Table 1
PAX6 polymorphisms detected in 285 autistic patients in the Japanese.

Polymorphism[a]	dbSNP ID	Allele frequency
ATG~−11,521C>T	Novel	1/570
IVS4-85T>C	Novel	1/570
IVS4-70~72Ins/DelTCT	Novel	1/570
IVS4-42C>T	Novel	1/570
117G>A [Pro(CCG)39Pro(CCA)]	Novel	1/570
136C>G [Leu(CTG)46Val(GTG)]	Novel	1/570
IVS5-163C>T	Novel	1/570
319C>T [Leu(CTG)107LeuTTG]	Novel	1/570
IVS6+28C>T	Novel	1/570
IVS8+14Ins/DelT	Novel	1/570
IVS9-12C>T	rs667773	68/570
867T>C [Ser(AGT)289Ser(AGC)]	Novel	4/570
IVS12+11(G>A)	Novel	1/570
IVS12+43T>G	rs3026393	261/570
1194C>T [Ser(TCC)398Ser(TCT)]	Novel	4/570

[a] Major allele > minor allele; intron Nos. are based on the NM_000280 (Fig. 1A).

in these subjects. We also examined 252 Autism Genetic Resource Exchange (AGRE) trios (756 samples; http://www.agre.org), but again did not detect it.

The autistic patient with the *PAX6* missense mutation (Leu46Val) is a daughter of nonconsanguineous parents (Fig. 2), and the mutation was transmitted from her father. However, her father does not have autism, major depression, anxiety and social awkwardness implying a sign of autism upon psychiatric interview, as well as her mother and older brother. The patient's pregnancy and birth history were without any problems. At 1.5 years of age, she was assessed as developing normally except for mild speech delay. During her preschool days, she used to spend many hours playing alone. She attended a normal elementary school and a normal junior high school. However, she attended a high school for handicapped children because of her difficulty in learning at a normal high school. When she was 10 years old, she was diagnosed as having autism by an expert in childhood psychiatry. She had deficits in all three areas of communication, reciprocal social interaction and behavior. Her ADI-R scores were 17 in the social domain (cutoff = 10), 15 in the language (verbal) domain (cutoff = 8), and 6 in the repetitive/restrictive behaviors domain (cutoff = 3). The Wechsler Intelligence Scale was administered with a full-scale WISC-III of 66 (verbal IQ 73, performance IQ 67). She sometimes looked anxious, which manifested intermittently and briefly. Therefore, she was administered psychotropic drugs from 10 years of age. She had no histories of any other neurological illnesses including seizure and head injury. In addition, MRI examination revealed normal morphologies of the corpus callosum, anterior commissure, grey matter in anterior cingulate cortex, medial temporal lobe, olfactory bulb, pineal gland and cerebellum. The blood karyotype analysis result was also normal and "fragile X" was excluded. With respect to ocular phenotypes, the patient displayed reduced vision, photophobia and eyelid ptosis, but no other ocular abnormalities including aniridia. Her father carrying the same mutation did not show abnormal ocular phenotypes except for reduced vision and age-related macular degeneration (Fig. 3). Recently it is also known that PAX6 regulates proinsulin processing and glucose metabolism via modulation of PC1/3 production [37]. Therefore, we also checked blood glucose levels and BMIs (body mass indexes) of the patient and her father (the patient's BMI is 16.8 and her father's BMI is 22.4), and these measures were within normal limits.

Val46 is deemed to elicit functional impairment, because Leu46 is conserved from Drosophila to human (Fig. 1) and Leu46Arg and Leu46Pro mutations are reported to affect ocular phenotypes in previous studies [5,7]. Additionally, this mutation may be important for the following reasons: (1) the Leu46Val mutation is located in the paired domain, (2) structural analysis of this mutation using the Sequence Analysis Software "GENETYX" (GENETYX Co., Tokyo, Japan) suggests that it may disrupt the helix-turn-helix motif, and as a consequence the DNA-binding properties of the resulting mutated protein may vary, (3) this variant is predicted to be possibly damaging using a tool-website "PolyPhen" that can estimate the possible impact of an amino acid substitution on the structure and function of a protein (http://coot.embl.de/PolyPhen/), and (4) it is also possible that this mutation may exert its effect by disrupting the activity of an exonic splicing enhancer (ESE), because the SC35 score matrix of this mutation (3.17473) is lower than that of the wild type (4.09004), which is calculated using a tool-website "ESE finder" (an online resource to identify ESEs in query sequences) (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home). Therefore, these predicted functional consequences may be relevant to the autism phenotype, although the cosegregation was not observed in the present nuclear family.

A network of protein–protein interactions underlies complex biological processes. We addressed this issue using the Genome Network Platform (http://genomenetwork.nig.ac.jp/). There are 36 proteins that can interact with Pax6 and it is reported that 5 proteins out of the 36 proteins correlate with autism, which include HoxB1 [15,22], Tbp [6,31], Diaph1 [24,33], Ifi16 [1,11] and Ep300 [14,29] (Supplementary Fig. 1). For example, Hoxb1 that plays an important role in morphogenesis in all multicellular organisms can interact with both the paired domain and homeodomain of Pax6

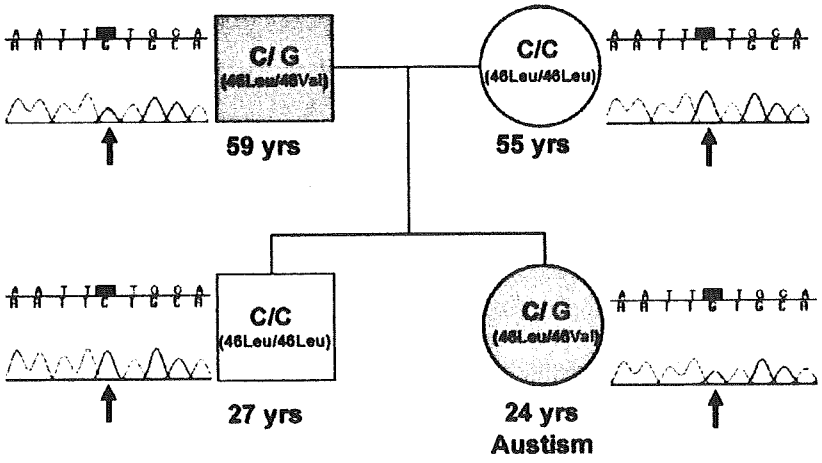

Fig. 2. Family structure of a patient with the *PAX6* missense mutation. Grey symbols indicate individuals carrying heterozygous *PAX6* Val46 allele. White symbols show the subjects with homozygous Leu46 alleles. Squares represent men and circles represent women. Genotype and sequence electrophelogram of each subject are also shown.

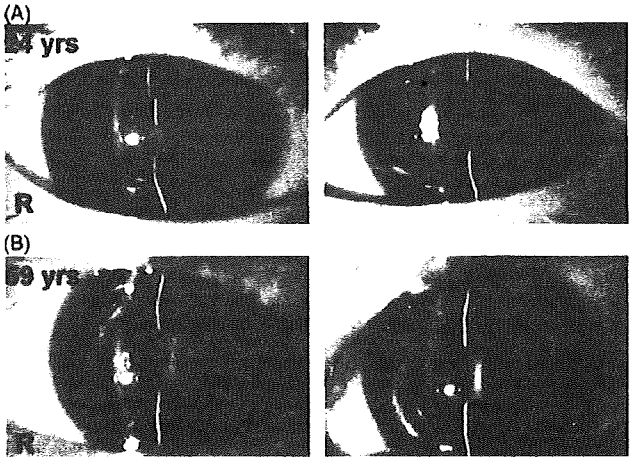

Fig. 3. Biomicroscopic observations of autistic patient (A) and her father (B) both of whom carry the mutant allele. (A) and (B) show the slit lamp aspects of ocular anterior segments as well as the lens of the subjects. Ages are also shown. R, right eye; L, left eye.

and can enhance Pax6-mediated transactivation of a minimal promoter that contains consensus Pax6 paired domain binding sites in *in vitro* experiments [22]. The Leu46Val missense mutation was located in the paired domain of PAX6. Therefore, the mutated PAX6 may affect the autism phenotype, owing to the attenuated interaction with HOXB1.

Concerning the penetrance of *PAX6* mutations in aniridia, it is known that *PAX6* missense mutations are a less frequent cause than expected [35], and they are not fully penetrant for other ocular anomalies [8]. Our current results that the Val46 allele did not cosegregate with autism or ocular phenotype in the family suggest that the Val46 of PAX6 may have only a modest effect, if any, on the development of autism and ocular abnormalities. It is also of note that in general autism is a multi-factorial disease caused by multiple susceptibility genes of small effect sizes, environmental factors and their interactions like other psychiatric illnesses [4].

MRI and functional MRI (fMRI) show that individuals with *PAX6* heterozygous mutations (haploinsufficiency) have structural abnormalities of grey matter in the anterior cingulated cortex, cerebellum and medial temporal lobe, as well as white matter deficits in the corpus callosum [9,10,13,23,30]. Additionally, patients of high-functioning autism with *PAX6* mutations (not haploinsufficiency) also have significant structural abnormalities [2,3,7]. In this study, we did not detect any abnormal brain structures in the patient. However, we previously reported that hippocampal neurogenesis is reduced in the *Pax6* heterozygous mutant rats that present behavioral abnormalities including decreased prepulse inhibition [18,19]. Therefore, we suspect that the autistic patient with the *PAX6* missense mutation may also have suffered from dampened hippocampal neurogenesis, potentially contributing to autism as one of the risk-conferring events [21].

In summary, we identified in this study a novel missense mutation of *PAX6* in one autistic patient with mild ocular abnormalities, and the mutation was not detected in 2120 nonautistic subjects. Further studies using larger samples and on the biological importance of this missense mutation are required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2009.07.021.

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CLINICAL INVESTIGATION

Central Corneal Thickness in Japanese Children

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Abstract

Purpose: To determine the central corneal thickness (CCT) in Japanese children and to investigate the changes in CCT with increasing age.

Methods: Pachymetry was performed on 338 eyes of 169 patients undergoing eye muscle surgery under general anesthesia, and the intraocular pressure (IOP) was measured on 312 eyes of 156 of those same patients. Patients with abnormalities other than refractive errors and strabismus were excluded. Patients were divided into four groups: group 1, ≤1 year of age; group 2, 2–4; group 3, 5–9; and group 4, 10–18 years of age. Analysis of variance (ANOVA) was performed to determine the significance of the changes in CCT.

Results: The average CCT of the right eye was 544.3 ± 36.9 μm. The CCT was thinner in group 1 than in groups 3 and 4 (ANOVA, $P = 0.02$). There was a positive but weak correlation between IOP and CCT (IOP = 6.253 + 0.014 × CCT; $r^2 = 0.047$, $P = 0.007$).

Conclusions: CCT reaches the adult thickness in Japanese children by age 5 years. The average CCT is thinner in Japanese children than in Caucasians but thicker than in African American children.
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Key Words: central corneal thickness, child, general anesthesia, intraocular pressure, ultrasound pachymeter

Introduction

Measuring central corneal thickness (CCT) has become increasingly important, particularly for the diagnosis and management of glaucoma. The Ocular Hypertension Treatment Study reported that subjects with ocular hypertension had greater CCT,[1] and subjects with smaller CCT had a higher risk of developing glaucoma.[2]

Goldmann applanation tonometer measurement is based on the assumption that CCT is 500 μm, a thickness obtained from measurements of cadaver eyes. Because a permanent thinning or flattening of the cornea induces lower intraocular pressure (IOP) after refractive surgery,[3,4] special attention has been paid to the variability of CCT in the healthy population and in patients with various eye diseases. To obtain an accurate IOP value, measurements with the Goldmann applanation tonometer should be corrected by the CCT value.[5] Thus, measuring CCT has become essential for determining true IOP for glaucoma management.

Children with congenital glaucoma also have significantly thinner CCT than healthy children.[6,7] However, aphakic[8,9] and pseudophakic[10] children with glaucoma have significantly thicker CCT than healthy children. These findings then raise the question of why up to 45% of aphakic children who have thicker than average CCT develop glaucoma.[11] Muir et al.[12] speculated that CCT increases after cataract surgery because of endothelial cell damage, or because increased IOP injures the endothelial cells. Thus, measuring CCT in children who are at high risk for glaucoma, such as children with aphakic or pseudophakic eyes, is important.

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Another important factor that influences the CCT is race or ethnicity. The CCT of African American adults is thinner than that of Caucasian adults,[2,13] and lower IOP in African Americans may delay the diagnosis of glaucoma and determination of an appropriate treatment target.[14] CCT in the Japanese population has been found to be thinner than in Chinese and Filipino populations.[15]

These racial differences are also found in the pediatric population. African American children have thinner CCT than do Caucasian[16] or Hispanic children.[17] A literature search on PubMed did not extract any CCT data regarding healthy Japanese children. Knowing the normal range of CCT of Japanese children is important for diagnosing and treating pediatric glaucoma.

Thus, the purpose of this study was to determine the CCT in Japanese children and to investigate the changes in CCT with increasing age. To accomplish this, we measured the CCT of 338 eyes of 169 children ≤18 years of age by ultrasound pachymetry under general anesthesia.

Subjects and Methods

All patients scheduled for strabismus surgery under general anesthesia were recruited from Hamamatsu University School of Medicine and Aichi Children's Health and Medical Center from December 2005 to August 2007. Patients with corneal disease, a history of intraocular surgery, glaucoma, cataract, or eyelid abnormalities were excluded. Patients known to have abnormally thin corneas such as those with Down syndrome[18] or with Marfan syndrome,[19] or abnormally thick corneas such as those with aniridia,[20] were also excluded.

This study was approved by the Institutional Review Board of the Hamamatsu University School of Medicine and Aichi Children's Health and Medical Center. Full explanation of the research, including the measurement procedures for CCT and IOP was given, and written informed consent was obtained from a parent or legal guardian of each of the patients.

For controls, we measured the CCT of eight healthy subjects aged 26 to 52 years under topical anesthesia.

CCT was measured between 9:00 and 16:00 in the operating room with an ultrasound pachymeter (SP-100 Handy, 1640 Hz; Tomey, Nagoya, Japan). Measurements started within 5 min of endotracheal intubation. All patients were sedated by inhalation or intravenously, and a muscle relaxant was given before insertion of the airway tube. Sevoflurane and nitrous oxide were used to maintain surgical anesthesia during the surgery. The patient's eyelid was held open manually, with special care taken not to press on the eye. One drop of topical anesthesia (4% oxybuprocaine) was administered, and the central cornea was defined as the center of the pupil. The pachymeter probe was placed perpendicularly on the center of the cornea, and the average of eight measurements was recorded as the CCT. Next, the IOP was measured with a Tono-Pen XL (Reichert, Depew, NY, USA). All measurements were performed first on the right eye and then on the left eye.

For statistical purposes, only the data from the right eye were used. The patients were divided into four groups: group 1, ≤1 year of age; group 2, 2–4; group 3, 5–9; and group 4, 10–18 years of age. Statistical analysis was performed using StatView version J-5.0 for Windows (SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) with a Bonferroni post hoc test was used to determine the significance of any differences among the age groups. Paired t tests were used for comparisons between eyes. Linear regression was used to determine the correlation between CCT and IOP. A P value of <0.05 was considered to be statistically significant.

Results

We measured the CCT of 338 eyes of 169 subjects (87 boys, 82 girls) with a mean age of 6.01 ± 3.87 years and an age range of 8 months to 18 years. The patient age distribution and the IOP and CCT measurements are summarized in Table 1.

The average CCT of the right eye was 544.3 ± 36.9 μm (range, 429–648 μm). The CCT distribution is shown in Fig. 1. The CCT was significantly different between age groups (ANOVA $P = 0.0198$); it was significantly thinner in group 1 than in groups 3 or 4 ($P = 0.0071$ and 0.0157, respectively, Bonferroni; Table 1). The average CCT in group 4 was 550.6 μm, which was not significantly different from the mean CCT of the eight healthy adult subjects (525–586 μm).

Table 1. Subjects' characteristics and measurement of CCT and IOP

	n (%)	CCT (μm)	IOP (mmHg)
All patients	169 (100)		
Age distribution (years)		(right eye)	(right eye)
0–1	14 (8)	522 ± 26.7	14.07 ± 2.89
2–4	50 (30)	538 ± 36.6 *	14.14 ± 2.55
5–9	77 (45)	550 ± 36.7 **	14.13 ± 2.13
10–18	28 (17)	550 ± 37.5	12.88 ± 2.45

Values are means ± SD.
CCT, central corneal thickness; IOP, intraocular pressure.
*$P = 0.0071$.
**$P = 0.0157$.

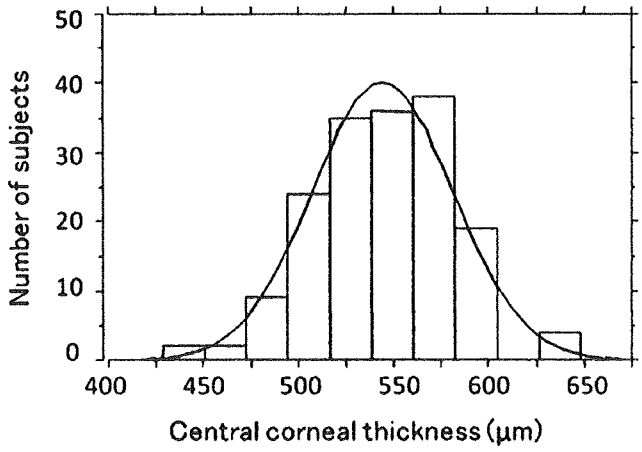

Figure 1. Distribution of central corneal thickness (CCT) in the right eye of children aged 0 to 18 years. CCT is normally distributed. The average CCT was 544.3 ± 36.9 μm.

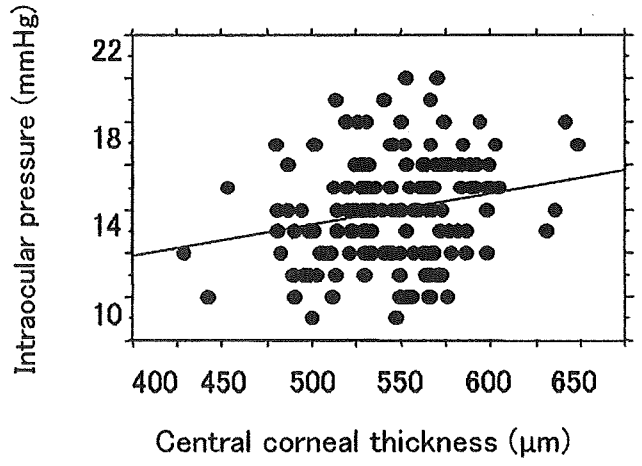

Figure 2. Relationship between CCT and intraocular pressure (IOP). There is a positive but weak correlation between CCT and IOP.

The mean CCT in our adults was comparable to the published average range for the adult Japanese population.[21,22]

The average IOP in the right eye was 13.9 ± 2.4 mmHg (range, 9.0–10.0 mmHg). The IOP did not differ significantly among the different age groups. IOP (y) and CCT (x) were positively correlated, but the correlation coefficient was low ($y = 6.253 + 0.014x$, $r^2 = 0.047$; $P = 0.007$; Fig. 2).

Discussion

Differences in CCT values measured with different instruments have been reported,[23–25] but the values obtained by ultrasound pachymetry and by noncontact optical low-coherence reflectometry are reported to be highly correlated.[26] Bovelle et al.[27] reported that the Topcon specular microscope gives significantly lower values than the ultrasound pachymeter. Suzuki et al.[21] compared CCT values obtained using Orbscan scanning-slit corneal topography/pachymetry, the Topcon SP-2000P, noncontact specular microscopy, and Tomey ultrasonic pachymetry in a Japanese population. The mean CCT was not significantly different between scanning-slit topography (546.9±35.4 μm) and ultrasonic pachymetry (548.1 ± 33.0 μm). However, contact specular microscopy gave a significantly smaller mean (525.3 ± 31.4 μm) than did the other two instruments.[21] Therefore, it is advisable not to compare CCT values obtained using different instruments.

Table 2. Comparison of CCT values of children by race

Race	Hussein[29] (μm)	Dai[17] (μm)	This study (μm)
Caucasians	551	563	
Hispanic	550	568	
Japanese			544
African Americans	532	523	

The SP100 ultrasonic pachymeter is compact and easy to use in the operating room. The measurements are accurate if the instrument is used appropriately. We took special care to place the probe on the center of the cornea because the cornea is thinnest at the center.[28,29]

In adults, CCT is negatively correlated with age in men,[30] or in both sexes.[31] In children, CCT is reported to decrease rapidly during the neonatal period,[32,33] and then to increase slowly and reach the adult level at 3[34] or 5 years of age.[29] Sawa[35] studied the Japanese population and found that the mean CCT of 1-month-old infants (534 ± 36 μm) is thicker than that of 3-month-old infants (508 ± 22 μm), but they found no difference between the 3-month-old infants and the 20- to 29-year-old adults (516 ± 17 μm). Muir et al.[36] suggested that CCT slowly increases in children up to the age of 5 and then decreases at around age 10–14 years. Hussein et al.[29] reported that CCT increases in children until age 9 years and then decreases between ages 10 and 14.[29] In our study, CCT was significantly less in group 1 than in groups 3 or 4, suggesting that adult CCT values are reached by 5 years of age.

Earlier studies have reported racial differences in CCT, not only in adults but also in children. Table 2 summarizes results from other countries for CCTs in pediatric populations from 0 to 18 years of age, measured with ultrasound pachymetry. We understand that it is not ideal to compare our data directly with those of previous reports, but as long as all the measurements were obtained with ultrasound pachymetry, it is reasonable to do so. Compared with the readings obtained from two different institutions,[17,29] the CCT of Japanese children still appears to be thicker than that of African American children and thinner than that of Caucasian or Hispanic children.

Studies focusing on the relationship between CCT and IOP have reported a significant correlation between IOP and CCT in children,[36] as in adults. Suzuki et al.[31] studied Japanese adults and found that IOP measured with the

Goldmann applanation tonometer was positively correlated with CCT. We found a positive correlation between CCT and IOP measured by Tono-Pen in children, but the correlation coefficient was low.

The Tono-Pen is generally used in children whose IOP is neither very high (>21 mmHg) nor very low (<9 mmHg).[37] IOPs obtained with Tono-Pen are significantly correlated with those obtained using the Goldmann tonotmeter.[38,39] IOPs measured with the Goldmann tonometer, the noncontact tonometer, and the Tono-Pen are known to be influenced by CCT, but IOPs measured by Tono-Pen are less affected by CCT than the other tonometers.[40,41]

We are aware that measured IOP differs significantly with the type and state of anesthesia; for example, succinylcholine can increase IOP, whereas halothane can reduce it.[42] In addition, IOP in the human infant depends strongly on the level of relaxation.[42]

In conclusion, the CCT of Japanese children increases up to age 5 years, when it does not differ significantly from that of adults. The CCT of Japanese children is thinner than that of Caucasian children but thicker than that of African American children. Knowing the average CCT value in the Japanese pediatric population will be useful when caring for not only congenital anomalies involving the cornea but also pediatric glaucoma.

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