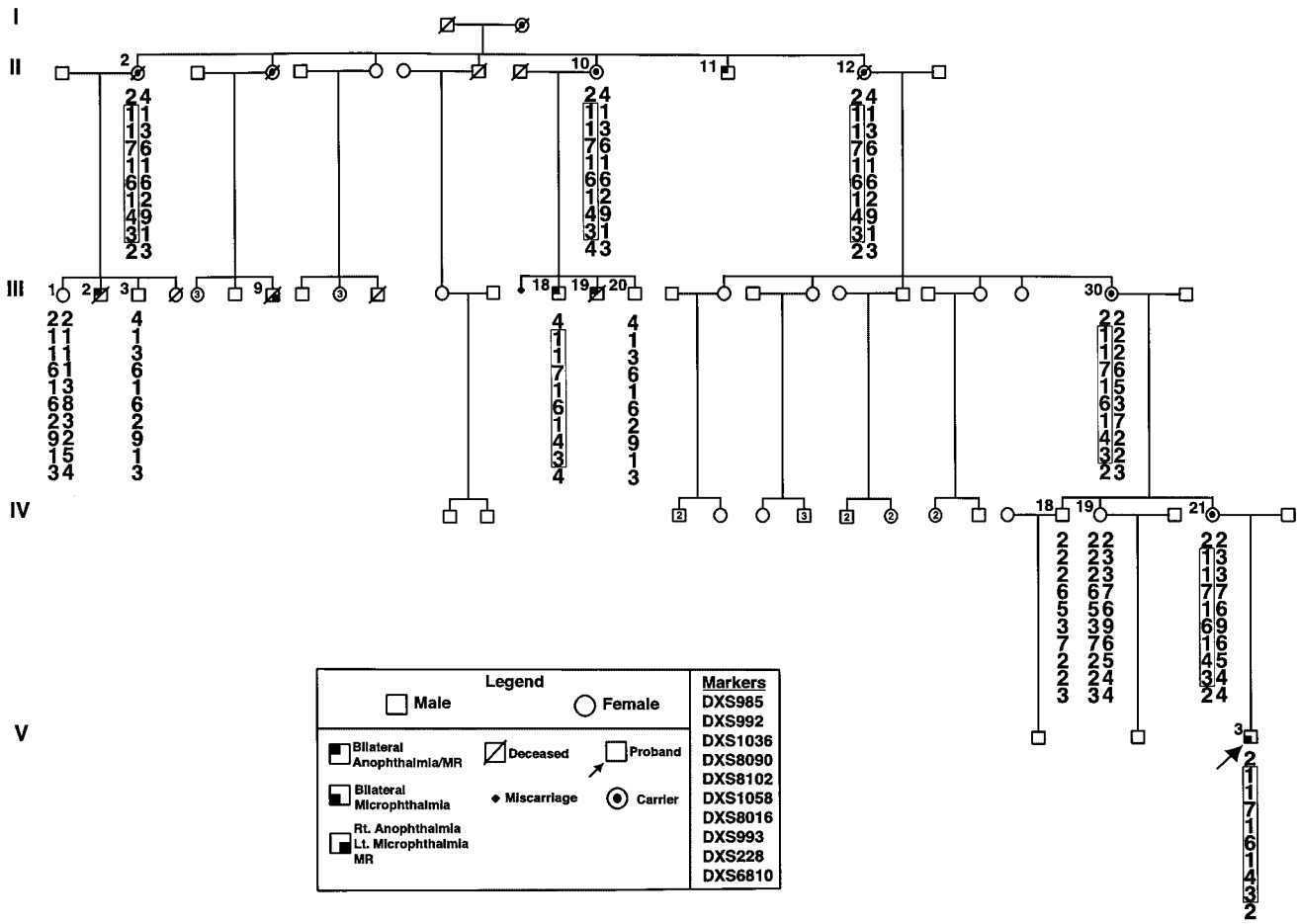

Fig. 1. Pedigree of family with XLSM. Blackened symbols represent affected individuals. A dot (●) indicates obligate carriers. An arrow points to the proband. The disease haplotype is denoted by a rectangle.

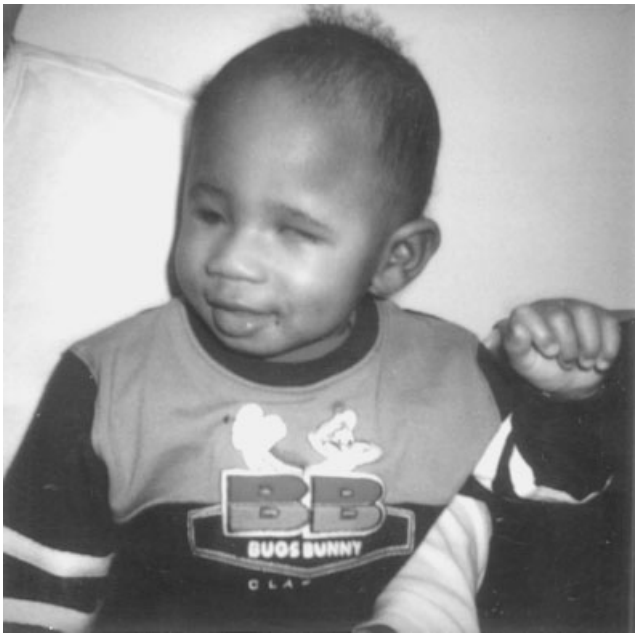

Fig. 2. Proband at 6 months of age with short palpebral fissures R > L, microcephaly, and fisted left hand.

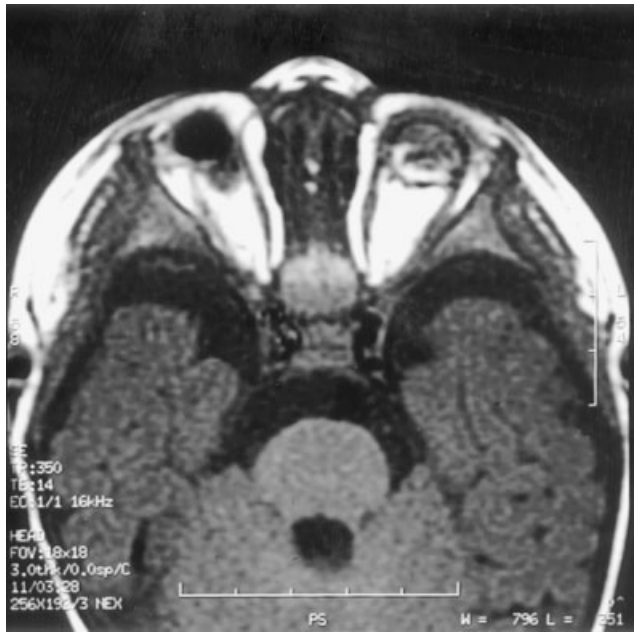

Fig. 4. Coronal view of probands cranial MRI showing small globes R > L and hypoplastic optic chiasm.

ceased to hold his hands in a fisted position, but continued to have mild to moderate axial hypotonia. His motor development is mildly delayed. He sat alone and crawled at 12 months, but is unable to walk independently at 15 months. His first word was spoken at 12 months. His sleep-wake patterns are mildly disturbed with naps during the day and early awakening during the night.

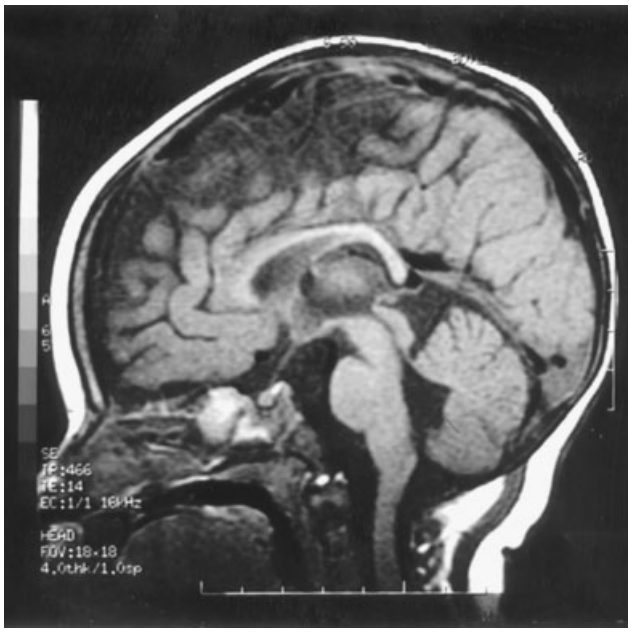

Fig. 3. Sagittal view of probands cranial MRI showing hypoplastic corpus callosum and cingulate gyrus.

MATERIALS AND METHODS

The research study was reviewed and approved by the National Human Genome Research Institute Institutional Review Board. After informed consent, molecular analysis was performed on the DNA samples of two affected males, five obligate carrier females, three unaffected males, and two female sibs of indeterminate carrier status. Genomic DNA was extracted from whole blood with QIAmp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA). STRP markers were selected from the Marshfield map (Research Genetics, Inc.), and analyzed as previously described [Broman et al., 1998]. The FASTLINK program v4.1P was used for linkage calculations. Allele frequencies were set to CEPH values when available. For markers where no CEPH values were available, equal allele frequencies were used. For markers where novel alleles were detected, the frequency of the novel alleles was set the same as the least frequent known allele. Penetrance was set to 0.99 for mutant males and homozygous females and to 0.01 for wild-type males and wild-type and heterozygous females. The mutant allele frequency was set to 0.0001.

RESULTS

Genotyping was performed at Xq27–q28 to exclude linkage to ANOP1 [MIM 301590] previously reported by Graham et al. [1991], and MAA, mapped by Forrester et al. [2001] for their respective microphthalmia families. Linkage was excluded to this region in the present family using markers GATA182EO4, DXS1215, DXS8086, and DXS1073 (data not shown). On the basis of this exclusion, we have designated the locus for the gene altered in this family as ANOP2. To map ANOP2,

an X chromosome scan was performed using Marshfield X-chromosome markers (spaced from 4–7 cM). Initial linkage was found to marker DXS8090 with a LOD score of 1.94 ($\theta = 0$). Two-point analyses with seven additional markers (Table I) showed a maximum LOD score of 2.34 at ($\theta = 0$) for DXS993. Five-point linkage analysis between the disease locus and markers DXS985, DXS1036, DXS8016, and DXS993 gave a maximum LOD score of 2.46. Haplotypes were constructed for the 12 individuals who were genotyped (Fig. 1). Two affected males had a crossover between DXS985 and DXS992 defining the telomeric boundary and between DXS228 and DXS6810 defining the centromeric boundary, indicating that the disease locus lies in this 10 cM interval. This candidate region includes 19 known genes: *DMD*, *TBCA*, *DKFZP761J7121*, *PRRG1*, *XK*, *TCTE1L*, *SRPX*, *OTC*, *TM4SF2*, *STRAIT11499*, *FLJ20285*, *APT6M8-9*, *MKRN4*, *USP9X*, *DDX3*, *NYX*, *CASK*, *ABCB7*, and *I-4* [OMIM, 2001]. Some of these genes are expressed in the eye and are currently being analyzed for mutations. In addition, there are several predicted or uncharacterized genes in the region and numerous expressed sequence tags.

DISCUSSION

Mild microphthalmia may be clinically difficult to distinguish from eyes of normal size without taking physical measurements. An age adjusted axial length of the globe below the 5th centile defines microphthalmia. For adults, a total axial length of less than 18.5 mm confirms the diagnosis [Weiss et al., 1989]. Total axial length is measured with A-scan ultrasonography and is subdivided into anterior (from the anterior edge of the corneal spike to the posterior edge of the lens spike), and posterior segments (from the posterior edge of the lens spike to the leading edge of the retinal spike). Thus, microphthalmia may affect the anterior or posterior segment in isolation or in total. Warburg [1993] estimates the frequency of congenital microphthalmos among newborn Caucasian infants to be 1.2–1.8 per 10,000. She proposed a phenotypic classification of microphthalmia based on ocular findings and separates this malformation into two groupings, partial and total. Total microphthalmos (both anterior and posterior segments are foreshortened) include A) congenital cystic eye (the optic vesicle fails to invaginate resulting in complete absence of the globe); (B) anophthalmos (a clinically severe form of microphthalmia where ocular structures can be found only by histopathologic sections); and C) nanophthalmos (microphthalmos without other major malformations). Phthisis of the globes as seen in Norrie disease (NDP, [MIM 310600])defines a separate grouping. In Norrie disease [MIM 310600], the globes are normal in size, structure, and function at birth. Postnatally, the globes shrink due to degeneration from pseudoglioma formation in the globe leading to hemorrhage and necrosis.

Lenz microphthalmia can include congenital cystic eye and anophthalmos. We tabulated 15 published cases of Lenz microphthalmia along with one case report of nonsyndromic colobomatous microphthalmia [Lehman et al., 2001]. The latter was included based on the linkage data that suggests it may be an allelic condition. In total, 36 individuals with X-linked recessive microphthalmia are listed with their extra-ocular findings in Table II. Review of these studies showed that the most constant associated feature is urogenital anomalies with an incidence of 77%. Mental retardation or developmental delay approached 63%. Extra-ocular manifestations such as ear, digital, and spine anomalies were present in more than half of the affected individuals. Presence of microcephaly and ear anomalies varied within families. In our kindred (see Fig. 1), individuals V-3 and III-19 have microcephaly whereas III-9 and III-19 have anteverted ears. In contrast, individual III-18 has neither of these anomalies (see Figs. 2, 5, 6). We conclude that the features of microcephaly, mental retardation, ear, spine, and urogenital anomalies in this family warrant a diagnosis of Lenz microphthalmia.

Based on the data reported here and the linkage studies reported by Graham et al. [1991] and Forrester et al. [2001], we conclude that there are at least two genetic loci that can cause what is currently considered to be Lenz microphthalmia. The studies by Graham et al. [1991] and Forrester et al. [2001] localize the candidate gene region in their families to Xq27–28 in contrast to the results here showing linkage to Xp11.4–21.2. Lehman et al. [2001] described a Mexican-American family with X-linked recessive nonsyndromic colobomatous microphthalmia [MIM 300345]. Affected

TABLE I. Two Point LOD Score Analysis Between Disease Locus and Chromosome X Markers

	LOD score at $\phi =$									
Marker	0.00	0.01	0.02	0.03	0.04	0.05	0.10	0.20	0.30	0.40
DXS985	−1.83	−0.80	−0.54	−0.39	−0.29	−0.21	0.00	0.12	0.12	0.70
DXS992	**1.32**	1.29	1.27	1.24	1.22	1.19	1.06	0.79	0.52	0.25
DXS1036	**2.18**	2.14	2.10	2.06	2.01	1.97	1.76	1.30	0.83	0.36
DXS8090	**1.94**	1.90	1.85	1.81	1.77	1.73	1.51	1.04	0.56	0.16
DXS8102	**1.15**	1.13	1.11	1.09	1.07	1.04	0.94	0.71	0.48	0.24
DXS1058	**1.54**	1.51	1.48	1.46	1.43	1.40	1.25	0.93	0.60	0.28
DXS8016	**2.10**	2.06	2.02	1.98	1.94	1.90	1.70	1.27	0.81	0.36
DXS993	**2.34**	2.29	2.25	2.21	2.16	2.12	1.89	1.40	0.89	0.39
DXS228	**2.23**	2.19	2.15	2.10	2.06	2.02	1.80	1.33	0.84	0.37
DXS6810	−2.03	0.48	0.74	0.88	0.97	1.03	**1.13**	1.01	0.73	0.37

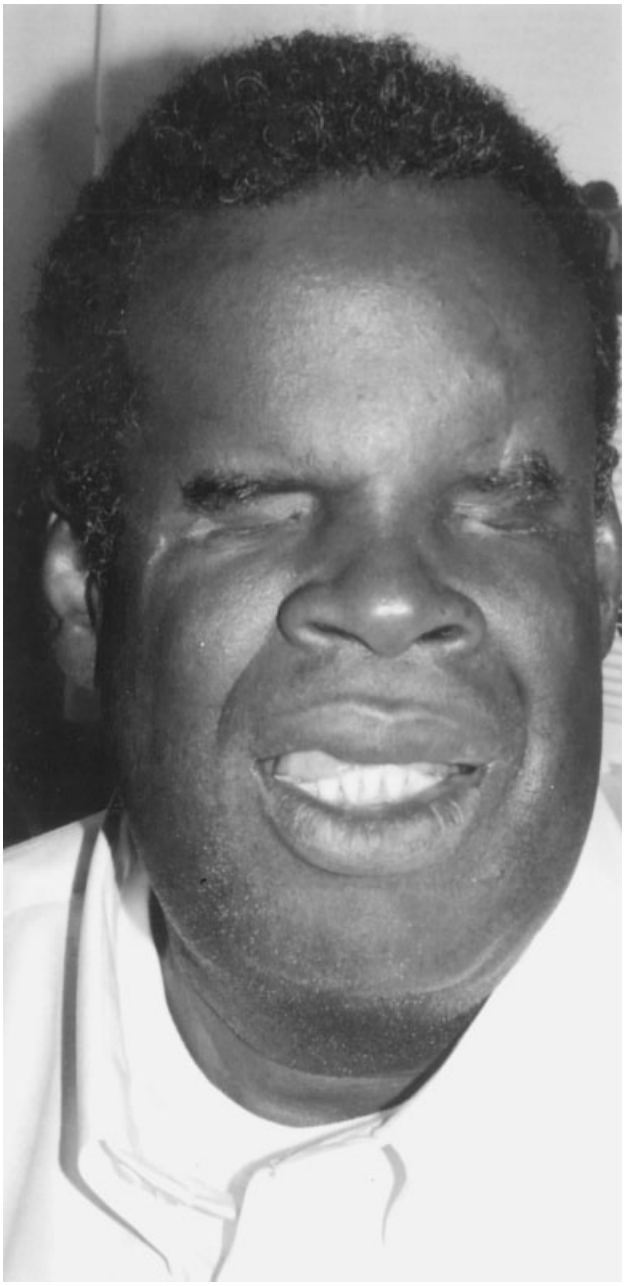

Fig. 5. Individual III-18 from kindred with bilateral short palpebral fissures and normocephaly (occipitofrontal circumference of 56 cm).

Fig. 6. Individual III-19 from kindred with bilateral microphthalmia, microcephaly, and anteverted ears.

males exhibited colobomatous microphthalmia without microcephaly, mental retardation, or other associated systemic anomalies as typically seen in XLSM families. The authors concluded that this family did not have Lenz syndrome due to the absence of associated congenital anomalies in the affected males and apparent male fertility (males with Lenz syndrome are typically infertile). Linkage analysis localized the region to a 28 cM interval with a maximum LOD score of 2.71 at ($\theta = 0$) with markers DXS1058, DXS6810, DXS1199, and DXS7132. These results overlap with our region of interest by approximately 3.9 Mb toward the centromeric end of Xp. We hypothesize that these two families have an alteration in the same gene in Xp and that isolated and syndromic microphthalmia represent two points of a phenotypic continuum attributable to mutations in this gene.

An interesting implication of these results is that Lenz microphthalmia and nonsyndromic microphthalmia appear to map to at least two loci on the X chromosome. Patients with syndromic microphthalmia, when inheritance is consistent with an X-linked recessive mode, are generally diagnosed with Lenz microphthalmia whereas nonsyndromic microphthalmia is considered a separate disorder. The current linkage data suggest that what we currently consider to be Lenz microphthalmia may instead be an amalgam of two phenotypes caused by mutations in ANOP1 and ANOP2. These two syndromes may have unappreciated phenotypic differences, or they may have sufficient phenotypic variability to include nonsyndromic variants. It is also possible that the phenotypes caused by mutations in ANOP1 and ANOP2 may be indistinguishable, as has been shown for several other phenotypes such as Bardet-Biedl syndrome (BBS, [MIM 209900]). It should be noted that the determination of linkage heterogeneity is tentative, however, because the previously reported linkage to Xq27–q28 does not meet formal criteria for linkage. The peak LOD score in the report of Forrester et al. [2001] was 1.83.

TABLE II. Associated Features of X-Linked Recessive Microphthalmia*

	Microcephaly	MR	Colaboma	Palate	Teeth	Ear	Extremities, spine	Urogenital
Lenz [1955]								
Individual III-8	−	+	−	High arch	Crowded	Antev	Clinodactyly	crpytorchidism aplastic kidney
Hermann and Opitz, 1969	+	+	−	High arch	Agenesis of incisors	hypoplastic, antev	clinodactyly/lumbar lordosis	cryptorchidism hypospadias
Goldberg and Mckusick, 1971	+	+	+	NR	wide-spaced	simple antev	scoliosis	NR
Individual III-4								
Individual III-18	+	+	+	NR	wide-spaced	simple, antev	NL	NR
Individual IV-3	+	+	+	NR	wide-spaced	simple, antev	NL	NR
Baraitser et al., 1982	+	+	−	high arch	crowded	simple, antev	NR	NL
Glanz et al., 1983	+	+	−	cleft	wide-spaced peg shaped	simple, antev	syndactyly	cryptorchidism hypospadias
Pallota, 1983	+	+	+	high arch	agenesis of incisors	antev, large	clinodactyly abnl L4, L5	cryptorchidism
Traboulsi et al., 1988								
Case 1	+	+	+	NL	wide-spaced	simple, antev	clinodactyly, abnl thumbs	NL
Case 2	−	+	+	NL	NL	cupped, skin tag	dupl thumbs, syndactyly	hypospadias
Graham et al., 1991	−	+	−	cleft	NL	skin tag	NL	NL
Antoniades et al., 1993	+	DD	+	high arch	delayed	simple cupped	syndactyly/lumbar lordosis	NL
Ozkinay et al., 1997	+	+	−	high arch	hypoplastic wide spaced	simple antev	abnormal thumbs	hypospadias
Temtamy et al., 2000								
Case 1	−	+	+	NL	hypoplastic wide spaced	low set	syndactyly, small 1st mcp	NR
Case 2	−	+	+	NL	hypoplastic, wide spaced	cupped	syndactyly	NR
Case 3	+	+	+	NL	crowded	cupped	abnl thumb, lumbar lordosis	ambiguous genitalia
Forrester et al., 2001								
Case 1	−	+	−	high arch	NL	overfolded	clinodactyly, scoliosis	duplicated L, renal system
Case 2	−	+	−	high arch	crowded	abnormally modeled	syndactyly, scoliosis	NL
Case 3	−	+	−	high arch	NL	abnormally modeled	syndactyly, clinodactyly	NL
Case 4	−	+	−	high arch	peg like	NL	syndactyly, brachydactyly, scoliosis	NR
Lehman et al., 2001	−	−	+	NL	NL	NL	NL	NL
Hoefnagel et al., 1963	NR	NR	−	NR	NR	NR	various deformity	renal agenesis
Ogunye et al., 1975								
Individual III-9	−	−	−	NR	NR	large antev	NR	cryptorchidism
Brunquell et al., 1984								
Individual III-19	+	+	−	NR	NR	large antev	scoliosis	cryptorchidism
Present study								
Individual III-18	−	+	−	NL	NL	NL	NL	cryptorchidism
Individual V-3	+	−	−	NL	NL	prearicular pit	NL	hypospadias
Incidence	13	22	21	12	16	22	18	23
N	35	35	36	30	33	35	34	30
Percentage	37	63	58	40	48	63	53	77

*NL, normal; NR, not reported; mcp, metacarpal; MR, mental retardation; antev, anteverted; abnl, abnormal; dupl, duplicated; DD, developmental delay; +, feature present, −, feature absent. Note that the present family was previously reported in Hoefnagel et al. [1963], Ogunye et al. [1975], and Brunquell et al. [1984].

The data presented in that study, however, showed negative LOD scores through the ANOP2 region, consistent with genetic heterogeneity. We conclude that X-linked microphthalmia comprises phenotypes caused by mutations at two X chromosome loci and may include nonsyndromic forms representing a phenotypic spectrum. What was formerly considered Lenz microphthalmia may be caused by mutations in at least two genes on the X chromosome. We hypothesize that there are two loci, each associated with a phenotypic spectrum ranging from isolated microphthalmia to XLSM.

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