

**Table 5. Association of the Axial Length Genes Identified in Gene-Based Tests with Spherical Equivalent in Independent Cohorts**

Gene	MIM Number	Chr	P <sub>gene-based</sub> Value <sup>a</sup>
CD55	125240	1	$4.5 \times 10^{-6}$
ALPPL2	171810	2	$8.3 \times 10^{-3}$
TIMELESS/MIP/SPRYD4/GLS2 <sup>b</sup>	603887	12	0.14

Abbreviation is as follows: Chr, chromosome.

<sup>a</sup>The association with spherical equivalent was assessed in 17 European ancestry cohorts of the 18 independent cohorts, with the HapMap 2 CEU population as the reference to estimate patterns of LD.

<sup>b</sup>Because of the  $\pm 50$  kb added to the definition for each gene and the close proximity of the genes, *MIP*, *SPRYD4*, *GLS2*, and *TIMELESS* all had similar gene-based p values (ranged from 0.14 to 0.20 for the combined analysis), and thus p value and MIM number for *TIMELESS* only is presented.

susceptibility genes do not overlap between primary angle closure glaucoma and eyes with shorter axial length.

In summary, we identified nine loci associated with AL. They fall into two groups, one also influencing common refractive error variation, and the other, which includes two genes in the Wnt signaling pathway, uniquely determining eye size with little effect on natural refractive status. Further elucidation and characterization of the causal variants underlying the growth of ocular component dimensions and the development of myopia may enable new pathway and target identification, leading to potential prevention and treatment development.

### Supplemental Data

Supplemental Data include six figures, ten tables, and Supplemental Acknowledgments and can be found with this article online at <http://www.cell.com/AJHG/>.

### Acknowledgments

The authors thank the staff and participants of all studies for their important contributions. Complete funding information and acknowledgements are provided in the Supplemental Data. ALSPAC thanks 23andMe for funding the generation of the ALSPAC GWA data.

Received: March 15, 2013

Revised: April 17, 2013

Accepted: June 12, 2013

Published: August 8, 2013

### Web Resources

The URLs for data presented herein are as follows:

HaploReg, <http://www.broadinstitute.org/mammals/haploreg/haploreg.php>

IMPUTE2, [http://mathgen.stats.ox.ac.uk/impute/impute\\_v2.html](http://mathgen.stats.ox.ac.uk/impute/impute_v2.html)

International HapMap Project, <http://hapmap.ncbi.nlm.nih.gov/>

LocusZoom, <http://csg.sph.umich.edu/locuszoom/>

MACH, <http://www.sph.umich.edu/csg/abecasis/MACH/>

METAL, <http://www.sph.umich.edu/csg/abecasis/metal/>

Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org/>

RegulomeDB, <http://RegulomeDB.org/>

UCSC Genome Browser, <http://genome.ucsc.edu>

VEGAS, <http://gump.qimr.edu.au/VEGAS/>

### References

- Lin, L.L., Shih, Y.F., Hsiao, C.K., and Chen, C.J. (2004). Prevalence of myopia in Taiwanese schoolchildren: 1983 to 2000. *Ann. Acad. Med. Singapore* 33, 27–33.
- Guo, Y.H., Lin, H.Y., Lin, L.L., and Cheng, C.Y. (2012). Self-reported myopia in Taiwan: 2005 Taiwan National Health Interview Survey. *Eye (Lond.)* 26, 684–689.
- Pascolini, D., and Mariotti, S.P. (2012). Global estimates of visual impairment: 2010. *Br. J. Ophthalmol.* 96, 614–618.
- Hsu, W.M., Cheng, C.Y., Liu, J.H., Tsai, S.Y., and Chou, P. (2004). Prevalence and causes of visual impairment in an elderly Chinese population in Taiwan: the Shihpai Eye Study. *Ophthalmology* 111, 62–69.
- Zheng, Y., Lavanya, R., Wu, R., Wong, W.L., Wang, J.J., Mitchell, P., Cheung, N., Cajucom-Uy, H., Lamoureux, E., Aung, T., et al. (2011). Prevalence and causes of visual impairment and blindness in an urban Indian population: the Singapore Indian Eye Study. *Ophthalmology* 118, 1798–1804.
- Wong, T.Y., Chong, E.W., Wong, W.L., Rosman, M., Aung, T., Loo, J.L., Shen, S., Loon, S.C., Tan, D.T., Tai, E.S., and Saw, S.M.; Singapore Malay Eye Study Team. (2008). Prevalence and causes of low vision and blindness in an urban malay population: the Singapore Malay Eye Study. *Arch. Ophthalmol.* 126, 1091–1099.
- Saw, S.M., Gazzard, G., Shih-Yen, E.C., and Chua, W.H. (2005). Myopia and associated pathological complications. *Ophthalmic Physiol. Opt.* 25, 381–391.
- Javitt, J.C., and Chiang, Y.P. (1994). The socioeconomic aspects of laser refractive surgery. *Arch. Ophthalmol.* 112, 1526–1530.
- Vitale, S., Cotch, M.F., Sperduto, R., and Ellwein, L. (2006). Costs of refractive correction of distance vision impairment in the United States, 1999–2002. *Ophthalmology* 113, 2163–2170.
- Shufelt, C., Fraser-Bell, S., Ying-Lai, M., Torres, M., and Varma, R.; Los Angeles Latino Eye Study Group. (2005). Refractive error, ocular biometry, and lens opalescence in an adult population: the Los Angeles Latino Eye Study. *Invest. Ophthalmol. Vis. Sci.* 46, 4450–4460.
- Pan, C.W., Wong, T.Y., Chang, L., Lin, X.Y., Lavanya, R., Zheng, Y.F., Kok, Y.O., Wu, R.Y., Aung, T., and Saw, S.M. (2011). Ocular biometry in an urban Indian population: the Singapore Indian Eye Study (SINDI). *Invest. Ophthalmol. Vis. Sci.* 52, 6636–6642.
- Ip, J.M., Huynh, S.C., Kifley, A., Rose, K.A., Morgan, I.G., Varma, R., and Mitchell, P. (2007). Variation of the contribution from axial length and other oculometric parameters to refraction by age and ethnicity. *Invest. Ophthalmol. Vis. Sci.* 48, 4846–4853.
- Lim, L.S., Yang, X., Gazzard, G., Lin, X., Sng, C., Saw, S.M., and Qiu, A. (2011). Variations in eye volume, surface area, and shape with refractive error in young children by magnetic resonance imaging analysis. *Invest. Ophthalmol. Vis. Sci.* 52, 8878–8883.
- Hsiang, H.W., Ohno-Matsui, K., Shimada, N., Hayashi, K., Moriyama, M., Yoshida, T., Tokoro, T., and Mochizuki, M. (2008). Clinical characteristics of posterior staphyloma in

- eyes with pathologic myopia. *Am. J. Ophthalmol.* *146*, 102–110.
15. Sanfilippo, P.G., Hewitt, A.W., Hammond, C.J., and Mackey, D.A. (2010). The heritability of ocular traits. *Surv. Ophthalmol.* *55*, 561–583.
  16. Lyhne, N., Sjølie, A.K., Kyvik, K.O., and Green, A. (2001). The importance of genes and environment for ocular refraction and its determiners: a population based study among 20-45 year old twins. *Br. J. Ophthalmol.* *85*, 1470–1476.
  17. Chen, C.Y., Scurrah, K.J., Stankovich, J., Garoufalidis, P., Dirani, M., Pertile, K.K., Richardson, A.J., Mitchell, P., and Baird, P.N. (2007). Heritability and shared environment estimates for myopia and associated ocular biometric traits: the Genes in Myopia (GEM) family study. *Hum. Genet.* *121*, 511–520.
  18. Klein, A.P., Sukhtipat, B., Duggal, P., Lee, K.E., Klein, R., Bailey-Wilson, J.E., and Klein, B.E. (2009). Heritability analysis of spherical equivalent, axial length, corneal curvature, and anterior chamber depth in the Beaver Dam Eye Study. *Arch. Ophthalmol.* *127*, 649–655.
  19. Fan, Q., Barathi, V.A., Cheng, C.Y., Zhou, X., Meguro, A., Nakata, I., Khor, C.C., Goh, L.K., Li, Y.J., Lim, W., et al. (2012). Genetic variants on chromosome 1q41 influence ocular axial length and high myopia. *PLoS Genet.* *8*, e1002753.
  20. Verhoeven, V.J., Hysi, P.G., Wojciechowski, R., Fan, Q., Guggenheim, J.A., Höhn, R., MacGregor, S., Hewitt, A.W., Nag, A., Cheng, C.Y., et al.; Consortium for Refractive Error and Myopia (CREAM); Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Research Group; Wellcome Trust Case Control Consortium 2 (WTCCC2); Fuchs' Genetics Multi-Center Study Group. (2013). Genome-wide meta-analyses of multiethnicity cohorts identify multiple new susceptibility loci for refractive error and myopia. *Nat. Genet.* *45*, 314–318.
  21. Verhoeven, V.J., Hysi, P.G., Saw, S.M., Vitart, V., Mirshahi, A., Guggenheim, J.A., Cotch, M.F., Yamashiro, K., Baird, P.N., Mackey, D.A., et al. (2012). Large scale international replication and meta-analysis study confirms association of the 15q14 locus with myopia. The CREAM consortium. *Hum. Genet.* *131*, 1467–1480.
  22. Boyd, A., Golding, J., Macleod, J., Lawlor, D.A., Fraser, A., Henderson, J., Molloy, L., Ness, A., Ring, S., and Davey Smith, G. (2013). Cohort Profile: the 'children of the 90s'—the index offspring of the Avon Longitudinal Study of Parents and Children. *Int. J. Epidemiol.* *42*, 111–127.
  23. Mackey, D.A., Mackinnon, J.R., Brown, S.A., Kearns, L.S., Ruddle, J.B., Sanfilippo, P.G., Sun, C., Hammond, C.J., Young, T.L., Martin, N.G., and Hewitt, A.W. (2009). Twins eye study in Tasmania (TEST): rationale and methodology to recruit and examine twins. *Twin Res. Hum. Genet.* *12*, 441–454.
  24. Mitchell, P., Smith, W., Attebo, K., and Wang, J.J. (1995). Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophthalmology* *102*, 1450–1460.
  25. Foran, S., Wang, J.J., and Mitchell, P. (2003). Causes of visual impairment in two older population cross-sections: the Blue Mountains Eye Study. *Ophthalmic Epidemiol.* *10*, 215–225.
  26. Vitart, V., Bencić, G., Hayward, C., Herman, J.S., Huffman, J., Campbell, S., Bućan, K., Zgaga, L., Kolčić, I., Polasek, O., et al. (2010). Heritabilities of ocular biometrical traits in two croatian isolates with extended pedigrees. *Invest. Ophthalmol. Vis. Sci.* *51*, 737–743.
  27. Aulchenko, Y.S., Heutink, P., Mackay, I., Bertoli-Avella, A.M., Pullen, J., Vaessen, N., Rademaker, T.A., Sandkuijl, L.A., Cardon, L., Oostra, B., and van Duijn, C.M. (2004). Linkage disequilibrium in young genetically isolated Dutch population. *Eur. J. Hum. Genet.* *12*, 527–534.
  28. Pardo, L.M., MacKay, I., Oostra, B., van Duijn, C.M., and Aulchenko, Y.S. (2005). The effect of genetic drift in a young genetically isolated population. *Ann. Hum. Genet.* *69*, 288–295.
  29. Hofman, A., van Duijn, C.M., Franco, O.H., Ikram, M.A., Janssen, H.L., Klaver, C.C., Kuipers, E.J., Nijsten, T.E., Stricker, B.H., Tiemeier, H., et al. (2011). The Rotterdam Study: 2012 objectives and design update. *Eur. J. Epidemiol.* *26*, 657–686.
  30. Vitart, V., Bencić, G., Hayward, C., Skunca Herman, J., Huffman, J., Campbell, S., Bućan, K., Navarro, P., Gunjaca, G., Marin, J., et al. (2010). New loci associated with central cornea thickness include COL5A1, AKAP13 and AVGR8. *Hum. Mol. Genet.* *19*, 4304–4311.
  31. Evans, S., Newnham, J., MacDonald, W., and Hall, C. (1996). Characterisation of the possible effect on birthweight following frequent prenatal ultrasound examinations. *Early Hum. Dev.* *45*, 203–214.
  32. Newnham, J.P., Evans, S.F., Michael, C.A., Stanley, F.J., and Landau, L.I. (1993). Effects of frequent ultrasound during pregnancy: a randomised controlled trial. *Lancet* *342*, 887–891.
  33. Williams, L.A., Evans, S.F., and Newnham, J.P. (1997). Prospective cohort study of factors influencing the relative weights of the placenta and the newborn infant. *BMJ* *314*, 1864–1868.
  34. Xu, L., Li, J., Cui, T., Hu, A., Fan, G., Zhang, R., Yang, H., Sun, B., and Jonas, J.B. (2005). Refractive error in urban and rural adult Chinese in Beijing. *Ophthalmology* *112*, 1676–1683.
  35. Lavanya, R., Jeganathan, V.S., Zheng, Y., Raju, P., Cheung, N., Tai, E.S., Wang, J.J., Lamoureux, E., Mitchell, P., Young, T.L., et al. (2009). Methodology of the Singapore Indian Chinese Cohort (SICC) eye study: quantifying ethnic variations in the epidemiology of eye diseases in Asians. *Ophthalmic Epidemiol.* *16*, 325–336.
  36. Saw, S.M., Tong, L., Chua, W.H., Chia, K.S., Koh, D., Tan, D.T., and Katz, J. (2005). Incidence and progression of myopia in Singaporean school children. *Invest. Ophthalmol. Vis. Sci.* *46*, 51–57.
  37. Foong, A.W., Saw, S.M., Loo, J.L., Shen, S., Loon, S.C., Rosman, M., Aung, T., Tan, D.T., Tai, E.S., and Wong, T.Y. (2007). Rationale and methodology for a population-based study of eye diseases in Malay people: The Singapore Malay eye study (SiMES). *Ophthalmic Epidemiol.* *14*, 25–35.
  38. Dirani, M., Chan, Y.H., Gazzard, G., Hornbeak, D.M., Leo, S.W., Selvaraj, P., Zhou, B., Young, T.L., Mitchell, P., Varma, R., et al. (2010). Prevalence of refractive error in Singaporean Chinese children: the strabismus, amblyopia, and refractive error in young Singaporean Children (STARS) study. *Invest. Ophthalmol. Vis. Sci.* *51*, 1348–1355.
  39. Rahi, J.S., Cumberland, P.M., and Peckham, C.S. (2011). Myopia over the lifecourse: prevalence and early life influences in the 1958 British birth cohort. *Ophthalmology* *118*, 797–804.
  40. Fraser, A., Macdonald-Wallis, C., Tilling, K., Boyd, A., Golding, J., Davey Smith, G., Henderson, J., Macleod, J., Molloy, L., Ness, A., et al. (2013). Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. *Int. J. Epidemiol.* *42*, 97–110.
  41. Burdon, K.P., Macgregor, S., Hewitt, A.W., Sharma, S., Chidlow, G., Mills, R.A., Danoy, P., Casson, R., Viswanathan,

- A.C., Liu, J.Z., et al. (2011). Genome-wide association study identifies susceptibility loci for open angle glaucoma at TMCO1 and CDKN2B-AS1. *Nat. Genet.* **43**, 574–578.
42. The Diabetes Control and Complications Trial Research Group. (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **329**, 977–986.
  43. Nelis, M., Esko, T., Mägi, R., Zimprich, F., Zimprich, A., Toncheva, D., Karachanak, S., Piskácková, T., Balascák, I., Peltonen, L., et al. (2009). Genetic structure of Europeans: a view from the North-East. *PLoS ONE* **4**, e5472.
  44. Louttit, M.D., Kopplin, L.J., Igo, R.P., Jr., Fondran, J.R., Tagliaferri, A., Bardenstein, D., Aldave, A.J., Croasdale, C.R., Price, M.O., Rosenwasser, G.O., et al.; FECD Genetics Multi-Center Study Group. (2012). A multicenter study to map genes for Fuchs endothelial corneal dystrophy: baseline characteristics and heritability. *Cornea* **31**, 26–35.
  45. Leibowitz, H.M., Krueger, D.E., Maunder, L.R., Milton, R.C., Kini, M.M., Kahn, H.A., Nickerson, R.J., Pool, J., Colton, T.L., Ganley, J.P., et al. (1980). The Framingham Eye Study monograph: An ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and visual acuity in a general population of 2631 adults, 1973-1975. *Surv. Ophthalmol. Suppl.* **24**, 335–610.
  46. Pärssinen, O., Jauhonen, H.M., Kauppinen, M., Kaprio, J., Koskenvuo, M., and Rantanen, T. (2010). Heritability of spherical equivalent: a population-based twin study among 63- to 76-year-old female twins. *Ophthalmology* **117**, 1908–1911.
  47. Wichmann, H.E., Gieger, C., and Illig, T.; MONICA/KORA Study Group. (2005). KORA-gen—resource for population genetics, controls and a broad spectrum of disease phenotypes. *Gesundheitswesen* **67** (Suppl 1), S26–S30.
  48. Holle, R., Happich, M., Löwel, H., and Wichmann, H.E.; MONICA/KORA Study Group. (2005). KORA—a research platform for population based health research. *Gesundheitswesen* **67** (Suppl 1), S19–S25.
  49. Oexle, K., Ried, J.S., Hicks, A.A., Tanaka, T., Hayward, C., Bruegel, M., Gögele, M., Lichtner, P., Müller-Myhsok, B., Döring, A., et al. (2011). Novel association to the proprotein convertase PCSK7 gene locus revealed by analysing soluble transferrin receptor (sTfR) levels. *Hum. Mol. Genet.* **20**, 1042–1047.
  50. Steffens, M., Lamina, C., Illig, T., Bettecken, T., Vogler, R., Entz, P., Suk, E.K., Toliat, M.R., Klopp, N., Caliebe, A., et al. (2006). SNP-based analysis of genetic substructure in the German population. *Hum. Hered.* **62**, 20–29.
  51. Biino, G., Palmas, M.A., Corona, C., Prodi, D., Fanciulli, M., Sulis, R., Serra, A., Fossarello, M., and Pirastu, M. (2005). Ocular refraction: heritability and genome-wide search for eye morphometry traits in an isolated Sardinian population. *Hum. Genet.* **116**, 152–159.
  52. Sim, X., Ong, R.T., Suo, C., Tay, W.T., Liu, J., Ng, D.P., Boehnke, M., Chia, K.S., Wong, T.Y., Seielstad, M., et al. (2011). Transferability of type 2 diabetes implicated loci in multi-ethnic cohorts from Southeast Asia. *PLoS Genet.* **7**, e1001363.
  53. Spector, T.D., and Williams, F.M. (2006). The UK Adult Twin Registry (TwinsUK). *Twin Res. Hum. Genet.* **9**, 899–906.
  54. Klein, R., Lee, K.E., Gangnon, R.E., and Klein, B.E. (2010). The 25-year incidence of visual impairment in type 1 diabetes mellitus the wisconsin epidemiologic study of diabetic retinopathy. *Ophthalmology* **117**, 63–70.
  55. Raitakari, O.T., Juonala, M., Rönnemaa, T., Keltikangas-Järvinen, L., Räsänen, L., Pietikäinen, M., Hutri-Kähönen, N., Taittonen, L., Jokinen, E., Marniemi, J., et al. (2008). Cohort profile: the cardiovascular risk in Young Finns Study. *Int. J. Epidemiol.* **37**, 1220–1226.
  56. Marchini, J., Howie, B., Myers, S., McVean, G., and Donnelly, P. (2007). A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat. Genet.* **39**, 906–913.
  57. Howie, B.N., Donnelly, P., and Marchini, J. (2009). A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.* **5**, e1000529.
  58. Li, Y., Willer, C.J., Ding, J., Scheet, P., and Abecasis, G.R. (2010). MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet. Epidemiol.* **34**, 816–834.
  59. Saw, S.M., Chua, W.H., Hong, C.Y., Wu, H.M., Chia, K.S., Stone, R.A., and Tan, D. (2002). Height and its relationship to refraction and biometry parameters in Singapore Chinese children. *Invest. Ophthalmol. Vis. Sci.* **43**, 1408–1413.
  60. Wong, T.Y., Foster, P.J., Johnson, G.J., Klein, B.E., and Seah, S.K. (2001). The relationship between ocular dimensions and refraction with adult stature: the Tanjong Pagar Survey. *Invest. Ophthalmol. Vis. Sci.* **42**, 1237–1242.
  61. Stephens, M., and Balding, D.J. (2009). Bayesian statistical methods for genetic association studies. *Nat. Rev. Genet.* **10**, 681–690.
  62. Higgins, J.P., Thompson, S.G., Deeks, J.J., and Altman, D.G. (2003). Measuring inconsistency in meta-analyses. *BMJ* **327**, 557–560.
  63. Liu, J.Z., McRae, A.F., Nyholt, D.R., Medland, S.E., Wray, N.R., Brown, K.M., Hayward, N.K., Montgomery, G.W., Visscher, P.M., Martin, N.G., and Macgregor, S.; AMFS Investigators. (2010). A versatile gene-based test for genome-wide association studies. *Am. J. Hum. Genet.* **87**, 139–145.
  64. Lu, Y., Vitart, V., Burdon, K.P., Khor, C.C., Bykhovskaya, Y., Mirshahi, A., Hewitt, A.W., Koehn, D., Hysi, P.G., Ramdas, W.D., et al.; NEIGHBOR Consortium. (2013). Genome-wide association analyses identify multiple loci associated with central corneal thickness and keratoconus. *Nat. Genet.* **45**, 155–163.
  65. Barathi, V.A., Boopathi, V.G., Yap, E.P., and Beuerman, R.W. (2008). Two models of experimental myopia in the mouse. *Vision Res.* **48**, 904–916.
  66. Barathi, V.A., Beuerman, R.W., and Schaeffel, F. (2009). Effects of unilateral topical atropine on binocular pupil responses and eye growth in mice. *Vision Res.* **49**, 383–387.
  67. Brink, N., Szamel, M., Young, A.R., Wittern, K.P., and Bergemann, J. (2000). Comparative quantification of IL-1beta, IL-10, IL-10r, TNFalpha and IL-7 mRNA levels in UV-irradiated human skin in vivo. *Inflamm. Res.* **49**, 290–296.
  68. Rozen, S., and Skaletsky, H. (2000). Primer3 on the WWW for general users and for biologist programmers. *Methods Mol. Biol.* **132**, 365–386.
  69. Solouki, A.M., Verhoeven, V.J., van Duijn, C.M., Verkerk, A.J., Ikram, M.K., Hysi, P.G., Despriet, D.D., van Koolwijk, L.M., Ho, L., Ramdas, W.D., et al. (2010). A genome-wide association study identifies a susceptibility locus for refractive errors and myopia at 15q14. *Nat. Genet.* **42**, 897–901.
  70. Kim, K.A., Wagle, M., Tran, K., Zhan, X., Dixon, M.A., Liu, S., Gros, D., Korver, W., Yonkovich, S., Tomasevic, N., et al.

- (2008). R-Spondin family members regulate the Wnt pathway by a common mechanism. *Mol. Biol. Cell* 19, 2588–2596.
71. Hao, H.X., Xie, Y., Zhang, Y., Charlat, O., Oster, E., Avello, M., Lei, H., Mickanin, C., Liu, D., Ruffner, H., et al. (2012). ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. *Nature* 485, 195–200.
  72. Fuhrmann, S. (2008). Wnt signaling in eye organogenesis. *Organogenesis* 4, 60–67.
  73. Ward, L.D., and Kellis, M. (2012). HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res.* 40 (Database issue), D930–D934.
  74. Boyle, A.P., Hong, E.L., Hariharan, M., Cheng, Y., Schaub, M.A., Kasowski, M., Karczewski, K.J., Park, J., Hitz, B.C., Weng, S., et al. (2012). Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res.* 22, 1790–1797.
  75. Brockschmidt, A., Todt, U., Ryu, S., Hoischen, A., Landwehr, C., Birnbaum, S., Frenck, W., Radlwimmer, B., Lichter, P., Engels, H., et al. (2007). Severe mental retardation with breathing abnormalities (Pitt-Hopkins syndrome) is caused by haploinsufficiency of the neuronal bHLH transcription factor TCF4. *Hum. Mol. Genet.* 16, 1488–1494.
  76. Baratz, K.H., Tosakulwong, N., Ryu, E., Brown, W.L., Branham, K., Chen, W., Tran, K.D., Schmid-Kubista, K.E., Heckenlively, J.R., Swaroop, A., et al. (2010). E2-2 protein and Fuchs's corneal dystrophy. *N. Engl. J. Med.* 363, 1016–1024.
  77. Shiels, A., Bassnett, S., Varadaraj, K., Mathias, R., Al-Ghoul, K., Kuszak, J., Donoviel, D., Lilleberg, S., Friedrich, G., and Zambrowicz, B. (2001). Optical dysfunction of the crystalline lens in aquaporin-0-deficient mice. *Physiol. Genomics* 7, 179–186.
  78. Dirani, M., Shekar, S.N., and Baird, P.N. (2008). Evidence of shared genes in refraction and axial length: the Genes in Myopia (GEM) twin study. *Invest. Ophthalmol. Vis. Sci.* 49, 4336–4339.
  79. Guggenheim, J.A., Zhou, X., Evans, D.M., Timpson, N.J., McMahon, G., Kemp, J.P., St Pourcain, B., Northstone, K., Ring, S.M., Fan, Q., et al. (2013). Coordinated genetic scaling of the human eye: shared determination of axial eye length and corneal curvature. *Invest. Ophthalmol. Vis. Sci.* 54, 1715–1721.
  80. Guggenheim, J.A., McMahon, G., Kemp, J.P., Akhtar, S., St Pourcain, B., Northstone, K., Ring, S.M., Evans, D.M., Smith, G.D., Timpson, N.J., and Williams, C. (2013). A genome-wide association study for corneal curvature identifies the platelet-derived growth factor receptor alpha gene as a quantitative trait locus for eye size in white Europeans. *Mol. Vis.* 19, 243–253.
  81. Vithana, E.N., Khor, C.C., Qiao, C., Nongpiur, M.E., George, R., Chen, L.J., Do, T., Abu-Amero, K., Huang, C.K., Low, S., et al. (2012). Genome-wide association analyses identify three new susceptibility loci for primary angle closure glaucoma. *Nat. Genet.* 44, 1142–1146.

## Consortia

**Consortium for Refractive Error And Myopia (CREAM):** 1958 British Birth Cohort: Jugnoo S. Rahi, Pirro G. Hysi; Aichi cohort: Nagahisa Yoshimura, Kenji Yamashiro, Masahiro Miyake; ALIENOR: Cécile Delcourt,

Cecilia Maubaret; ALSPAC: Cathy Williams, Jeremy A. Guggenheim, Kate Northstone, Susan M. Ring, George Davey-Smith; ANZRAG: Jamie E. Craig, Kathryn P. Burdon, Rhys D. Fogarty; AREDS1a: Sudha K. Iyengar, Robert P. Igo, Jr., Emily Chew, Sarayut Janmahasathian; AREDS1b: Sudha K. Iyengar, Robert P. Igo, Jr., Emily Chew, Sarayut Janmahasathian; AREDS1c: Dwight Stambolian, Joan E. Bailey Wilson; BATS: Stuart MacGregor, Yi Lu; Beijing Eye Study: Jost B. Jonas, Liang Xu, Seang-Mei Saw; BMES: Paul N. Baird, Elena Rochtchina, Paul Mitchell, Jie Jin Wang; CIEMS: Jost B. Jonas, Vinay Nangia; CROATIA-Korčula: Caroline Hayward, Alan F. Wright, Veronique Vitart; CROATIA-Split: Ozren Polasek, Harry Campbell, Veronique Vitart; CROATIA-Vis: Igor Rudan, Zoran Vataavuk, Veronique Vitart; DCCT: Andrew D. Paterson, S. Mohsen Hosseini; Duke FECD Fuchs Dystrophy GWAS: Sudha K. Iyengar, Robert P. Igo Jr, Jeremy R. Fondran; Duke Myopia Study: Terri L. Young, Sheng Feng; Erasmus Rucphen Family Study: Virginie J.M. Verhoeven, Caroline C. Klaver, Cornelia M. van Duijn; Estonian Genome Project / EGCUT: Andres Metspalu, Toomas Haller, Evelin Mihailov; FITSA: Olavi Pärssinen, Juho Wedenoja; Framingham Eye Study: Joan E. Bailey Wilson, Robert Wojciechowski; GEMT: Paul N. Baird, Maria Schache; Gutenberg Health Study: Norbert Pfeiffer, René Höhn; Hong Kong cohort study: Chi Pui Pang, Peng Chen; KORA: Thomas Meitinger, Konrad Oexle, Aharon Wegner; Kyoto high myopia: Nagahisa Yoshimura, Kenji Yamashiro, Masahiro Miyake; LIKI: Olavi Pärssinen; Myopia Genomics Study (Hong Kong HTI): Shea Ping Yip, Daniel W. H. Ho; Ogliastra Genetic Park Study: Mario Pirastu, Federico Murgia, Laura Portas, Genevra Biino; ORCADES: James F. Wilson, Brian Fleck, Veronique Vitart; Penn Family Studies: Dwight Stambolian, Joan E. Bailey Wilson; RAINE: Alex W. Hewitt, Wei Ang; Rotterdam Study: Virginie J.M. Verhoeven, Caroline C. Klaver, Cornelia M. van Duijn; SCES: Seang-Mei Saw, Tien-Yin Wong, Yik-Ying Teo, Qiao Fan, Ching-Yu Cheng, Xin Zhou, M. Kamran Ikram; SCORM: Seang-Mei Saw, Yik-Ying Teo, Qiao Fan, Ching-Yu Cheng, Xin Zhou, M. Kamran Ikram; SIMES: Seang-Mei Saw, Tien-Yin Wong, Yik-Ying Teo, Qiao Fan, Ching-Yu Cheng, Xin Zhou, M. Kamran Ikram; SINDI: Seang-Mei Saw, Tien-Yin Wong, Yik-Ying Teo, Qiao Fan, Ching-Yu Cheng, Xin Zhou, M. Kamran Ikram; SP2: Seang-Mei Saw, E-Shyong Tai, Yik-Ying Teo, Qiao Fan, Ching-Yu Cheng, Xin Zhou, M. Kamran Ikram; STARS: Seang-Mei Saw, Yik-Ying Teo, Qiao Fan, Ching-Yu Cheng, Xin Zhou, M. Kamran Ikram; TEST: David A. Mackey, Stuart MacGregor; TwinsUK: Christopher J. Hammond, Pirro G. Hysi; Utah Timorese: Margaret M. Deangelis, Margaux Morrison; Wenzhou: Xiangtian Zhou, Wei Chen; WESDR: Andrew D. Paterson, S. Mohsen Hosseini; Yokohama Study: Nobuhisa Mizuki, Akira Meguro; Young Finns Study: Terho Lehtimäki, Kari-Matti Mäkelä, Olli Raitakari, Mika Kähönen

**The Fuchs' Genetics Multi-Center Study Group:** William Reinhart, Michael W. Belin, Robert L. Schultze, Todd Morason, Alan Sugar, Shahzad Mian, Hunson Kaz Soong, Kathryn Colby, Ula Jurkunas, Richard Yee, Mark

Vital, Eduardo Alfonso, Carol Karp, Yunhee Lee, Sonia Yoo, Kristin Hammersmith, Elisabeth Cohen, Peter Laibson, Christopher Rapuano, Brandon Ayres, Christopher Croasdale, James Caudill, Sanjay Patel, Keith Baratz, William Bourne, Leo Maguire, Joel Sugar, Elmer Tu, Ali Djalilian, Vinod Mootha, James McCulley, Wayne Bowman, H. Dwight Cavanaugh, Steven Verity, David Verdier, Ann Renucci, Matt Oliva, Walter Rotkis, David R. Hardten, Ahmad Fahmy, Marlene Brown, Sherman Reeves, Elizabeth A. Davis, Richard Lindstrom, Scott Hauswirth, Stephen Hamilton, W. Barry Lee, Francis Price, Marianne Price, Kathleen Kelly, Faye Peters, Michael Shaughnessy, Thomas Steinemann, B.J. Dupps, David M. Meisler, Mark Mifflin, Randal Olson, Anthony Aldave, Gary Holland, Bartly J. Mondino, George Rosenwasser, Mark Gorovoy, Steven P. Dunn, David G. Heidemann, Mark Terry, Neda Shamie, Steven I. Rosenfeld, Brandon Suedekum, David Hwang, Donald Stone, James Chodosh, Paul G. Galentine, David Bardenstein, Katrina Goddard, Hemin Chin, Mark Mannis, Rohit Varma, and Ingrid Borecki

**Wellcome Trust Case Control Consortium 2: Management Committee:** Peter Donnelly (Chair), Ines Barroso (Deputy Chair), Jenefer M. Blackwell, Elvira Bramon, Matthew A. Brown, Juan P. Casas, Aiden Corvin, Panos Deloukas, Audrey Duncanson, Janusz Jankowski, Hugh S. Markus, Christopher G Mathew, Colin N.A. Palmer, Robert Plomin, Anna Rautanen, Stephen J. Sawcer, Richard C. Trembath, Ananth C. Viswanathan, and Nicholas W. Wood; *Data and Analysis Group:* Chris C.A. Spencer, Gavin Band, Céline Bellenguez, Colin Freeman, Garrett Hellenenthal, Eleni Giannoulatou, Matti Pirinen, Richard Pearson, Amy Strange, Zhan Su, Damjan Vukcevic, and Peter Donnelly; *DNA, Genotyping, Data QC and Informatics Group:* Cordelia Langford, Sarah E. Hunt, Sarah Edkins, Rhian Gwilliam, Hannah Blackburn, Suzannah J. Bumpstead, Serge Dronov, Matthew Gillman, Emma Gray, Naomi Hammond, Alagurevathi Jayakumar, Owen T. McCann, Jennifer Liddle, Simon C. Potter, Radhi Ravindrarajah, Michelle Ricketts, Matthew Waller, Paul Weston, Sara Widaa, Pamela Whittaker, Ines Barroso, and Panos Deloukas; *Publications Committee:* Christopher G. Mathew (Chair), Jenefer M. Blackwell, Matthew A. Brown, Aiden Corvin, and Chris C.A. Spencer

**The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group:** *Study Chairmen:* S. Genuth, D.M. Nathan, B. Zinman (vice-chair), and O. Crofford (past); *Members:* J. Crandall, M. Reid, J. Brown-Friday, S. Engel, J. Sheindlin, H. Martinez (past), H. Shamoon (past), H. Engel (past), M. Phillips R. Gubitosi-Klug, L. Mayer, S. Pendegast, H. Zegarra, D. Miller, L. Singerman, S. Smith-Brewer, M. Novak, J. Quin (past), W. Dahms (deceased), Saul Genuth (past), M. Palmert (past), D. Brillon, M. E. Lackaye, S. Kiss, R. Chan, V. Reppucci (past), T. Lee (past), M. Heinemann (past), F. Whitehouse, D. Kruger, J.K. Jones, M. McLellan (past), J. D. Carey, E. Angus, A. Thomas, A. Galprin (past), R. Bergenstal,

M. Johnson, M. Spencer (past), K. Morgan, D. Etwiler, (deceased), D. Kendall (past), Lloyd Paul Aiello, E. Golden, A. Jacobson (past), R. Beaser, O. Ganda, O. Hamdy, H. Wolpert, G. Sharuk, P. Arrigg, D. Schlossman, J. Rosenzweig (past), L. Rand (past), D.M. Nathan, M. Larkin, M. Ong, J. Godine, E. Cagliero, P. Lou, K. Folino, S. Fritz (past), S. Crowell (past), K. Hansen (past), C. Gauthier-Kelly (past), J. Service, G. Ziegler, L. Luttrell, S. Caulder, M. Lopes-Virella (past), J. Colwell (past), J. Soule (past), J. Fernandes, K. Hermayer, S. Kwon, M. Brabham (past), A. Blevins, J. Parker, D. Lee (past), N. Patel, C. Pittman, P. Lindsey (past), M. Bracey (past), K. Lee, M. Nutaitis, A. Farr (past), S. Elsing (past), T. Thompson (past), J. Selby (past), T. Lyons (past), S. Yacoub-Wasef (past), M. Szpiech (past), D. Wood (past), R. Mayfield (past), M. Molitch, B. Schaefer, L. Jampol, A. Lyon, M. Gill, Z. Strugula, L. Kaminski, R. Mirza, E. Simjanoski, D. Ryan, O. Kolterman, G. Lorenzi, M. Goldbaum, W. Sivitz, M. Bayless, D. Counts, S. Johnsonbaugh, M. Hebdon (past), P. Salemi, R. Liss, T. Donner (past), J. Gordon (past), R. Hemady (past), A. Kowarski (past), D. Ostrowski (past) S. Steidl (past), B. Jones (past), W.H. Herman, C.L. Martin, R. Pop-Busui, A. Sarma, J. Albers, E. Feldman, K. Kim, S. Elner, G. Comer, T. Gardner, R. Hackel, R. Prusak, L. Goings, A. Smith, J. Gothrup, P. Titus, J. Lee, M., Brandle, L. Prosser, D.A. Greene (past), M.J. Stevens (past), A.K. Vine (past), J. Bantle, N. Wimmergren, A. Cochrane, T. Olsen (past), E. Steuer (past), P. Rath (past), B. Rogness (past), D. Hainsworth, D. Goldstein, S. Hitt, J. Giangiacomo, D.S. Schade, J.L., Canady, J.E. Chapin, L.H. Ketai C, S. Braunstein, P.A. Bourne, S. Schwartz (past), A. Brucker, B.J. Maschak-Carey (past), L. Baker (deceased), T. Orchard, N. Silvers, C. Ryan, T. Songer, B. Doft, S. Olson, R.L. Bergren, L. Lobes, P. Paczan Rath, D. Becker, D. Rubinstein, P.W. Conrad, S. Yalamanchi, A. Drash (past), A. Morrison, M.L. Bernal, J. Vaccaro-Kish (past), J. Malone, P.R. Pavan, N. Grove, M.N. Iyer, A.F. Burrows, E.A. Tanaka (past), R. Gstalder (past), S. Dagogo-Jack, C. Wigley, H. Ricks, A. Kitabchi, M.B. Murphy (past), S. Moser (past), D. Meyer (past), A. Iannacone (past), E. Chaum, S. Yoser (past), M. Bryer-Ash (past), S. Schussler (past), H. Lambeth (past), P. Raskin, S. Stowig, B. Zinman, A. Barnie, R. Devenyi, M. Mandelcorn, M. Brent, S. Rogers, A. Gordon, J. Palmer, S. Catton, J. Brunzell, H. Wessells, I.H. de Boer, J. Hokanson, J. Purnell, J. Ginsberg, J. Kinyoun, S. Deeb, M. Weiss, G. Meekins, J. Distad, L. Van Ottingham (past), J. Dupre, J. Harth, D. Nicolle, M. Driscoll, J. Mahon, C. Canny, M. May, J. Lipps, A. Agarwal, T. Adkins, L. Survant, R.L. Pate, G.E. Munn, R. Lorenz (past), S. Feman (past) N. White, L. Levandoski, I. Boniuk, G. Grand, M. Thomas, D.D. Joseph, K. Blinder, G. Shah, Boniuk (past), Burgess (past), J. Santiago (deceased), W. Tamborlane, P. Gatcomb, K. Stoessel, K. Taylor (past), J. Goldstein (past), S. Novella (past), H. Mojibian (past), D. Cornfeld (past), J. Lima, D. Bluemke, E. Turkbey, R. J. van der Geest, C. Liu, A. Malayeri, A. Jain, C. Miao (past), H. Chahal (past), R. Jarboe (past), and J. Maynard; *Clinical Coordinating Center:* R. Gubitosi-Klug,

J. Quin, P. Gaston, M. Palmert (past), R. Trail (past), and W. Dahms (deceased); *Data Coordinating Center*: J. Lachin, P. Cleary, J. Backlund, W. Sun, B. Braffett, K. Klumpp, K. Chan (past), L. Diminick, D. Rosenberg (past), B. Petty (past), A. Determan (past), D. Kenny (past), B. Rutledge (past), Naji Younes (past), L. Dews, and M. Hawkins; *National Institute of Diabetes and Digestive and Kidney Disease Program Office*: C. Cowie, J. Fradkin, C. Siebert (past), and R. Eastman (past); *Central Fundus Photograph Reading Center*: R. Danis, S. Gangaputra, S. Neill, M. Davis (past), L. Hubbard (past), H. Wabers, M. Burger, J. Dingle-dine, V. Gama, and R. Sussman; *Central Biochemistry Laboratory*: M. Steffes, J. Bucksa, M. Nowicki, and B. Chavers; *Central Carotid Ultrasound Unit*: D. O'Leary, J. Polak, A. Harrington, and L. Funk (past); *Central ECG Reading Unit*: R. Crow (past), B. Gloeb (past), S. Thomas (past), and C. O'Donnell (past); *Central ECG Reading Unit*: E. Soliman, Z.M. Zhang, R. Prineas (past), and C. Campbell; *Central Neuropsychological Coding Unit*: C. Ryan, D. Sandstrom, T. Williams, M. Geckle, E. Cupelli, F. Thoma, B. Burzuk, and T. Woodfill; *Central ANS Reading Unit*: P. Low, C. Sommer, and K. Nickander; *Computed Tomography Reading Center*: M. Budoff, R. Detrano (past), N. Wong, M. Fox, L. Kim (past), and R. Oudiz; *External Evaluation Committee*: G. Weir (Chairman), M. Espeland, T. Manolio, L. Rand, D. Singer, M. Stern, A.E. Boulton, C. Clark (past), and R. D'Agostino (past); *Molecular Risk Factors Program Project*: M. Lopes-Virella, W.T. Garvey (past), T.J. Lyons, A. Jenkins, G. Virella, A. Jaffa, Rickey Carter, D. Lackland (past), M. Brabham (past), D. McGee (past), D. Zheng (past), and R.K. Mayfield (past); *Genetic Studies Group*: A. Boright, S. Bull, L. Sun, S. Scherer (past), and B. Zinman (past); *Epigenetics*: R. Natarajan, F. Miao, L. Zhang, and Z. Chen; *Editor, EDIC Publications*: D.M. Nathan

# Association Between the Cholesteryl Ester Transfer Protein Gene and Polypoidal Choroidal Vasculopathy

Isao Nakata,<sup>1,2</sup> Kenji Yamashiro,<sup>1</sup> Takahisa Kawaguchi,<sup>2</sup> Norimoto Gotoh,<sup>1,2</sup> Hideo Nakanishi,<sup>1,2</sup> Yumiko Akagi-Kurashige,<sup>1,2</sup> Masahiro Miyake,<sup>1,2</sup> Akitaka Tsujikawa,<sup>1</sup> Akio Oishi,<sup>1</sup> Masaaki Saito,<sup>3</sup> Tomohiro Iida,<sup>4</sup> Ryo Yamada,<sup>2</sup> Fumihiko Matsuda,<sup>2</sup> and Nagahisa Yoshimura<sup>1</sup> for the Nagahama Study Group

<sup>1</sup>Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan

<sup>2</sup>Center for Genomic Medicine/Institut National de la Santé et de la Recherche Médicale Unité 852, Kyoto University Graduate School of Medicine, Kyoto, Japan

<sup>3</sup>Department of Ophthalmology, Fukushima Medical University School of Medicine, Fukushima, Japan

<sup>4</sup>Department of Ophthalmology, Tokyo Women's Medical University School of Medicine, Tokyo, Japan

Correspondence: Kenji Yamashiro, Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, 54 Kawahara, Shogoin, Sakyo, Kyoto 606-8507, Japan; yamashro@kuhp.kyoto-u.ac.jp.

See the Appendix for the members of the Nagahama Study Group.

Submitted: January 4, 2013

Accepted: August 8, 2013

Citation: Nakata I, Yamashiro K, Kawaguchi T, et al; for the Nagahama Study Group. Association between the cholesteryl ester transfer protein gene and polypoidal choroidal vasculopathy. *Invest Ophthalmol Vis Sci*. 2013;54:6068-6073. DOI:10.1167/iov.13-11605

**PURPOSE.** To determine whether genetic variants in the lipid-associated genes are related to the risk of developing polypoidal choroidal vasculopathy (PCV) in a Japanese population.

**METHODS.** Five hundred eighty-one patients with PCV and 793 controls were enrolled in the study. Association analysis of allele and genotype frequencies was performed for the following single-nucleotide polymorphisms (SNPs) that are associated with high-density lipoprotein cholesterol levels in blood: rs493258 at the hepatic lipase gene (*LIPC*), rs3764261 at the cholesteryl ester transfer protein gene (*CETP*), and rs12678919 at the lipoprotein lipase gene (*LPL*). A further model adjusting for age-related maculopathy susceptibility 2 (*ARMS2*) A69S, complement factor H (*CFH*) I62V, age, sex, and smoking status was used to confirm the independent association of these SNPs with other covariates.

**RESULTS.** *CETP* rs3764261 was significantly associated with the development of PCV; the frequency of the minor allele *A* was higher in the PCV cases (24.0%) than in the control subjects (18.5%) ( $P = 0.0025$ ; odds ratio [OR], 1.41; 95% confidence interval, 1.13-1.75). Furthermore, we found an independent association of *CETP* variants with age, sex, smoking status, and genetic background of *ARMS2* A69S, *CFH* I62V, *LIPC* rs493258, and *LPL* rs12678919 ( $P = 0.0013$ ; OR, 1.50). *LIPC* rs493258 and *LPL* rs12678919 did not show significant associations with the development of PCV ( $P > 0.05$ ).

**CONCLUSION.** *CETP* variants are associated a risk of developing PCV among the Japanese population.

**Keywords:** PCV, lipid, *CETP*, case-control study

Polypoidal choroidal vasculopathy (PCV) is characterized by aneurysmal dilations with interconnecting vessels that are best demonstrated by indocyanine green angiography.<sup>1-3</sup> Clinically, PCV is classified into a specific subtype of age-related macular degeneration (AMD), and the incidence of PCV in Asian populations has been reported to be higher than that in Caucasians.<sup>4-6</sup> Controversies exist about the pathogenesis of PCV; whether this condition represents inner choroidal vascular abnormalities or a particular variety of choroidal neovascularization (CNV) remains undetermined. However, because there are apparent differences in the demographic risk profile, clinical course, and visual prognosis, PCV is thought to be a distinct clinical entity.<sup>7</sup> For example, the response to treatment, particularly in photodynamic therapy for PCV, is completely different from that for typical AMD and CNV.<sup>8,9</sup>

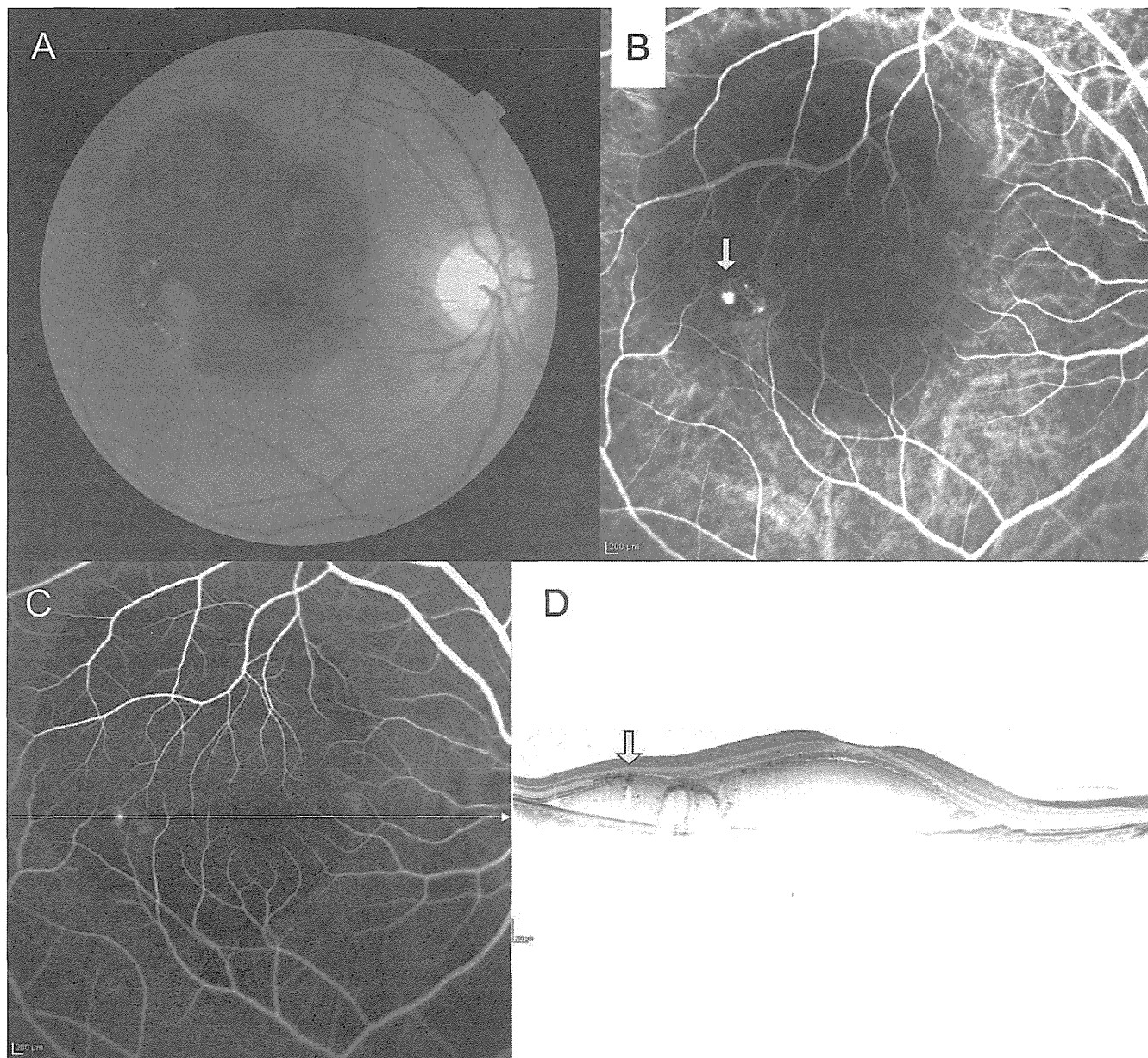
Cholesterol and lipids are reported to accumulate underneath the retinal pigment epithelium (RPE) with age. When sufficient debris, including lipids, accumulates and forms a mound between the RPE cell and its basement membrane, it

can be seen clinically as drusen. Because many population-based studies have shown the association between drusen and the progression of AMD, drusen is thought to be one of the determinants of both early and late AMD. In fact, an association between high-density lipoprotein (HDL) cholesterol level and the development of AMD has been reported in several studies.<sup>10-12</sup>

Previous studies<sup>13-15</sup> showed that the prevalence of drusen under RPE was reported to be lower in PCV than in AMD. Therefore, the absence of drusen was thought to be one of the criteria necessary to diagnose PCV.<sup>6,15,16</sup> However, the results of a clinical study<sup>16</sup> suggested that drusen is frequently seen in PCV eyes, and several studies<sup>6,17,18</sup> reported that drusen were observed in 20% to 27% of unaffected, fellow eyes in patients with unilateral PCV. Therefore, whether drusen has a functional role in the development of PCV remains controversial.

While previous investigations showed a lower prevalence of drusen among patients with PCV, lipid deposits that distribute from the inner retina to the outer retina are known to be the paramount features of PCV (Figure). Some recent investiga-





**FIGURE.** A 64-year-old woman with a typical case of PCV in the right eye. (A) Fundoscopic examination shows massive subretinal hemorrhage, lipid deposits, and reddish orange nodules. (B) Indocyanine green angiography demonstrates a small branching vascular network terminating in polypoidal lesions (*white arrow*). The speckle noise-reduced spectral-domain optical coherence tomography image of a horizontal section corresponding to the arrow indicated in fluorescein angiography (C) shows hyperreflective foci, indicating lipids (D), *arrowhead*), in the outer retina beside the polyp (D), *white arrow*).

tions, including a study<sup>19</sup> in a large cohort of Caucasians, showed significant associations between the lipid-associated genes and the development of AMD. These discoveries of genetic variants in the lipid pathway provided new insight into the pathogenesis of AMD. However, there are limited reports evaluating the association between the lipid-associated genes and the development of PCV. Although several genes are thought to be involved in regulating susceptibility to the development of PCV,<sup>20-23</sup> almost all are identical to those involved in the development of AMD, including the age-related maculopathy susceptibility 2 and high-temperature requirement factor A1 genes (*ARMS2/HTRA1*) locus<sup>24,25</sup> and the complement factor H gene (*CFH*).<sup>26-29</sup> Considering that several studies<sup>13-15</sup> reported a difference in the clinical

features of drusen between AMD and PCV, there could be different roles of the lipid-associated genes in these subtypes. Thus, we aimed in this study to determine whether genetic variants in the lipid-associated genes, including variants affecting HDL cholesterol levels, are related to the risk of developing PCV in a Japanese population.

## METHODS

All procedures in this study adhered to the tenets of the Declaration of Helsinki, and the ethics committee of each institution involved approved the study protocols. All patients were fully informed about the purpose and procedures of this study, with each patient providing written consent.



TABLE 1. Characteristics of the Study Population

Variable	Cases, n = 581	Controls, n = 793	P Value
Age, y			
Mean ± SD	72.59 ± 8.13	65.99 ± 4.33	<0.0001
Range	48–92	60–75	
Sex, n (%)			
M	420 (72.3)	326 (41.1)	<0.0001
F	161 (27.7)	467 (58.9)	
Smoking status, n (%)			
Never	200 (38.5)	509 (64.3)	<0.0001
Former	195 (37.6)	176 (22.3)	
Current	124 (23.9)	106 (13.4)	

Five hundred eighty-one patients with PCV were recruited from the departments of ophthalmology at Kyoto University Hospital, Fukushima Medical University Hospital, and Kobe City Medical Center General Hospital. The diagnosis of PCV was based on indocyanine green angiography, which showed a branching vascular network terminating in polypoidal swelling (Figure), and was confirmed by three retina specialists (KY, AT, AO); a fourth specialist (NY) was consulted when the diagnosis could not be agreed on by the initial three reviewers. Patients who had both typical CNV and polypoidal lesions were excluded from this study. The control group consisted of 793 unrelated individuals 60 years or older recruited in the Nagahama Prospective Genome Cohort for Comprehensive Human Bioscience (the Nagahama Study).<sup>30</sup> Fundoscopic photographs of both eyes confirmed the absence of any signs of AMD (large drusen or pigment change) using the Age-Related Eye Disease Study<sup>31</sup> severity scale, with grading by two independent ophthalmologists (IN, YAK), followed by grading by a senior reviewer (KY).

We targeted three single-nucleotide polymorphisms (SNPs) of three genes reported to be associated with HDL cholesterol levels in blood, including rs493258 at the hepatic lipase gene (*LIPC*), rs3764261 at the cholesteryl ester transfer protein gene (*CETP*), and rs12678919 at the lipoprotein lipase gene (*LPL*).<sup>32</sup> Genomic DNA was prepared from peripheral blood using a DNA extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan). All case samples were genotyped using the Taqman SNP assay with an ABI PRISM 7700 system (Applied Biosystems, Foster City, CA). Controls were genotyped using Human610-Quad BeadChips and HumanOmni2.5 BeadChips (Illumina, Inc., San Diego, CA). *ARMS2* A69S (rs10490924) and *CFH* I62V (rs800292) were also genotyped in the same manner. Fasting serum samples from the control subjects were analyzed for HDL cholesterol level, measured using a direct assay system with the selective inhibitory method on an automatic analyzer (LABOSPECT 008; Hitachi, Ltd., Tokyo, Japan). We did not have HDL cholesterol data for the case samples.

Information on smoking status was obtained via a self-reported questionnaire with three categories of never smoker, former smoker, and current smoker. The never smokers were

TABLE 3. Logistic Regression Analysis, Including Major Factors Associated With PCV

Variable	P Value*	OR (95% CI)
Age	<0.0001	1.18 (1.16–1.21)
F:M sex	<0.0001	3.16 (2.20–4.52)
<i>ARMS2</i> rs10490924 (G/T)	<0.0001	2.27 (1.86–2.77)
<i>CFH</i> rs800292 (A/G)	<0.0001	1.77 (1.43–2.19)
<i>LIPC</i> rs493258 (G/A)	0.689	1.05 (0.82–1.35)
<i>CETP</i> rs3764261 (C/A)	0.0013	1.50 (1.17–1.92)
<i>LPL</i> rs12678919 (A/G)	0.948	0.99 (0.72–1.35)
Smoking (never, former, or current)	0.0107	1.35 (1.07–1.69)

\* A logistic regression model was used for covariate adjustment.

those who had smoked fewer than 100 cigarettes in the past, current smokers were those who had smoked in the past year, and former smokers were those who had quit smoking more than 1 year earlier.

Deviations in genotype distributions from the Hardy-Weinberg equilibrium (HWE) of the controls were assessed with the HWE exact test. Statistical differences in the observed allelic distribution were identified using logistic regression analyses with age and sex adjustments, under the assumption of an additive genetic effect where the genotypes of each SNP are coded numerically as 0, 1, and 2 for the number of minor alleles carried. A linear regression analysis was performed to assess the association between HDL cholesterol level and genotype. R software (<http://www.r-project.org/> in the public domain) was used for statistical analyses.  $P < 0.05$  was considered statistically significant.

## RESULTS

Demographics of the study population are given in Table 1. Genotype and allele frequencies of the three SNPs were analyzed in 581 patients with PCV and compared with those of 793 age-matched individuals without any signs of AMD or PCV. The genotyping of all evaluated SNPs had a success rate exceeding 99.4%.

Table 2 gives details of genotype and allele frequencies and summary statistics. The distributions of the genotypes for all evaluated SNPs were in HWE ( $P > 0.05$ ). We found that *CETP* rs3764261 was significantly associated with the development of PCV; the frequency of the minor allele A in the patients with PCV (24.0%) was higher than that in the controls (18.5%) ( $P = 0.0025$ ; odds ratio [OR], 1.41; 95% confidence interval [CI], 1.13–1.75). This significant association remained even after a correction for multiple testing ( $P = 0.0075$ ). *LIPC* rs493258 and *LPL* rs12678919 did not show significant associations with the development of PCV ( $P > 0.05$ ).

Next, we conducted a logistic regression analysis that included the effects of the most robust Japanese variants associated with AMD and PCV, *ARMS2* A69S (rs10490924) and *CFH* I62V (rs800292), as well as age, sex, smoking status, *LIPC*

TABLE 2. Distribution of Genotypes and Results of the Association Tests

Gene	SNP	Allele		Cases, n = 581				Controls, n = 793				Association Results*	
		1	2	11	12	22	MAF	11	12	22	MAF	P Value	OR (95% CI)
<i>LIPC</i>	rs493258	G	A	32	185	354	0.22	37	259	497	0.21	0.706	1.04 (0.84–1.30)
<i>CETP</i>	rs3764261	C	A	332	210	33	0.24	528	237	28	0.19	0.0025	1.41 (1.13–1.75)
<i>LPL</i>	rs12678919	A	G	439	135	3	0.12	602	179	12	0.13	0.883	1.02 (0.77–1.35)

MAF, minor allele frequency.

\* Adjusted for age and sex.

rs493258, and *LPL* rs12678919 in the regression model. Table 3 gives the results of the logistic regression analysis. *CETP* rs3764261 remained significant for the development of PCV even after including the effects of these covariates ( $P = 0.0013$ ; OR, 1.50; 95% CI, 1.17–1.92).

Finally, we investigated the role of *CETP* rs3764261 in blood HDL cholesterol level using fasting serum samples from 793 control subjects. The mean  $\pm$  SD HDL cholesterol level of the control samples was  $61.3 \pm 16.1$  mg/dL. In this analysis, we found that the *A* allele of rs3764261 was associated with the following increases in HDL cholesterol: 59.3 mg/dL for the *CC* genotype, 64.8 mg/dL for the *CA* genotype, and 67.2 mg/dL for the *AA* genotype ( $P < 0.0001$ ).

## DISCUSSION

Plasma CETP was first described as a high-molecular-weight protein stimulating the transfer of cholesteryl ester between lipoproteins in plasma of hypercholesterolemic rabbits.<sup>33</sup> Other studies demonstrated various roles of CETP in the lipid pathway: CETP facilitates the transfer of triglycerides and phospholipids<sup>34</sup>; it is an important component of reverse cholesterol transport, which is chiefly characterized by the transport of cholesterol from peripheral tissues to the liver; and it regulates the concentration of HDL cholesterol.<sup>35,36</sup>

After the discovery of the association between HDL cholesterol level and cardiovascular diseases,<sup>37</sup> studies<sup>38,39</sup> evaluated the functional role of the lipid-associated genes that can affect the HDL cholesterol level. Among those genes, the *A* allele of *CETP* rs3764261 was associated with an increase in HDL cholesterol by 5.6 mg/dL among the Japanese population.<sup>40</sup> Herein, we confirmed the role of rs3764261 in increased HDL cholesterol levels among 793 healthy Japanese individuals.

In the present study comparing the allelic distributions of *CETP* variants in a sample of 581 patients with PCV and 793 control subjects, the *A* allele of *CETP* rs3764261 was significantly associated with a risk of developing PCV (OR, 1.41; 95% CI, 1.13–1.75), which indicates a higher level of HDL cholesterol in patients with PCV. In addition, the association of *CETP* variants remained significant even when we adjusted for the effects of other established risk factors for developing AMD and PCV (age, sex, smoking status, and genetic background of *ARMS2* A69S, *CFH* I62V, *LIPC* rs493258, and *LPL* rs12678919). Although the effect of *CETP* variants (OR, 1.50) was not as large as the effects of the major genes associated with AMD and PCV (ORs, 2.27 for *ARMS2* and 1.77 for *CFH*) in this regression analysis, we were able to confirm that *CETP* variants have a significant role in the development of PCV. Our findings for *CETP* rs3764261 were similar to the associations already documented in AMD among Caucasians,<sup>41,42</sup> which suggests that a higher HDL cholesterol level may be a risk factor in both PCV and Caucasian AMD. The hypothesis that a higher level of HDL cholesterol is associated with the development of PCV might appear contradictory to the fact that a lower level of HDL cholesterol is associated with an increased risk of cardiovascular disease. However, despite the well-known antiatherogenic properties of HDL cholesterol, some studies<sup>10,11,43</sup> found elevated levels of HDL cholesterol in Caucasian patients with AMD.

Recently, Zhang et al.<sup>44</sup> reported an investigation of lipid-associated SNPs for PCV and neovascular AMD in a Chinese population. In that article, they showed a significant association of *CETP* with PCV, while no association was found with neovascular AMD. Thus, they concluded that the HDL cholesterol pathway in the pathogenesis of PCV likely differs

from that of neovascular AMD. However, the sample size evaluated in their article was small (204 controls, 250 patients with PCV, and 157 patients with neovascular AMD), which suggests that the negative result of the association between *CETP* and neovascular AMD could have been due to insufficient power to detect the association. To confirm whether the observed association of *CETP* with PCV exists for neovascular AMD as well, we performed an additional analysis using another Japanese cohort of neovascular AMD cases ( $n = 452$ ). In this evaluation, we found a significant association between *CETP* and neovascular AMD ( $P = 0.0246$ ; OR, 1.35).

Adenosine triphosphate-binding cassette, subfamily A member 1 (*ABCA1*) is also known to be associated with the lipid pathway. Because *ABCA1* has been reported to be another susceptible gene for the development of AMD in Caucasians,<sup>19</sup> we also evaluated whether *ABCA1* rs1883025 has a significant role in the development of PCV but found no significant association with PCV ( $P > 0.05$ ). In previous genome-wide association analyses for HDL cholesterol, the strongest and most consistently associated SNPs have been reported in the *CETP* locus.<sup>45,46</sup> Study<sup>32</sup> findings also suggest that *LIPC* rs493258 and *LPL* rs12678919 are associated with HDL cholesterol level in Caucasians, so the lack of association in the present study could be due to insufficient statistical power or racial/ethnic differences. Further study that includes a larger number of participants is needed to clarify the association between genetic variants of HDL cholesterol-associated genes and the development of PCV.

In the present study, there was a large sex difference between the PCV cases and the general population controls. It remains unknown why there is such a high prevalence of PCV among men. In a previous meta-analysis by Kawasaki et al.,<sup>47</sup> the prevalence of late AMD among Asian women was reported to be much lower than that among Asian men. In contrast, a male predominance was reported in PCV.<sup>4</sup> Considering the high prevalence of PCV among Asian populations, these results suggest that men are more likely to develop PCV. In our study, genetic factors had an enormous influence on whether participants developed PCV (Table 3). However, sex had the largest effect among all covariates on the development of PCV (OR, 3.16). A previous genetic study<sup>23</sup> among Japanese may provide insight into this question because the results suggested that differences in sex would affect phenotypic differences in AMD. Another limitation of the present study was the age difference between cases and controls. Although we enrolled only controls who were 60 years or older, the average age of the control cohort was still younger than that of the case cohort, which means that some of the young controls may develop PCV in the future. To exclude a potential confounder of genetic background with age, a logistic regression analysis adjusting for age and sex was performed in the present study. However, given that the prevalence of late AMD among the Japanese population is reported to be 0.5%,<sup>48</sup> the magnitude of statistical bias of the association analysis is negligible. In addition, considering that case-control association analyses among such subjects are less likely to be statistically significant, our positive results should be acceptable.

Overall, this study provides the first evidence to date that *CETP* variants have a significant role in the risk of developing PCV among the Japanese population. Our study also indicates the same role of HDL cholesterol in both PCV and Caucasian AMD, although the role of fatty acids in Japanese AMD is reported to be different from that in Caucasian AMD.<sup>49</sup> Further studies are needed to increase the understanding of the genetic backgrounds of PCV, as well as the molecular pathogenesis, particularly the role of lipids.

### Acknowledgments

The authors thank the participants of the Nagahama Study, the Nagahama City Office, and the nonprofit organization Zero-ji Club for Health Promotion.

Supported by grants-in-aid from the following organizations: the Ministry of Education, Culture, Sports, Science, and Technology of Japan (2006–2012); Grants 19390442, 22791706, and 22791653 from the Japan Society for the Promotion of Science; the Japanese National Society for the Prevention of Blindness; and Takeda Science Foundation (2008–2012).

Disclosure: I. Nakata, None; K. Yamashiro, None; T. Kawaguchi, None; N. Gotoh, None; H. Nakanishi, None; Y. Akagi-Kurashige, None; M. Miyake, None; A. Tsujikawa, None; A. Oishi, None; M. Saito, None; T. Iida, None; R. Yamada, None; F. Matsuda, None; N. Yoshimura, None

### References

1. Yannuzzi LA, Sorenson J, Spaide RF, Lipson B. Idiopathic polypoidal choroidal vasculopathy (IPCVC). *Retina*. 1990;10:1–8.
2. Spaide RF, Yannuzzi LA, Slakter JS, Sorenson J, Orlach DA. Indocyanine green videoangiography of idiopathic polypoidal choroidal vasculopathy. *Retina*. 1995;15:100–110.
3. Ross RD, Gitter KA, Cohen G, Schomaker KS. Idiopathic polypoidal choroidal vasculopathy associated with retinal arterial macroaneurysm and hypertensive retinopathy. *Retina*. 1996;16:105–111.
4. Ciardella AP, Donsoff IM, Huang SJ, Costa DL, Yannuzzi LA. Polypoidal choroidal vasculopathy. *Surv Ophthalmol*. 2004;49:25–37.
5. Liu Y, Wen F, Huang S, et al. Subtype lesions of neovascular age-related macular degeneration in Chinese patients. *Graefes Arch Clin Exp Ophthalmol*. 2007;245:1441–1445.
6. Maruko I, Iida T, Saito M, Nagayama D, Saito K. Clinical characteristics of exudative age-related macular degeneration in Japanese patients. *Am J Ophthalmol*. 2007;144:15–22.
7. Yannuzzi LA, Wong DW, Sforzolini BS, et al. Polypoidal choroidal vasculopathy and neovascularized age-related macular degeneration. *Arch Ophthalmol*. 1999;117:1503–1510.
8. Gomi F, Ohji M, Sayanagi K, et al. One-year outcomes of photodynamic therapy in age-related macular degeneration and polypoidal choroidal vasculopathy in Japanese patients. *Ophthalmology*. 2008;115:141–146.
9. Tsuchiya D, Yamamoto T, Kawasaki R, Yamashita H. Two-year visual outcomes after photodynamic therapy in age-related macular degeneration patients with or without polypoidal choroidal vasculopathy lesions. *Retina*. 2009;29:960–965.
10. Klein R, Klein BE, Franke T. The relationship of cardiovascular disease and its risk factors to age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology*. 1993;100:406–414.
11. Hyman L, Schachat AP, He Q, Leske MC; Age-Related Macular Degeneration Risk Factors Study Group. Hypertension, cardiovascular disease, and age-related macular degeneration. *Arch Ophthalmol*. 2000;118:351–358.
12. Reynolds R, Rosner B, Seddon JM. Serum lipid biomarkers and hepatic lipase gene associations with age-related macular degeneration. *Ophthalmology*. 2010;117:1989–1995.
13. Ciardella AP, Donsoff IM, Yannuzzi LA. Polypoidal choroidal vasculopathy. *Ophthalmol Clin North Am*. 2002;15:537–554.
14. Hiram Y, Mandai M, Takahashi M, Teramukai S, Tada H, Yoshimura N. Association of clinical characteristics with disease subtypes, initial visual acuity, and visual prognosis in neovascular age-related macular degeneration. *Jpn J Ophthalmol*. 2009;53:396–407.
15. Uyama M, Matsubara T, Fukushima I, et al. Idiopathic polypoidal choroidal vasculopathy in Japanese patients. *Arch Ophthalmol*. 1999;117:1035–1042.
16. Iwama D, Tsujikawa A, Sasahara M, Hiram Y, Tamura H, Yoshimura N. Polypoidal choroidal vasculopathy with drusen. *Jpn J Ophthalmol*. 2008;52:116–121.
17. Ladas ID, Rouvas AA, Moschos MM, Synodinos EE, Karagiannis DA, Koutsandrea CN. Polypoidal choroidal vasculopathy and exudative age-related macular degeneration in Greek population. *Eye (Lond)*. 2004;18:455–459.
18. Scassellati-Sforzolini B, Mariotti C, Bryan R, Yannuzzi LA, Giuliani M, Giovannini A. Polypoidal choroidal vasculopathy in Italy. *Retina*. 2001;21:121–125.
19. Neale BM, Fagerness J, Reynolds R, et al. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). *Proc Natl Acad Sci U S A*. 2010;107:7395–7400.
20. Gotoh N, Nakanishi H, Hayashi H, et al. *ARMS2* (*LOC387715*) variations in Japanese patients with exudative age-related macular degeneration and polypoidal choroidal vasculopathy. *Am J Ophthalmol*. 2009;147:1037–1041, 1041.e1–e2.
21. Kondo N, Honda S, Kuno S, Negi A. Coding variant I62V in the complement factor H gene is strongly associated with polypoidal choroidal vasculopathy. *Ophthalmology*. 2009;116:304–310.
22. Hayashi H, Yamashiro K, Gotoh N, et al. *CFH* and *ARMS2* variations in age-related macular degeneration, polypoidal choroidal vasculopathy, and retinal angiomatous proliferation. *Invest Ophthalmol Vis Sci*. 2010;51:5914–5919.
23. Nakata I, Yamashiro K, Yamada R, et al. Significance of *C2/CFB* variants in age-related macular degeneration and polypoidal choroidal vasculopathy in a Japanese population. *Invest Ophthalmol Vis Sci*. 2012;53:794–798.
24. Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. Susceptibility genes for age-related maculopathy on chromosome 10q26. *Am J Hum Genet*. 2005;77:389–407.
25. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical *LOC387715* is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum Mol Genet*. 2005;14:3227–3236.
26. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science*. 2005;308:385–389.
27. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science*. 2005;308:419–421.
28. Edwards AO, Ritter R III, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science*. 2005;308:421–424.
29. Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (*HF1/CFH*) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2005;102:7227–7232.
30. Yoshimura K, Nakayama T, Sekine A, et al; Nagahama Cohort Research Group. B-type natriuretic peptide as an independent correlate of nocturnal voiding in Japanese women. *Neurorol Urodyn*. 2012;31:1266–1271.
31. Ferris FL, Davis MD, Clemons TE, et al; Age-Related Eye Disease Study (AREDS) Research Group. A simplified severity scale for age-related macular degeneration: AREDS report No. 18. *Arch Ophthalmol*. 2005;123:1570–1574.
32. Sarzynski MA, Jacobson P, Rankinen T, et al. Association of GWAS-based candidate genes with HDL-cholesterol levels before and after bariatric surgery in the Swedish obese subjects study. *J Clin Endocrinol Metab*. 2011;96:E953–E957.

33. Zilversmit DB, Hughes LB, Balmer J. Stimulation of cholesterol ester exchange by lipoprotein-free rabbit plasma. *Biochim Biophys Acta*. 1975;409:393-398.
34. Swenson TL, Brocia RW, Tall AR. Plasma cholesteryl ester transfer protein has binding sites for neutral lipids and phospholipids. *J Biol Chem*. 1988;263:5150-5157.
35. Chajek T, Fielding CJ. Isolation and characterization of a human serum cholesteryl ester transfer protein. *Proc Natl Acad Sci U S A*. 1978;75:3445-3449.
36. Glomset JA. The plasma lecithins: cholesterol acyltransferase reaction. *J Lipid Res*. 1968;9:155-167.
37. Pekkanen J, Linn S, Heiss G, et al. Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. *N Engl J Med*. 1990;322:1700-1707.
38. Wallace C, Newhouse SJ, Braund P, et al. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum urate and dyslipidemia. *Am J Hum Genet*. 2008;82:139-149.
39. Willer CJ, Sanna S, Jackson AU, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat Genet*. 2008;40:161-169.
40. Hiura Y, Shen CS, Kokubo Y, et al. Identification of genetic markers associated with high-density lipoprotein-cholesterol by genome-wide screening in a Japanese population: the Suita Study. *Circ J*. 2009;73:1119-1126.
41. Yu Y, Bhangale TR, Fagerness J, et al. Common variants near *FRK/COL10A1* and *VEGFA* are associated with advanced age-related macular degeneration. *Hum Mol Genet*. 2011;20:3699-3709.
42. Chen W, Stambolian D, Edwards AO, et al. Genetic variants near *TIMP3* and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2010;107:7401-7406.
43. van Leeuwen R, Klaver CC, Vingerling JR, et al. Cholesterol and age-related macular degeneration: is there a link? *Am J Ophthalmol*. 2004;137:750-752.
44. Zhang X, Li M, Wen F, et al. Different impact of high-density lipoprotein-related genetic variants on polypoidal choroidal vasculopathy and neovascular age-related macular degeneration in a Chinese Han population. *Exp Eye Res*. 2013;108:16-22.
45. Chasman DI, Pare G, Zee RY, et al. Genetic loci associated with plasma concentration of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B among 6382 white women in genome-wide analysis with replication. *Circ Cardiovasc Genet*. 2008;1:21-30.
46. Kooner JS, Chambers JC, Aguilar-Salinas CA, et al. Genome-wide scan identifies variation in *MLXIPL* associated with plasma triglycerides. *Nat Genet*. 2008;40:149-151.
47. Kawasaki R, Yasuda M, Song SJ, et al. The prevalence of age-related macular degeneration in Asians: a systematic review and meta-analysis. *Ophthalmology*. 2010;117:921-927.
48. Kawasaki R, Wang JJ, Ji GJ, et al. Prevalence and risk factors for age-related macular degeneration in an adult Japanese population: the Funagata Study. *Ophthalmology*. 2008;115:1376-1381, 1381.e1-e2.
49. Kabasawa S, Mori K, Horie-Inoue K, et al. Associations of cigarette smoking but not serum fatty acids with age-related macular degeneration in a Japanese population. *Ophthalmology*. 2011;118:1082-1088.

#### APPENDIX

The following investigators were core members of the Nagahama Study Group: Takeo Nakayama (Department of Health Informatics, Kyoto University School of Public Health, Kyoto, Japan), Akihiro Sekine (Department of Genome Informatics, Kyoto University School of Public Health, Kyoto, Japan), Shinji Kosugi (Department of Medical Ethics, Kyoto University School of Public Health, Kyoto, Japan), and Yasuharu Tabara (Center for Genomic Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan).

# Quantitative Variation in Plasma Angiotensin-I Converting Enzyme Activity Shows Allelic Heterogeneity in the ABO Blood Group Locus

Chikashi Terao<sup>1</sup>, Nervana Bayoumi<sup>2</sup>, Colin A. McKenzie<sup>3</sup>, Diana Zelenika<sup>4,5</sup>, Shigeo Muro<sup>6</sup>, Michiaki Mishima<sup>6</sup>, The Nagahama Cohort Research Group<sup>7</sup>, John M. C. Connell<sup>8</sup>, Mark A. Vickers<sup>9</sup>, G. Mark Lathrop<sup>4,5,10</sup>, Martin Farrall<sup>11,12</sup>, Fumihiko Matsuda<sup>1</sup> and Bernard D. Keavney<sup>13,14\*</sup>

<sup>1</sup>The Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan

<sup>2</sup>Physiology Department, College of Medicine, King Saud University, Riyadh, Saudi Arabia

<sup>3</sup>Tropical Metabolism Research Unit, University of the West Indies, Mona, Jamaica

<sup>4</sup>Commisariat à l'énergie Atomique (CEA), Institut Genomique, Centre National de Genotypage, Evry, France

<sup>5</sup>Fondation Jean Dausset, Centre d'Etude du Polymorphisme Humain, Paris, France

<sup>6</sup>Department of Respiratory Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan

<sup>7</sup>The following investigators belonging to Kyoto University Graduate School of Medicine (Kyoto, Japan) were core members of the Nagahama Study Group: Takeo Nakayama (Department of Health Informatics); Shinji Kosugi (Department of Medical Ethics); Akihiro Sekine, Takahisa Kawaguchi, Ryo Yamada and Yasuharu Tabara (The Center for Genomic Medicine)

<sup>8</sup>College of Medicine, Dentistry and Nursing, University of Dundee, UK

<sup>9</sup>School of Medicine, University of Aberdeen, UK

<sup>10</sup>McGill University and Genome Quebec Innovation Center, Montreal, Canada

<sup>11</sup>Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

<sup>12</sup>Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK

<sup>13</sup>Institute of Genetic Medicine, Newcastle University, UK

<sup>14</sup>Institute of Cardiovascular Sciences, Manchester University, UK

## Summary

Angiotensin-I converting enzyme (ACE) occupies a pivotal role in cardiovascular homeostasis. Major loci for plasma ACE have been identified at *ACE* on Chromosome 17 and at *ABO* on Chromosome 9. We sought to characterise the genetic architecture of plasma ACE at finer resolution in two populations. We carried out a GWAS in 1810 individuals of Japanese ethnicity; this identified signals at *ACE* and *ABO* that together accounted for nearly half of the population variability of the trait. We conducted measured haplotype analysis at the *ABO* locus in 1425 members of 248 British families using haplotypes of three SNPs, which together tagged the alleles responsible for the principal blood group antigens A1, A2, B and O. Type O alleles were associated with intermediate plasma ACE activity compared to Type A1 alleles (in whom plasma ACE activity was ~36% lower) and Type B alleles (in whom plasma ACE activity was ~36% higher). We demonstrated heterogeneity among A alleles: A2 alleles were associated with plasma ACE activity that was very similar to the O alleles. Variation at *ACE* accounted for 35% of the trait variance, and variation at *ABO* accounted for 15%. A further 10% could be ascribed to polygenic effects.

Keywords: ABO blood group, angiotensin-I converting enzyme, genome wide association study, QTL

## Introduction

The renin-angiotensin system plays a critical role in cardiovascular homeostasis, regulating blood pressure, arterial tone and renal salt excretion. The angiotensin I-converting enzyme (ACE) converts circulating angiotensin-I, which is

\*Corresponding author: Bernard D. Keavney, Institute of Genetic Medicine, Newcastle University, Central Parkway, NE1 3BZ, UK. Tel: +44 191 241 8615; Fax: +44 191 241 8666; E-mail: bernard.keavney@newcastle.ac.uk

biologically inactive, to the active angiotensin-II and degrades the vasodilator bradykinin. ACE occupies a pivotal position in the renin-angiotensin system: drugs which inhibit ACE, or which block the cellular receptor for the angiotensin-II generated by the action of ACE, are among the most widely prescribed agents in patients with coronary artery disease, hypertension and chronic renal disease (Yusuf et al., 2000). Plasma ACE activity is related to tissue ACE activity, and is strongly influenced by genetic factors, the largest such influence being due to polymorphic variation in the *ACE* (also known as *DCP1*) gene, which encodes ACE (Rigat et al., 1990; Keavney et al., 1998). Previous segregation and linkage analysis suggested the existence of a second major quantitative trait locus (QTL) influencing plasma ACE activity (McKenzie et al., 1995). A genome wide association study (GWAS) in a population of hypertensive patients of Han Chinese ancestry subsequently found this to be located at the *ABO* gene which encodes glycosyltransferases A and B (Chung et al., 2010). Global variation in the distribution of the alleles responsible for ABO blood groups is well described; we therefore sought to confirm the identity of the second principal locus influencing ACE activity and to estimate the proportions of phenotypic variance attributable to major gene effects in two additional populations from Japan and the United Kingdom.

## Methods

### Study Populations and ACE Phenotyping

The discovery cohort included 1830 volunteers recruited as a part of the Nagahama Prospective Genome Cohort for Comprehensive Human Bioscience (the Nagahama Study), a community-based prospective multiomics cohort study. The study has been described in detail elsewhere (Yoshimura et al., 2012); demographics of the cohort are summarised in Table S1. In brief, a total of 9809 volunteers from Nagahama City, Shiga Prefecture, Japan, were recruited for this study from 2008 to 2010. All participants completed a detailed health questionnaire. DNA, serum and plasma samples from all participants were obtained and stored for further analysis. Samples were kept on ice immediately after they were obtained from the participants and were promptly processed. Plasma was stored at  $-80^{\circ}\text{C}$ . ACE activity was quantified by the method reported by Kasahara & Ashihara (1981). Patients receiving ACE inhibitor therapy were excluded from the analyses.

The replication cohort comprised 248 British families of Northern and Western European ancestry who participated in a quantitative genetic study of cardiovascular risk factors (Palomino-Doza et al., 2008). The population collection strategy has been previously described in detail (Gaukrodger et al., 2005). In brief, families were ascertained via a hyperten-

sive proband between 1993 and 1996, and any sibship in the family (in the generation of the proband or his/her offspring) greater than three members quantitatively assessable for blood pressure was collected. Families were extended where additional hypertensives were encountered during collection; a total of 1425 individuals participated. Families underwent detailed cardiovascular phenotyping including a questionnaire, electrocardiographic and echocardiographic measurement and measurement of 24-hour ambulatory blood pressure using an automated monitor (Keavney et al., 2000; Mayosi et al., 2008). Demographics of the cohort are presented in Table S2. Blood was drawn into multiple anticoagulants, immediately put on ice and transported rapidly to a central facility for processing. ACE activity was assessed by HPLC using a synthetic substrate, as previously described (Chiknas, 1979).

### Genotyping

The 1830 volunteers in the Japanese cohort were genotyped using the Infinium Human 610-Quad Bead Chip carrying 592,044 SNP markers on a Bead Station 500G Genotyping System (Illumina, Inc., San Diego, CA, USA). There were no subjects showing call rates lower than 0.99. Kinship analysis was performed using PLINK. Of the 20 pairs of samples showing high degrees of kinship (PI-HAT > 0.4), the sample with the lower call rate in each pair was removed. 165,591 SNPs were removed either due to call rate lower than 0.95, minor allele frequency of less than 0.05, or distorted Hardy-Weinberg equilibrium ( $P < 10^{-7}$ ). Finally, the results of 426,453 SNP markers in 1810 subjects were used for the analysis.

Three SNPs at the ABO locus (rs505922, rs8176746 and rs8176750) were typed in the entire British family cohort using matrix-assisted laser desorption/ionisation—time of flight mass spectrometry (MALDI-TOF) on a Sequenom instrument (Sequenom, San Diego, CA, USA). ABO blood group in the British families was studied in a subset of 734 individuals by multiplex polymerase chain reaction (PCR). Two pairs of primers were used to amplify exons 6 and 7 of the *ABO* gene; the amplified fragments were digested with restriction endonucleases *HpaII* and *KpnI* and separated by gel electrophoresis. This enabled us to call genotypes at the SNPs rs8176719, rs1053878, rs8176743 and rs8176472. As previously reported, genotypes at these SNPs, considered together, identify the A1, A2, B, O1 and O2 blood group alleles (Seltsam et al., 2003).

### Statistical Methods

A quantitative linear regression analysis was first performed in the Japanese cohort to find the polymorphisms associated with ACE activity. SNP genotype imputation for SNPs within and



flanking the *ACE* (20 SNPs) and *ABO* (43 SNPs) loci was performed in the Japanese samples using the MaCH (version 1.0.10) computer program with 500 Markov sampler rounds and 200 haplotype states (Li et al., 2010). A forward-selection stepwise regression analysis was performed to identify a parsimonious subset of associated SNPs from the *ACE* and *ABO* loci in the Japanese population. This analysis was based on imputed SNP dosages using linear regression models based on marginal sums of squares and the *stepwise* procedure in Stata™ v10.1 (Stata Corp, College Station, TX, USA) using a  $P < 0.01$  criterion for adding SNPs to the model. Variance component proportions ( $R^2$ ) were calculated from a supplementary analysis of variance based on sequential sums of squares.

Haplotyping of *ABO* in the British samples was performed using PHASE (version 2.1.1) specifying a parent-independent multiallelic model for both SNP and blood group variation. (Stephens et al., 2001; Stephens & Scheet, 2005) Pedigree analysis was performed using the Pedigree Analysis Package (PAP version 5.0) to fit maximum likelihood models including polygenic variance components (Hasstedt, 1993) to extended families; missing data is efficiently incorporated into this analysis (Elston & Stewart, 1971). For the measured haplotype analysis, the PAP quantitative major gene subroutine *qmlprmv* was modified to parametrise an additive (codominant) genetic model. Likelihoods were maximised with simultaneous estimation of haplotype frequencies assuming Hardy–Weinberg equilibrium, haplotype-specific effects on ACE activity, covariate effects, polygenic effects and residual individual-specific random (i.e. environmental) effects and estimates of standard errors were calculated with the bundled quasi-Newton nonlinear optimisation function GEMINI (Lalouel, 1979). Variance component proportions were calculated by hand using a standard additive genetic variance formula.

## Results

### GWAS for ACE Activity

The SNPs with the strongest association with ACE activity genome-wide are presented in Table S3. Two loci, *ACE* on Chromosome 17, and *ABO* on Chromosome 9, showed genome-wide significant association ( $P < 5 \times 10^{-8}$ ) with plasma ACE activity (Fig. S1). SNPs mapping to the *ACE* (20 SNPs) and *ABO* (43 SNPs) loci and their immediate upstream and downstream flanking regions (50 kb, respectively) were selected for fine-mapping analysis and any missing genotype data was imputed. Stepwise linear regression then identified three SNPs with independent significant effects ( $10^{-213} < P < 10^{-40}$ ): rs4362 at *ACE*; and rs495828 and rs8176746 at

**Table 1** Forward selection stepwise regression analysis of plasma ACE activity and GWAS SNPs in the Japanese cohort.  $R^2$  shows the proportion of variance explained by each variable.

Locus	Variable	Beta	SE	F-statistic	P-value	$R^2$
	Age	0.0363	0.0040	84.32	1.13E-19	0.0229
ACE	rs4362	−3.0450	0.0849	1286.30	9.60E-213	0.3494
ABO	rs495828	1.4762	0.0982	225.75	3.81E-48	0.0613
ABO	rs8176746	1.5698	0.1154	184.97	3.66E-40	0.0502

**Table 2** *ABO* haplotype analysis using PHASE 2.1.1 in British families. Common haplotypes assessed in the measured haplotype analysis are shown in bold.

rs505922	rs8176746	rs8176750	Blood group allele	Conditional probability	Frequency
C	<b>G</b>	<b>G</b>	<b>A1</b>	<b>0.9235</b>	<b>0.1719</b>
C	G	G	A2	0.0049	0.0009
C	G	G	B	0.0139	0.0026
C	G	G	O1	0.0271	0.0050
C	G	G	O2	0.0307	0.0057
C	G	del	A1	0.1541	0.0096
C	<b>G</b>	<b>del</b>	<b>A2</b>	<b>0.8333</b>	<b>0.0517</b>
C	G	del	O1	0.0011	0.0001
C	G	del	O2	0.0115	0.0007
C	<b>T</b>	<b>G</b>	<b>B</b>	<b>1.0000</b>	<b>0.0513</b>
C	T	del	A2	0.2244	0.0004
C	T	del	B	0.7756	0.0014
T	G	G	A1	0.0068	0.0047
T	G	G	B	0.0055	0.0038
<b>T</b>	<b>G</b>	<b>G</b>	<b>O1</b>	<b>0.9618</b>	<b>0.6709</b>
T	G	G	O2	0.0259	0.0180
T	G	del	A1	0.0377	0.0000
T	G	del	O1	0.9623	0.0002
T	T	G	O1	1.0000	0.0007

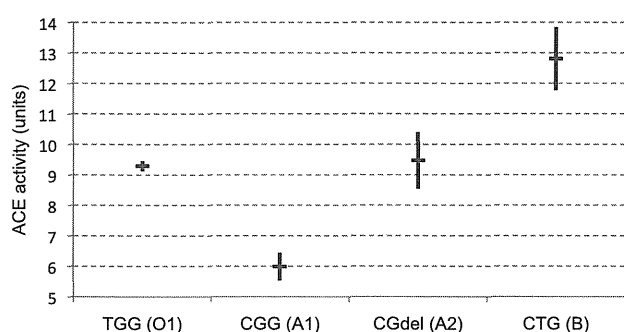
*ABO*, that together accounted for nearly half of the population variation in the trait (Table 1), with the *ACE* locus accounting for 35% and the *ABO* locus accounting for 11%.

### Association between Haplotypes Defining Blood Groups and ACE Activity

Haplotypes of rs505922, rs8176746 and rs8176750 showed strong associations with alleles defining the different blood groups in the subset of the British families (734 samples) where blood groups were available (conditional tagging probabilities range from 0.83 to 1.00; Table 2). Haplotypes of these three SNPs that occurred at a frequency of  $>0.05$  in the population, and accurately tagged alleles responsible for A1, A2, B and O1 phenotypes, were taken forward to the

**Table 3** Measured haplotype analysis of plasma ACE activity in British families. The *ABO* haplotype is defined by rs505922, rs8176746 and rs8176750; the most strongly tagged blood group allele is shown in parentheses.

<i>ABO</i> haplotype (blood group allele)	Frequency	Mean	SE
TGG (O1)	0.647	9.295	0.084
CGG (A1)	0.212	5.989	0.231
CGdel (A2)	0.079	9.473	0.473
CTG (B)	0.063	12.808	0.528



**Figure 1** Measured haplotype analysis of plasma ACE activity in British families. The mean activities and 95% confidence intervals for each *ABO* haplotype are indicated by horizontal and vertical lines, respectively. *ABO* haplotypes are defined by rs505922, rs8176746 and rs8176750; the most strongly tagged blood group allele is shown in parentheses.

measured haplotype analysis of ACE activity in the total UK cohort. In the measured haplotype analyses, SNPs rs4295 and rs4392, previously shown in this cohort to tag the principal haplotype blocks influencing ACE activity at the *ACE* locus, were included as covariates. (Keavney et al., 1998) The measured haplotype analysis showed that the TGG haplotype of rs505922/rs8176746/rs8176750, which accurately tagged type O alleles and occurred at a frequency of ~65% in the population, was associated with an intermediate plasma ACE activity (Table 3). The CGG haplotype (frequency ~21%), which tagged type A1 alleles, was associated with a 36% lower plasma ACE activity than the TGG haplotype. The CTG haplotype (frequency ~6%), which tagged type B alleles, was associated with a 36% higher plasma ACE activity than the TGG haplotype. The CGdel haplotype (frequency ~8%), which tagged type A2 alleles, had a plasma ACE activity very similar to the TGG haplotype (Fig. 1). The measured haplotype analysis in the British families provided very similar estimates of variance components to the GWAS in the Japanese cohort: 35.4% of the variance was due to the *ACE* locus, and 13.0% to the *ABO* locus, with a further 9.6% attributable to polygenes and 42.1% to nongenetic residual variation.

## Discussion

We have shown strong evidence for association between plasma ACE activity and genotypes at the *ABO* gene that define the major blood groups. Genome-wide analyses in a cohort of unrelated Japanese people confirmed that the strongest genetic influence on plasma ACE levels is located at the *ACE* gene itself on Chromosome 17 ( $P = 1.55 \times 10^{-164}$  for rs4362) and demonstrated two further independent SNP effects at *ABO* (rs495828 and rs8176746). Stepwise regression suggested that the association at *ACE* accounted for 35% of the variability in the Japanese population and the two SNPs at *ABO* accounted for 11%. To confirm this finding, we typed SNPs at the *ABO* locus in a cohort of British families previously extensively genotypically characterised at the *ACE* locus. (McKenzie et al., 2001) Measured haplotype analysis in these families indicated that the haplotype characterizing group A1 was associated with the lowest plasma ACE level, the haplotypes characterizing groups O and A2 were associated with intermediate plasma ACE level, and the haplotype characterizing group B was associated with the highest plasma ACE level. The proportions of variance accounted for by the *ACE* and *ABO* loci in the British families were highly concordant with the Japanese cohort (35.4% and 13.0%, respectively) with an additional 9.6% attributable to polygenic effects. The *ACE* and *ABO* loci make the most substantial contribution to population variance in plasma ACE levels; also, there is appreciable heterogeneity in plasma ACE levels among the alleles specifying the two subgroups A1 and A2.

The *ABO* gene encodes a glycosyltransferase. Genetic variation in *ABO* results in the production of two differently named protein products: glycosyltransferase-A and glycosyltransferase-B. Glycosyltransferase-A transfers N-acetylgalactosamine to an acceptor glycoconjugate on the glycosphingolipid H-antigen, which is strongly present on the surface of red blood cells and more weakly present on a wide range of other cell types. Glycosyltransferase-B transfers D-galactose to the same position on the H-antigen. These glycosyltransferase activities define the blood group antigens A and B, respectively. AB heterozygotes have molecules with both A and B antigens present on the red cell surface. Mutations which inactivate the glycosyltransferase encoded by *ABO* result in nonmodification of the H-antigen, which characterises blood group O. A and B alleles are both dominant to O.

Previous studies have shown association between either *ABO* genotypes or *ABO* blood groups and plasma ACE activity. Cidl et al. found blood groups A and O to have similar levels of plasma ACE activity and groups AB and B to have progressively higher levels among 197 Caucasian subjects of Eastern European origin. (Cidl et al., 1996) Chung et al. performed a GWAS for plasma ACE activity among 1023 subjects with young-onset hypertension, replicating their findings in a

study of 428 hypertension pedigrees, all of self-reported Han Chinese ethnicity (Chung et al., 2010). By contrast with the findings of Cidl et al., these authors found plasma ACE activity in blood group A individuals to be 86% of that in the reference group O, and plasma ACE activity in blood group B individuals to be 114% of that in the reference group O. However, neither of these previous studies carried out measured haplotype analyses to determine the effect of the combination of SNPs defining the principal blood group antigens on plasma ACE levels, and neither conducted formal two-locus genetic analyses to determine the relative contribution of *ABO* haplotypes and *ACE* haplotypes on phenotypic variance. Moreover, the reason for the discordance between the two reports with respect to the relative levels of plasma ACE activity in type A and type O individuals remained unclear. Our findings illustrate significant differences in plasma ACE activity associated with the haplotypes defining the type A subgroups A1 and A2. The A1-defining haplotype had ~60% of the plasma ACE activity of the O-defining haplotype, whereas the plasma ACE activity of the A2-defining haplotype was not significantly different from the O-defining haplotype. In common with the previous studies, we found that the B-defining haplotype was associated with a ~40% higher plasma ACE activity than the O-defining haplotype.

The effects of the different *ABO* haplotypes on plasma ACE activity were assessed in a codominant (allelic association) model. This may explain the substantially smaller proportion of variation that could be accounted for by ABO blood grouping in the study of Cidl et al. (1996), when compared to the present study (since A and B alleles are dominant to O with respect to the determination of blood group). Differences in the proportions of A1 and A2 alleles in the previously studied populations may also contribute to the discrepancy in the results of previous studies: among Asian populations such as those studied by Chung et al. (2010), the A2 allele is extremely rare, while among European populations it comprises up to 25% of all A alleles. The A2 allele shows a weaker activity than the A1 allele in adding N-acetylgalactosamine to the acceptor glycoconjugate of the H antigen, which is present on red blood cells. At the DNA level, the A2 allele differs from the A1 in two ways: a single amino acid change (Pro156Leu) and a 1061delC mutation causing a frameshift that extends the reading frame by 64 nucleotides (Yamamoto et al., 1992).

Recently, several significant associations between the *ABO* locus and complex diseases have been reported. Association between ABO blood group and the risk of myocardial infarction (MI) was first observed decades ago; a GWAS approach recently confirmed strong association between non-O blood group and the risk of MI, although not of coronary artery disease without MI (Reilly et al., 2011). The likely principal mechanism is a differential susceptibility to thrombosis: people of blood group O have 25% lower plasma levels of

von Willebrand factor and Factor VIII, both members of the blood coagulation cascade, and SNPs defining group O are associated with less coagulable blood on standard laboratory coagulation tests. (Tang et al., 2012) Congruent with this observation, *ABO* genotypes are the strongest risk factor genome-wide for venous thromboembolism (Qi et al., 2010). Nevertheless, other potential mechanisms whereby the pleiotropic *ABO* locus could contribute to MI risk have been identified through GWAS approaches. *ABO* genotypes are associated with plasma levels of lipids, and of other plasma factors associated with coronary artery disease including soluble ICAM-1, E-selectin and P-selectin (Barbalic et al., 2010; Qi et al., 2010; Pare et al., 2011). GWAS studies have also identified SNPs at *ABO* responsible for the long-standing epidemiological observations of association between blood group O and higher risk of duodenal ulceration; (Tanikawa et al. 2012) and lower risks of gastric and pancreatic cancer (Amundadottir et al., 2009). Plasma ACE activity has not, in general, been systematically studied as a risk factor for any of the conditions associated with ABO blood groups. Since the *ABO* haplotype defining type O in our study was associated with intermediate levels of plasma ACE activity compared with types A and B, it is unlikely that plasma ACE activity lies in the causal pathway for any of these conditions, in which type O lies at the upper or lower extreme of the risk distribution. Further research will be required to define the mechanism whereby *ABO* haplotypes are associated with plasma ACE activity, which remains uncertain. A plausible hypothesis is that it may involve differential affinities of receptors involved in the clearance of ACE, a heavily glycosylated protein, for the A B and H glycosylation motifs that depend upon *ABO* genotype.

Although evidence remains inconclusive, it has been suggested that blood group O protects against severe malaria, and that this may have constituted a selective advantage favouring the alleles responsible for the group O phenotype throughout human evolutionary history (Anstee, 2010). In this regard it is of interest that angiotensin-II has recently been shown to reduce erythrocyte invasion *in vitro* by *P. falciparum* in a dose-dependent manner, and that the deletion allele of the *ACE* insertion/deletion polymorphism, which is associated with higher plasma ACE activity, has been reported to be protective against cerebral malaria (Dhangadamajhi et al., 2010; Saraiva et al., 2011). Further research into the question of whether the major genetic effects on plasma ACE activity at the *ACE* and *ABO* loci contribute significantly to differential susceptibility to severe malaria would be of interest.

In conclusion, this study has confirmed the existence of two major genetic loci influencing plasma levels of ACE, and quantified the contribution of the two loci to the population variability of the trait in two populations. We have also demonstrated previously unrecognised heterogeneity among

alleles responsible for group A antigens with respect to their effect on plasma ACE.

## Acknowledgements

The principal acknowledgement is to the participants in the Japanese and British cohorts. We are grateful to the Nagahama City Office and the nonprofit organisation “Zeoji Club” for their help in performing the Nagahama Study. The Nagahama Study is in part supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan; and by the Takeda Science Foundation. The collection and genotyping of the British families was funded by the Wellcome Trust and the British Heart Foundation. BK holds a British Heart Foundation Personal Chair. MF acknowledges support from the Wellcome Trust core award (090532/Z/09/Z) and the BHF Centre of Clinical Research Excellence in Oxford. ML acknowledges support from Genome Québec, le Ministère de l'Enseignement supérieur, de la Recherche, de la Science et de la Technologie (MESRST) Québec and McGill University, and from the Agence Nationale de la Recherche (ANR), France, for the Labex project “Medical Genomics.” No author has a conflict of interest to declare in respect of this work.

## References

- Amundadottir, L., Kraft, P., Stolzenberg-Solomon, R. Z., Fuchs, C. S., Petersen, G. M., Arslan, A. A., Bueno-De-Mesquita, H. B., Gross, M., Helzlsouer, K., Jacobs, E. J., Lacroix, A., Zheng, W., Albanes, D., Bamlet, W., Berg, C. D., Berrino, F., Bingham, S., Buring, J. E., Bracci, P. M., Canzian, F., Clavel-Chapelon, F., Clipp, S., Cotterchio, M., De Andrade, M., Duell, E. J., Fox, J. W., Jr., Gallinger, S., Gaziano, J. M., Giovannucci, E. L., Goggins, M., Gonzalez, C. A., Hallmans, G., Hankinson, S. E., Hassan, M., Holly, E. A., Hunter, D. J., Hutchinson, A., Jackson, R., Jacobs, K. B., Jenab, M., Kaaks, R., Klein, A. P., Kooperberg, C., Kurtz, R. C., Li, D., Lynch, S. M., Mandelson, M., McWilliams, R. R., Mendelsohn, J. B., Michaud, D. S., Olson, S. H., Overvad, K., Patel, A. V., Peeters, P. H., Rajkovic, A., Riboli, E., Risch, H. A., Shu, X. O., Thomas, G., Tobias, G. S., Trichopoulos, D., Van Den Eeden, S. K., Virtamo, J., Wactawski-Wende, J., Wolpin, B. M., Yu, H., Yu, K., Zeleniuch-Jacquotte, A., Chanock, S. J., Hartge, P., & Hoover, R. N. (2009) Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. *Nat Genet* **41**, 986–990.
- Anstee, D. J. (2010) The relationship between blood groups and disease. *Blood* **115**, 4635–4643.
- Barbalic, M., Dupuis, J., Dehghan, A., Bis, J. C., Hoogeveen, R. C., Schnabel, R. B., Nambi, V., Bretler, M., Smith, N. L., Peters, A., Lu, C., Tracy, R. P., Aleksic, N., Heeriga, J., Keaney, J. F., Jr., Rice, K., Lip, G. Y., Vasan, R. S., Glazer, N. L., Larson, M. G., Uitterlinden, A. G., Yamamoto, J., Durda, P., Haritunians, T., Psaty, B. M., Boerwinkle, E., Hofman, A., Koenig, W., Jenny, N. S., Witteman, J. C., Ballantyne, C., & Benjamin, E. J. (2010) Large-scale genomic studies reveal central role of ABO in sP-selectin and sICAM-1 levels. *Hum Mol Genet* **19**, 1863–1872.
- Chiknas, S. G. (1979) A liquid chromatography-assisted assay for angiotensin-converting enzyme (peptidyl dipeptidase) in serum. *Clin Chem* **25**, 1259–1262.
- Chung, C. M., Wang, R. Y., Chen, J. W., Fann, C. S., Leu, H. B., Ho, H. Y., Ting, C. T., Lin, T. H., Sheu, S. H., Tsai, W. C., Chen, J. H., Jong, Y. S., Lin, S. J., Chen, Y. T., & Pan, W. H. (2010) A genome-wide association study identifies new loci for ACE activity: potential implications for response to ACE inhibitor. *Pharmacogenomics J* **10**, 537–544.
- Cidl, K., Strelcova, L., Znojil, V., & Vachi, J. (1996) Angiotensin I-converting enzyme (ACE) polymorphism and ABO blood groups as factors codetermining plasma ACE activity. *Exp Hematol* **24**, 790–794.
- Dhangadamajhi, G., Mohapatra, B. N., Kar, S. K., & Ranjit, M. (2010) Gene polymorphisms in angiotensin I converting enzyme (ACE I/D) and angiotensin II converting enzyme (ACE2 C→T) protect against cerebral malaria in Indian adults. *Infect Genet Evol* **10**, 337–341.
- Elston, R. C., & Stewart, J. (1971) A general model for the genetic analysis of pedigree data. *Hum Hered* **21**, 523–542.
- Gaukrodger, N., Mayosi, B. M., Imrie, H., Avery, P., Baker, M., Connell, J. M., Watkins, H., Farrall, M., & Keavney, B. (2005) A rare variant of the leptin gene has large effects on blood pressure and carotid intima-medial thickness: a study of 1428 individuals in 248 families. *J Med Genet* **42**, 474–478.
- Hasstedt, S. J. (1993) Variance components/major locus likelihood approximation for quantitative, polychotomous, and multivariate data. *Genet Epidemiol* **10**, 145–158.
- Kasahara, Y., & Ashihara, Y. (1981) Colorimetry of angiotensin-I converting enzyme activity in serum. *Clin Chem* **27**, 1922–1925.
- Keavney, B., Bird, R., Caiazza, A., Casadei, B., & Conway, J. (2000) Measurement of blood pressure using the auscultatory and oscillometric methods in the same cuff deflation: validation and field trial of the A&D TM2421 monitor. *J Hum Hypertens* **14**, 573–579.
- Keavney, B., Mckenzie, C. A., Connell, J. M., Julier, C., Ratcliffe, P. J., Sobel, E., Lathrop, M., & Farrall, M. (1998) Measured haplotype analysis of the angiotensin-I converting enzyme gene. *Hum Mol Genet* **7**, 1745–1751.
- Lalouel, J.-M. (1979) GEMINI: A computer program for optimization of general nonlinear functions. Technical Reports. Honolulu.
- Li, Y., Willer, C. J., Ding, J., Scheet, P., & Abecasis, G. R. (2010) MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol* **34**, 816–834.
- Mayosi, B. M., Avery, P. J., Farrall, M., Keavney, B., & Watkins, H. (2008) Genome-wide linkage analysis of electrocardiographic and echocardiographic left ventricular hypertrophy in families with hypertension. *Eur Heart J* **29**, 525–530.
- McKenzie, C. A., Abecasis, G. R., Keavney, B., Forrester, T., Ratcliffe, P. J., Julier, C., Connell, J. M., Bennett, F., Mcfarlane-Anderson, N., Lathrop, G. M., & Cardon, L. R. (2001) Trans-ethnic fine mapping of a quantitative trait locus for circulating angiotensin I-converting enzyme (ACE). *Hum Mol Genet* **10**, 1077–1084.
- McKenzie, C. A., Julier, C., Forrester, T., Mcfarlane-Anderson, N., Keavney, B., Lathrop, G. M., Ratcliffe, P. J., & Farrall, M. (1995) Segregation and linkage analysis of serum angiotensin I-converting enzyme levels: evidence for two quantitative-trait loci. *Am J Hum Genet* **57**, 1426–1435.
- Palomino-Doza, J., Rahman, T. J., Avery, P. J., Mayosi, B. M., Farrall, M., Watkins, H., Edwards, C. R., & Keavney, B. (2008) Ambulatory blood pressure is associated with polymorphic variation in P2X receptor genes. *Hypertension* **52**, 980–985.

- Pare, G., Ridker, P. M., Rose, L., Barbalic, M., Dupuis, J., Dehghan, A., Bis, J. C., Benjamin, E. J., Shiffman, D., Parker, A. N., & Chasman, D. I. (2011) Genome-wide association analysis of soluble ICAM-1 concentration reveals novel associations at the NFKB1K, PNPLA3, RELA, and SH2B3 loci. *PLoS Genet* **7**, e1001374.
- Qi, L., Cornelis, M. C., Kraft, P., Jensen, M., Van Dam, R. M., Sun, Q., Girman, C. J., Laurie, C. C., Mirel, D. B., Hunter, D. J., Rimm, E., & Hu, F. B. (2010) Genetic variants in ABO blood group region, plasma soluble E-selectin levels and risk of type 2 diabetes. *Hum Mol Genet* **19**, 1856–1862.
- Reilly, M. P., Li, M., He, J., Ferguson, J. F., Stylianou, I. M., Mehta, N. N., Burnett, M. S., Devaney, J. M., Knouff, C. W., Thompson, J. R., Horne, B. D., Stewart, A. F., Assimes, T. L., Wild, P. S., Allayee, H., Nitschke, P. L., Patel, R. S., Martinelli, N., Girelli, D., Quyyumi, A. A., Anderson, J. L., Erdmann, J., Hall, A. S., Schunkert, H., Quertermous, T., Blankenberg, S., Hazen, S. L., Roberts, R., Kathiresan, S., Samani, N. J., Epstein, S. E., & Rader, D. J. (2011) Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. *Lancet* **377**, 383–392.
- Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P., & Soubrier, F. (1990) An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* **86**, 1343–1346.
- Saraiva, V. B., De Souza Silva, L., Ferreira-Dasilva, C. T., Da Silva-Filho, J. L., Teixeira-Ferreira, A., Perales, J., Souza, M. C., Henriques, M., Caruso-Neves, C., & De Sa Pinheiro, A. A. (2011) Impairment of the Plasmodium falciparum erythrocytic cycle induced by angiotensin peptides. *PLoS One* **6**, e17174.
- Seltsam, A., Hallensleben, M., Kollmann, A., & Blasczyk, R. (2003) The nature of diversity and diversification at the ABO locus. *Blood* **102**, 3035–42.
- Stephens, M., & Scheet, P. (2005) Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. *Am J Hum Genet* **76**, 449–462.
- Stephens, M., Smith, N. J., & Donnelly, P. (2001) A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* **68**, 978–989.
- Tang, W., Schwienbacher, C., Lopez, L. M., Ben-Shlomo, Y., Oudot-Mellakh, T., Johnson, A. D., Samani, N. J., Basu, S., Gogele, M., Davies, G., Lowe, G. D., Tregouet, D. A., Tan, A., Pankow, J. S., Tenesa, A., Levy, D., Volpato, C. B., Rumley, A., Gow, A. J., Minelli, C., Yarnell, J. W., Porteous, D. J., Starr, J. M., Gallacher, J., Boerwinkle, E., Visscher, P. M., Pramstaller, P. P., Cushman, M., Emilsson, V., Plump, A. S., Matijevic, N., Morange, P. E., Deary, I. J., Hicks, A. A., & Folsom, A. R. (2012) Genetic associations for activated partial thromboplastin time and prothrombin time, their gene expression profiles, and risk of coronary artery disease. *Am J Hum Genet* **91**, 152–162.
- Tanikawa, C., Urabe, Y., Matsuo, K., Kubo, M., Takahashi, A., Ito, H., Tajima, K., Kamatani, N., Nakamura, Y., & Matsuda, K. (2012) A genome-wide association study identifies two susceptibility loci for duodenal ulcer in the Japanese population. *Nat Genet* **44**, 430–434.
- Yamamoto, F., Mcneill, P. D., & Hakomori, S. (1992) Human histo-blood group A2 transferase coded by A2 allele, one of the A subtypes, is characterized by a single base deletion in the coding sequence, which results in an additional domain at the carboxyl terminal. *Biochem Biophys Res Commun* **187**, 366–374.
- Yoshimura, K., Nakayama, T., Sekine, A., Matsuda, F., Kosugi, S., Yamada, R., Shimizu, Y., Kanematsu, A., & Ogawa, O. (2012) B-type natriuretic peptide as an independent correlate of nocturnal voiding in Japanese women. *NeuroUrol Urodyn* **31**, 1266–1271.
- Yusuf, S., Sleight, P., Pogue, J., Bosch, J., Davies, R., Dagenais, G., & The Heart Outcomes Prevention Evaluation Study Investigators. (2000) Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* **342**, 145–153.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1** Demographics of the Japanese discovery cohort.

**Table S2** Demographics of the British replication cohort.

**Table S3** Single-SNP analyses of GWAS SNPs at *ACE* and *ABO* loci in the Japanese cohort.

**Figure S1** Manhattan plot of GWAS for plasma ACE in Japanese cohort, showing significant evidence of association at the *ACE* locus on Chromosome 17 and the *ABO* locus on Chromosome 9.

Received: 26 February 2013

Accepted: 16 May 2013

## Two Susceptibility Loci to Takayasu Arteritis Reveal a Synergistic Role of the *IL12B* and *HLA-B* Regions in a Japanese Population

Chikashi Terao,<sup>1,2,\*</sup> Hajime Yoshifuji,<sup>2</sup> Akinori Kimura,<sup>3</sup> Takayoshi Matsumura,<sup>4</sup> Koichiro Ohmura,<sup>2</sup> Meiko Takahashi,<sup>1</sup> Masakazu Shimizu,<sup>1</sup> Takahisa Kawaguchi,<sup>1</sup> Zhiyong Chen,<sup>3</sup> Taeko K. Naruse,<sup>3</sup> Aiko Sato-Otsubo,<sup>5</sup> Yusuke Ebana,<sup>6</sup> Yasuhiro Maejima,<sup>7</sup> Hideyuki Kinoshita,<sup>8</sup> Kosaku Murakami,<sup>9</sup> Daisuke Kawabata,<sup>2</sup> Yoko Wada,<sup>10</sup> Ichiei Narita,<sup>10</sup> Junichi Tazaki,<sup>11</sup> Yasushi Kawaguchi,<sup>12</sup> Hisashi Yamanaka,<sup>12</sup> Kimiko Yurugi,<sup>13</sup> Yasuo Miura,<sup>13</sup> Taira Maekawa,<sup>13</sup> Seishi Ogawa,<sup>5</sup> Issei Komuro,<sup>4</sup> Ryozo Nagai,<sup>14</sup> Ryo Yamada,<sup>1</sup> Yasuharu Tabara,<sup>1</sup> Mitsuaki Isobe,<sup>7</sup> Tsuneyo Mimori,<sup>2</sup> and Fumihiko Matsuda<sup>1</sup>

Takayasu arteritis (TAK) is an autoimmune systemic vasculitis of unknown etiology. Although previous studies have revealed that HLA-B\*52:01 has an effect on TAK susceptibility, no other genetic determinants have been established so far. Here, we performed genome scanning of 167 TAK cases and 663 healthy controls via Illumina Infinium Human Exome BeadChip arrays, followed by a replication study consisting of 212 TAK cases and 1,322 controls. As a result, we found that the *IL12B* region on chromosome 5 (rs6871626, overall  $p = 1.7 \times 10^{-13}$ , OR = 1.75, 95% CI 1.42–2.16) and the *MLX* region on chromosome 17 (rs665268, overall  $p = 5.2 \times 10^{-7}$ , OR = 1.50, 95% CI 1.28–1.76) as well as the *HLA-B* region (rs9263739, a proxy of HLA-B\*52:01, overall  $p = 2.8 \times 10^{-21}$ , OR = 2.44, 95% CI 2.03–2.93) exhibited significant associations. A significant synergistic effect of rs6871626 and rs9263739 was found with a relative excess risk of 3.45, attributable proportion of 0.58, and synergy index of 3.24 ( $p \leq 0.00028$ ) in addition to a suggestive synergistic effect between rs665268 and rs926379 ( $p \leq 0.027$ ). We also found that rs6871626 showed a significant association with clinical manifestations of TAK, including increased risk and severity of aortic regurgitation, a representative severe complication of TAK. Detection of these susceptibility loci will provide new insights to the basic mechanisms of TAK pathogenesis. Our findings indicate that *IL12B* plays a fundamental role on the pathophysiology of TAK in combination with HLA-B\*52:01 and that common autoimmune mechanisms underlie the pathology of TAK and other autoimmune disorders such as psoriasis and inflammatory bowel diseases in which *IL12B* is involved as a genetic predisposing factor.

### Introduction

Takayasu arteritis (TAK [MIM 207600]) is an autoimmune systemic vasculitis that was first reported from Japan.<sup>1</sup> It is estimated that TAK affects around 0.004% of the population in Japan, especially young women aged between 15 and 35. Although TAK was originally thought to affect individuals of mainly Asian origin, individuals with TAK have been identified worldwide, though with lower prevalence compared to Asia.<sup>2</sup> TAK is characterized by the involvement of large arteries, especially the aorta and its large branches, and is grouped into “vasculitis affecting large vessels” according to the Chapel Hill classification.<sup>3</sup> Individuals with TAK develop a wide range of symptoms such as fatigue, syncope, and lowering of vision in addition to its characteristic complications including aortic regurgitation (AR), pulselessness, and difference of blood

pressure between right and left upper limbs. Previous studies have revealed that genetic components are involved in the pathogenesis of TAK, and HLA-B\*52:01 is so far the only established genetic factor across the world.<sup>4–7</sup> Other genetic components especially outside of the HLA locus have not been confirmed to date. Establishment of association with non-HLA regions would lead to a deeper understanding of the basics of TAK pathology and the development of a novel therapy for this vasculitis. Here, we performed a genome-scanning study of TAK to identify the genetic predisposing factors for TAK.

### Subjects and Methods

#### Study Subjects

A total of 379 TAK cases and 1,985 controls were enrolled in this study. All the cases were diagnosed based on the criteria of

<sup>1</sup>Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan; <sup>2</sup>Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan; <sup>3</sup>Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 113-8510, Japan; <sup>4</sup>Department of Cardiovascular Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8655, Japan; <sup>5</sup>Cancer Genomics Project, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8655, Japan; <sup>6</sup>Department of Bioinformatical Pharmacology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 113-8510, Japan; <sup>7</sup>Department of Cardiovascular Medicine, Tokyo Medical and Dental University, Tokyo 113-8510, Japan; <sup>8</sup>Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan; <sup>9</sup>Osaka Red Cross Hospital, Osaka 543-8555, Japan; <sup>10</sup>Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata 951-8510, Japan; <sup>11</sup>Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan; <sup>12</sup>Institute of Rheumatology, Tokyo Women's Medical University, Tokyo 162-0054, Japan; <sup>13</sup>Department of Transfusion Medicine and Cell Therapy, Kyoto University Hospital, Kyoto 606-8507, Japan; <sup>14</sup>Jichi Medical University, Tochigi 329-0498, Japan

\*Correspondence: a0001101@kuhp.kyoto-u.ac.jp

http://dx.doi.org/10.1016/j.ajhg.2013.05.024. ©2013 by The American Society of Human Genetics. All rights reserved.

