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(54) **USING GENETIC POLYMORPHISMS OF THE BICD1 GENE AS A METHOD FOR DIAGNOSING AND TREATING MYOPIA**

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      C07H 21/02   (2006.01)
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      See application file for complete search history.

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(57)   **ABSTRACT**

Using the BICD1 gene as a method for diagnosing myopia and/or myopia related complications is provided. The method includes obtaining a biological sample from a subject, and determining at least one SNP genotype in the BICD1 gene in the biological sample, wherein the presence of the SNP genotype indicates that the subject is susceptible to myopia. The SNP genotype is selected from the group consisting of SNPs rs7966276, rs1151029, rs2650122, and rs10771923. In addition, the present invention also provides a method of screening a material for preventing, treating myopia, and a method of assessing a subject for probability of response to a myopia therapeutic agent.
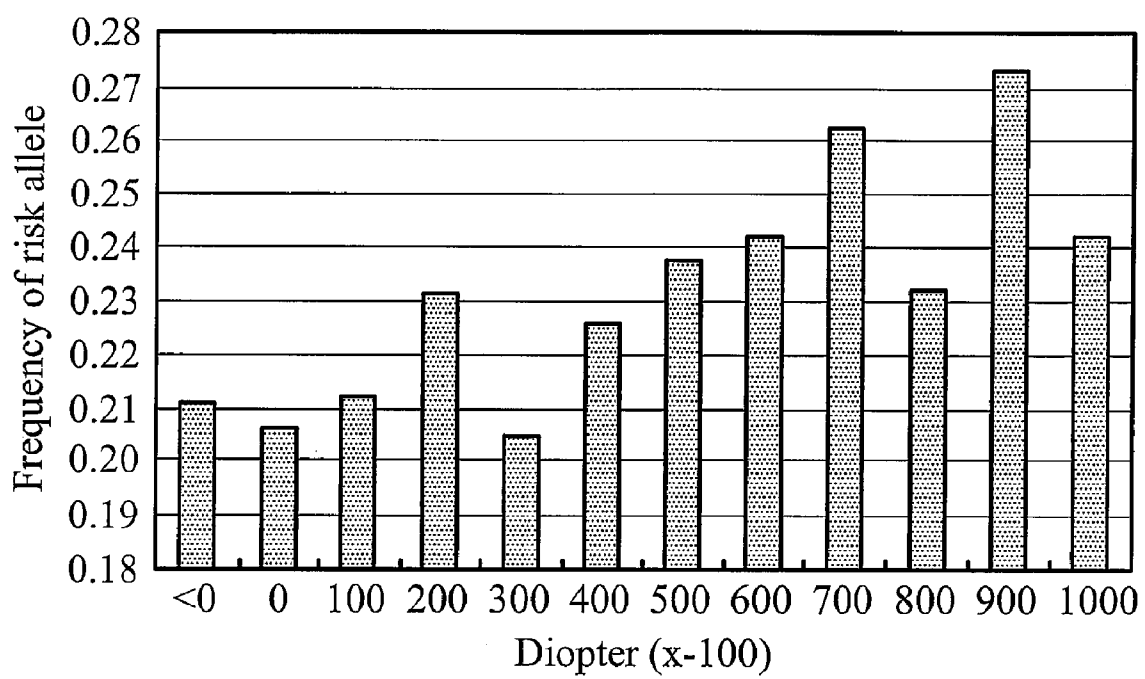
2 Claims, 1 Drawing Sheet

FIG. 1

1

USING GENETIC POLYMORPHISMS OF THE BICD1 GENE AS A METHOD FOR DIAGNOSING AND TREATING MYOPIA

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a method for diagnosing myopia, and in particular relates to a method for diagnosing myopia and/or myopia related complications by determining SNP (single nucleotide polymorphism) genotype in the BICD1 gene. Further, the present invention relates to a method for screening a material for treating myopia and/or myopia related complications.

2. Description of the Related Art

Myopia, also called near- or short-sightedness, is a refractive defect of the eye in which collimated light produces image focus in front of the retina when accommodation is relaxed. Those with myopia see nearby objects clearly but distant objects appear blurred. With myopia, the eyeball is too long, or the cornea is too steep, so images are focused in the vitreous inside the eye rather than on the retina at the back of the eye.

Myopia is a common eye condition worldwide. The prevalence of the condition varies widely among populations, genders, and ages (Invest Ophthalmol Vis Sci 1997; 38:334-40; Optom Vis Sci 2001; 78:234-9; J Formos Med Assoc 2001; 100:68-91). In the USA, the prevalence of myopia was estimated to be approximately 25% between ages of 12 to 54 years (Arch Ophthalmol 1983; 101:405-7). In the Baltimore Eye Survey, myopia was less common in blacks (19.4%) compared with whites (28.1%) (Invest Ophthalmol Vis Sci 1997; 38:334-40). High myopia (defined as refractive dioptric power$\leqq$−5.0 D in this study) accounted for 27% to 33% of all myopic eyes, corresponding to a prevalence of 1.7% to 2% in the general population in the USA (Arch Ophthalmol 1983; 101:405-7). Taiwan is among the highest risk areas in the world for myopia. Using the definition of less than −6.0 D for high myopia, high myopia is much more common in Asia. The percentage of myopia in Taiwan is 18% among Taiwanese school boys and 24% among Taiwanese school girls (J Formos Med Assoc 2001; 100:684-91). The totals are even higher than the 13.1% reported among young men in Singapore (Optom Vis Sci 2001; 78:234-9). Furthermore, the prevalence of myopia is increasing in Taiwan based on two large nationwide surveys (participant number>10,000) conducted in 1995 and 2000.

High myopia is associated with potential blinding conditions such as retinal detachment, macular degeneration, and glaucoma. It has been estimated that 5.6% of blindness among school children in the USA is attributable to myopia. Substantial resources are required for optical correction of myopia such as spectacles, contact lenses, orthokeratology, photorefractive keratectomy and laser in situ keratomileusis (LASIK). However, these corrections do not prevent the ocular complications mentioned above. Furthermore, complications arising from the use of contact lenses (Curr Opin Ophthalmol 1998; 9:66-71), orthokeratology (Cornea 2003; 22:262-4) and surgical procedures (J Refract Surg 2003; 19:S247-9) also impose additional risks to myopes. In the USA, treatment of myopia costs an estimated $250 million per year (Arch Ophthalmol 1994; 112:1526-30).

While studies have found that several risks were attributed to environmental factors, twin studies have indicated a strong genetic influence on myopia with the estimates of heritability ranging from 58 to 90% (Invest Ophthalmol Vis Sci 2001; 42:1232-6; Genet Epidemiol 1988; 5:171-81; Hum Hered

2

1991; 41:151-6; Br J Ophthalmol 2001; 85:1470-6). Using family data, it has been reported that a family history was a significant risk factor for high myopia (Invest Ophthalmol Vis Sci 2004; 45:3446-52). Several studies also demonstrated a similar finding (Optom Vis Sci 1996; 73:279-82; JAMA 1994; 271:1323-7; Invest Ophthalmol Vis Sci 2002; 43:3633-40; Optom Vis Sci 1999; 76:387-92; Invest Ophthalmol Vis Sci 2004; 45:2873-8). However, while few papers reported identifying susceptible myopia genes, none of the studies have been replicated, thus making the identification highly questionable.

Accordingly, what is needed in the art, is a method for diagnosing, treating, preventing or ameliorating myopia and/or myopia related complications.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a method for diagnosing myopia and/or myopia related complications in a subject, comprising, obtaining a biological sample from a subject; and determining at least one SNP genotype in the BICD1 gene in the biological sample, wherein the presence of the SNP genotype indicates that the subject is susceptible to myopia.

The present invention also provides a method for screening a material for treating myopia and/or myopia related complications in a subject, comprising, contacting a test material with a cell expressing the BICD1 gene, and selecting a material that modulates the expression of the BICD1 gene as compared with a control.

The present invention further provides a method for treating, preventing or ameliorating myopia and/or myopia related complications, comprising, administering to a subject a pharmaceutically effective amount of the material obtained by the method disclosed above.

The present invention further provides a method of assessing a subject for probability of response to a myopia therapeutic agent, comprising detecting at least one SNP genotype in the BICD1 gene, wherein the presence of the SNP genotype is indicative of a probability of a positive response to a myopia therapeutic agent.

The present invention further provides a kit for assaying a sample to detect a susceptibility for myopia, comprising one or more reagents for detecting one or more SNP genotype in the BICD1 gene.

A detailed description is given in the following embodiments with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention can be more fully understood by reading the subsequent detailed description and examples with references made to the accompanying drawings, wherein:

FIG. 1 shows the frequency of the risk T allele of SNP rs1151029 separated by different diopters.

DETAILED DESCRIPTION OF THE INVENTION

The following description is of the best-contemplated mode of carrying out the invention. This description is made for the purpose of illustrating the general principles of the invention and should not be taken in a limiting sense. The scope of the invention is best determined by reference to the appended claims.

In one aspect of the invention, the present invention provides a novel method for diagnosing myopia and/or myopia related complications in a subject, comprising, obtaining a

3

biological sample from the subject, and determining at least one SNP genotype in the BICD1 gene in the biological sample, wherein the presence of a particular allele in the SNP genotype indicates that the subject is susceptible to myopia. The diagnosis of the present invention is made by detecting a polymorphism in a BICD1 nucleic acid, such as the alleles in SNPs rs7966276, rs1151029, rs2650122, and rs10771923. The polymorphism can be a change in the BICD1 sequence, such as the change of a single nucleotide, which may cause a difference in the polypeptide encoded by the BICD1 gene, or may cause a different transcription activity.

In the method for diagnosing myopia and/or myopia related complications of the present invention, primer extension (PinPoint assay, Massextend™, SPC-SBE, or GOOD assay), hybridization (TaqMan assay, bead arry, or SNP chip), ligation (combinatorial fluorescence energy transfer (CFET) tags), and enzymatric cleavage (RFLP, Invader® assay), PCR-SSCP (single-strand conformation polymorphism), MRD (mismatch repair dection), BeadArray™, or SNPlex™ can be used. Firstly, a biological sample containing nucleic acid (DNA) is collected from a subject. The subject can be a mammalian, preferably, a human or an individual having myopia and/or myopia related complications. Examples of the subject include, but are not limited to, an adult, child, or fetus. The biological sample can be isolated or collected from any source which contains genomic DNA, such as a blood sample, sample of amniotic fluid, sample of cerebrospinal fluid, or tissue sample from skin, muscle, buccal or conjunctival mucosa, placenta, gastrointestinal tract or other organs. The DNA sample is then examined to determine whether a polymorphism of the BICD1 gene is present, and/or to determine the presence of the risk genotype in the BICD1 gene. In one embodiment, the presence of the polymorphism in the BICD1 gene can be indicated by TaqMan assay. Briefly, the PCR primers and TaqMan MGB probes are designed with Primer Express version 2.0. Reactions can be performed in 96-well microplates with GeneAmp 9700 thermal cyclers. Fluorescence can be measured with an ABI Prism 7500 sequence detection system and analyzed with the ABI Prism 7500 SDS software version 1.0. In another embodiment, the presence of SNP can be determined by genotyping as described in Mutat Res 2005; 573:70-82. Genotyping can be performed by the Illumina BeadArray technology (Sentrix® Array Matrix) [Shen, 2005 #135]. DNA is annealed to allelic-specific oligonucleotides and amplified by polymerase chain reaction (PCR). Array-based hybridization takes place and genotyping are achieved by Cy-3 and Cy-5 labeled primers. Alternately, a commercial gene chip also can be used to determine presence of SNP in BICD1 gene. The Affymetrix GeneChip® Human Mapping 500K Array Set includes two arrays, each capable of genotyping on average 250,000 SNPs (approximately 262,000 for Nsp arrays and 238,000 for Sty arrays). Genomic DNA is hybridized in accordance with the manufacturer's standard recommendations. Genotypes are determined using BRLMM clustering algorithm.

In addition, if the polymorphism results in the creation or elimination of a restriction site, a restriction digestion can be used to determine the polymorphism in the BICD1 gene. Firstly, the PCR can be used to amplify the BICD1 gene in the biological sample of genomic DNA from the subject. Next, an RFLP analysis is performed. The digestion pattern of the relevant DNA fragment indicates the presence or absence of a particular allele (or genotype) in the BICD1 gene, and therefore indicates the presence or absence for a susceptibility to myopia or a decreased susceptibility to myopia. In another embodiment, a sequence analysis can be used to determine the polymorphism in the BICD1 gene. PCR or other appropriate methods can be used to amplify the gene or nucleic acid, and/or its flanking sequences, if desired. The sequence of a BICD1 nucleic acid, or a fragment of the

4

nucleic acid, or the BICD1 genomic DNA, or fragment of the BICD1 genomic DNA is determined, using standard methods. The sequence of the nucleic acid, nucleic acid fragment, genomic DNA, or genomic DNA fragment is compared with the known nucleic acid sequence of the gene. The presence a particular allele or genotype of a polymorphism indicates that the subject has a susceptibility to myopia and/or myopia related complications.

There are many methods for determining whether a polymorphism in the BICD1 gene is present. One of ordinary skill in the art will select the appropriate method and protocol to use. These and many other methods will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the present invention.

The term "susceptibility to myopia" of the invention refers to either an increased risk or a decreased risk of myopia, when a certain allele or SNP genotype is present. The term "decreased susceptibility to myopia" of the invention indicates that the relative risk has accordingly decreased, when a certain other allele or SNP genotype is present. The term "increased susceptibility to myopia" of the invention indicates that the relative risk has accordingly increased when a certain other allele or SNP genotype is present. In one embodiment, when the G allele in SNP rs10771923 or the T allele in SNP rs1151029 is preset, it indicates that the subject has increased susceptibility to myopia. The increased susceptibility is characterized by a relative risk of at least 1.2. In another embodiment, when the A allele in SNP rs7966276 is present, it indicates that the subject has decreased susceptibility to myopia. The decreased susceptibility is characterized by a relative risk of at least 0.6. It is understood however, that identifying whether an increased or decreased risk is medically significant may also depend on a variety of factors, including the specific disease, the marker and environmental factors.

In another aspect of the invention, the present invention also provides a method for screening a material (candidate therapeutic agents) for treating myopia and/or myopia related complications, comprising, contacting a test material with a cell expressing the BICD1 gene, and selecting a material that modulates the expression of the BICD1 gene as compared to a control.

The polymorphisms of the BICD1 gene (e.g. SNP rs7966276, rs1151029, rs2650122, and rs10771923) can also be used to identify candidate therapeutic agents for treating myopia and/or myopia related complications. The method is based on screening a candidate therapeutic agent to determine if it alters an expression profile of the BICD1 gene. For example, a cell is exposed to a test material or a combination of test materials (sequentially or consequentially) and the expression of the BICD1 gene in the cell is measured. The expression profile of the BICD1 gene in the test cell population is compared to an expression level of the BICD1 gene in a reference cell population that is not exposed to the test material. The test material can be a compound not previously described or can be a previously known compound which is not known to be an anti-myopia agent. Examples of the test materials include, but are not limited to, chemical compounds, small molecule pharmaceutical substances, carbohydrates, nucleotides, proteins, or a peptide, etc. For example, the materials can be a small interfering RNA. In one embodiment, the test material can influence or modulate the expression of the BICD1 gene through the effect of SNP rs10771923, SNP rs1151029, or SNP rs7966276 leading to prevent, reduce and/or treat myopia and/or myopia related complications.

In yet another aspect of the invention, the present invention further provides a method for treating, preventing or amelio-

US 7,906,289 B2

5

rating myopia and/or myopia related complications, comprising, administering to a subject a pharmaceutically effective amount of the myopia therapeutic agents.

As discussed in detail above, by controlling the expression levels or activities of the BICD1 gene, the prevention, progression of myopia and/or myopia related complications can be controlled. Thus, candidate agents, which are potential targets in the treatment or prevention of myopia and/or myopia related complications, can be identified by screening test compounds using the expression levels and/or activities of the BICD1 gene.

In yet another aspect of the invention, the present method further provides a method of assessing a subject for probability of response to a myopia therapeutic agent, comprising detecting a SNP genotype in the BICD1 gene, wherein the presence of a particular allele or genotype in the SNP is indicative of a probability for a positive response to a myopia therapeutic agent.

In this method, genetic markers relating to the BICD1 gene are assessed, as described above in relation to assessing an individual for susceptibility to myopia and/or myopia related complications. The presence of an allele or SNP genotype associated with increased risk for myopia (e.g., the G allele in SNP rs10771923 or the T allele in SNP rs1151029), indicates a probability for a positive response to a myopia therapeutic agent. The term "Probability of a positive response" of the present invention refers to the concept that the subject is more likely to have a positive response to a myopia therapeutic agent than a subject not having an allele or SNP, and the subject is associated with an increased risk for myopia and/or myopia related complications.

In yet another aspect of the invention, the present invention further provides a kit for assaying a biological sample from a subject to detect susceptibility for myopia, comprising one or more reagents for detecting one or more SNP genotypes in the BICD1 gene.

The terms "kits" as used herein in the context of SNP detection reagents, are intended to refer to such things as combinations of multiple SNP detection reagents, or one or more SNP detection reagents in combination with one or more other types of elements or components (e.g., other types of biochemical reagents, containers, packages such as packaging intended for commercial sale, substrates to which SNP detection reagents are attached, electronic hardware components, etc.). Accordingly, the present invention further provides SNP detection kits, including but not limited to, packaged probe and primer sets (e.g., TaqMan probe/primer sets), arrays/microarrays of nucleic acid molecules, and beads that contain one or more probes, primers, or other detection reagents for detecting one or more SNPs of the present invention. The kits can optionally include various electronic hardware components; for example, arrays (DNA chips) and microfluidic systems ("lab-on-a-chip" systems) provided by various manufacturers typically comprise hardware components. Other kits (e.g., probe/primer sets) may not include electronic hardware components, but may be comprised of, for example, one or more SNP detection reagents (along with, optionally, other biochemical reagents) packaged in one or more containers.

A SNP detection kit typically contains one or more detection reagents and other components (e.g., a buffer, enzymes such as DNA polymerases or ligases, chain extension nucleotides such as deoxynucleotide triphosphates, and in the case of Sanger-type DNA sequencing reactions, chain terminating nucleotides, positive control sequences, negative control sequences, and the like) necessary to carry out an assay or reaction, such as amplification and/or detection of a SNP-

6

containing nucleic acid molecule. A kit may further contain means for determining the amount of a target nucleic acid, and means for comparing the amount with a standard, and can comprise instructions for using the kit to detect the SNP-containing nucleic acid molecule of interest. In one embodiment, kits are provided which contain the necessary reagents to carry out one or more assays to detect one or more SNPs disclosed herein. In another embodiment, SNP detection kits are in the form of nucleic acid arrays, or compartmentalized kits, including microfluidic/lab-on-a-chip systems.

Any number of probes, such as allele-specific probes, can be implemented in an array, and each probe or pair of probes can hybridize to a different allele position. Polynucleotide probes can be synthesized at designated areas (or synthesized separately and then affixed to designated areas) on a substrate using a light-directed chemical process. Each DNA chip can contain, for example, thousands to millions of individual synthetic polynucleotide probes arranged in a grid-like pattern and miniaturized (e.g., to the size of a dime). Preferably, probes are attached to a solid support in an ordered, addressable array.

In the present invention, the kits include for example, one or more reagents for detecting one or more SNP genotypes in the BICD1 gene, wherein the reagent includes at least one material or instrument to discriminate and detect the SNP genotypes, a buffer, and an enzyme. Examples of the materials include, but are not limited to, a nucleotide sequence that is completely complementary to a region comprising at least one SNP genotype in the BICD1 gene, a restriction enzyme (e.g. endonuclease enzyme) for recognizing a specific sequence, a labeled sequence primer, or a labeled allele-specific probe. In one embodiment, the kit for diagnosing a susceptibility to myopia can comprise primers for nucleic acid amplification of a region in the BICD1 gene comprising the SNPs rs7966276, rs1151029, rs2650122, and/or rs10771923 where the risk alleles or genotypes are more frequently present in a subject having myopia. The primers can be designed using portions of the nucleic acids flanking SNPs rs7966276, rs1151029, rs2650122, and/or rs10771923. Further, after the analysis for allelic differences, mass the mass spectrometry, fluorescence, or chemiluminescence can be used to detect the allelic differences.

EXAMPLES

Example 1

Genome Scan in the Initial Step

A total of about 4000 subjects were recruited for the myopia study. There were multiple sources for participants, whom included the following: (1) young men in military conscripts, (2) university students, (3) hospital personnel, and (4) patients from ophthalmology clinics. Individuals with spherical refraction$\geq$−6.0 D in one eye and $\leq$−4.0 D in the other eye were classified as high myopia. A subject was defined as a control if the worse eye had a spherical refraction$\geq$—1.5 D. All subjects were between the ages of 17-45 years old. All the participants were of Chinese descent. All participants gave informed consents. The study was approved by the Institutional Review Board at the Kaohsiung Medical University, Kaohsiung, Taiwan.

In the initial step, Affymetrix GeneChip® Human Mapping 500K Array Set was used. It comprised two arrays, each capable of genotyping on average 250,000 SNPs (approximately 262,000 for Nsp arrays and 238,000 for Sty arrays). To ensure the quality of DNA sample, all DNA were required to

7

have the OD 260/280 between 1.7 and 2.0, and 260/230>1.0. About 1.5 µg (30 µl of 50 ng/µl) genomic DNA was required for genotyping. The images were analyzed using BRLMM algorithm to obtain the genotyping data. To remove an SNP or a sample which might have genotyping problems, the following criteria was used: per sample call rate of at least 90%, per SNP call rate of at least 90%, an SNP with the minor allele frequency of at least 1%, and genotypes in Hardy-Weinberg equilibrium (p>0.001). Taken together, 380619 SNPs were considered for further analysis.

To test for allelic and genotypic association between each SNP and the high myopia status, PLINK program (*Am J Hum Genet* 2007; 81:559-75) was used to calculate genotype and allele frequencies, and to perform χ^2 test. To investigate the genotypic association under different inheritance models, genotype data were further encoded into dominant, recessive, and additive modes. In addition, the trend test was also performed. The genetic effect along with covariates (sex and age) was included in the logistic regression. A linear regression model was applied to test for the association between each SNP and the refraction errors. Since the refraction errors greater than –3 D were common in the Taiwanese population, the subjects as normal/mild myopia ($\geq$–3 D) and high myopia ($\leq$–6 D) was also dichotomized, and the discrete phenotype was tested in the logistic regression model. For logistic regression analysis, subjects with refraction between –3 and –6 D were not included in the analysis. Hap-clustering was employed to perform haplotype analysis.

For the Affymetrix 500K SNP chips, the average call rate was 98.7 (ranging from 97.4 to 99.5). The initial analysis indicated that the best 10 SNPs had the highest p value=0.0002. The 10 SNPs are on chromosome 1, 2, 4, 6, 12, 16, 17 and 21, and none of them are closely located.

Example 2

Genome Scan in the Second Step

In the second step, firstly the 10 best SNPs as the centers was used, and a genomic region of 200 kb surrounding each of the 10 best SNPs as our candidate region was selected. A total of 384 tagging SNPs were selected for follow-up fine mapping in independent 1536 subjects whose refraction errors were either <–6 D or >–1.5 D. Genotyping was performed by the Illumina BeadArray technology (Sentrix® Array Matrix) (Mutat Res 2005; 573:70-82). DNA was annealed to allelic-specific oligonucleotides and amplified by polymerase chain reaction. Array-based hybridization took place and genotyping were achieved by Cy-3 and Cy-5 labeled primers. Thirty replicates of each SNP were done to ensure the highest quality of genotype calling.

The overall call rate in the second stage was 97.1%. The most significant SNP (rs7966276) was at the BICD1 gene on chromosome 12p11 with a p value <1.13×10^{-4}. Another three

8

SNPs at BICD1 were also significant (SNP rs1151029, p=4.78×10^{-4}; SNP rs2650122, p=3.65×10^{-2}; SNP rs10771923, p=2.70×10^{-2}).

Example 3

Genome Scan in the Third Step

In the third step, the most promising SNPs based on the stage II result were genotyped by using the TaqMan technology (Applied Biosystems [ABI], Foster City, USA). Briefly, PCR primers and TaqMan MGB probes were designed with Primer Express version 2.0. Reactions were performed in 96-well microplates with GeneAmp 9700 thermal cyclers. Fluorescence was measured with an ABI Prism 7500 sequence detection system and analyzed with the ABI Prism 7500 SDS software version 1.0. The subjects with refraction errors between –6 D and –1.5 D were used in the stage III study.

In the third step, the BICD1 gene was focused and the genetic effect was analyzed using different inheritance models for the three SNPs in a larger dataset including, SNP rs10771923 in 3273 subjects, SNP rs1151029 in 3917 subjects and SNP rs7966276 in 2962 subjects. The allele frequencies of the three SNPs are listed in Table 1. Referring to Table 2, the most significant results were from the dichotomized phenotype (>–3 D as control and $\leq$6 D as case) when minor G allele of rs10771923 had an additive effect (p=0.00088, OR=1.22) or the minor allele T of rs1151029 had a dominant deleterious effect (p=0.00088, OR=1.3). SNP rs7966276 had less significant results when compared with the other two SNPs. When the phenotype was treated as a continuous trait, the three SNPs showed different significant results as illustrated in Table 3. Referring to FIG. **1**, the frequency of the risk T allele of rs1151029 separated by different diopters was calculated. The a-x-axis is the negative diopter (×100).

TABLE 1

Allele frequency and the number of genotyped individuals for the BICD1 gene

Refraction errors	rs10771923		rs1151029		rs7966276	
	sample size	MAF	sample size	MAF	sample size	MAF
$\geq$–3 D	1305	0.375	0630	0.211	1215	0.040
–3 D to –6 D	941	0.400	1148	0.228	953	0.027
$\leq$–6 D	1027	0.423	1139	0.247	794	0.025
Total	3273	0.397	3917	0.226	2962	0.032

MAF: minor allele frequency

TABLE 2

results of the dichotomized trait (normal/mild vs. high myopia) from logistic regression

	Genotype				OR (p value)		
	N (%)	N (%)	N (%)	Total	Additive	Dominant	Recessive
rs10771923	AA	AG	GG				
$\geq$–3 D	509 (39.0)	614 (47.1)	182 (14.0)	1305	1.22 (0.00088)	1.30 (0.0024)	1.30 (0.021)
$\leq$–6 D	338 (32.9)	510 (49.7)	179 (17.4)	1027			

TABLE 2-continued

results of the dichotomized trait (normal/mild vs. high myopia) from logistic regression							
rs1151029	AA	AT	TT				
≧−3 D	1027 (63.0)	519 (31.8)	84 (5.15)	1630	1.22 (0.0017)	1.30 (0.00088)	1.21 (0.263)
≦−6 D	646 (56.7)	423 (37.1)	70 (6.14)	1139			
rs7966276	TT	AT	AA				
≧−3 D	1122 (92.3)	90 (7.40)	3 (0.24)	1215	0.61 (0.0114)	0.62 (0.0161)	
≦−6 D	755 (95.0)	39 (4.91)	0 (0)	794			

TABLE 3

Results based on the refractive errors in the linear regression model						
Genotype (categorical)		Additive		Dominant		Recessive
	p value	beta × 100	p value	beta × 100	p value	beta × 100	p value
rs10771923	0.0128	48.4	0.0032	29.2	0.0115	35.4	0.0230
rs1151029	0.020	46.9	0.0060	28.8	0.0060	29.6	0.181
rs7966276	0.022	−125.1	0.0060	−62.7	0.0077	−173.8	0.260

While the invention has been described by way of example and in terms of the preferred embodiments, it is to be understood that the invention is not limited to the disclosed embodiments. To the contrary, it is intended to cover various modifications and similar arrangements (as would be apparent to those skilled in the art). Therefore, the scope of the appended claims should be accorded the broadest interpretation so as to encompass all such modifications and similar arrangements.

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<223> OTHER INFORMATION: Bicaudal D Homolog 1 (Drosophila) (BICD1) gene

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aagactgaga tagagaggct aaccaaggag ctcacggaga ccacccacga gaagatccag     180

gctgccgagt acgggctggt ggtgctggag gagaagctga ccctcaaaca gcagtatgat     240

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tgcctgtcac ctctcccttt cctggccctc actcccccca cccgcaaggc ccactcatca     360

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aaatgaatat ataagctgga atttgggagg caatggctgt ttggcctgcg ggggagggag     540

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US 7,906,289 B2

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US 7,906,289 B2

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US 7,906,289 B2

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US 7,906,289 B2

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US 7,906,289 B2

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US 7,906,289 B2

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US 7,906,289 B2

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US 7,906,289 B2

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US 7,906,289 B2

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US 7,906,289 B2

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What is claimed is:

1. A method of determining an increased risk of developing myopia in a human, comprising detecting in a sample from the human the SNP rs7966276 (A/T) in the BICD1 gene, and determining the risk of developing myopia in the human, wherein the presence of the TT genotype is detected at the SNP rs7966276 (A/T) and indicates that the human has an increased risk of developing high myopia, and wherein the high myopia comprises a spherical refraction ≤ -6 D.

2. The method as claimed in claim 1, wherein the sample comprises blood, amniotic fluid, cerebrospinal fluid, tissue from skin, muscle, buccal or conjunctival mucosa, placenta, or gastrointestinal tract.

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