尿酸の排泄トランスポーターをコードすることがわかってきた[27-29]．ヒトにおいては，血清尿酸の2/3が腎臓から尿中へ，残りの1/3が主に小腸から便中へ排泄されることが古くから知られていた[30]．ABCG2トランスポーターが腎臓，肝臓および小腸に発現していることから，これらの臓器における尿酸排泄機序に中心的な役割を果たしていることが示唆される．尿酸トランスポーターに関する一連の仕事により，我々は，教科書的に記載されながらその詳細が不明であった腎外排泄を含む尿酸排泄の分子機構モデルを提唱することができた（図1）[31]．

一方，*SLC17A3-SLC17A1-SLC17A4*のように，GWASで同定された領域が複数のトランスポーター遺伝子を含む領域にまたがっている場合には，連鎖不平衡という大きな問題がある．すなわち，GWASで同定されたSNPが複数の遺伝子のうちどの遺伝子の影響を反映しているかという課題については，遺伝学的解析のみでは解決が困難である．これを解決して血清尿酸値の生理学的な調節において真に重要な遺伝子を同定するためには，GWAS後のさらなる詳細な解析が必要である．このようななか，*SLC17A3-SLC17A1-SLC17A4*のうち，*NPT1/SLC17A1*遺伝子のSNPと痛風発症が関連しているという報告がある[32]．また，NPT1/SLC17A1およびNPT4/SLC17A3がともに尿酸を輸送することが報告された[33,34]．これらの知見をもとに，*SLC17A3-SLC17A1-SLC17A4*遺伝子領域中のどのトランスポーター遺伝子が血清尿酸値の生理学的な調節に重要なのか，今後解明されていくものと思われる．

これまでのGWASの成果をもとに，近年，2万8000人以上を対象としたメタ解析が実施され，尿酸値の変動に関わるさらに多くの遺伝子群が報告された[35]．この報告では，*GLUT9*，*ABCG2*，*SLC17A3*の3つの遺伝子領域のほかに，新たに6つの遺伝子領域が見出された．そのうち，*URAT1/SLC22A12*，*OAT4/SLC22A11*，*MCT9/SLC16A9*がトランスポーター遺伝子の領域としてあげられ，*PDZK1*，*GCKR*，*LRRC16A-SCGN*はその他の機能を担う遺伝子領域として報告されている（表2）．このGWASメタ解析において，*URAT1*遺伝子のSNPと尿酸値変動との関わりが初めて確認された．さらに，*LRRC16A-SCGN*以外の5つは，その後のreplication studyにおいても血清尿酸値への影響の再現性が認められている[36]．OAT4については尿酸輸送活性があることが既に示されており[37,38]，高尿酸血症や低尿酸血症などとの関連が解明されていくものと考えられる．また，PDZドメインタンパク質PDZK1は，URAT1をはじめとするトランスポーターと結合してその機能を高めるため[39]，尿酸トランスポートソーム（尿酸輸送分子複合体）における尿酸輸送調節機構の解明につながることが期待される．2010年には日本人を対象としたGWASの結果も発表されており[40]，日本人においても*GLUT9*，*URAT1*，*ABCG2*が血清尿酸値と関連することが示されたほか，新たな関連遺伝子候補として*LRP2*が報告された．さらに，欧米の白人を対象としたメタ解析では，*INHBC*，*RREB1*が新たな関連遺伝子として浮上してきている（表2）[41]．これらの遺伝子と尿酸関連疾患との関係についても，今後の研究の進展が期待される．

むすび

本稿では，腎性低尿酸血症の病因遺伝子探索のこれまでの経緯について振り返ってみた．ヒトゲノム情報の解読後に，腎臓の尿酸再吸収トランスポーターをコードする*URAT1*が腎性低尿酸血症1型の病因遺伝子であることが同定された．また，重要なポストゲノム研究の1つとも言えるGWASと関連研究の進展により，腎性低尿酸血症2型の病因遺伝子*GLUT9*や痛風・高尿酸血症の主要病因遺伝子*ABCG2*が同定された．血清尿酸値の変

動に関わるGWASでは，*URAT1*，*GLUT9*，*ABCG2*以外にも複数の尿酸トランスポーター候補遺伝子が示されており，今後，これらの遺伝子についても生理機能や尿酸関連疾患との関係が明らかになるものと期待される．痛風・高尿酸血症治療薬であるベンズブロマロンの標的分子がURAT1であることからもわかるように，腎性低尿酸血症の病因遺伝子の解析は，痛風や高尿酸血症に対する新たな分子標的薬の開発にも繋がる．腎性低尿酸血症3型の病因遺伝子の同定を含めた今後の尿酸トランスポーター研究の展開は，尿酸代謝関連疾患の診断や治療に大いに寄与するであろう．

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RESEARCH ARTICLE

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Ophthalmic Features of CHARGE Syndrome With CHD7 Mutations

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Coloboma and various ocular abnormalities have been described in CHARGE syndrome, although the severity of visual impairment varies from case to case. We conducted a multicenter study to clarify the ophthalmic features of patients with molecularly confirmed CHARGE syndrome. Thirty-eight eyes in 19 patients with CHARGE syndrome and confirmed CHD7 mutations treated at four centers were retrospectively studied. Colobomata affected the posterior segment of 35 eyes in 18 patients. Both retinochoroidal and optic disk colobomata were bilaterally observed in 15 patients and unilaterally observed in 3 patients. The coloboma involved the macula totally or partially in 21 eyes of 13 patients. We confirmed that bilateral large retinochoroidal colobomata represents a typical ophthalmic feature of CHARGE syndrome in patients with confirmed CHD7 mutations; however, even eyes with large colobomata can form maculas. The anatomical severity of the eye defect was graded according to the presence of colobomata, macula defect, and microphthalmos. A comparison of the severity in one eye with that in the other eye revealed a low-to-moderate degree of agreement between the two eyes, reflecting the general facial asymmetry of patients with CHARGE syndrome. The location of protein truncation and the anatomical severity of the eyes were significantly correlated. We suggested that the early diagnosis of retinal morphology and function may be beneficial to patients, since such attention may determine whether treatment for amblyopia, such as optical correction and patching, will be effective in facilitating the visual potential or whether care for poor vision will be needed.

Key words: CHARGE syndrome; *CHD7*; coloboma; ophthalmic features

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INTRODUCTION

CHARGE syndrome is a multiple malformation syndrome named from the acronym of its major features: coloboma, heart defects, atresia of the choanae, retarded growth and/or development, genital anomalies, and ear abnormalities [Pagon et al., 1981; Zentner et al., 2010]. The major ocular feature of CHARGE syndrome is coloboma, and a previous investigation by ophthalmologists revealed an incidence of up to 86%, although the severity

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of coloboma and visual impairment varied from case to case [Russell-Eggitt et al., 1990].

Recently, the gene *Chromodomain helicase DNA-binding protein-7* (*CHD7*) at chromosome 8q12.1 was identified as a causative gene of CHARGE syndrome [Vissers et al., 2004]. Up to 70% of patients clinically diagnosed as having CHARGE syndrome exhibit mutations in the *CHD7* gene [Aramaki et al., 2006a; Jongmans et al., 2006; Lalani et al., 2006]. Although the exact function of this gene product remains unknown, it may have an important effect on an early stage of ocular morphogenesis.

We conducted the present multicenter study to clarify the ophthalmic features of patients with molecularly confirmed CHARGE syndrome and to explore the role of *CHD7* in ocular development.

PATIENTS AND METHODS

Thirty-eight eyes in 19 patients clinically diagnosed as having CHARGE syndrome at the National Center for Child Health and Development, the Osaka Medical Center and Research Institute for Maternal and Child Health, the Kanagawa Children's Medical Center, or the Institute for Developmental Research, Aichi Human Service Center were retrospectively studied. All the patients had been molecularly confirmed to carry *CHD7* mutations at the Keio University School of Medicine [Aramaki et al., 2006a]. The clinical diagnosis of CHARGE syndrome was made based on the Blake criteria [Blake et al., 1998]. Molecular screening for mutations in the *CHD7* gene was conducted as reported previously [Aramaki et al., 2006b]. Ophthalmic features were examined using slit-lamp biomicroscopy and binocular indirect ophthalmoscopy. Two patients were also examined using a spectral domain optical coherence tomography (SD-OCT). The SD-OCT images were obtained with RS-3000 (NIDEK Co., Ltd., Gamagori, Japan). The best-corrected visual acuity (BCVA) was measured with a standard Japanese VA chart using Landolt rings or pictures at 5 m, then converted to Snellen VA.

The anatomical severity of the eye defect was classified as follows: Grade 1, Normal; Grade 2, colobomata with macular formation; Grade 3, colobomata including the macula; and Grade 4, colobomata, macular defect, and microphthalmos. Then, Cohen's kappa coefficient [Cohen, 1960] was used to measure the agreement of the severity in the two eyes among 19 CHD7-mutation positive patients. The potential correlation between the anatomical severity of the eyes in an individual and the amino acid position where the truncation of the *CHD7* protein occurred in the same individual was evaluated among 14 patients with protein-truncating mutations.

This study was approved by the institutional ethics committee; the patients or the parents of the patients provided informed consent prior to enrollment in the study.

RESULTS

The characteristics of the 38 eyes of the 19 patients with CHARGE syndrome carrying *CHD7* mutations are summarized in Table I. Ten patients (53%) were male and 9 (47%) were female. The age of the patients at the time of the examination ranged from 1 to 21 years (mean 7.9 ± 5.0 years). Ocular abnormalities were found in 18 patients (94.7%), bilateral abnormalities were observed in 17 patients (89.4%), and unilateral abnormalities were observed in 1 patient (5.3%). Among these 18 patients, all 35 abnormal eyes had varying severities of colobomata.

TABLE I. Characteristics of Patients of CHARGE Syndrome With CHD7 Mutations (n = 9)

Variable	Number
Gender	
Male	10 (53%)
Female	9 (47%)
Age at examination	1–21 years
Mean	7.9 ± 5.0 years
Ocular abnormalities (colobomata)	
Bilateral	17 (89.4%)
Unilateral	1 (5.3%)
None	1 (5.3%)
BCVA	
<20/400	4 (21.1%)
20/400 to <20/60	7 (36.8%)
20/60 to 20/20	6 (31.6%)
Not measured	2 (10.5%)

BCVA, best-corrected visual acuity.

The ocular features of the individual patients are summarized in Table II. Colobomata affected the posterior segment in 35/38 eyes (92.1%), retinochoroidal coloboma was present in 33 eyes (86.8%), and optic disk coloboma was present in 33 eyes (86.8%). Both retinochoroidal coloboma and optic disk coloboma were bilaterally present in 15 patients (78.9%) and unilaterally present in 3 patients (15.8%). The coloboma involved the macula totally or partially in 21 eyes (55.3%) of the 13 patients (68.4%): bilaterally in 8 patients

TABLE II. Ocular Features of the Patients (n = 19 patients, 38 eyes)

	Number of patients (%)			Number of
Findings	Bilateral	Unilateral	Total	eyes (%)
Colobomata	17 (89.5)	1 (5.3)	18 (94.7)	35 (92.1)
Retinochoroidal	15 (78.9)	3 (15.8)	18 (94.7)	33 (86.8)
Optic disk	15 (78.9)	3 (15.8)	18 (94.7)	33 (86.8)
Macula	8 (42.1)	5 (26.3)	13 (68.4)	21 (55.3)
Iris	1 (5.3)	0 (0.0)	1 (5.3)	2 (5.3)
Lens	0 (0.0)	1 (5.3)	1 (5.3)	1 (2.6)
Microphthalmos	3 (15.8)	2 (10.5)	5 (26.3)	8 (21.1)
Microcornea	3 (15.8)	1 (5.3)	4 (21.1)	7 (18.4)
Ptosis	1 (5.3)	1 (5.3)	2 (10.5)	3 (7.9)
PFV	0 (0.0)	1 (5.3)	1 (5.3)	1 (2.6)
Cataract	0 (0.0)	1 (5.3)	1 (5.3)	1 (2.6)
High myopia (>6.0 D)	2 (10.5)	1 (5.3)	3 (15.8)	5 (13.2)

PFV, persistent fetal vasculature.

(42.1%) and unilaterally in 5 patients (26.3%). The SD-OCT demonstrated a partially formed macula and cystic changes in the colobomatous area in 1 case (Fig. 1).

Only 2 eyes of 1 patient (5.3%) were identified as having iris colobomata, and 1 eye (2.6%) of another patient was revealed by examination under general anesthesia to have a dislocated and colobomatous lens. No cases of eyelid colobomata were seen, but congenital ptosis was present in 3 eyes (7.9%) of 2 patients who had undergone surgical treatment. All the cases of ptosis were not pseudoptosis associated with microphthalmos and/or cranial nerve palsy, but were true congenital ptosis associated with poor levator function. We evaluated the levator muscle function in each case. None of the patients had a history of acquired causes or signs of oculomotor palsy, such as paralytic strabismus and limited ocular movement.

Microphthalmos was found in 8 eyes (21.1%) of 5 patients (26.3%): bilaterally in 3 patients (15.8%) and unilaterally in 2 patients (10.5%). Microcornea was also present in 7 eyes (18.4%) of 4 patients (21.1%): bilaterally in 3 patients (15.8%) and unilaterally in 1 patient (5.3%). Persistent fetal vasculature was identified in 1 eye (2.6%). Cataracts had developed in 1 eye (2.6%), but neither glaucoma nor retinal detachment was observed in this series.

The refraction could be estimated in 23 eyes of 12 patients (63.2%). Of these eyes, 10 were myopic, 7 were emmetropic, and 6 were hypermetropic. High myopia (−6.00 diopters or more) was found in 5 eyes (13.2%) of 3 patients (15.8%).

The BCVA are shown in Table I. The measurement of VA was possible in 17 patients (89.5%) older than 3 years of age. The remaining 2 patients were infants or mentally retarded. The binocular BCVA or BCVA in the better eye was less than 20/400 in 4 patients (21.1%), less than 20/60 but no less than 20/400 in 7 patients (36.8%), and 20/60 to 20/20 in 6 patients (31.6%) with macular formation (Fig. 1). The overall prevalence of blindness and visual impairment (less than 20/60) [World Health Organization, 1992] among the 17 patients was 65%.

The agreement of anatomical severity between the 2 eyes in each of the 19 patients was evaluated using Cohen's Kappa statistics. The κ statistic of 0.41 suggested a moderate degree of agreement, per the guidelines by Landis and Koch [1977]. Because there was a moderate, if not a substantial, agreement between the severity of the 2 eyes, the severity grading of the more severely affected eye was used as the representative grade for the severity of the eyes in an individual. The correlation between the anatomical severity of the eyes in an individual and the amino acid position where the truncation of the CHD7 protein occurred in the same individual is illustrated in Figure 2. Patients with truncated protein devoid of the SANT domain tended to have severer anatomical defects of the eyes. Subcategorization of the patients according to the presence or absence of the SANT domain (4 cases with intact SANT domain and 10 other cases), and the subcategorization of the anatomical severity of the eyes in an individual (7 cases classified as Grade 1 or 2 vs. 7 cases classified as Grade 3 or 4) revealed a statistically

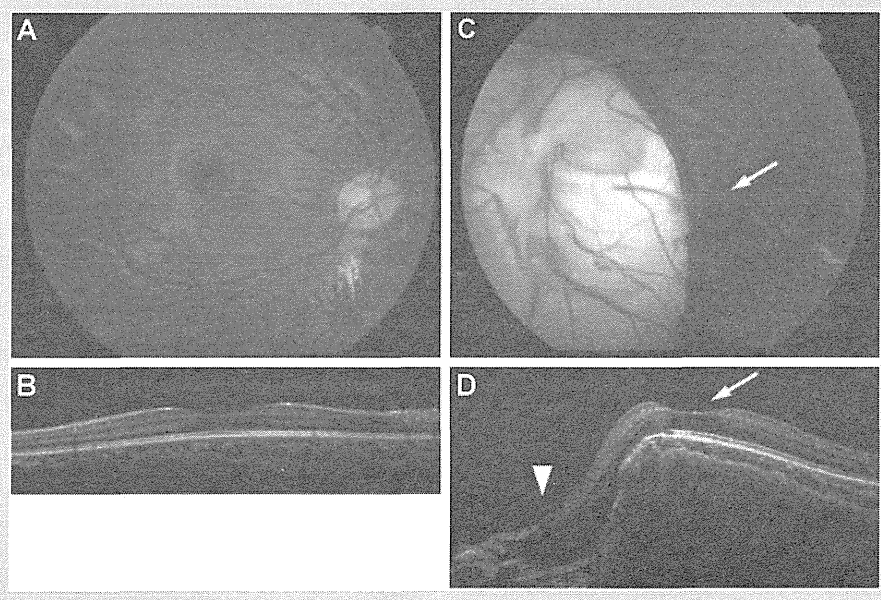

FIG. 1. Fundus photographs and spectral domain optical coherence tomography (SD-OCT) scan of the retina in the right eye (A,B) and the left eye (C,D) in a 6-year-old girl. A: Retinochoroidal colobomata inferior to the optic disk is visible in the right eye. B: The SD-OCT shows a good macular formation in the right eye, resulting in a BCVA of 20/20. C: Retinochoroidal and optic disk coloboma are seen in the left eye. The colobomata partially involved the macula (arrow). D: The SD-OCT shows a partially formed macula (arrow) and cystic changes in the colobomatous area (arrow head) in the left eye, resulting in a BCVA of 20/50 after amblyopia treatment.

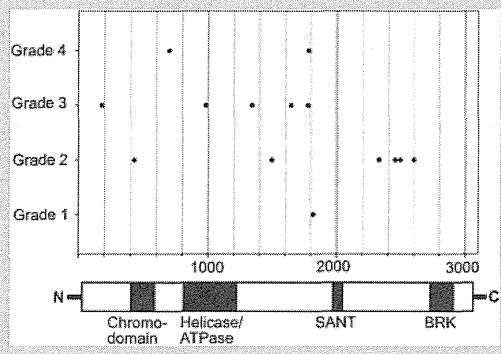

FIG. 2. The correlation between the anatomical severity of the eyes in an individual and the amino acid position where the truncation of the *CHD7* protein occurred in the same individual. Horizontal axis indicates amino acid position of the *CHD7* protein together with the domains of the protein. Vertical axis indicates the anatomical severity of the eye defect classified as follows: Grade 1, Normal; Grade 2, colobomata with macular formation; Grade 3, colobomata including the macula; and Grade 4, colobomata, macular defect, and microphthalmos.

significant correlation between the location of protein truncation and the anatomical severity of the eyes ($P = 0.02$, chi-squared test).

DISCUSSION

In the current series, the incidence of coloboma, the major ocular feature of CHARGE syndrome, was 94.7% (18/19), which was much higher than the previously reported incidence. Since most of the authors were ophthalmologists, the number of cases without eye defects might have been underrepresented. Hence, this high incidence should be viewed with caution. Nevertheless, attending clinical geneticists were on duty at all the participating children's hospitals, and thus the bias from such underrepresentation may be relatively small. The finding that there was one mutation-positive patient who did not have abnormal eye findings confirms that no finding in CHARGE syndrome has a 100% penetrance as is sometimes surmised.

Both retinochoroidal and optic disk coloboma occurred in 94.7% of the cases, mostly bilaterally, while the incidence of iris coloboma was only 5.3% (1/19). Coloboma also affected the macula in 68.4% of the cases. We confirmed that bilateral large retinochoroidal colobomata represent a typical ophthalmic feature of CHARGE syndrome with *CHD7* mutations.

The incidence of anomalies in the anterior segment was lower than that in the posterior segment, although microphthalmos, microcornea, PFV, and cataracts were present in some cases bilaterally or unilaterally. The presence of characteristic large retinochoroidal coloboma indicates the essential role of *CHD7* in the closure of the fetal fissure posteriorly between 5 and 6 weeks of gestation, and the malfunction of *CHD7* may have an effect so severe as to influence the entire ocular morphogenesis to some degree. Although most cases had bilateral colobomata in the posterior segment, the severity and associated features often differed between the two eyes. Other associated features in this series were ptosis in 10.5% and high myopia in 15.8%. Subtle-associated anomalies and refractive errors may have been underestimated in examinations that were not performed under general anesthesia.

The anatomical severity grading of the eye defect was evaluated in two ways: a comparison between the severity in one eye in comparison with that in the other eye and the correlation between the severity and the genotype. The low-to-moderate degree of agreement between the two eyes (i.e., left and right) reflects the general facial asymmetry in patients with CHARGE syndrome [Zentner et al., 2010]. In other words, the lack of substantial or perfect agreement between the anatomical severity of the right and the left eyes indicates a variable phenotypic effect of the same mutation. Yet, the location of protein truncation and the anatomical severity of the eyes were significantly correlated: if the chromodomain, helicase/ATP domain, and SANT domains are intact, the severity of the eyes tends to be milder. Interestingly, all four cases in which those domains were intact had less severe eye defects with intact macula. Further studies are warranted to verify this potential genotype–phenotype correlation.

The visual acuities of the eyes ranged between no light perception and 20/20, and the prevalence of blindness and visual impairment (less than 20/60) was 65% among 17 patients. A poor visual prognosis depended on the presence of a large coloboma involving the macula in the posterior segment and associated microphthalmos or microcornea, as reported previously [Russell-Eggitt et al., 1990; Hornby et al., 2000]. On the other hand, even eyes with large colobomata as a result of *CHD7* mutations were capable of forming maculas, resulting in good central visual acuity with superior visual field defects. As shown in the case illustrated in Figure 1, even a partially formed macula will enable useful vision following the adequate treatment of amblyopia as optical correction and patching during the earlier age of visual development. A recent report of a case examined using OCT revealed additional morphologic characteristics of eyes in patients with CHARGE syndrome carrying *CHD7* mutations [Holak et al., 2008]. Further investigation of retinal morphology and function using OCT and electroretinograms (ERG) may help to clarify the function of *CHD7* in ocular morphogenesis, including macular formation.

We suggested that the early diagnosis of retinal morphology and function, especially of macular lesions by way of OCT and ERG, may be beneficial to patients, since such attention may determine whether treatment for amblyopia, such as optical correction and patching, will be effective in facilitating the visual potential or whether care for poor vision will be needed. An infant's visual acuity rapidly develops during its first 2–3 years and continues up until 7–8 years of age, but plasticity decreases progressively thereafter. Thus, a better visual prognosis can be obtained with the earlier treatment of amblyopia during the critical period of visual development.

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