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References

Ahola V, Aittokallio T, Vihinen M, Uusipaikka E. 2006. A statistical score for assessing the quality of multiple sequence alignments. BMC Bioinformatics 7:484.

Ahola V, Aittokallio T, Vihinen M, Uusipaikka E. 2008. Model-based prediction of sequence alignment quality. Bioinformatics 24:2165–2171.

Amberger A, Bocchini CA, Scott AF, Hamosh A. 2009. McKusick's Online Mendelian Inheritance in Man (OMIM). Nucleic Acids Res 37:D793–D796.

Axton M. 2008. Human variome microattribution reviews. Nat Genet 40:1.

Baird PA, Anderson TW, Newcombe HB, Lowry RB. 1988. Genetic disorders in children and young adults: a population study. Am J Hum Genet 42:677–693.

Beall CM. 2007. Two routes to functional adaptation: Tibetan and Andean high-altitude natives. Proc Natl Acad Sci USA 104(Suppl 1):8655–8660.

Becker KG, Barnes KC, Bright TJ, Wang SA. 2004. The genetic association database. Nat Genet 36:431–432.

Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, Hall KP, Evers DJ, Barnes CL, Bignell HR, Boutell JM, Bryant J, and many others. 2008. Accurate whole human genome sequencing using reversible terminator chemistry. Nature 456:53–59.

Beroud C, Collod-Beroud G, Boileau C, Soussi T, Junien C. 2000. UMD (Universal mutation database): a generic software to build and analyze locus-specific databases. Hum Mutat 15:86–94.

Bhan A, Singh JA, Upshur RE, Singer PA, Daar AS. 2007. Grand challenges in global health: engaging civil society organizations in biomedical research in developing countries. PLoS Med 4:e272.

Bittles A. 2001. Consanguinity and its relevance to clinical genetics. Clin Genet 60:89–98.

Bittles AH. 2002. Endogamy, consanguinity and community genetics. J Genet 81:91–98.

Brenner SE. 2007. Common sense for our genomes. Nature 449:783–784.

Bruford EA, Lush MJ, Wright MW, Sneddon TP, Povey S, Birney E. 2008. The HGNC Database in 2008: a resource for the human genome. Nucleic Acids Res 36:D445–D458.

Cambon-Thomsen A. 2003. Assessing the impact of biobanks. Nat Genet 34:25–26.

Chan PA, Duraisamy S, Miller PJ, Newell JA, McBride C, Bond JP, Raevaara T, Ollila S, Nyström M, Grimm AJ, Christodoulou J, Oetting WS, Greenblatt MS. 2007. Interpreting missense variants: comparing computational methods in human disease genes CDKN2A, MLH1, MSH2, MECP2, and tyrosinase (TYR). Hum Mutat 28:683–693.

Chao EC, Velasquez JL, Witherspoon MS, Rozek LS, Peel D, Ng P, Gruber SB, Watson P, Rennert G, Anton-Culver H, Lynch H, Lipkin SM. 2008. Accurate classification of MLH1/MSH2 missense variants with multivariate analysis of protein polymorphisms-mismatch repair (MAPP-MMR). Hum Mutat 29:852–860.

Cohen ER, Masum H, Berndtson K, Saunders V, Hadfield T, Panjwani D, Persad DL, Minhas GS, Daar AS, Singh JA, Singer PA. 2008. Public engagement on global health challenges. BMC Public Health 8:168.

Cotton RG, Appelbe W, Auerbach AD, Becker K, Bodmer W, Boone DJ, Boulyjenkov V, Brahmachari S, Brody L, Brookes A, Brown AF, Byers P, Cantu JM, Cassiman JJ, Claustres M, Concannon P, Cotton RG, den Dunnen JT, Flicek P, Gibbs R, Hall J, Hasler J, Katz M, Kwok PY, Laradi S, Lindblom A, Maglott D, Marsh S, Masimirembwa CM, Minoshima S, de Ramirez HZ, Scriver CR, Sherry S, Shimizu N, Shimizu N, Stein L, Tadmouri GO, Taylor G, Watson M. 2007a. Recommendations of the 2006 Human Variome Project meeting. Nat Genet 39:433–436.

Cotton RG, Auerbach AD, Brown AF, Carrera P, Christodoulou J, Claustres M, Compton J, Cox DW, De Baere E, den Dunnen JT, Greenblatt M, Fujiwara M, Hilbert P, Jani A, Lehvasiaiho H, Nebert DW, Verma I, Vihinen M, Members of the Human Genome Variation Society, Human Variome Project diagnostic Laboratory Working Group. 2007b. A structured simple form for ordering genetic tests is needed to ensure coupling of clinical detail (phenotype) with DNA variants (genotype) to ensure utility in publication and databases. Hum Mutat 28:931–932.

Cotton RG, Horaitis O. 2000. Human Genome Variation Society. In: Cooper DN, editor. Nature encyclopedia of the human genome. London: Nature Publishing Group. p 361–362.

Cotton RG, Sallee C, Knoppers BM. 2005. Locus-specific databases: from ethical principles to practice. Hum Mutat 26:489–493.

Cotton RGH, Auerbach AD, Axton M, Barash CI, Berkovic SF, Brookes AJ, Burn J, Cutting G, den Dunnen JT, Flicek P, Freimer N, Greenblatt MS, Howard HJ, Katz M, Macrae FA, Maglott D, Möslein G, Povey S, Ramesar RS, Richards CS, Seminara D, Smith TD, Sobrido M-J, Soibakk JH, Tanzi RE, Tavtigian SV, Taylor GR, Utsunomlya J, Watson M. 2008. GENETICS: the human variome project. Science 322:861–862.

Couch FJ, Rasmussen LJ, Hofstra R, Monteiro AN, Greenblatt MS, de Wind N. 2008. Assessment of functional effects of unclassified genetic variants. Hum Mutat 29:1314–1326.

Craig DW, Pearson JV, Szelinger S, Sekar A, Redman M, Corneveaux JJ, Pawlowski TL, Laub T, Nunn G, Stephan DA, Homer N, Huentleman MJ. 2008. Identification of genetic variants using bar-coded multiplexed sequencing. Nat Methods 5:887–893.

Crawford DC, Nickerson DA. 2005. Definition and clinical importance of haplotypes. Annu Rev Med 56:303–320.

Daar AS, Thorsteinsdottir H, Martin DK, Smith AC, Nast S, Singer PA. 2002. Top ten biotechnologies for improving health in developing countries. Nat Genet 32:229–232.

Dantzer J, Moad C, Heiland R, Mooney S. 2005. MutDB services: interactive structural analysis of mutation data. Nucleic Acids Res 33:W311–W314.

den Dunnen JT, Antonarakis SE. 2000. Mutation nomenclature extensions and suggestions to describe complex mutations: A discussion. Hum Mutat 15:7–12.

Eisinger F. 2008. Genetic testing for familial cancer. The French National Report (year 2003). Community Genet 11:63–67.

Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Jarvela I. 2002. Identification of a variant associated with adult-type hypolactasia. Nat Genet 30:233–237.

Fenstermacher D, Street C, McSherry T, Nayak V, Overby C, Feldman M. 2005. The Cancer Biomedical Informatics Grid (caBIG™). Conf Proc IEEE Eng Med Biol Soc 1:743–746.

Fokkema IF, den Dunnen JT, Taschner PE. 2005. LOVD: easy creation of a locus-specific sequence variation database using an "LSDB-in-a-box" approach. Hum Mutat 26:63–68.

Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, Belmont JW, Boudreau A, Hardenbol P, Leal SM, Pasternak S, Wheeler DA, and many others. 2007. A second generation human haplotype map of over 3.1 million SNPs. Nature 449:851–861.

Giardine B, Riemer C, Hefferon T, Thomas D, Hsu F, Zielenski J, Sang Y, Elnitski L, Cutting G, Trumbower H, Kern A, Kuhn R, Patrinos GP, Hughes J, Niggs D, Chul D, Scriver C, Phiommarinh M, Patnaik SK, Blumenfeld O, Gottlieb B, Vihinen M, Väliaho J, Kent J, Miller W, Hardison RC. 2007. PhenCode: connecting ENCODE data with mutations and phenotype. Hum Mutat 28:554–562.

Hancock AM, Witonsky DB, Gordon AS, Eshel G, Pritchard JK, Coop G, Di Rienzo A. 2008. Adaptations to climate in candidate genes for common metabolic disorders. PLoS Genet 4:e32.

Hinds DA, Stuve LL, Nilsen GB, Halperin E, Eskin E, Ballinger DG, Frazer KA, Cox DR. 2005. Whole-genome patterns of common DNA variation in three human populations. Science 307:1072–1079.

Hoffman R. 2008. A wiki for the life sciences where authorship matters. Nat Genet 40:1047–1051.

Horaitis O, Talbot Jr CC, Phommarinh M, Phillips KM, Cotton RG. 2007. A database of locus-specific databases. Nat Genet 39:425.

International HapMap Consortium. 2003. The International HapMap Project. Nature 426:789–796.

International HapMap Consortium. 2004. Integrating ethics and science in the International HapMap Project. Nat Rev Genet 5:467–475.

Jorde LB, Wooding SP. 2004. Genetic variation, classification and "race". Nat Genet 36(Suppl 1):S28–S33.

Kaput J. 2008. Nutrigenomics research for personalized nutrition and medicine. Curr Opin Biotechnol 19:110–120.

Kaput J, Ordovas JM, Ferguson L, van Ommen B, Rodriguez RL, Allen L, Ames BN, Dawson K, German B, Krauss R, Malyj W, Archer MC, and many others. 2005. The case for strategic international alliances to harness nutritional genomics for public and personal health. Br J Nutr 94:623–632.
Kathiresan S, Musunuru K, Orho-Melander M. 2008. Defining the spectrum of alleles that contribute to blood lipid concentrations in humans. Curr Opin Lipidol 19:122–127.
Kauffmann F, Cambon-Thomsen A. 2008. Tracing biological collections: between books and clinical trials. JAMA 299:2316–2318.
Knoppers BM, Fortier I, Legault D, Burton P. 2008. The Public Population Project in Genomics (P3G): a proof of concept? Eur J Hum Genet 16:664–665.
Kwiatkowski DP. 2005. How malaria has affected the human genome and what human genetics can teach us about malaria. Am J Hum Genet 77:171–192.
Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, and many others, International Human Genome Sequencing Consortium. 2001. Initial sequencing and analysis of the human genome. Nature 409:860–921.
Lang L. 2008. Three sequencing companies join the 1000 genomes project. Gastroenterology 135:336–337.
Lao O, Lu TT, Nothnagel M, Junge O, Freitag-Wolf S, Caliebe A, Balascakova M, Bertranpetit J, Bindoff LA, Comas D, Holmlund G, Kouvatsi A, Macek M, Mollet I, Parson W, Palo J, Ploski R, Sajantila A, Tagliabracci A, Gether U, Werge T, Rivadeneria F, Hofman A, Uitterlinden AG, Geiger C, Wichmann HE, Ruther A, Schreiber S, Becker C, Nürnberg P, Nelson MR, Krawczak M, Kayser M. 2008. Correlation between genetic and geographic structure in Europe. Curr Biol 18:1241–1248.
Levy S, Sutton G, Ng PC, Feuk L, Halpern AL, Walenz BP, Axelrod N, Huang J, Kirkness EF, Denisov G, Lin Y, MacDonald JR, Pang AW, Shago M, Stockwell TB, Tsiamouri A, Bafna V, Bansal V, Kravitz SA, Busam DA, Beeson KY, McIntosh TC, Remington KA, Abril JF, Gill J, Berman J, Rogers YH, Frazier ME, Scherer SW, Strausberg RL, Venter JC. 2007. The diploid genome sequence of an individual human. PLoS Biol 5:e254.
Lim U, Wang SS, Hartge P, Cozen W, Kelemen LE, Chanock S, Davis S, Blair A, Schenk M, Rothman N, Lan Q. 2007. Gene-nutrient interactions among determinants of folate and one-carbon metabolism on the risk of non-Hodgkin lymphoma: NCI-SEER case-control study. Blood 109:3050–3059.
Lomer MC, Parkes GC, Sanderson JD. 2008. Review article: lactose intolerance in clinical practice—myths and realities. Aliment Pharmacol Ther 27: 93–103.
Mailman MD, Feolo M, Jin Y, Kimura M, Tryka K, Bagoutdinov R, Hao L, Kiang A, Paschall J, Phan L, Popova N, Pretel S, Ziyabari L, Lee M, Shao Y, Wang ZY, Sirotkin K, Ward M, Kholodov M, Zbiez K, Beck J, Kimelman M, Shevelev S, Preuss D, Yaschenko E, Graeff A, Ostell J, Sherry ST. 2007. The NCBI dbGaP database of genotypes and phenotypes. Nat Genet 39:1181–1186.
Makinen VP, Soininen P, Forsblom C, Parkkonen M, Ingman P, Kaski K, Groop PH, Ala-Korpela M. 2008. 1H NMR metabonomics approach to the disease continuum of diabetic complications and premature death. Mol Syst Biol 4:167.
Marini NJ, Gin J, Ziegle J, Keho KH, Ginzinger D, Gilbert DA, Rine J. 2008. The prevalence of folate-remedial MTHFR enzyme variants in humans. Proc Natl Acad Sci USA 105:8055–8060.
McCabe-Sellers B, Lovera D, Nuss H, Wise C, Green B, Teitel C, Ning B, Clark B, Bogle M, Kaput J. 2008. Community based participatory research and omics technologies. Omics 12:263–272.
McDonald CJ, Huff SM, Suico JG, Hill G, Leavelle D, Aller R, Forrey A, Mercer K, DeMoor G, Hook J, Williams W, Case J, Maloney P. 2003. LOINC, a universal standard for identifying laboratory observations: A 5-year update. Clin Chem 49:624–633.
McKusick VA. 2006. A 60-year tale of spots, maps, and genes. Annu Rev Genomics Hum Genet 7:1–27.
McKusick VA. 2007. Mendelian Inheritance in Man and its online version, OMIM. Am J Hum Genet 80:588–604.
Metcalfe KA, Birenbaum-Carmeli D, Lubinski J, Gronwald J, Lynch H, Moller P, Ghadirian P, Foulkes WD, Klijn J, Friedman E, Kim-Sing C, Ainsworth P, Rosen B, Domchek S, Wagner T, Tung N, Manoukian S, Couch F, Sun P, Narod SA,Hereditary Breast Cancer Clinic Study Group. 2008. International variation in rates of uptake of preventive options in BRCA1 and BRCA2 mutation carriers. Int J Cancer 122:2017–2022.
Montgomery RK, Krasinski SD, Hirschhorn JN, Grand RJ. 2007. Lactose and lactase—who is lactose intolerant and why? J Pediatr Gastroenterol Nutr 45(Suppl 2):S131–S137.
Myles S, Davison D, Barrett J, Stoneking M, Timpson N. 2008a. Worldwide population differentiation at disease-associated SNPs. BMC Med Genomics 1:22.
Myles S, Tang K, Somel M, Green RE, Kelso J, Stoneking M. 2008b. Identification and analysis of genomic regions with large between-population differentiation in humans. Ann Hum Genet 72(Pt 1):99–110.
Nalla VK, Rogan PK. 2005. Automated splicing mutation analysis by information theory. Hum Mutat 25:334–342.
Nelson MR, Bryc K, King KS, Indap A, Boyko AR, Novembre J, Briley LP, Maruyama Y, Waterworth DM, Waeber G, Vollenweider P, Oksenberg JR, Hauser SL, Sitrnadel HA, Kooner JS, Chambers JC, Jones B, Mooser V, Bustamanta CD, Roses AD, Burns DK, Ehm MG, Lia EH. 2008. The population reference sample, POPRES: a resource for population, disease, and pharmacological genetics research. Am J Hum Genet 83:347–358.
Novembre J, Johnson T, Bryc K, Kutalik Z, Boyko AR, Auton A, Indap A, King KS, Bergmann S, Nelson MR, Stephens M, Bustamanta CD. 2008. Genes mirror geography within Europe. Nature 455:861.
Ordovas JM, Corella D. 2006. Gene–environment interactions: defining the playfield. In: Kaput J, Rodriguez RL, editors. Nutritional genomics. Discovering the path to personalized nutrition. Hoboken, NJ: John Wiley and Sons. p 57–76.
Ou J, Niessen RC, Lutzen A, Sijmons RH, Kleibeuker JH, de Wind N, Rasmussen LJ, Hofstra RM. 2007. Functional analysis helps to clarify the clinical importance of unclassified variants in DNA mismatch repair genes. Hum Mutat 28:1047–1054.
Pagon RA, Tarczy-Hornoch P, Baskin PK, Edwards JE, Covington ML, Espeseth M, Beahler C, Bird TD, Popovich B, Nesbitt C, Dolan C, Marymee K, Hanson NB, Neufeld-Kaiser W, Grohs GM, Kicklighter T, Abair C, Malmin A, Barclay M, Palepu RD. 2002. GeneTests-GeneClinics: genetic testing information for a growing audience. Hum Mutat 19:501–509.
Patrinos G, Petricoin E. 2009. A new scientific journal linked to a genetic database: towards a novel publication modality. Hum Genomics Proteomics (in press).
Patrinos GP, Brookes AJ. 2005. DNA, diseases and databases: disastrously deficient. Trends Genet 21:333–338.
Peltomaki P, Vasen HF. 1997. Mutations predisposing to hereditary nonpolyposis colorectal cancer: database and results of a collaborative study. The International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer. Gastroenterology 113:1146–1158.
Perry GH, Dominy NJ, Claw KG, Lee AS, Fiegler H, Redon R, Werner J, Villanea FA, Mountain JL, Misra R, Carter NP, Lee C, Stone AC. 2007. Diet and the evolution of human amylase gene copy number variation. Nat Genet 39:1256–1260.
Plon SE, Eccles DM, Easton D, Foulkes WD, Genuardi M, Greenblatt MS, Hogervorst FB, Hoogerbrugge N, Spurdle AB, Tavtigian SV. 2008. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum Mutat 29:1282–1291.
Price A, Butler J, Patterson N, Capelli C, Pascali V, Scarnicci F, Ruiz-Linares A, Groop L, Saetta A, Korkolopoulou P, Seligsohn U, Waliszewska A, Schirmer C, Ardlie K, Ramos A, Nemesh J, Arbeitman L, Goldstein DB, Reich D, Hirschhorn JN. 2008. Discerning the ancestry of European Americans in genetic association studies. PLoS Genetics 4:e236.
Ring HZ, Kwok PY, Cotton RG. 2006. Human Variome Project: an international collaboration to catalogue human genetic variation. Pharmacogenomics 7:969–972.
Ropers HH. 2007. New perspectives for the elucidation of genetic disorders. Am J Hum Genet 81:199–207.
Rosenzweig JL, Weinger K, Poirier-Solomon L, Rushton M. 2002. Use of a disease severity index for evaluation of healthcare costs and management of comorbidities of patients with diabetes mellitus. Am J Manag Care 8:950–958.
Saadallah AA, Rashed MS. 2007. Newborn screening: experiences in the Middle East and North Africa. J Inherit Metab Dis 30:482–489.
Schulz LO, Bennett PH, Ravussin E, Kidd JR, Kidd KK, Esparza J, Valencia ME. 2006. Effects of traditional and western environments on prevalence of type 2 diabetes in Pima Indians in Mexico and the U.S. Diabetes Care 29:1866–1871.
Seguin B, Hardy BJ, Singer PA, Daar AS. 2008. Genomic medicine and developing countries: creating a room of their own. Nat Rev Genet 9:487–493.
Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, Sirotkin K. 2001. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 29:308–311.
Singer PA, Daar AS. 2001. Harnessing genomics and biotechnology to improve global health equity. Science 294:87–89.
Siva N. 2008. 1000 Genomes project. Nat Biotechnol 26:256.
Spurdle AB, Couch FJ, Hogervorst FB, Radice P, Sinilnikova OM. 2008. Prediction and assessment of splicing alterations: implications for clinical testing. Hum Mutat 29:1304–1313.
Stein LD. 2008. Towards a cyberinfrastructure for the biological sciences: progress, visions and challenges. Nat Rev Genet 9:678–688.
Stenson PD, Ball E, Howells K, Phillips A, Mort M, Cooper DN. 2008. Human Gene Mutation Database: towards a comprehensive central mutation database. J Med Genet 45:124–126.
Stoffel EM, Ford B, Mercado RC, Punglia D, Kohlmann W, Conrad P, Blanco A, Shannon KM, Powell M, Gruber SB, Terdiman J, Chung DC, Syngal S. 2008. Sharing genetic test results in Lynch syndrome: communication with close and distant relatives. Clin Gastroenterol Hepatol 6:333–338.

Tavtigian SV, Greenblatt MS, Lesueur F, Byrnes GB. 2008. In silico analysis of missense substitutions using sequence-alignment based methods. Hum Mutat 29:1327–1336.

Taylor CF. 2007. Standards for reporting bioscience data: a forward look. Drug Discov Today 12:527–533.

Taylor CF, Field D, Sansone SA, Aerts J, Apweiler R, Ashburner M, Ball CA, Binz PA, Bogue M, Booth T, Brazma A, Brinkman RR, and many others. 2008. Promoting coherent minimum reporting guidelines for biological and biomedical investigations: the MIBBI project. Nat Biotechnol 26:889–896.

Taylor JG, Choi EH, Foster CB, Chanock SJ. 2001. Using genetic variation to study human disease. Trends Mol Med 7:507–512.

Tindana PO, Singh JA, Tracy CS, Upshur RE, Daar AS, Singer PA, Frohlich J, Lavery JV. 2007. Grand challenges in global health: community engagement in research in developing countries. PLoS Med 4:e273.

Tishkoff SA, Reed FA, Ranciaro A, Voight BF, Babbitt CC, Silverman JS, Powell K, Mortensen HM, Hirbo JB, Osman M, Ibrahim M, Omar SA, Lema G, Nyambo TB, Ghori J, Bumpstead S, Pritchard JK, Wray GA, Deloukas P. 2007. Convergent adaptation of human lactase persistence in Africa and Europe. Nat Genet 39:31–40.

Topel T, Hofestadt R, Scheible D, Trefz F. 2006. RAMEDIS: the rare metabolic diseases database. Appl Bioinformatics 5:115–118.

Tracy RP. 2008. "Deep phenotyping": characterizing populations in the era of genomics and systems biology. Curr Opin Lipidol 19:151–157.

van Baal S, Kaimakis P, Phommarinh M, Koumbi D, Cuppens H, Riccardino F, Macek Jr M, Scriver CR, Patrinos GP. 2007. FINDbase: a relational database recording frequencies of genetic defects leading to inherited disorders worldwide. Nucleic Acids Res 35:D690–D695.

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, and many others. 2001. The sequence of the human genome. Science 291: 1304–13051.

Wang J, Wang W, Li R, Li Y, Tian G, Goodman L, Fan W, Zhang J, Li J, Guo Y, Feng B, Li H, Lu Y, Feng X, Liang H, Du Z, Li D, Zhao Y, Hu Y, Yang Z, Zheng H, Hellmann I, Inouye M, Pool J, Yi X, Zhao J, Duan J, Zhou Y, Qin J, Ma L, Li G, Yang Z, Zhang G, Liu D, Lu Z, Li N, Guo G, Zhang J, Ye J, Fang L, Hao Q, Chen Q, Liang Y, Su Y, San A, Ping C, Yang S, Chen F, Li L, Zhou K, Zheng H, Ren Y, Yang L, Gao Y, Yang G, Li Z, Feng X, Kristiansen K, Wong GK, Nielsen R, Durbin R, Bolund L, Zhang X, Li S, Yang M, Wang J. 2008. The diploid genome sequence of an Asian individual. Nature 456:60–65.

Wheeler DA, Srinivasan M, Egholm M, Shen Y, Chen L, McGuire A, He W, Chen YJ, Makhijani V, Roth GT, Gomes X, Tartaro K, Niazi F, Turcotte CL, Irzyk GP, Lupski JR, Chinault C, Song XZ, Liu Y, Yuan Y, Nazareth L, Qin X, Muzny DM, Marqulies M, Weinstock GM, Gibbs RA, Rothberg GM. 2008. The complete genome of an individual by massively parallel DNA sequencing. Nature 452:872–876.

Wong ML, Chia KS, Wee S, Chia SE, Lee J, Koh WP, Shen HM, Thumboo J, Sofjan D. 2004. Concerns over participation in genetic research among Malay-Muslims, Chinese and Indians in Singapore: a focus group study. Community Genet 7:44–54.

Wong ND. 2006. Screening and risk stratification of patients with the metabolic syndrome and diabetes. Expert Rev Cardiovasc Ther 4:181–190.

Zaninotto M, Mion MM, Novello E, Altinier S, Plebani M. 2007. New biochemical markers: from bench to bedside. Clin Chim Acta 381:14–20.

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Cytochrome P450 Oxidoreductase Deficiency: Identification and Characterization of Biallelic Mutations and Genotype-Phenotype Correlations in 35 Japanese Patients

Maki Fukami, Gen Nishimura, Keiko Homma, Toshiro Nagai, Keiichi Hanaki, Ayumi Uematsu, Tomohiro Ishii, Chikahiko Numakura, Hirotake Sawada, Mariko Nakacho, Takanori Kowase, Katsuaki Motomura, Hidenori Haruna, Mihoko Nakamura, Akira Ohishi, Masanori Adachi, Toshihiro Tajima, Yukihiro Hasegawa, Tomonobu Hasegawa, Reiko Horikawa, Kenji Fujieda, and Tsutomu Ogata*

Context: Cytochrome P450 oxidoreductase (POR) deficiency is a rare autosomal recessive disorder characterized by skeletal dysplasia, adrenal dysfunction, disorders of sex development (DSD), and maternal virilization during pregnancy. Although multiple studies have been performed for this condition, several matters remain to be clarified, including the presence of manifesting heterozygosity and the underlying factors for clinical variability.

Objective: The objective of the study was to examine such unresolved matters by detailed molecular studies and genotype-phenotype correlations.

Patients: Thirty-five Japanese patients with POR deficiency participated in the study.

Results: Mutation analysis revealed homozygosity for R457H in cases 1–14 (group A), compound heterozygosity for R457H and one apparently null mutation in cases 15–28 (group B), and other combinations of mutations in cases 29–35 (group C). In particular, FISH and RT-PCR sequencing analyses revealed an intragenic microdeletion in one apparent R457H homozygote, transcription failure of apparently normal alleles in three R457H heterozygotes, and nonsense mediated mRNA decay in two frameshift mutation-positive cases examined. Genotype-phenotype correlations indicated that skeletal features were definitely more severe, and adrenal dysfunction, 46,XY DSD, and pubertal failure were somewhat more severe in group B than group A, whereas 46,XX DSD and maternal virilization during pregnancy were similar between two groups. Notable findings also included the contrast between infrequent occurrence of 46,XY DSD and invariable occurrence of 46,XX DSD and pubertal growth pattern in group A mimicking that of aromatase deficiency.

Conclusions: The results argue against the heterozygote manifestation and suggest that the residual POR activity reflected by the R457H dosage constitutes the underlying factor for clinical variability in some features but not other features, probably due to the simplicity and complexity of POR-dependent metabolic pathways relevant to each phenotype. (*J Clin Endocrinol Metab* **94:** **1723–1731, 2009)**

Cytochrome P450 oxidoreductase (POR) deficiency (PORD) is a rare autosomal recessive disorder caused by mutations in the gene encoding an electron donor for all microsomal P450 enzymes and several non-P450 enzymes (1–4). Salient clinical features of PORD include skeletal dysplasia referred to as Antley-Bixler syndrome (ABS), adrenal dysfunction, 46,XY and 46,XX disorders of sex development (DSD), and maternal virilization during pregnancy (3, 4). Such features are primarily ascribed to impaired activities of POR-dependent CYP51A1 (lanosterol 14α-demethylase) and SQLE

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.
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doi: 10.1210/jc.2008-2816 Received December 29, 2008. Accepted February 24, 2009.
First Published Online March 3, 2009
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Abbreviations: ABS, Antley-Bixler syndrome; CHX, cycloheximide; DSD, disorders of sex development; E_2, estradiol; FISH, fluorescent *in situ* hybridization; hCG, human chorionic gonadotropin; M, metabolite; NMD, nonsense-mediated mRNA decay; PCO, polycystic ovary; POR, cytochrome P450 oxidoreductase; PORD, POR deficiency; 17-OHP, 17α-hydroxyprogesterone; T, testosterone.

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<zshorttitle>Cytochrome P450 Oxidoreductase Deficiency: Genotype-Phenotype Correlations in Japanese

(squalene monooxigenase) involved in cholesterologenesis and CYP17A1 (17α-hydroxylase and 17,20 lyase), CYP21A2 (21-hydroxylase), and CYP19A1 (aromatase) involved in steroidogenesis (3, 4).

PORD has been identified in multiple patients (4). Mutations are diverse, including missense, nonsense, frameshift, and splice site mutations (4). Notably, however, A287P is the most common mutation in Caucasian patients, and R457H is the most prevalent founder mutation in Japanese patients (1–8). In addition, there is no patient with two apparently null mutations, suggesting that absence of a residual POR activity is incompatible with life (4–6). Clinical features are also variable, with a wide range of expressivity and penetrance. Indeed, ABS-compatible skeletal features and DSD are severely manifested by some patients and apparently absent in other patients (4–6). In addition, adrenal crisis remains relatively rare (4, 6), and maternal virilization is not a consistent feature (5, 6, 9).

To date, however, several critical matters remain to be clarified. First, although about 12% of patients have one apparently normal *POR* allele (4), it is uncertain whether such patients represent manifesting heterozygotes or have hidden aberrations in nonexamined region(s) (4, 10). Second, the underlying factors for the clinical diversity remain to be determined, although variable supporting activities of different POR mutants for target enzymes would have a certain role (5, 11, 12). Third, pubertal development and longitudinal growth have poorly been investigated.

To examine these matters, we analyzed the *POR* gene in affected patients and performed genotype-phenotype correlations in terms of the dosage effect of the R457H mutant.

Patients and Methods

Patients

This study consisted of 35 Japanese patients aged 0.1–23.8 yr (16 patients with 46,XY and 19 patients with 46,XX), including previously reported 23 cases (6, 8, 9) (Table 1). Of the 35 patients, 25 were sporadic cases and the remaining 10 were familial cases from families A–D. Twenty-three sporadic cases and four probands (cases 10, 15, 30, and 35) were ascertained by skeletal features and/or DSD, two sporadic cases (cases 1 and 5) by newborn mass screening for 21-hydroxylase deficiency, and the remaining six cases by familial studies.

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Molecular analysis

This study was approved by the Institutional Review Board Committee at National Center for Child Health and Development. The primers used in this study are shown in supplementary Table 1, published as supplemental data on The Endocrine Society's Journals Online Web site at http://jcem.endojournals.org. After taking written informed consent, peripheral blood samples were obtained from all the patients and the parents of 19 sporadic cases and two familial cases (families A and C). Subsequently, genomic DNA samples were subjected to direct sequencing for the *POR* exons 1–16, together with their flanking splice sites. To confirm a heterozygous mutation, the corresponding PCR products were subcloned with a TOPO TA cloning kit (Invitrogen, Carlsbad, CA), and the two alleles were sequenced separately.

AQ: A

AQ: B

When lymphoblastoid cell lines were available, fluorescent *in situ* hybridization (FISH) analysis was performed with two long PCR products spanning exons 4–7 (probe 1) and exons 8–12 (probe 2). The two probes were labeled with digoxigenin and detected by rhodamine anti-digoxigenin. A spectrum green-labeled probe for *D7Z1* (CEP7) (Abbott, Abbott Park, IL) was used as an internal control. For a case with a probable microdeletion, RT-PCR was performed with a variety of primers, to determine the deletion size. Furthermore, to examine the occurrence of transcription failure in cases with apparent heterozygosity and that of the nonsense-mediated mRNA decay (NMD) in cases with premature truncation mutations, the lymphoblastoid cell lines available were incubated for 8 h with and without an NMD inhibitor cycloheximide (CHX; 100 μg/ml; Sigma, St. Louis, MO), and direct sequencing was performed for RT-PCR products (13, 14).

AQ: C

In addition to disease-causing mutations, we also examined the presence or absence of a common A503V variant that has been shown to have a mildly decreased supporting activity at least for CYP17A1 (~60%) (15), to investigate whether the A503V variant can function as a modifier of the clinical phenotype. To examine whether the A503V variant resides on the same allele carrying R457H, PCR products encompassing both the 457th and 503rd codons were subcloned and subjected to direct sequencing.

Clinical assessment

Skeletal features were assessed by bone survey. Adrenal function was evaluated by basal and ACTH-stimulated blood hormone values [250 μg/m^2 (maximum 250 μg) bolus iv; blood sampling at 0 and 60 min] and by urine steroid profiles determined by the gas chromatography/mass spectrometry using first morning urine samples in cases aged older than 6 months (16) (several urine steroid metabolites cannot be measured precisely during the first 6 months of age due to interference of unknown steroids derived from the fetal adrenocortex). DSD was clinically evaluated, as was pubertal development in boys aged older than 14.3 yr (mean +2 SD age for pubic stage 2) and in girls aged older than 12.8 yr (mean +2 SD age for breast stage 2) (17). When possible, basal blood pituitary-gonadal hormone values were also obtained as well as human chorionic gonadotropin (hCG)-stimulated testosterone (T) values (3000 IU/m^2 per dose im for 3 consecutive days; blood sampling on d 1 and 4). In addition, clinical records were surveyed for the data of 17-hydroxyprogesterone (17-OHP) values at the newborn mass screening, adrenal crisis, maternal virilization during pregnancy, polycystic ovary (PCO) in female cases, and body measurement.

Penile length, clitoral size, Tanner stage, testis size, age of menarche, and statural growth were assessed by age- and sex-matched Japanese reference data (17–20), as were hormone values (21–23). Because urine steroid metabolites (Ms) expressed in a logarithm scale grossly followed the normal distribution and showed marked change with age in control

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TABLE 1. Summary of molecular analyses

Patients				*POR* mutations	
Case	Karyotype	Age (yr)	Inheritance	Nucleotide changes[a]	Aminoacid changes
Group A: homozygotes for R457H					
1	46,XY	5.0	Sporadic	1370G>A/1370G>A	R457H/R457H
2	46,XY	23.8	Familial-A	1370G>A/1370G>A	R457H/R457H
3	46,XY	22.6	Familial-A	1370G>A/1370G>A	R457H/R457H
4	46,XY	6.7	Sporadic	1370G>A/1370G>A	R457H/R457H
5	46,XY	0.4	Sporadic	1370G>A/1370G>A	R457H/R457H
6	46,XX	0.4	Sporadic	1370G>A/1370G>A	R457H/R457H
7	46,XX	0.4	Sporadic	1370G>A/1370G>A	R457H/R457H
8	46,XX	2.0	Sporadic	1370G>A/1370G>A	R457H/R457H
9	46,XX	14.1	Sporadic	1370G>A/1370G>A	R457H/R457H
10	46,XX	15.0	Familial-A (P)	1370G>A/1370G>A	R457H/R457H
11	46,XX	3.0	Sporadic	1370G>A/1370G>A	R457H/R457H
12	46,XX	0.2	Sporadic	1370G>A/1370G>A	R457H/R457H
13	46,XX	0.1	Sporadic	1370G>A/1370G>A	R457H/R457H
14	46,XX	18.0	Sporadic	1370G>A/1370G>A	R457H/R457H
Group B: compound heterozygotes for R457H and an apparently null mutation					
15	46,XY	16.8	Familial-B (P)	1370G>A/601C>T	R457H/Q201X
16	46,XY	15.7	Familial-B	1370G>A/601C>T	R457H/Q201X
17	46,XY	14.8	Sporadic	1370G>A/1329-1330insC	R457H/I444fsX449
18	46,XY	17.5	Sporadic	1370G>A/(15A>G)	R457H/Non-transcribed (G5G)[b]
19	46,XY	2.1	Sporadic	1370G>A/143delG	R457H/R48fsX63
20	46,XY	0.2	Sporadic	1370G>A/1665delG	R457H/Q555fsX612
21	46,XY	13.1	Sporadic	1370G>A/(−)[c]	R457H/DeltaExons 2–13[d]
22	46,XX	9.0	Sporadic	1370G>A/IVS7 + 1G>A	R457H/IVS7 + 1G>A
23	46,XX	14.8	Sporadic	1370G>A/1698-1699insC	R457H/Y567fsX574
24	46,XX	13.2	Sporadic	1370G>A/1329-1330insC	R457H/I444fsX449
25	46,XX	12.9	Familial-B	1370G>A/601C>T	R457H/Q201X
26	46,XX	6.6	Sporadic	1370G>A/(−)[c]	R457H/Non-transcribed[b]
27	46,XX	4.2	Sporadic	1370G>A/(−)[c]	R457H/Non-transcribed[b]
28	46,XX	17.0	Sporadic	1370G>A/1329-1330insC	R457H/I444fsX449
Group C: other compound heterozygotes					
29	46,XY	0.4	Sporadic	1370G>A/1386-1387insATCGCC	R457H/A462-S463insIA
30	46,XY	23.5	Familial-C (P)	1370G>A/1835-1858del[e]	R457H/L612-W620delinsR
31	46,XY	18.0	Familial-C	1370G>A/1835-1858del[e]	R457H/L612-W620delinsR
32	46,XY	17.9	Familial-D	1733A>G/1329-1330insC	Y578C/I444fsX449
33	46,XX	0.8	Sporadic	1370G>A/1738G>C	R457H/E580Q
34	46,XX	0.7	Sporadic	1370G>A/1042-1044delGTC	R457H/348delV
35	46,XX	0.5	Familial-D (P)	1733A>G/1329-1330insC	Y578C/I444fsX449

The genomic position corresponding to each mutation based on NC_000007.12 sequence at the National Center for Biotechnology Information database (Bethesda, MD) is as follows: R457H, 75452433G>A; Q201X, 75448386C>T; I444fsX449, 75452391-2insC; G5G, 75421261A>G; R48fsX63, 75421389delG; Q555fsX612, 75453099delG; IVS7 + 1G>A, 75448861G>A; Y567fsX574, 75453205-6insC; A462-S463insIA, 75452349-50insATCGCC; L612-W620delinsR, 75453432-55delTAAAGCAAGACCGAGAGCACCTGT; Y578C, 75453237A>G; E580Q, 75453245G>C; and 348delV, 75451086-88delGTC. Cases 1–3, 6–10, 15–18, 22–26, 29–33, and 35 have been reported previously (6, 8, 9), and the remaining 12 cases were first examined in this study. P, Proband.

[a] The A of the ATG encoding the initiator methionine residue of the predicted translation product is denoted position +1.

[b] The allele with G5G and the apparently normal alleles are not trasncribed into mRNA.

[c] The (−) symbol indicates the absence of a recognizable mutation on the exonic sequences.

[d] An intragenic microdeletion involving exons 2–13.

[e] 1835-1858delTAAAGCAAGACCGAGAGCACCTGT.

subjects of both sexes (854 males and 909 females), the M data of the patients were expressed as the SD score to allow for the comparison among patients of different sexes and ages.

Statistical analysis

Statistical significance of the frequency of clinical features was analyzed by the Fisher's exact probability test, and that of the median of nonpaired and paired variables was examined by the Mann-Whitney's *U* test and the Wilcoxon signed-rank test, respectively. $P < 0.05$ was considered significant.

Results

POR mutations

The results are summarized in Table 1. Direct sequencing revealed 12 types of mutations and one silent substitution (G5G) (Fig. 1A), with R457H being identified in 40 of the 58 alleles (~70%) in 25 sporadic cases and four probands of families A–D. Of the 12 mutations, R48fsX63, Q555fsX612, and 348delV were first identified in this study. These mutations were absent in 100 control subjects.

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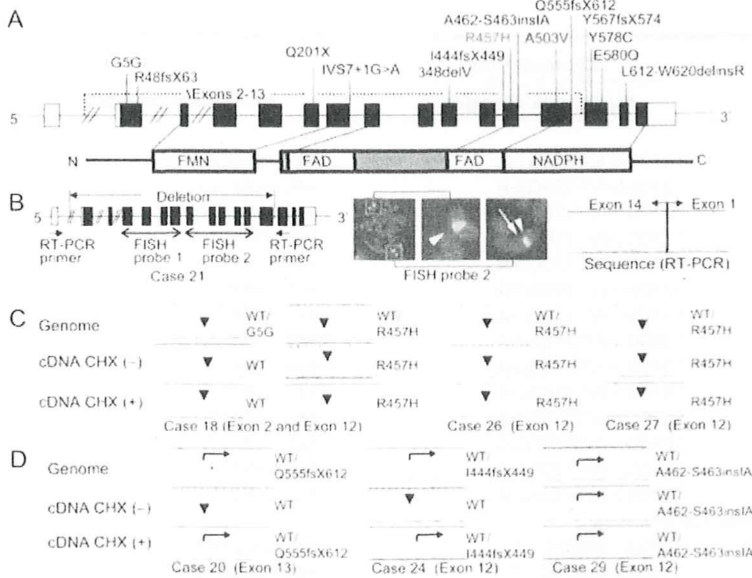

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FIG. 1. Mutation analysis of *POR*. A, Schematic representation of the *POR* gene and the positions of identified mutations. The Japanese founder mutation R457H is shown in *red*, other disease-causing mutations in *black*, and the common A503V variant in *blue*. *Upper diagram*, The genomic structure comprising 16 exons. The *black* and *white boxes* denote the coding and the untranslated regions, respectively. *Lower diagram*, The protein structure consisting of the cofactor binding domains (FMN: flavin mononucleotide; FAD: flavin-adenine dinucleotide; and NADPH: nicotinamide-adenine dinucleotide phosphate, reduced) and the connecting domain (*stippled area*). B, FISH and RT-PCR sequencing analyses in case 21. *Left diagram*, The positions of the two FISH probes and those of the primers for RT-PCR. *Middle diagram*, FISH findings showing two signals for *D7Z1* (*arrowheads*) and a single signal for *POR* (*arrow*) delineated by the FISH probe 2. *Right diagram*, RT-PCR sequencing indicating the fusion between exons 1 and 14 (the deletion of exons 2–13). C, Transcription failure in cases 18, 26, and 27. Although heterozygosity for R457H is delineated for the genomic DNA, RT-PCR sequencing indicates absent expression of the wild-type (WT) alleles in the three cases. Similarly, although heterozygosity for G5G is shown for the genomic DNA of case 18, RT-PCR sequencing reveals no expression of the G5G allele. Such lack of transcripts is not recovered by CHX. D, Nonsense-mediated mRNA decay in cases 20 and 24 but not case 29. Although heterozygosity for the mutations is shown for the genomic DNA, RT-PCR sequencing delineates the WT alleles only before CHX treatment and the heterozygosity after CHX treatment in cases 20 and 24. The NMD is not observed in case 29.

Fifteen cases were apparently homozygous for R457H, and hemizygosity was excluded in 14 of the 15 cases by parental analysis indicating heterozygosity for R457H in both parents (cases 1–3, 6–11, and 13) and by FISH analysis with two FISH probes (cases 4, 5, 12, and 14). Notably, however, FISH analysis delineated a heterozygous microdeletion in case 21, and RT-PCR sequencing analysis revealed loss of exons 2–13 in this case (Fig. 1B). The mother was heterozygous for R457H, and the father was heterozygous for the intragenic microdeletion.

Three cases were apparently heterozygous for R457H (cases 18, 26, and 27), although case 18 also had G5G. However, RT-PCR sequencing analysis using lymphoblastoid cell lines showed nearly complete absence of mRNA derived from the apparently normal alleles in the three cases (Fig. 1C). The mRNA remained undetected after CHX treatment, indicating transcription failure.

Of the 11 other types of mutations, the nonsense and four frameshift mutations (Q201X, R48fsX63, I444fsX449, Q555fsX612, and Y567fsX574) leading to premature termination and the conserved splice donor site mutation (IVS7+1G>A) appeared to be null mutations, whereas the remaining five mutations (Y578C, E580Q, 348delV, A462-S463insIA, and L612-W620delinsR) were unknown for residual activities. Indeed, RT-PCR sequencing analysis performed before and after CHX treatment in three cases with available lymphoblastoid cell lines demonstrated that the alleles carrying Q555fsX612 and I444fsX449 underwent NMD, whereas the allele harboring A462-S463insIA escaped NMD (Fig. 1D).

The common A503V variant was absent from cases of group A and was identified in four cases of group B (cases 22, 23, 26, and 27) and four cases of group C (cases 29–31, and 34). The eight cases with A503V were all compound heterozygotes with R457H and another mutation, and direct sequencing for subcloned PCR products encompassing both 457th and 503rd codons revealed lack of coexistence of R457H and A503V. Thus, it was indicated that the A503V variant was absent from all of the 47 alleles carrying R457H and was present on alleles carrying IVS7+1G>A, Y567fsX574, A462-S463insIA, L612-W620delinsR, and 348delV and on the two nontranscribed alleles.

Classification of the patients

On the basis of the above results, the 35 cases were classified into three groups: group A, homozygotes for R457H (cases 1–14); group B, compound heterozygotes for R457H and one apparently null mutation (cases 15–28); and group C, other types of compound heterozygotes (cases 29–35) (Table 1). The residual POR activity was predicted to be higher in group A than group B, although it was unknown for group C. In addition, group B was subclassified into A503V-positive cases (cases 22, 23, 26, and 27) and negative cases (cases 15–21, 24, 25, and 28).

Clinical features

The prevalence of each clinical feature in groups A–C is summarized in Table 2, together with its comparison between groups A and B. The sex ratio was similar between groups A and B, as was the median age. T2,AQ:D

ABS-compatible skeletal features were definitely more prevalent in group B than group A (Table 2 and supplementary Fig. 1, published as supplemental data on The Endocrine Society's Journals Online Web site at http://jcem.endojournals.org). In particular, severe brachycephaly, elbow joint synostosis, and choanal stenosis were exclusively identified in group B.

Adrenal steroidogenic dysfunction was biochemically identified in all cases, with some difference between groups A and B. Blood ACTH was normal or elevated at the baseline, 17-OHP was normal or elevated at the baseline and above the normal range after ACTH stimulation, and cortisol was normal at the baseline but barely responded to ACTH stimulation (Fig. 2A). Significant difference between groups A and B was identified for basal 17-OHP value ($P = 0.044$) and basal and ACTH-stimulated cortisol values ($P = 0.018$ and $P = 0.022$). Urine Ms of progesterone and 17-OHP were elevated, whereas those of an- F2

TABLE 2. The prevalence of each clinical feature in groups A–C and its comparison between groups A and B

	Group A (n = 14)	Group B (n = 14)	Group C (n = 7)	Groups A *vs.* B (*P* value)
Sex (male:female)	5:9	7:7	4:3	0.35
Age (median, range, yr)	4.0 (0.1–23.8)	13.1 (0.2–17.5)	0.8 (0.4–23.5)	0.19
Skeletal features				
Any skeletal feature	7/14	14/14	7/7	0.0029
Brachycephaly (overt)	0/14	14/14	6/7[a]	0.000000025
Elbow joint synostosis[b]	0/14	7/14	4/7	0.0029
Arachnodactyly (overt)	5/14	14/14	7/7	0.048
Choanal stenosis	0/14	5/14	1/7	0.020
Joint contracture	7/14	14/14	7/7	0.0029
Adrenal dysfunction				
Adrenal crisis	0/14	4/14	1/7[c]	0.049
Detection by mass screening[d]	5/8	3/8	2/4	0.31
46,XY DSD				
Any genital feature at birth	1/5[e]	3/7[f]	3/4	0.42
Hypospadias	0/5	2/7	1/4	0.32
Cryptorchidism	0/5	3/7	2/4	0.16
Micropenis	1/5	2/7	3/4	0.64
46,XX DSD				
Any genital feature at birth	9/9[e]	7/7[f]	3/3	1.0
Clitoromegaly	8/9	5/7	3/3	0.40
Labial fusion	8/9	5/7	2/3	0.40
Common urogenital sinus	2/9	2/7	0/3	0.61
Maternal virilization	8/14	5/14	4/7	0.22
Pubertal failure, 46,XY				
Delayed (>2 SD) or no pubertal sign	0/2[g]	3/4[h]	2/3	0.20
Small testis (<2 SD)	0/2	2/4	1/3	0.40
Primary hypogonadism[i]	0/2	2/2	3/3	0.17
Pubertal failure, 46,XX				
Delayed (>2 SD) or no pubertal sign	3/3[g]	4/4[h]		1.0
Delayed (>2 SD) or no menses	0/2[j]	2/2		0.17
Primary hypogonadism[i]	3/3	3/3		1.0
Polycystic ovary	4/9	3/6	1/3	0.62

The denominators indicate the number of patients examined for the presence or absence of each feature, and the numerators represent the number of patients assessed to be positive for that feature; thus, the differences between the denominators and numerators denote the number of patients evaluated to be negative for that feature.

[a] Severe craniosynostosis is absent in case 33 with two missense mutations.

[b] Humeroradial, humeroulnar, or radioulnar synostosis.

[c] Adrenal crisis has been manifested by case 35 with Y578C and I444fsX449.

[d] The measurement of 17-OHP in the mass screening for 21-hydroxylase deficiency has been performed since 1988 in Japan.

[e,f] DSD is more frequent in 46,XX cases than 46,XY cases in groups A ($P = 0.0050$) and B ($P = 0.035$).

[g,h] The *P* values between 46,XY and 46,XX cases are 0.19 for group A and 0.50 for group B.

[i] Elevated gonadotropins (LH and/or FSH) and/or decreased T or E_2, as compared with age- and sex-matched reference data.

[j] Only a few vaginal spottings.

drostenedione, 11-deoxycortisol, cortisol, and aldosterone grossly remained within the normal range (Fig. 2B). The M ratio indicating 17α-hydroxylase activity remained almost normal, consistent with the elevation of both substrates and products, whereas the M ratios indicating 17,20 lyase and 21-hydroxylase activities were grossly decreased. Significant difference between groups A and B was identified for MS of progesterone ($P = 0.044$), those of 17-OHP ($P = 0.022$), those of aldosterone ($P = 0.0084$), and M ratio indicating 17,20 lyase activity ($P = 0.011$). Adrenal crisis was observed only in group B with a significant difference between groups A and B, whereas the detection frequency of elevated 17-OHP in mass screening was similar between groups A and B (Table 2).

DSD was more prevalent in 46,XX cases than 46,XY cases in both groups A and B (Table 2, footnote, and supplementary Fig. 2). 46,XY DSD in group A was micropenis in one case, and that in group B included more severe phenotypes. By contrast, 46,XX DSD was invariably identified in both groups A and B. Maternal virilization during pregnancy was often found in groups A and B with a similar prevalence. Serum T of case 20, aged 0.2 yr in group B, was 6.5 and 7.6 nmol/liter (1.9 and 2.2 ng/ml) before and after hCG stimulation, respectively.

Pubertal development was apparently normal in two 46,XY cases of group A and one of four 46,XY cases in group B and was invariably affected in 46,XX cases in both groups A and B (Table 2). In family A of group A, cases 2 and 3 exhibited full pubertal development with testis volume of 20 ml, whereas case 10 had obvious pubertal failure with Tanner B2 stage. T value of case 18, aged 17.5 yr in group B, was low at the baseline (0.7 nmol/liter,

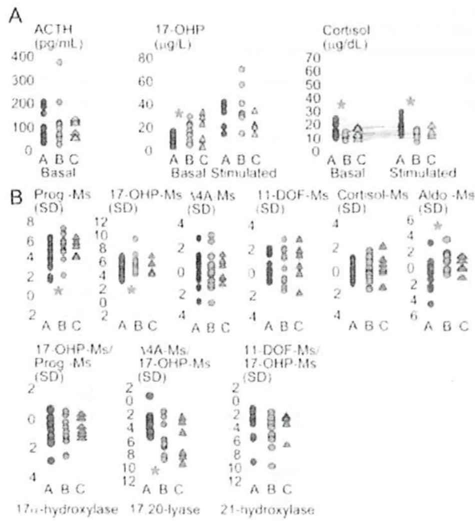

FIG. 2. Adrenal steroidogenic dysfunctions in groups A–C. *Light blue areas* represent the normal ranges. *Red asterisks* indicate the presence of significant differences between groups A and B. A, Basal and ACTH-stimulated blood hormone values. B, Basal urine steroid M values. Prog, Progesterone; Δ4A, androstenedione; 11DOF, 11-deoxycortisol; Aldo, aldosterone.

0.2 ng/ml) and poorly responded to hCG stimulation (1.0 nmol/liter, 0.3 ng/ml). PCO was observed in infantile or pubertal cases with a similar frequency between groups A and B, and cases 22 and 24 had ovarian torsion. Notably, bilateral ovarian cysts of case 10 markedly reduced in size after treatment with estradiol (E_2) (supplementary Fig. 3).

Long-term growth patterns were obtained in eight cases (Fig. 3). Whereas childhood heights tended to be high in both groups A and B, pubertal growth was different between the two groups. Cases in group A lacked obvious pubertal growth spurt but continued to grow for a long term, attaining tall adult heights, whereas those in group B showed rather compromised pubertal growth with worsening of scoliosis (supplementary Fig. 1).

There was no phenotypic difference between A503V-positive and -negative cases of group B (supplementary Table 2). In addition, the phenotypes in group C were grossly similar to those in group B (Table 2). In particular, craniosynostosis was identified in all cases except for case 33 with R457H and E580Q, and adrenal crisis was manifested by case 35 with Y578C and I444fsX449.

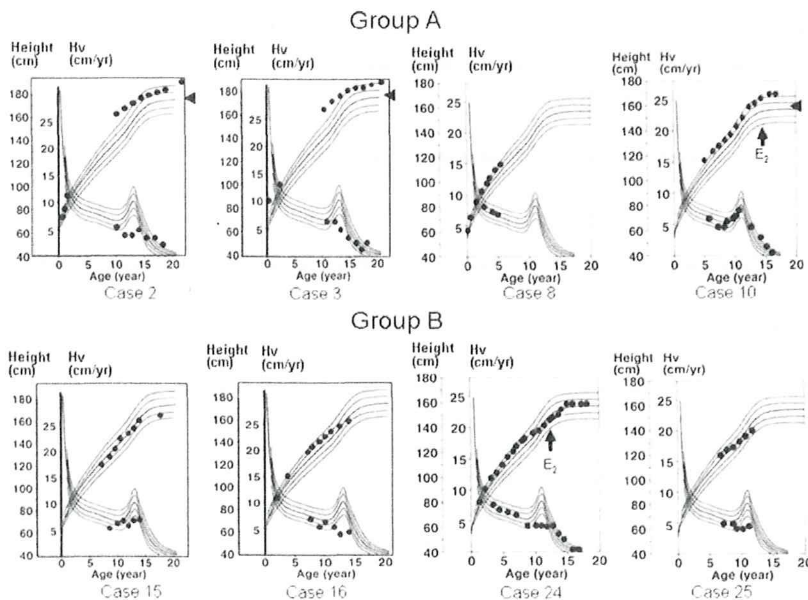

FIG. 3. Growth charts of eight cases plotted on the sex-matched longitudinal growth curves for the normal Japanese children (+2 SD, +1 SD, the mean, −1 SD, and −2 SD). The *triangles* in cases 2, 3, and 10 represent the target heights. Cases 10 and 24 are placed on E_2 replacement therapy. Hv, Height velocity.

Discussion

Molecular studies

Detailed molecular studies were performed in this study, providing two notable findings. First, all 35 cases were found to be homozygotes or compound heterozygotes for *POR* mutations including intragenic microdeletion and transcription failure. Because the microdeletion was found in case 21 with apparent R457H homozygosity, such a microdeletion might be hidden in the previously reported patients with apparent homozygosity (1, 5). Similarly, because transcription failure was invariably identified in cases 18, 26, and 27 with apparent heterozygosity, it may also underlie in the previously reported patients with apparent heterozygosity (4, 5, 10). In this regard, it is likely that the three cases carry a mutation in a hitherto unidentified *cis*-regulatory sequence(s) for the transcription of *POR*, as has been reported for several genes (24).

Second, RT-PCR sequence analysis indicated the occurrence of NMD in the two frameshift mutations (I444fsX449 and Q555fsX612). In this context, all the premature termination codons caused by the nonsense and the four frameshift mutations satisfy the positional conditions for the occurrence of NMD that functions as an mRNA surveillance mechanism to prevent the formation of aberrant proteins (13, 14). Thus, it is likely that the remaining three mutations (Q201X, R48fsX63, and Y567fsX574) are also null mutations subject to NMD *in vivo*.

Genotype-phenotype correlations

Genotype-phenotype correlations also provide several informative findings. Skeletal features were clearly different between groups A and B. Because cholesterol production in skeletal tissues is carried out in a simple one way manner (Fig. 4), this would explain why the skeletal phenotype is obviously dependent on the R457H dosage, reflecting the residual activity. It is likely that the threshold level for the development of severe skeletal phenotypes resides between a single copy and two copies of the R457H residual activity.

Adrenal steroidogenic dysfunction was grossly similar between groups A and B, although it was somewhat milder in group A than group B. Such a relatively minor role of R457H dosage in adrenal steroidogenesis

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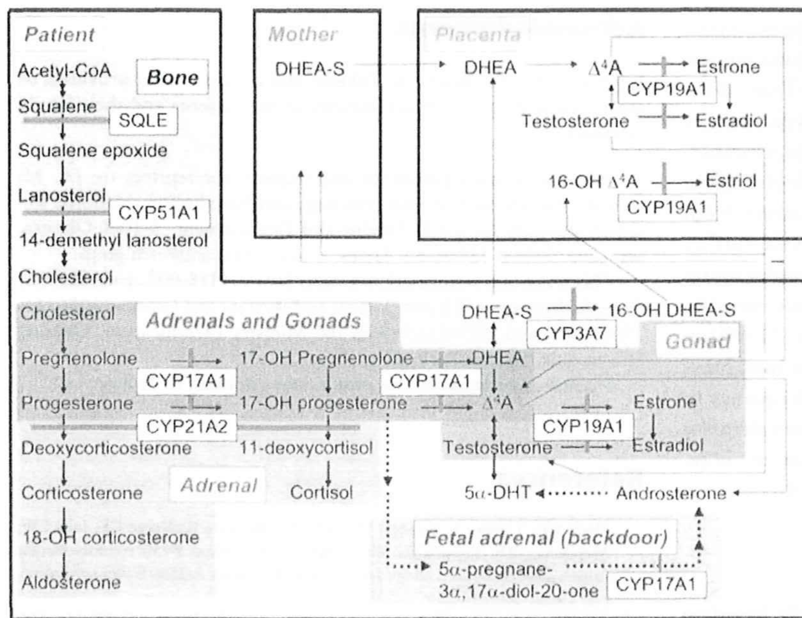

FIG. 4. Simplified schematic representation indicating impaired cholesterologenesis and steroidogenesis in PORD. DHEA, Dehydroepiandrosterone; DHEA-S, DHEA sulfate; Δ^4A, androstenedione; DHT, dihydrotestosterone. SQLE, CYP51A1, CYP17A1, CYP21A2, CYP19A1, and CYP3A7 are POR-dependent enzymes. The important Ms only are shown, and the reaction steps in which some Ms are omitted are indicated by two tandem *arrows*. Note that the amount of estriol synthesized in the placenta far exceeds the total amount of estrone and E_2 (~10 times) (34).

may primarily be due to the complexity of steroidogenesis in PORD (Fig. 4). For example, both production and degradation of 17-OHP are carried out by POR-dependent enzymes, and such enzymatic reactions would depend on the R457H dosage and the differential supporting activity of the R457H protein for target enzymes as well as the amount of substrates and products. Furthermore, the basal cortisol values imply that the baseline steroidogenic capacity can grossly be sustained, even in group B. Indeed, whereas basal blood 17-OHP values were significantly higher in group B than group A, some of them remained within the normal range, and several cases of both groups were not detected in neonatal mass screening. Nevertheless, the R457H dosage would have important clinical relevance, because the ACTH-stimulated blood cortisol was drastically reduced especially in group B, and adrenal crisis was observed only in group B. Furthermore, because 17,20 lyase activity alone was significantly different between groups A and B (Fig. 2B), this would provide further support for the previous finding that 17,20 lyase activity is the most sensitive index of defective POR activity (5, 15).

46,XY DSD was not so remarkable, whereas 46,XX DSD was invariably identified. This suggests a mildly reduced androgen production in genetic males and a definitely excessive androgen production in genetic females. In this context, there are three androgen sources during the fetal life in PORD, *i.e.* the fetal testis, backdoor pathway, and placenta (3, 4, 9, 25, 26) (Fig. 4). For fetal testicular T production specific to 46,XY cases, placental hCG-stimulated T production around the critical period for sex development would be more compromised in group B than group A because testicular T production is performed in a simple one-way manner, as in cholesterologenesis. Furthermore, because T responses to hCG stimulation were reduced, at least in the two examined cases of group B, this implies the compromised maximum T production capacity. By contrast, the backdoor- and placenta-derived androgen productions common to both 46,XY and 46,XX cases may be similar between groups A and B: 1) whereas 17-OHP as the source metabolite for the backdoor pathway is higher in group B than group A, the supporting activity for fetal adrenal CYP17A1 involved in the backdoor pathway would be lower in group B than group A; and 2) whereas fetal adrenal derived dehydroepiandrosterone as the source metabolite for placental androgens would be lower in group B than group A (4, 9, 25), the residual supporting activity for placental CYP19A1 would be lower in group B than group A. Thus, the total amount of androgens would be relatively well preserved in 46,XY cases with a mild difference in the fetal testis-derived T between groups A and B and invariably and similarly increased in 46,XX cases of both groups A and B. Furthermore, this notion explains why maternal virilization during pregnancy was similar between groups A and B because it is primarily due to androgens of the placental origin rather than the fetal gonadal or the backdoor origin (3, 4, 25).

Assessment of pubertal development was possible in a limited number of patients. However, pubertal development appeared to differ between groups A and B and between 46,XY and 46,XX cases. In this regard, T and E_2 biosynthesis during puberty is also performed in a simple one-way manner, and T production is mediated by CYP17A1 and E_2 production is mediated by both CYP17A1 and CYP19A1 (Fig. 4). Thus, gonadal steroid production would depend on the R457H dosage, with T production being less compromised than E_2 production. In addition, our observation suggests the frequent occurrence of PCO in infancy and puberty when gonadotropins are physiologically elevated (27) and the beneficial effect of estrogen replacement therapy in the amelioration of PCO.

Evaluation of growth pattern also remained fragmentary. However, two implications are possible. First, the intrinsic skeletal abnormalities may be relevant to the growth pattern. Indeed, relative tall stature in childhood may be compatible with the elongation of long bones as indicated by arachnodactyly and dolichostenomelia, and worsening of scoliosis during puberty in group B would also be consistent with the low POR activity (supplementary Fig. 1). Second, the spontaneous pubertal growth pattern of cases 2 and 3 without scoliosis is considered to represent a mild form of that of male patients with aromatase deficiency (28, 29). Such a qualitatively similar but quantitatively different pubertal growth pattern would be explained by assuming a drastically attenuated but not abolished *in vivo* supporting function of the R457H protein for aromatase.

Lastly, clinical features were similar between A503V-positive and -negative cases in group B. However, this would not argue

against a possible phenotypic effect of mildly hypomorphic A503V, because A503V of the four cases in group B was present on the alleles carrying apparently null mutations. Thus, it remains unknown whether A503V can modify phenotypic features in PORD, although the previous study argues against a modifying effect of A503V on clinical phenotypes in 21-hydroxylase deficiency (30). Furthermore, because A503V was absent from all of 47 alleles carrying R457H, this would provide further support for the previous notion that R457H is a founder mutation accompanied by a specific haplotype (6, 7). Thus, whereas A503V was identified in only eight of the 70 alleles (11.4%) in this study, this frequency is obviously biased by the high prevalence of R457H in Japanese patients. Rather, the frequency of A503V in R457H-negative alleles suggests that the prevalence of A503V is considerably high in the Japanese population, as reported in other populations (from 19.1% in African American to 36.7% in Chinese American) (15).

Remarks and conclusion

It should be pointed out that the results are totally based on the studies of Japanese patients. In this regard, A287P is common in Caucasian patients (4, 5), and clinical studies in 10 A287P-positive patients including three homozygotes (five with 46,XY and five with 46,XX) have suggested phenotypic similarities and differences between R457H-positive patients and A287P-positive patients: 1) skeletal phenotype is usually obvious and appears to be grossly dependent on the A287P dosage; 2) 46,XY DSD is variable and is apparently independent of the A287P dosage; 3) 46,XX DSD is also variable and absent in one A287P homozygote and one of four compound heterozygotes with A287P; and 4) maternal virilization during pregnancy is not described (1, 2, 5, 31, 32). Thus, skeletal phenotype would be explained by assuming that both R457H and A287P have drastically lost supporting activities for CYP51A1 and/or SQLE involved in cholesterologenesis, although functional studies have not been performed. Furthermore, clinical features relevant to steroidogenic dysfunction would be grossly consistent with the previous *in vitro* functional data. It has been reported that R457H yields only 1–3% supporting activities for 17α-hydroxylase and aromatase, and virtually no activity for 17,20 lyase, whereas A287P provides supporting activities of about 40% for 17α-hydroxylase, about 20% for 17,20 lyase, about 70% for 21-hydroxylase, and about 100% for aromatase (1, 5, 11, 33). Thus, the relative activities of frontdoor and backdoor pathways would be different largely between R457H-positive and A287P-positive patients, and placental T production would remain minor, if any, in A287P-positive patients. Collectively, the Japanese data would not apply simply to other populations.

In conclusion, the present study in Japanese patients argues against the heterozygote manifestation and suggests that the residual POR activity reflected by the R457H dosage constitutes the underlying factor for the clinical variability in some features but not other features, probably because of the simplicity and the complexity of the POR-dependent metabolic pathways relevant to each phenotype. Further studies including genotype-phenotype analyses in various ethnic groups will permit a better clarification of the molecular and clinical characteristics of PORD.

Acknowledgments

We thank Drs. T. Ohashi, R. Takeda, and I. Fujiwara for providing us with clinical data and blood samples of the patients and their family members.

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This work was supported by Grants 20C-2, H18-005, and H20-004 from the Ministry of Health, Labor, and Welfare and Grants 16086215, 19390290, and 20390265 from the Ministry of Education, Culture, Sports, Science, and Technology.

Disclosure Summary: The authors have nothing to declare.

References

1. Flück CE, Tajima T, Pandey AV, Arlt W, Okuhara K, Verge CF, Jabs EW, Mendonca BB, Fujieda K, Miller WL 2004 Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. Nat Genet 36:228–230
2. Arlt W, Walker EA, Draper N, Ivison HE, Ride JP, Hammer F, Chalder SM, Borucka-Mankiewicz M, Hauffa BP, Malunowicz EM, Stewart PM, Shackleton CH 2004 Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. Lancet 363:2128–2135
3. Miller WL 2004 P450 oxidoreductase deficiency: a new disorder of steroidogenesis with multiple clinical manifestations. Trends Endocrinol Metab 15: 311–315
4. Scott RR, Miller WL 2008 Genetic and clinical features of P450 oxidoreductase deficiency. Horm Res 69:266–275
5. Huang N, Pandey AV, Agrawal V, Reardon W, Lapunzina PD, Mowat D, Jabs EW, Van Vliet G, Sack J, Fluck CE, Miller WL 2005 Diversity and function of mutations in P450 oxidoreductase in patients with Antley-Bixler syndrome and disordered steroidogenesis. Am J Hum Genet 76:729–749
6. Fukami M, Horikawa R, Nagai T, Tanaka T, Naiki Y, Sato N, Okuyama T, Nakai H, Soneda S, Tachibana K, Matsuo N, Sato S, Homma K, Nishimura G, Hasegawa T, Ogata T 2005 Cytochrome P450 oxidoreductase gene mutations and Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. J Clin Endocrinol Metab 90:414–426
7. Adachi M, Asakura Y, Matsuo M, Yamamoto T, Hanaki K, Arlt W 2006 POR R457H is a global founder mutation causing Antley-Bixler syndrome with autosomal recessive trait. Am J Med Genet A 140:633–635
8. Fukami M, Hasegawa T, Horikawa R, Ohashi T, Nishimura G, Homma K, Ogata T 2006 Cytochrome P450 oxidoreductase deficiency in three patients initially regarded as having 21-hydroxylase deficiency and/or aromatase deficiency: diagnostic value of urine steroid hormone analysis. Pediatr Res 59: 276–280
9. Homma K, Hasegawa T, Nagai T, Adachi M, Horikawa R, Fujiwara I, Tajima T, Takeda R, Fukami M, Ogata T 2006 Urine steroid hormone profile analysis in cytochrome P450 oxidoreductase deficiency: implication for the backdoor pathway to dihydrotestosterone. J Clin Endocrinol Metab 91:2643–2649
10. Scott RR, Gomes LG, Huang N, Van Vliet G, Miller WL 2007 Apparent manifesting heterozygosity in P450 oxidoreductase deficiency and its effect on coexisting 21-hydroxylase deficiency. J Clin Endocrinol Metab 92:2318–2322
11. Pandey AV, Kempna P, Hofer G, Mullis PE, Flück CE 2007 Modulation of human CYP19A1 activity by mutant NADPH P450 oxidoreductase. Mol Endocrinol 21:2579–2595
12. Agrawal V, Huang N, Miller WL 2008 Pharmacogenetics of P450 oxidoreductase: effect of sequence variants on activities of CYP1A2 and CYP2C19. Pharmacogenet Genomics 18:569–576
13. Carter MS, Doskow J, Morris P, Li S, Nhim RP, Sandstedt S, Wilkinson MF 1995 A regulatory mechanism that detects premature nonsense codons in T-cell receptor transcripts *in vivo* is reversed by protein synthesis inhibitors *in vitro*. J Biol Chem 270:28995–29003
14. Kuzmiak HA, Maquat LE 2006 Applying nonsense-mediated mRNA decay research to the clinic: progress and challenges. Trends Mol Med 12:306–316
15. Huang N, Agrawal V, Giacomini KM, Miller WL 2008 Genetics of P450 oxidoreductase: sequence variation in 842 individuals of four ethnicities and activities of 15 missense mutations. Proc Natl Acad Sci USA 105:1733–1738
16. Homma K, Hasegawa T, Masumoto M, Takeshita E, Watanabe K, Chiba H,

Kurosawa T, Takahashi T, Matsuo N 2003 Reference values for urinary steroids in Japanese newborn infants: gas chromatography/mass spectrometry in selected ion monitoring. Endocr J 50:783–792
17. Matsuo N 1993 Skeletal and sexual maturation in Japanese children. Clin Pediatr Endocrinol 2(Suppl):1–4
18. Fujieda K, Matsuura N 1987 Growth and maturation in the male genitalia from birth to adolescence. I: change of testicular volume; II: change of penile length. Acta Paediatr Jpn 29:214–223
19. Yokoya S, Kato K, Suwa S 1983 Penile and clitoral sizes in premature newborns, full term neonates, infants, and children. Horumon to Rinsho 31:1215–1220 (in Japanese)
20. Suwa S, Tachibana K, Maesaka H, Tanaka T, Yokoya S 1992 Longitudinal standards for height and height velocity for Japanese children from birth to maturity. Clin Pediatr Endocrinol 1:5–13
21. Lashansky G, Saenger P, Fishman K, Gautier T, Mayes D, Berg G, Di Martino-Nardi J, Reiter E 1991 Normative data for adrenal steroidogenesis in a healthy pediatric population: age- and sex-related changes after adrenocorticotropin stimulation. J Clin Endocrinol Metab 73:674–686
22. Lashansky G, Saenger P, Dimartino-Nardi J, Gautier T, Mayes D, Berg G, Reiter E 1992 Normative data for the steroidogenic response of mineralocorticoids and their precursors to adrenocorticotropin in a healthy pediatric population. J Clin Endocrinol Metab 75:1491–1496
23. Hasegawa Y 2003 Normal range of GnRH and hCG stimulation tests. In: Let's enjoy pediatric endocrinology. 3rd ed. Tokyo: Sindan-to-tiryou-sha; 260–262 (in Japanese)

AQ: E

24. Kleinjan DJ, van Heyningen V 1998 Position effect in human genetic disease. Hum Mol Genet 7:1611–1618
25. Shackleton C, Marcos J, Arlt W, Hauffa BP 2004 Prenatal diagnosis of P450 oxidoreductase deficiency (ORD): a disorder causing low pregnancy estriol, maternal and fetal virilization, and the Antley-Bixler syndrome phenotype. Am J Med Genet A 129:105–112
26. Auchus RJ 2004 The backdoor pathway to dihydrotestosterone. Trends Endocrinol Metab 15:432–438
27. Achermann JC, Hughes, IA 2008 Disorders of sex development. In: Kronenberg HM, Melmed M, Polonsky KS, Larsen PR, eds. Williams textbook of endocrinology. 11th ed. Philadelphia: Saunders; 783–848
28. Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K 1995 Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 80:3689–3698
29. Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS, Simpson ER 1997 Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med 337:91–95
30. Gomes LG, Huang N, Agrawal V, Mendonça BB, Bachega TA, Miller WL 2008 The common P450 oxidoreductase variant A503V is not a modifier gene for 21-hydroxylase deficiency. J Clin Endocrinol Metab 93:2913–2916
31. Wudy SA, Hartmann MF, Draper N, Stewart PM, Arlt W 2004 A male twin infant with skull deformity and elevated neonatal 17-hydroxyprogesterone: a prismatic case of P450 oxidoreductase deficiency. Endocr Res 30:957–964
32. Williamson L, Arlt W, Shackleton C, Kelley RI, Braddock SR 2006 Linking Antley-Bixler syndrome and congenital adrenal hyperplasia: a novel case of P450 oxidoreductase deficiency. Am J Med Genet A 140A:1797–1803
33. Dhir V, Ivison HE, Krone N, Shackleton CH, Doherty AJ, Stewart PM, Arlt W 2007 Differential inhibition of CYP17A1 and CYP21A2 activities by the P450 oxidoreductase mutant A287P. Mol Endocrinol 21:1958–1968
34. Sarda IR, Gorwill RH 1976 Hormonal studies in pregnancy. I. Total unconjugated estrogens in maternal peripheral vein, cord vein, and cord artery serum at delivery. Am J Obstet Gynecol 124:234–238

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Received 21 August 2009; Accepted 16 July 2009

Campomelic dysplasia (CD; OMIM 114290) is a rare skeletal disorder characterized by hypoplastic scapulae, 11 pairs of ribs, pelvic abnormalities, and bowing of the lower limb bones [Maroteaux et al., 1971]. Affected patients often die shortly after birth due to respiratory distress, and roughly two-thirds of affected genetic males have disorders of sex development (DSD) due to dysgenetic testes [Mansour et al., 1995]. Acampomelic campomelic dysplasia (ACD) is associated with similar but milder skeletal features and lacks long bone curvature [MacPherson et al., 1989].

SOX9 on chromosome 17q24 is a member of SRY-related gene family [Harley et al., 2003]. It encodes a 509-amino acid protein that harbors a high mobility group (HMG) domain with a DNA-binding capacity and a proline/glutamine/serine-rich domain with a transactivation function [Harley et al., 2003]. Furthermore, putative *cis*-control elements have been mapped within the 1 Mb region upstream of *SOX9* [Hill-Harfe et al., 2005].

To date, it has been shown that both CD and ACD can be caused by heterozygous intragenic *SOX9* mutations or chromosomal aberrations (translocations, inversions, or deletions) affecting *SOX9* or the putative enhancer region [Pfeifer et al., 1999; Thong et al., 2000; Moog et al., 2001; Harley et al., 2003; Pop et al., 2004; Leipoldt et al., 2007]. However, the frequency and the type of mutations and chromosomal aberrations are quite different

Wada Y, Nishimura G, Nagai T, Sawai H, Yoshikata M, Miyagawa S, Hanita T, Sato S, Hasegawa T, Ishikawa S, Ogata T. 2009. Mutation analysis of *SOX9* and single copy number variant analysis of the upstream region in eight patients with campomelic dysplasia and acampomelic campomelic dysplasia.

Am J Med Genet Part A 9999:1–4.

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Published online 00 Month 2009 in Wiley InterScience (www.interscience.wiley.com)
DOI 10.1002/ajmg.a.33107

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AJMA-08-0591.R2(33107)

	Campomelic dysplasia				Acampomelic campomelic dysplasia			
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Patient								
Gastational age (weeks)	25	42	38	38	39	40	42	38
Birth weight (g)	625	2490	2670	2060	3400	2700	2680	2306
Present age (y:m)	Stillbirth	0:11	(0:5)[a]	(1:5)[a]	11:6	19:8	3:2	3:9
Karyotype	46,XY	46,XX	46,XX	46,XX	46,XY	46,XY	46,XX	46,XX
Phenotype								
Cleft palate	−	−	+	−	+	+	+	+
Micrognathia	+	+	+	+	+	−	−	+
Scapular hypoplasia	+	+	+	+	+	+	+	+
Tibial bowing	+	+	+	+	−	−	−	−
Femoral bowing	+	+	+	+	−	−	−	−
11 pairs of ribs	−	+	+	+	−	+	+	+
Small thoracic cage	+	+	+	+	+	+	+	−
NM thoracic pedicles	+	+	+	+	−	−	+	+
Scoliosis	−	−	−	−	+	+	+	−
Narrow iliac wings	±	+	+	+	±	±	±	+
Clubfeet	+	+	+	+	−	−	−	+
46,XY DSD	+	−	−	−	−	−	−	−
Mutation								
cDNA	771_772insGGCGC	1330_1333delGACC	T338C	G442T	C509T	−	−	−
Amino acids	G257fsX296	T443fsX468	M113T	E148X	P170L	−	−	−

NM: non-mineralized; DSD: disorders of sex development.
[a]Deceased at 5 months and 1 year and 5 months, respectively.

between CD and ACD. CD is predominantly caused by nonsense or frameshift mutations or by chromosomal aberrations disrupting *SOX9*, although missense mutations and chromosomal aberrations impairing the enhancer region are also occasionally identified. By contrast, ACD is almost exclusively caused by missense mutations or by chromosomal aberrations affecting the enhancer region. Thus, while missense mutations are exclusively identified within the HMG box in both CD and ACD [Kwok et al., 1995; Cameron and Sinclair, 1997; Meyer et al., 1997; Hageman et al., 1998; Moog et al., 2001; Thong et al., 2000], these findings imply that severe mutations usually result in CD whereas mild mutations usually lead to ACD.

However, the underlying causes remain to be determined in several patients, especially those with ACD, and such patients may have hidden perturbation in the putative enhancer region. Thus, we performed mutation analysis of *SOX9* in eight patients with CD or ACD and single copy number variant (CNV) analysis [Redon et al., 2006] of the upstream region in *SOX9* mutation negative patients.

Clinical features of the eight patients are summarized in Table I, and representative roentgenograms are shown in Figure 1. Patients 1–4 showed CD-compatible severe clinical features, whereas patients 5–8 exhibited relatively mild ACD-compatible clinical features. In addition, patient 1 ended in a stillbirth, and patients 3 and 4 died of respiratory insufficiency during infancy, although patient 2 aged 11 months was alive. By contrast, patients 5–8 have survived a relatively long period. Among genetic males, patient 1 exhibited DSD with nearly complete female external genitalia, while patients 5 and 6 showed male external genitalia.

We first performed mutation analysis of *SOX9*. This study was approved by the Institutional Review Board Committees at National Center for Child Health and Development, and performed after obtaining written informed consent. Genomic DNA samples

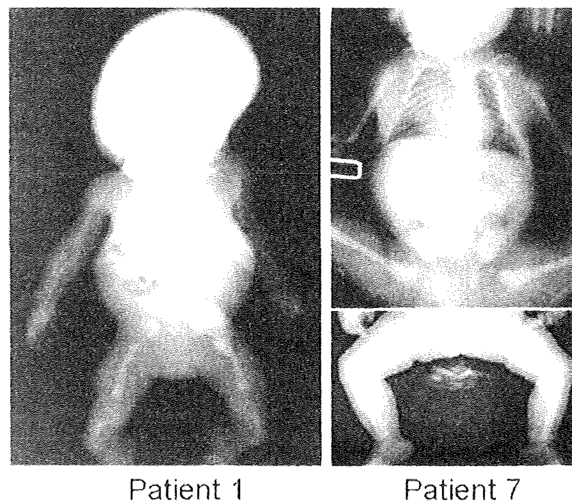

FIG. 1. Representative roentgenograms indicating CD in patient 1 at birth and ACD in patient 7 at 3 months of age.

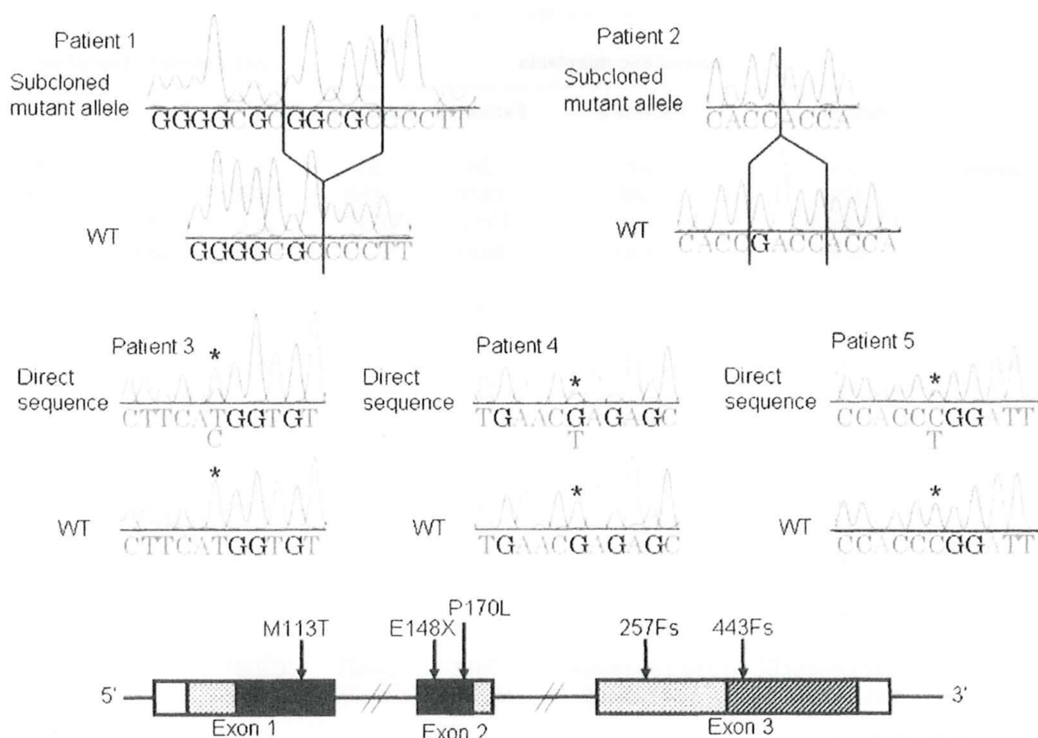

FIG. 2. Molecular findings in patients 1–5 with *SOX9* mutations. Upper part: Electrochromatograms showing the mutations in patients 1–5. In patients 1 and 2, the subcloned mutant alleles and the corresponding wildtype (WT) alleles are shown. In patients 3–5, the direct sequences are shown, together with the corresponding wildtype sequences; the asterisks indicate the mutant and the corresponding wildtype nucleotides. Lower part: The position of the mutations on the genomic sequences. Exons 1–3 are depicted with boxes; the black, the striped, the stippled, and the white areas indicate the HMG domain, the transactivation domain, other translated regions, and the untranslated regions, respectively.

extracted from cord blood cells (patient 1) or peripheral blood cells (patients 2–8) were amplified by PCR for all the three coding exons and were subjected to direct sequencing on a CEQ 8000 autosequencer (Beckman Coulter, Fullerton, CA) (the primer sequences are available on request). To confirm frameshift mutations, the corresponding PCR products were subcloned with TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA) and normal and mutant alleles were sequenced separately.

Consequently, we identified a novel heterozygous 5-bp insertion mutation at exon 3 that is predicted to cause a frameshift at the 257th glycine codon and resultant termination at the 296th codon (G257fsX296) in patient 1, a novel heterozygous 4-bp deletion mutation at exon 3 that is predicted to cause a frameshift at the 443rd threonine codon and resultant termination at the 468th codon (T443fsX468) in patient 2, a novel heterozygous missense mutation at exon 1 (M113T) in patient 3, a recurrent heterozygous nonsense mutation at exon 2 (E148X) in patient 4, and a novel heterozygous missense mutation at exon 2 (P170L) in patient 5 (Fig. 2). The two missense mutations resided within the HMG. The mutations of patients 1–4 were absent in their parents. In addition, while mutation analysis was refused by the parents of patient 5, the P170L missense mutation was absent in 200 control subjects. No mutations were identified in patients 6–8.

Then, to examine for a small deletion, we carried out the whole genome CNV analysis in patients 6–8 and their parents, using custom high density oligonucleotide microarray based on Affymetrix platform [Redon et al., 2006]. In brief, 25 bp oligonucleotide probes are designed on 1,330,354 *Nsp* I restriction fragments with average and median spacing of 2,271 and 776 bp. The experimental protocol is the same as the Affymetrix 500K arrays. Ninety microgram of target was hybridized overnight to the arrays [Fujii et al., 2007]. The signal intensity ratio of the sample to reference was calculated by Genome Imbalance Map Algorithm [Ishikawa et al., 2005], using NA10851 HapMap DNA samples from Coriell Cell Repositories (Camden, NJ) as the reference samples. Consequently, no deletion was indicated in the whole genome including the 5′ region of *SOX9* in patients 6–8.

The results are primarily consistent with the previous data. Three of four patients with CD died during fetal life or infancy, whereas patients 5–8 with ACD survived into childhood or puberty. 46,XY with DSD was observed in patient 1 with CD but not in patients 5 and 6 with ACD. Similarly, truncating mutations of *SOX9* were identified in patients 1–3 with CD, together with a missense mutation in patient 4 with CD, whereas only one missense mutation was found in patients with ACD.

We could not detect a microdeletion in patients 6–8 with ACD in whom no intragenic mutations were identified. Although the underlying causes remain to be clarified in patients 6–8, there are several possible explanations for the development of ACD in patients 6–8. First, a mutation(s) may exist in the unexamined intronic or the downstream region. Second, a tiny deletion may remain undetected. Third, there may be a mutation in some gene(s) other than *SOX9*. Further studies will identify underlying mechanisms involved in the development of ACD in *SOX9* mutation negative patients.

Cameron FJ, Sinclair AH. 1997. Mutation in SRY and SO X9: Testis-determining genes. Hum Mutat 9:388–395.

Fujii K, Ishikawa S, Uchikawa H, Komura D, Shapero MH, Shen F, Hung J, Arai H, Tanaka Y, Sasaki K, Kohno Y, Yamada M, Jones KW, Aburatani H, Miyashita T. 2007. High-density oligonucleotide array with sub-kilobase resolution reveals breakpoint information of submicroscopic deletions in nevoid basal cell carcinoma syndrome. Hum Genet 122:459–466.

Hageman RM, Cameron FJ, Sinclair AH. 1998. Mutation analysis of the SOX9 gene in a patient with campomelic dysplasia[Q1]. Hum Mutat S112–S113.

Harley VR, Clarkson MJ, Argentaro A. 2003. The molecular action and regulation of the testis-determining factors, SRY (sex-determining region on the Y chromosome) and SOX9 [SRY-related high-mobility group (HMG) box 9]. Endocr Rev 24:466–487.

Hill-Harfe KL, Kaplan L, Stalker HJ, Zori RT, Pop R, Scherer G, Wallace MR. 2005. Fine mapping of chromosome 17 translocation breakpoints >900 kb upstream of *SOX9* in acampomelic campomelic dysplasia and a mild, familial skeletal dysplasia. Am J Hum Genet 76:663–671.

Ishikawa S, Komura D, Tsuji S, Nishimura K, Yamamoto S, Panda B, Huang J, Fukayama M, Jones KW, Aburatani H. 2005. Allelic dosage analysis with genotyping microarrays. Biochem Biophys Res Commun 333:1309–1314.

Kwok C, Weller PA, Guioli S, Foster JW, Mansour S, Zuffardi O, Punnett HH, Dominguez-Steglich MA, Brook JD, Young ID, Goodfellow PN, Schafer AJ. 1995. Mutations in SOX9, the gene responsible for Campomelic dysplasia and autosomal sex reversal. Am J Hum Genet 57: 1028–1036.

Leipoldt M, Erdel M, Bien-Willner GA, Smyk M, Theurl M, Yatsenko SA, Lupski JR, Lane AH, Shanske AL, Stankiewicz P, Schere G. 2007. Two novel translocation breakpoints upstream of SOX9 define borders of the proximal and distal breakpoint cluster region in campomelic dysplasia. Clin Genet 71:67–75.

MacPherson RI, Skinner SA, Donnenfeld AE. 1989. Acampomelic campomelic dysplasia. Pediatr Radiol 20:90–93.

Mansour S, Hall CM, Pembery ME, Young ID. 1995. A clinical and genetic study of campomelic dysplasia. J Med Genet 32:145–420.

Maroteaux P, Spranger J, Opitz J, Kucera J, Lowry R, Schimke R, Kagan S. 1971. Le syndrome campomelique. Presse Med 22:1157–1162.

Meyer J, Südbeck P, Held M, Wagner T, Schmitz ML, Bricarelli FD, Eggermont E, Friedrich U, Haas OA, Kobelt A, Leroy JG, Van Maldergem L, Michel E, Mitulla B, Pfeiffer RA, Schinzel A, Schmidt H, Scherer G. 1997. Mutational analysis of the SOX9 gene in campomelic dysplasia and autosomal sex reversal: Lack of genotype/phenotype correlations. Hum Mol Genet 6:91–98.

Moog U, Jansen NJ, Scherer G, Schrander-Stumpel CT. 2001. Acampomelic campomelic syndrome. Am J Med Genet 104:239–245.

Pfeifer D, Kist R, Dewar K, Devon K, Lander ES, Brirren B, Korniszewski L, Back E, Scherer G. 1999. Campomelic dysplasia translocation breakpoints are scattered over 1 Mb proximal to *SO* X9: Evidence for an extended control region. Am J Hum Genet 65:111–124.

Pop R, Conz C, Lindenberg KS, Blesson S, Schmalenberger B, Briault S, Pfeifer D, Scherer G. 2004. Screening of the 1 Mb SOX9 5' control region by array CGH identifies a large deletion in a case of campomelic dysplasia with XY sex reversal. J Med Genet 41:e47.

Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, González JR, Gratacòs M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. 2006. Global variation in copy number in the human genome. Nature 444:444–454.

Thong MK, Scherer G, Kozlowski K, Haan E, Morris L. 2000. Acampomelic campomelic dysplasia with SOX9 mutation. Am J Med Genet 93:421–425.

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ORIGINAL RESEARCH

Abnormal Basiocciput Development in CHARGE Syndrome

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BACKGROUND AND PURPOSE: The causative gene of the common congenital malformation referred to as CHARGE syndrome is *CHD7*. Affected individuals often undergo head and neck imaging to assess abnormalities of the olfactory structures, hypothalamus-pituitary axis, and inner ear. We encountered a few children with severe hypoplasia of the basiocciput during a radiologic assessment of patients with CHARGE syndrome. To our knowledge, this anomaly has not been reported. Our purpose was to evaluate the incidence and severity of this anomaly in this syndrome.

MATERIALS AND METHODS: Sagittal MR images of 8 patients with CHARGE syndrome were retrospectively reviewed by 2 radiologists who consensually evaluated the status of the basiocciput of the patients with CHARGE syndrome, as either normal or hypoplastic, and associated anomalies, which include basilar invagination, Chiari type I malformation, and syringomyelia, as either present or absent. The length between the basion (Ba) and the endo-sphenobasion (Es) and between the basion and the exo-sphenobasion (Xs) was measured on midsagittal MR images of the 8 patients and 70 age-matched controls. We searched for trends related to age in the length of Ba-Es and Ba-Xs of the control children by using a matched *t* test.

RESULTS: Basioccipital hypoplasia was identified in 7 of the 8 patients with CHARGE syndrome and was severe in 6. Of those, 5 had associated basilar invagination and 1 had Chiari type I malformation with syringomyelia.

CONCLUSIONS: Basioccipital hypoplasia and basilar invagination are prevalent in patients with CHARGE syndrome.

CHARGE syndrome is a widespread malformation that was originally described independently by Hall [1] and Hittner et al.[2] The mnemonic acronym CHARGE represents the major anomalies associated with the disorder: coloboma of the eye, heart defects, choanal atresia, retarded growth and development, genital hypoplasia, and ear anomalies.[3] Other cardinal features include facial palsy or facial asymmetry, anomalies of the inner ear and laryngotracheoesophagus, anosmia, hypogonadotropic hypogonadism, and orofacial clefts.[3-6] The phenotypic diversity of affected individuals has raised the notion that CHARGE is not a genuine syndrome but an association of various anomalies occurring in a nonrandom but inconsistent fashion. However, this hypothesis has been disputed by the recent discovery of a CHARGE syndrome gene, *CHD7*, which encodes the chromodomain helicase deoxyribonucleic acid (DNA)-binding protein 7.[7] Nevertheless, locus heterogeneity of CHARGE syndrome might exist because only 60%–70% of patients with CHARGE syndrome have *CHD7* mutations.[8,9] Therefore, a more discriminating syndromic delineation and further studies of the genotype-phenotype correlation are required to understand this syndrome thoroughly.

Malformations of the inner ear, olfactory structures, and hypothalamus/pituitary axis in patients with CHARGE syndrome have usually been evaluated by using imaging techniques.[10-12] We encountered a few children with severe hypoplasia of the basiocciput during a radiologic assessment of patients with CHARGE syndrome. Basiocciput hypoplasia results in shortening of the clivus and is always associated with basilar invagination.[13] There is an increased prevalence of neural dysgenesis, such as the Chiari malformation or syringohydromyelia, reported to occur in 25%–35% of patients with basilar invagination.[14] Here, we evaluated the incidence and severity of basioccipital hypoplasia in CHARGE syndrome.

Received August 6, 2008; accepted after revision September 25

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DOI 10.3174/ajnr.A1380

Materials and Methods

Patients with CHARGE Syndrome

We retrospectively reviewed 8 patients who were diagnosed with CHARGE syndrome according to the clinical criteria of Blake et al (Table 1),[15] in which patients with all 4 major criteria or with 3 major and 3 minor criteria are considered to have definitive CHARGE syndrome, whereas those with 1 or 2 major criteria and several minor criteria possibly have the syndrome. Five of our patients had definitive and 3 had possible CHARGE syndrome. Patients 3 and 5 fulfilled 2 major and 5 minor criteria, and patient 8 fulfilled 2 major and 3 minor criteria. Our institutional review board did not require its approval or informed consent for the retrospective evaluation of patients' records and images. High-performance liquid chromatography DNA screening[16] and direct sequencing proved that 7 of the 8 patients harbored heterozygous mutations in *CHD7* (Table 2).

Controls

Seventy age-matched controls comprising 10 individuals per group, 3, 6, 7, 8, 9, 11, and 21 years of age, were randomly selected from patients with normal findings on MR imaging at our hospital between 2006 and 2008. Patients with known or suggested abnormalities in-

Table 1: Diagnostic criteria of CHARGE syndrome according to Blake et al[15]

Criteria	
Major	
Coloboma	Coloboma of iris, retina, choroid, disk, microphthalmia
Choanal atresia	Unilateral/bilateral, membranous/bony, stenosis/atresia
Characteristic ear abnormalities	External ear (loop or cup-shaped), middle ear (ossicular malformations, chronic serous otitis), mixed deafness, cochlear defects
Cranial nerve dysfunction	I, Anosmia; VII, facial palsy (unilateral or bilateral); VIII, sensorineural deafness and vestibular problems; IX and/or X, swallowing problems
Minor	
Genital hypoplasia	Males: micropenis, cryptorchidism; Females: hypoplastic labia; Both: delayed incomplete pubertal development
Developmental delay	Delayed motor milestones, hypotonia, mental retardation
Cardiovascular malformations	All types: conotruncal defects (eg, tetralogy of Fallot), arteriovenous canal defects, and aortic arch anomalies
Growth deficiency	Short stature
Orofacial cleft	Cleft lip and/or palate
Tracheoesophageal fistula	Tracheoesophageal defects of all types
Distinctive face	Characteristic facial features

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volving the skull base or bone marrow were excluded. Patients with systemic disease or who had undergone previous radiation or chemotherapy were also excluded.

Image Analysis of the Basiocciput

Sagittal MR images were obtained from all patients and all control children by using a 1.5T scanner. The images were reviewed on a PACS workstation. The length between the basion (Ba) and the endo-sphenobasion (Ba-Es) and between the Ba and the exo-sphenobasion (Ba-Xs) was measured in accordance with the definitions of Ba, Es, and Xs on midsagittal images (Fig 1). The definitions of Ba, Es, and Xs are the midpoint on the anterior margin of the foramen magnum, the anteriormost midpoint on the dorsal aspect of the basiocciput, and the anteriormost midpoint on the ventral aspect of the basiocciput, respectively. The midsagittal image is defined as that on which the clivus, pituitary infundibulum, and aqueduct of the cerebrum are delineated in a single plane. Measurements were obtained from 2- or 3-mm-thick sections of T1-weighted midsagittal spin-echo (SE) images (TR/TE/NEX, 400–500 ms/10–15 ms/2) from patients with CHARGE syndrome. Control measurements were obtained from 4-mm-thick sections of T2-weighted midsagittal half-Fourier acquired single-shot turbo spin-echo images (TR/TE/flip angle, 1500 ms/101 ms /170°). Each measurement was obtained 5 times by 1 radiologist (K.F.).

Evaluation of the presence or absence of basioccipital hypoplasia, basilar invagination, Chiari type I malformation, and syringomyelia among the patients with CHARGE syndrome was performed by using 2- or 3-mm-thick sections of T1-weighted SE sagittal images (TR/TE/NEX, 400–500 ms/10–15 ms/2) in all patients, T2-weighted fast SE sagittal images (TR/TE/NEX, 2000–3000 ms/92–94 ms/1) in all the patients except patient 1, and 4-mm-thick sections of T1-weighted fast SE sagittal images (TR/TE/NEX, 337 ms/12 ms/1) and T2-weighted fast SE images (TR/TE/NEX, 1600 ms/170 ms/1) of the whole spine of patient 4. The MR images of the patients were consensually reviewed by 2 board-certified radiologists (K.F. and N.A.).

Basioccipital hypoplasia was defined as follows: Mild hypoplasia defined in patients in whom the length of either the Ba-Es or the Ba-Xs was <2 SDs compared with the normal values for each age group and with a maintained triangular shape. Moderate hypoplasia was defined as patients in whom the length of either Ba-Es or Ba-Xs was <2 SDs and without maintenance of a triangular shape; and severe hypoplasia was defined as patients in whom the lengths of both Ba-Es and Ba-Xs were <2 SDs and without maintenance of a triangular shape. The basilar invagination was when the tip of the odontoid lay 5 mm above the Chamberlain line, which joins the posterior margin of the foramen magnum to the posterior margin of the hard palate. Herniation of at least 1 cerebellar tonsil ≥5 mm below the foramen magnum was considered to be Chiari type I malformation. A cavity and/or a dilated central canal of the spinal cord was considered to be the syringomyelia.

Statistical analysis was performed by using a matched *t* test to search for trends related to age in the length of Ba-Es and Ba-Xs of the control children. A *P* value of < .05 was considered to represent a statistically significant difference.

Results

Table 3 and Fig 2 show the lengths of the Ba-Es and Ba-Xs in the controls. The lengths of Ba-Es and Ba-Xs in the control individuals of the 3-year-old group were significantly shorter than those at other ages ($P = .023$, $P = .0015$). The Ba-Es was significantly elongated in the 8- and 9-year-olds ($P = .0479$), but none of the other values significantly differed among the age groups.

Table 2 and Fig 2 summarize the Ba-Es and Ba-Xs lengths and imaging findings from patients with CHARGE syndrome. The basiocciput was hypoplastic in 7 of the 8 patients (6/7 patients with *CHD7* mutations and patient 8 who did not undergo molecular analysis) and was accompanied by a shorter Ba-Es and Ba-Xs. Basioccipital hypoplasia was severe in 6 and mild in 1 patient (Fig 3*A*). Basilar invagination was identified in 5 of 6 patients with severe basioccipital hypoplasia (Fig 3*B*). One of the 5 patients had a Chiari type I malformation with syringomyelia that involved the lower cervical and whole thoracic spinal cord. This patient also had atrophy of the hands.

Four of the 8 patients had orofacial clefts, and 3 of these 4 had severe basioccipital hypoplasia.

Discussion

The anatomy of the clival region has been described from various morphologic or clinical viewpoints. Lang and Issing[17]

Table 2: Characteristics and basioccipital findings of patients with CHARGE syndrome

	Patient No.							
	1	2	3	4	5	6	7	8
Sex	Male	Male	Male	Female	Female	Female	Male	Female
CHARGE features								
C	+	+	–	+	–	+	+	–
H	+	+	–	–	+	+	–	+
A	–	–	–	–	–	–	–	–
R	+	+	+	+	+	+	+	+
G	+	+	+	–	–	–	+	+
E	+	+	+	+	+	+	+	+
Others, CL	–	–	+	+	+	–	+	–
Genetics								
Mutation	*7367C→G*	*550C→T*	*4171delC*	*4480C→T*	*5050G→A*	*4036C→T*	*5355G→A*	NE
Amino acid	S2456X	Q184X	1391 fs X1403	R1494X	G1684S	Q1346X	W1785X	
Age at occipital evaluation (yr)	3.7	6.7	6.9	7.6	8.5	9.6	11.0	21.0
Basiocciput*	Mild hypoplasia	Severe hypoplasia	Normal	Severe hypoplasia	Severe hypoplasia	Severe hypoplasia	Severe hypoplasia	Severe hypoplasia
Basilar invagination	–	–	–	+	+	+	+	+
Chiari I malformation	–	–	–	+	–	–	–	–
Syringomyelia	–	–	–	+	–	–	–	–
Neurologic sequelae	–	–	–	+	–	–	–	–
Length (mm)								
Ba-Es (SD)	15.0 (–2.2)	12.5 (–3.7)	23.1 (–0.4)	9.5 (–5.0)	12.1 (–5.1)	7.3 (–15.8)	10.9 (–6.1)	16.0 (–2.7)
Ba-Xs (SD)	16.0 (–1.4)	9.9 (–4.8)	22.5 (–0.2)	6.0 (–6.8)	9.8 (–6.5)	6.4 (–7.8)	8.8 (–7.4)	11.3 (–3.5)

Note:—C indicates coloboma; H, congenital heart defect; A, atresia or stenosis of choanae; R, retarded growth or development and/or central nervous system anomalies; G, genital hypoplasia; E, ear anomalies and/or deafness; CL, cleft lip and/or palate; +, present; –, absent; NE, not evaluated; Ba, basion; Es, endo-sphenobasion; Xs, exo-sphenobasion.

* Mild hypoplasia, length of either Ba-Es or Ba-Xs <2 SDs with a maintained triangular shape; moderate hypoplasia, length of either Ba-Es or Ba-Xs <2 SDs without a maintained triangular shape; severe hypoplasia, length of either Ba-Es and Ba-Xs <2 SDs compared with age-matched control values without a maintained triangular shape.

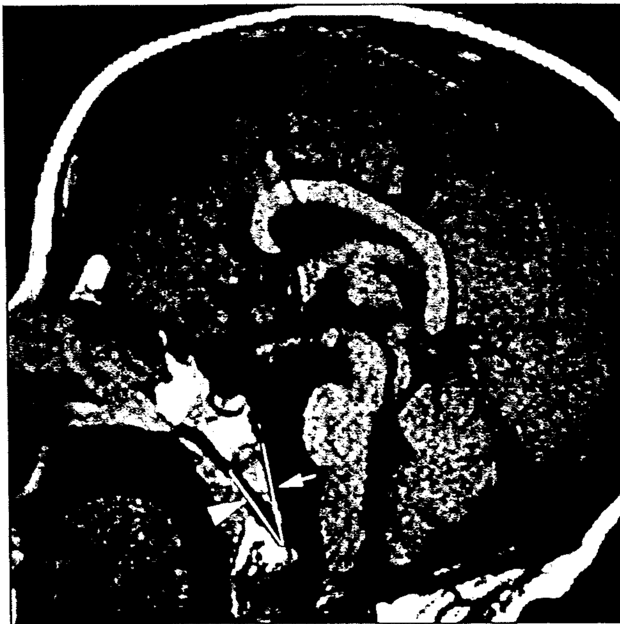

Fig 1. Measurement of the lengths of Ba-Es (*arrow*) and Ba-Xs (*arrowhead*) on a T1-weighted sagittal image (patient 3).

(cited by Krompotić-Nemanić et al[18]) reported that the width and length of the adult clivus is about 28 mm and from 52 to 54 mm, respectively, and Krompotić-Nemanić et al reported postnatal changes in the dimensions of the clivus.[18] However, postnatal changes in the length of the basiocciput, a component of the clivus, have not been reported as far as we can determine. Our study of control individuals ranging from 3 to 21 years of age showed that the occiput became significantly elongated between 3 and 6 years of age and again between 8 and 9 years of age, but not after 10 years of age. Our findings are compatible with anatomic findings showing that the length of the clivus is maximal before puberty and the second growth spurt.[18]

The present study showed that basioccipital hypoplasia and basilar invagination are prevalent in CHARGE syndrome. However, our series was biased, and we analyzed only a small number of patients with this syndrome because patients with CHARGE syndrome and *CHD7* mutations were more likely to undergo imaging.[19] Thus, additional studies of the incidence and severity of CHARGE syndrome without *CHD7* mutations are mandatory; however, we tentatively concluded that routine assessment of the basiocciput in patients with CHARGE syndrome is helpful to exclude potentially life-threatening basilar invagination regardless of the presence or absence of *CHD7* mutations.

CHARGE syndrome accompanied by basilar invagination is classified as the primary type as a result of a hypoplastic basiocciput, in contrast to the secondary type that is associated with soft bone conditions, such as hyperparathyroidism, osteomalacia, and Paget disease. Primary basilar invagination results from a congenital anomaly of the chondrocranium.[20,21] The malformed clivus and/or translocated odontoid peg impinges on the anterior craniospinal neuroaxis and causes neurologic symptoms, including upper motor neuron deficits, cranial nerve abnormalities, hydrocephalus, cerebellar dysfunction, syringomyelia, and even sudden death.[22-24] One of the patients with basilar invagination had Chiari type I malformation with syringomyelia and developed neurologic symptoms. Symptomatic patients will require surgical procedures for relief of symptoms because the ability to reduce basilar invagination is age-related.[25]

The pathogenesis of the hypoplastic basiocciput in CHARGE syndrome remains elusive. The sole known causative gene (*CHD7*) for the syndrome encodes chromodomain helicase DNA-binding protein 7 (*CHD7* protein), which belongs to the chromodomain family. In general, chromodomain family proteins are involved in the maintenance of chromatin structures and are expressed in mesenchymal cells derived from the neural crest. *CHD7* protein is mainly expressed in epithelial cells, olfactory epithelium, and eye, ear, and kidney tissues as well as the vascular system.[26] During the early embryonic stage, *CHD7* protein is preferentially expressed in the undifferentiated neuroepithelium and mesenchyme of neural crest origin.[27] These facts closely correspond with the clinical manifestations of CHARGE syndrome.

However, the basiocciput, unlike the facial bones, derives from the mesodermal cells of the occipital somites and not from the neural crest (Fig 4).[13,28] Thus far, the *CHD7* protein has not been identified in the occipital somites. To understand the association between *CHD7* mutations and the hypoplastic basiocciput, one must assume interaction between the neural crest and somite cells during development. Cleft lip and palate, which are thought to result from impaired neural crest cells, are associated with shortening of the clivus.[29] In addition, deviation of the cranial base in dimension and shape is described in complete cleft lip and palate.[29-31] These facts might support the notion of a developmental link between the facial bones and the basiocciput. However, orofacial clefts and hypoplastic basiocciput did not closely correspond in our series; only 3 of our 8 patients with severe basioccipital hypoplasia had orofacial clefts, whereas 1 without basioccipital hypoplasia had the anomaly. Thus, the relationship between basioccipital hypoplasia and maldevelopment of neural crest cells in CHARGE syndrome remains elusive.

With increasing experience, an expert group of geneticists

Table 3: Lengths of Ba-Es and Ba-Xs in control individuals at various ages

	Age Group (yr)						
	3	6	7	8	9	11	21
Mean (yr)	3.5	6.6	7.3	8.4	9.6	11.5	21.4
Range (yr)	3.2–3.9	6.2–6.9	7.0–7.9	8.0–8.9	9.3–9.9	11.1–11.8	21.1–21.8
Number	10	10	10	10	10	10	10
Length (mean ± SD)							
Ba-Es (mm)	20.2 ± 2.3	24.4 ± 3.2	24.0 ± 2.9	25.4 ± 2.6	27.8 ± 1.3	26.9 ± 2.6	26.9 ± 4.1
Ba-Xs (mm)	19.4 ± 2.4	23.1 ± 2.7	23.1 ± 2.5	24.2 ± 2.2	26.0 ± 2.5	25.9 ± 2.3	25.7 ± 4.1