Extent and Distribution of Linkage Disequilibrium in the Old Order Amish

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Knowledge of the extent and distribution of linkage disequilibrium (LD) is critical to the design and interpretation of gene mapping studies. Because the demographic history of each population varies and is often not accurately known, it is necessary to empirically evaluate LD on a population-specific basis. Here we present the first genome-wide survey of LD in the Old Order Amish (OOA) of Lancaster County Pennsylvania, a closed population derived from a modest number of founders. Specifically, we present a comparison of LD between OOA individuals and US Utah participants in the International HapMap project (abbreviated CEU) using a high-density single nucleotide polymorphism (SNP) map. Overall, the allele (and haplotype) frequency distributions and LD profiles were remarkably similar between these two populations. For example, the median absolute allele frequency difference for autosomal SNPs was 0.05, with an inter-quartile range of 0.02–0.09, and for autosomal SNPs 10–20 kb apart with common alleles (minor allele frequency ≥0.05), the LD measure $r^2$ was at least 0.8 for 15 and 14% of SNP pairs in the OOA and CEU, respectively. Moreover, tag SNPs selected from the HapMap CEU sample captured a substantial portion of the common variation in the OOA (>88%) at $r^2 ≥ 0.8$. These results suggest that the OOA and CEU may share similar LD profiles for other common but untyped SNPs. Thus, in the context of the common variant-common disease hypothesis, genetic variants discovered in gene mapping studies in the OOA may generalize to other populations. Genet. Epidemiol. 34:146–150, 2010. © 2009 Wiley-Liss, Inc.

Key words: single nucleotide polymorphism; population genetics; human genetics; founder population; linkage disequilibrium; haplotypes

INTRODUCTION

Many genetic studies of complex traits and diseases are being conducted in population isolates, including the Old Order Amish (OOA) of Lancaster County Pennsylvania [Ginns et al., 1998; Hsueh et al., 2000; Mitchell et al., 2001, 2008; Streeten et al., 2006; Post et al., 2007; Douglas et al., 2008; Wang et al., 2009]. Whether results from these studies will generalize to other populations is dependent (in part) on the similarity of allele frequencies and patterns of linkage disequilibrium (LD) between populations. To inform future genetic studies of the OOA and facilitate comparisons of findings with other populations, we conducted the first genome-wide survey of LD in the OOA and compared our findings to the International HapMap project [Frazer et al., 2007].

Most of the present-day OOA of Lancaster County are the descendants of approximately 200 individuals [Cross, 1976] from central western Europe who immigrated to the United States in the early eighteenth century [McKusick et al., 1964]. Although recent data indicate that the differences in LD between isolated and cosmopolitan populations for common alleles are modest [Bonnen et al., 2006; Service et al., 2006], the uncertain but unique demographic history of the OOA necessitates empirical evaluation of LD.

SUBJECTS AND METHODS

OOA study subjects were recruited and genotyped (n = 861) in the course of the Heredity and Phenotype Intervention (HAPI) Heart study [Mitchell et al., 2008], which was designed to identify gene-environment interactions influencing cardiovascular traits. Because many closely related individuals were deliberately ascertained, we used a simulated annealing algorithm [Douglas and Sandefur, 2008] to select a set of minimally related individuals (30 men and 30 women). The median (range) pairwise kinship coefficient was 0.03 (0.01–0.04) for the set of 60 vs. 0.03 (0.01–0.03) for the entire sample of 861. For comparison with the OOA, we also utilized 30 men and 30 women (or 60 unrelated parents) from a US Utah...
population with northern and western European ancestry (abbreviated CEU) in the International HapMap project [Frazer et al., 2007].

GENOTYPING AND QC METHODS

DNA was extracted from whole blood by standard methods as described previously [Mitchell et al., 2008]. The Affymetrix GeneChip® Human Mapping 500K Array Set was used for the comparison of LD patterns in both the OOA and CEU samples. Genotype calls were made using a Bayesian Robust Linear Model with Mahalanobis (BRLMM) distance classifier [Affymetrix, 2006]. Genotype data for the CEU sample and corresponding annotation for the platform, including chromosome and genomic positions for all single nucleotide polymorphisms (SNPs) on the array, were obtained from the Affymetrix website (www.affymetrix.com).

Individuals with >5% missing genotypes, and/or for men >1% heterozygous genotypes on the X chromosome, were excluded. A subset of autosomal SNPs (2,068), which were selected to have high information content (minor allele frequency (MAF) ≥0.3), low pair-wise LD (maximum $r^2$ of 0.44), and coverage across all autosomes (average intermarker spacing of 1.3 cm) in the OOA, were used to infer relationships using the maximum likelihood method implemented in Relpair [Epstein et al., 2000]. We excluded individuals who had an inferred relationship that differed from the pedigree relationship with a likelihood ratio greater than 10$^6$. Based on these combined criteria, a total of 24 individuals (out of 861) were excluded from further analysis.

SNPs were required to satisfy the following quality control criteria in both samples: (1) ≤5% uncalled genotypes; (2) ≤5 and ≤1 Mendelian inconsistencies in OOA and CEU samples, respectively, using pedigree diagnostics as implemented in PedCheck [O'Connell and Weeks, 1998]; and (3) Hardy Weinberg Equilibrium $P$-value $≥10^{-6}$ by Fisher’s exact test [Wigginton et al., 2005] as implemented in Haploview [Barrett et al., 2005]. To assess genotyping accuracy, we used duplicate genotype data for 61 of the 861 OOA subjects for whom data from the Affymetrix Genome-Wide Human SNP Array 6.0 (overlap of 482,235 SNPs with Affymetrix GeneChip® Human Mapping 500K Array Set) were also available. Only SNPs with <2 duplicate inconsistencies were retained for analysis. Of the 500,447 genotypes that mapped to a single location in the human genome, 82,404 failed at least one QC measure in at least one sample. Those SNPs were removed, leaving a total of 409,071 autosomal (Table I) and 8,972 X chromosome (Table AI in the Appendix) SNPs. For the SNPs that passed our quality control criteria, the genotype consistency rate among 61 duplicate pairs was 99.4%.

STATISTICAL ANALYSES

Fisher’s exact test was used to compare allele frequency distributions between the OOA and CEU. For common SNPs (MAF $≥0.05$) on the same chromosome and within 10 Mb of each other, we used the expectation-maximization (EM) algorithm to obtain maximum likelihood estimates of two-SNP haplotype frequencies and measured pair-wise LD by the $r^2$ and $D'$ statistics [Lewontin, 1964]. Based on common SNPs, we also identified haplotype blocks in the CEU using an extension of the four-gamete rule [Wang et al., 2002] and estimated haplotype frequencies in both the CEU and OOA using the EM algorithm with a partition-ligation method [Qin et al., 2002] for blocks with $>10$ SNPs as implemented in Haploview [Barrett et al., 2005]. For each sample, we then calculated and compared the effective number of haplotypes in each block, i.e., $\Sigma p_i r_i^2$, where $p_i$ is the frequency of the $i$th haplotype in the block. As a measure of redundancy, we identified the number of SNPs (or proxies) that were in strong LD with each SNP at various thresholds of $r^2$ in each sample. To evaluate the extent to which SNPs selected to tag variation in the CEU capture common variation in the OOA, we selected common tag SNPs in the CEU using the greedy algorithm [Carlson et al., 2004] implemented in Haploview [Barrett et al., 2005] such that every unselected SNP had an $r^2 ≥0.8$ with one or more selected SNPs. We then calculated $r^2$ between the tag SNPs and the remaining “non-tagged” but typed SNPs in the OOA. Unless specified otherwise, all analyses were carried out using a combination of in-house R, Perl, and C programs.

RESULTS

For the 418,043 SNPs that passed QC, mean heterozygosity was 0.26 and 0.27 for the autosomes in the OOA and CEU, respectively, and 0.23 and 0.24 for the X chromosome. The slightly lower heterozygosity in the OOA reflects the larger number of monomorphic SNPs in the OOA relative to the CEU, e.g., 68,869 vs. 57,669 for the autosomes (Table I). Among all SNPs that

| TABLE I. Summary of autosomal SNPs |
|------------------------------------|
|                                   |
| OOA  | CEU  | Overlap |
|------|------|---------|
| Total genotyped                    | 489,922 | 489,922 | 489,922 |
| > 1 duplicate inconsistency        | 51,459  | NA      | NA      |
| >5% missing data                   | 50,085  | 16,896  | 8,973   |
| Mendelian inconsistencies           | 3,188   | 1,168   | 202     |
| $P < 10^{-6}$ for HWE test          | 379     | 217     | 116     |
| Passed QC filter                   | 415,440 | 472,851 | 409,071 |
| Passed QC in both OOA and CEU      | 68,869  | 57,669  | 52,467  |
| Monomorphic                       |         |         |         |
| Polyorphic                         |         |         |         |
| MAF $≥0.05$                        | 297,605 | 310,704 | 287,476 |
| MAF $≥0.10$                        | 256,614 | 267,149 | 240,375 |
| MAF $≥0.20$                        | 182,941 | 189,133 | 161,062 |

OOA, Old Order Amish; CEU, US Utah residents from HapMap; MAF, minor allele frequency; SNPs that failed a QC measure in either sample were excluded from further analysis, and SNPs with MAF $>0.05$ passing QC in both samples ($σ = 287,476$) were used for LD analysis.

aBased on the 61 OOA individuals who were also genotyped on the Affymetrix 6.0 array; SNPs with more than one duplicated genotype discrepancy were excluded.

bBased on 837 OOA and 90 CEU individuals (30 trios).

bSNPs with $>5$ and $>1$ Mendelian inconsistencies in OOA and CEU, respectively.

bBased on 60 unrelated individuals (30 men and 30 women) from each sample.

bSNPs may fail QC in more than one way, so rows do not sum to the subtotal passing QC.

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were polymorphic in at least one sample, the median absolute allele frequency difference was 0.05 for the autosomes and 0.07 for the X chromosome. At \( P \)-value < 10\(^{-6} \), OOA and CEU allele frequencies were significantly different for 799 autosomal and 137 X chromosome SNPs.

The percentage of SNP pairs within 10 Mb of each other and between which strong LD was observed was remarkably similar between the OOA and CEU for the autosomes (Table II) and the X chromosome (Table AII in the Appendix). For example, for autosomal SNPs at an inter-marker distance of <10 kb, no evidence of recombination \((D' = 1)\) was observed for 79 and 75\% of SNP pairs, perfect LD \((r^2 = 1)\) was observed for 20 and 19\% of SNP pairs, and useful LD \((r^2 \geq 0.8)\) was observed for 30 and 29\% of SNP pairs in the OOA and CEU, respectively. Based on the CEU sample, we identified 58,097 autosomal haplotype blocks, with a median of three SNPs per block and an inter-quartile range of [3, 4].

Among all autosomal blocks, the median effective number of haplotypes \((n_e)\) was 2.43 and 2.47 in the OOA and CEU, respectively, and the median of the differences in \(n_e\) (CEU minus OOA) per block was 0.04, with an inter-quartile range of –0.2 to 0.3, suggesting modestly greater haplotype diversity in the CEU. Results based on haplotype blocks defined in the OOA did not qualitatively differ from those based on blocks defined in the CEU (data not shown).

Of common autosomal SNPs, 72 and 64\% had at least one proxy at \( r^2 \geq 0.8 \) and 55 and 44\% had at least one perfect proxy \((r^2 = 1)\) in the OOA and CEU, respectively, indicating that fewer independent SNPs are required to represent variation in the OOA relative to the CEU. At \( r^2 \geq 0.8 \), 170,979 of 310,704 common SNPs in the CEU were selected as tag SNPs and captured \( \approx 88\% \) of the “non-tagged” SNPs in OOA, suggesting that SNPs selected to tag common variation in the CEU capture much of the same variation in the OOA. SNPs not captured by the CEU tag SNPs tended to be of lower MAF (data not shown). Results for the X chromosome were qualitatively similar.

### TABLE II. Percentage of autosomal SNP pairs\(^a\) showing no evidence of recombination \((D' = 1)\), perfect LD \((r^2 = 1)\), or where useful LD is observed \((r^2 \geq 0.8)\)

| Inter-SNP distance (kb) | OOA | CEU | OOA | CEU | OOA | CEU |
|------------------------|-----|-----|-----|-----|-----|-----|
| ≤10                    | 79  | 75  | 20  | 19  | 30  | 29  |
| 10–20                  | 60  | 53  | 9   | 7   | 15  | 14  |
| 20–50                  | 43  | 34  | 4   | 3   | 9   | 7   |
| 50–100                 | 28  | 20  | 1   | 1   | 3   | 2   |
| 100–200                | 20  | 11  | 0   | 0   | 1   | 1   |
| 200–500                | 14  | 7   | 0   | 0   | 0   | 0   |
| 500–1,000              | 12  | 6   | 0   | 0   | 0   | 0   |
| 1,000–2,000            | 11  | 5   | 0   | 0   | 0   | 0   |
| 2,000–5,000            | 10  | 5   | 0   | 0   | 0   | 0   |
| 5,000–10,000           | 8   | 5   | 0   | 0   | 0   | 0   |

OOA, Old Order Amish \((n = 60)\); CEU, US Utah residents from HapMap \((n = 60)\).

\(\text{aRestricted to SNPs with minor allele frequency } \geq 0.05 \text{ in both samples } (n = 287,476).\)

## DISCUSSION

In general, we found a high degree of similarity in allele frequencies and LD patterns in the OOA and CEU samples. Allele frequencies were not significantly different between the OOA and CEU for >99\% of SNPs. Based on common SNPs, which comprised 74 and 66\% of autosomal SNPs in the OOA and CEU, respectively, the distribution and extent of LD were remarkably similar between these two samples. These data are consistent with previous theoretical predictions \[Kruglyak, 1999; Pritchard and Przeworski, 2001\] and recent empirical data \[Bonnen et al., 2006; Service et al., 2006; Navarro et al., 2009; Thompson et al., 2009\], all of which point to modest differences in LD between isolated and cosmopolitan populations for common alleles. The situation for rare alleles, however, is likely to be different as has been demonstrated in applications of LD mapping for monogenic diseases and traits.

Demographic and historical information indicate that the OOA were founded relatively recently (~10–15 generations ago) by a modest number of individuals (several hundred) and then expanded rapidly to a current census population size exceeding 30,000 \[Lancaster County Amish, 2002\]. Though the precise demographic details are unknown, it is apparent that the number of founders and rate of growth were sufficient and that the subsequent isolation of the OOA was too short for genetic drift and/or recombination to have meaningfully altered the common allele or haplotype frequency spectrum. Our recent study of variation on the Y chromosome supports these observations that in much of the diversity observed in non-isolated populations of similar ancestry is present in the OOA \[Pollin et al., 2008\]. It appears that inbreeding due to the finite population size of the OOA was also insufficient to meaningfully alter the allele frequency distribution or extent of LD. Based on the 60 OOA individuals included in our analyses, the average inbreeding coefficient \(F\) \[Wright, 1922\] was 0.026 (range of 0.0003–0.046), which is too weak to generate substantial differences in LD relative to a non-isolated population \[Hill and Robertson, 1968\].

Owing to similar allele frequencies and LD patterns in the OOA and CEU, CEU-derived tag SNPs performed well in capturing common variation in the OOA, consistent with previous studies in other samples of European ancestry, including those from isolated populations \[Willer et al., 2006; Service et al., 2007\]. These results suggest that the OOA and CEU samples may also share similar LD profiles for other common but untyped SNPs. Thus, findings from gene mapping studies in the OOA may generalize to other populations in the context of the common variant-common disease hypothesis.

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REFERENCES

Affymetrix. 2006. BRLMM: an Improved Genotype Calling Method for the GeneChip Human Mapping 500K Array Set. [http://www.affymetrix.com/support/technical/whitepapers/brlmm_whitepaper.pdf].

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265.

Bonnen PE, Pe'er I, Plenge RM, Salit J, Lovse JK, Shapero MH, Liiffon RP, Breslow JL, Daly MJ, Reich DE, Jones KW, Stoffel M, Altshuler D, Friedman JM. 2006. Evaluating potential for whole-genome studies in Kosrae, an isolated population in Micronesia. Nat Genet 38:214–217.

Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. 2004. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 74:106–120.

Cross HE. 1976. Population studies and the Old Order Amish. Nature 261:398–400.

Douglas JA, Sandefur CI. 2008. PedMine—a simulated annealing algorithm to identify maximally unrelated individuals in population isolates. Bioinformatics 24:1106–1108.

Douglas JA, Roy-Gagnon MH, Zhou C, Mitchell BD, Shuldiner AR, Chan HP, Helvie MA. 2008. Mammographic breast density—evidence for genetic correlations with established breast cancer risk factors. Cancer Epidemiol Biomarkers Prev 17:3509–3516.

Epstein MP, Duren WL, Bohneke M. 2000. Improved inference of relationship for pairs of individuals. Am J Hum Genet 67:1219–1231.

Frazier KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, Epstein MP, Duren WL, Boehnke M, Spiegel J, Sun, LM, Zhang L, Zhang H, Gabriel SB, Barry M, Gibbs RA, Altshuler D, Ziaugra L, Sun L, Fruchter J, Benjamin E, Lander E, Schork NJ, Fudenberg G, Glessner J, Goldstein DB, Hirschhorn J, Hillier LW, Hinds DA, Hovatta I, Jackson AU, Jhingran A, Keinan S, Lebovic I, Lieberman A, Lindblad-Toh K, Linton LN, MacKinnon A, Mauer AM, Mosley TH Jr, Pfeifer JD, Reedy-Miller L, Rennert H, Schaffner SF, Sherry ST, Sweeny H, Tucker K, Wheeler DL, Zhao H, Zhao H, Zhang Y, Zhang Z, Zinman L, Zody MC, McCall K, PL, Ross ST, Sims SK, Willey DL, Chen Z, Han H, Kang L, Godbout M, Wallenburg JC, L’Archevêque P, Bellemare G, Saeki K, Wang H, An D, Fu H, Li Q, Wang Z, Wang R, Holden AL, Brooks LD, McEwen JE, Guerrier MS, Wang VQ, Peterson JL, Shi M, Spiegel J, Sung LM, Zacharia LF, Collins FS, Kennedy K, Jamieson R, Stewart J. 2007. A second generation human haplotype map of over 3.1 million SNPs. Nature 449:851–861.

Gims EI, St Jean P, Philibert RA, Galdiczka M, Danschroder-Williams P, Thiel B, Long RT, Ingraham LJ, Dalwaldhi H, Murray MA, Ehert M, Paul S, Remortel BG, Patel AP, Anderson MC, Shaio C, Lau E, Dymarska I, Martin BM, Stubblefield B, Falls KM, Carulli JP, Keith TP, Fann CS, Lacy LG, Allen CR, Hostetter AM, Elston RC, Schork NJ, Egekand JAL, Paul SM. 1998. A genome-wide search for chromosomal loci linked to mental health wellness in relatives at high risk for bipolar affective disorder among the Old Order Amish. Proc Natl Acad Sci USA 95:15331–15336.

Hill WG, Robertson A. 1968. Linkage disequilibrium in finite populations. Theor Appl Genet 38:226–231.

Hsuie WC, Mitchell BD, Abouronia R, Pollin T, Sakul H, Gelder Ehm M, Michelsen BK, Wagner MJ, St Jean PL, Knowler WC, Burns DK, Bell CJ, Shuldiner AR. 2000. Diabetes in the Old Order Amish: characterization and heritability analysis of the Amish Family Diabetes Study. Diabetes Care 23:595–601.

Kruglyak L. 1999. Prospects for whole-genome linkage disequilibrium mapping of common disease genes. Nat Genet 22:139–144.

Lancaster County Amish. 2002. Church Directory of the Lancaster County Amish. Gordonville, PA: The Diary.

Lewontin RC. 1964. The interaction of selection and linkage. II. Optimum models. Genetics 50:757–782.

McKusick VA, Hostetler JA, Egekand JA. 1964. Genetic studies of the Amish, background and potentialities. Bull Johns Hopkins Hosp 115:203–222.

Mitchell BD, Hsuie WC, King TM, Pollin Tj, Sorkin J, Agarwala R, Schaffer AA, Shuldiner AR. 2001. Heritability of life span in the Old Order Amish. Am J Med Genet 102:346–352.

Mitchell BD, McCarron PD, Shen H, Rampersaud E, Pollin Tj, Bielak LF, Jaquish C, Douglas JA, Roy-Gagnon MH, Sack P, Naglieri R, Hines S, Horenstein RB, Chang YP, Post W, Ryan KA, Breerton NH, Pakyz RE, Sorkin J, Damcott CM, O’Connell JR, Mangano C, Corretti M, Vogel R, Herzog W, Weir MR, Peyer PA, Shuldiner AR. 2008. The genetic response to short-term interventions affecting cardiovascular function: rationale and design of the Heredity and Phenotype Intervention (HAPI) Heart Study. Am Heart J 155:823–828.

Navarro P, Vitart V, Hayward C, Tenesa A, Zgapa L, Juricic D, Polasek O, Hastie ND, Rudan I, Campbell H, Wright AF, Haley CS, Knott SA. 2009. Genetic comparison of a Croatian isolate and CEPH European Founders. Epidemiol Genet. DOI: 10.1002/gepi.20443.

O’Connell JR, Weeks DE. 1998. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 63:259–266.

Pollin TI, McBride DJ, Agarwala R, Schaffer AA, Shuldiner AR, Mitchell BD, O’Connell JR. 2008. Investigations of the Y chromosome, male founder structure and YSTR mutation rates in the Old Order Amish. Hum Hered 65:91–104.

Post W, Bielak LF, Ryan KA, Cheng YC, Shen H, Rumberger JA, Sheedy 2nd F, Shuldiner AR, Peyer PA, Mitchell BD. 2007. Determinants of coronary artery and aortic calcification in the Old Order Amish. Circulation 115:717–724.

Pritchard JK, Przeworski M. 2001. Linkage disequilibrium in humans: models and data. Am J Hum Genet 69:1–14.

Qin ZS, Niu T, Liu JS. 2002. Partition-ligation-expectation-maximization algorithm for haplotype inference with single-nucleotide polymorphisms. Am J Hum Genet 71:1242–1247.

Service S, DeYoung J, Karayiorgou M, Roos JL, Pretorius H, Bedoya G, Osipina J, Ruiz-Linares A, Macedo A, Palha JA, Heutink P, Aulchenko Y, Oostra B, van Duijn C, Jarvelin MR, Varilo T, Peddle L, Rahman P, Piras G, Monne M, Murray S, Galver L, Peltonen L, Sabatti C, Collins A, Freimer N. 2006. Magnitude and distribution of linkage disequilibrium in

Genet. Epidemiol.
population isolates and implications for genome-wide association studies. Nat Genet 38:556–560.

Service S, Sabatti C, Freimer N. 2007. Tag SNPs chosen from HapMap perform well in several population isolates. Genet Epidemiol 31:189–194.

Streeten EA, McBride DJ, Pollin TI, Ryan K, Shapiro J, Ott S, Mitchell BD, Shuldiner AR, O’Connell JR. 2006. Quantitative trait loci for BMD identified by autosomal linkage scan to chromosomes 7q and 21q in men from the Amish Family Osteoporosis Study. J Bone Miner Res 21:1433–1442.

Thompson EE, Sun Y, Nicole D, Ober C. 2009. Shades of gray: a comparison of linkage disequilibrium between Hutterites and Europeans. Genet Epidemiol, DOI: 10.1002/gepi.20442.

Wang N, Akey JM, Zhang K, Chakraborty R, Jin L. 2002. Distribution of recombination crossovers and the origin of haplotype blocks: the interplay of population history, recombination, and mutation. Am J Hum Genet 71:1227–1234.

Wang Y, O’Connell JR, McArdle PF, Wade JB, Dorff SE, Shah SJ, Shi X, Pan L, Rampersaud E, Shen H, Kim JD, Subramanya AR, Steinle NI, Parsa A, Ober CC, Wellin PA, Chakravarti A, Weder AB, Cooper RS, Mitchell BD, Shuldiner AR, Chang YP. 2009. Whole-genome association study identifies STK39 as a hypertension susceptibility gene. Proc Natl Acad Sci USA 106:6.

Wigginton JE, Cutler DJ, Abecasis GR. 2005. A note on exact tests of Hardy–Weinberg equilibrium. Am J Hum Genet 76:887–893.

Willer CJ, Scott LJ, Bonnycastle LL, Jackson AU, Chines P, Pruim R, Bark CW, Tsai YY, Pugh EW, Doheny KF, Kinnunen L, Mohlke KL, Valle TT, Bergman RN, Tuomilehto J, Collins FS, Boehnke M. 2006. Tag SNP selection for Finnish individuals based on the CEPH Utah HapMap database. Genet Epidemiol 30:180–190.

Wright S. 1922. Coefficients of inbreeding and relationship. Am Nat 56:330–338.

APPENDIX

Summary and percentage of X chromosomes are given in Tables AI and AII.

### TABLE AI. Summary of X chromosome SNPs

| OOA | CEU | Overlap |
|-----|-----|---------|
| Total genotyped | 10,525 | 10,525 | 10,525 |
| >1 duplicate inconsistency<sup>a</sup> | 1,061 | NA | NA |
| >5% missing data<sup>b</sup> | 547 | 461 | 261 |
| Mendelian inconsistencies<sup>b</sup><sup>c</sup> | 44 | 246 | 10 |
| P<10<sup>−6</sup> for HWE test<sup>d</sup> | 0 | 0 | 0 |
| Passed QC filter<sup>e</sup> | 9,139 | 10,064 | 8,972 |
| Passed QC in both OOA and CEU | | |
| Monomorphic<sup>d</sup> | 2,272 | 1,905 | 1,805 |
| Polymorphic<sup>d</sup> | 5,763 | 6,106 | 5,516 |
| MAF>0.05 | 4,971 | 5,376 | 4,449 |
| MAF>0.10 | 3,571 | 3,925 | 2,929 |

<sup>a</sup>Based on the 61 OOA individuals who were also genotyped on the Affymetrix 5.0 array; SNPs with more than one duplicated genotype discrepancy were excluded.

<sup>b</sup>Based on 837 OOA and 90 CEU individuals (30 trios).

<sup>c</sup>SNPs with >5 and >1 Mendelian inconsistencies in OOA and CEU, respectively.

<sup>d</sup>Based on 60 unrelated individuals (30 men and 30 women) from each sample.

<sup>e</sup>SNPs may fail QC in more than one way, so rows do not sum to the subtotal passing QC.

### TABLE AII. Percentage of X chromosome SNP pairs<sup>4</sup> showing no evidence of recombination (D<sup>2</sup> = 1), perfect LD (r<sup>2</sup> = 1), or where useful LD is observed (r<sup>2</sup> ≥ 0.8)

| Inter-SNP distance (kb) | OOA | CEU | OOA | CEU | OOA | CEU |
|-------------------------|-----|-----|-----|-----|-----|-----|
| D<sup>2</sup> = 1        |     |     |     |     |     |     |
| 10                      | 88  | 85  | 39  | 35  | 51  | 49  |
| 10–20                   | 72  | 64  | 23  | 19  | 34  | 31  |
| 20–50                   | 60  | 48  | 12  | 9   | 21  | 18  |
| 50–100                  | 44  | 31  | 6   | 3   | 11  | 10  |
| 100–200                 | 31  | 19  | 3   | 1   | 6   | 4   |
| 200–500                 | 22  | 11  | 1   | 0   | 2   | 1   |
| 500–1,000               | 18  | 7   | 0   | 0   | 0   | 0   |
| 1,000–2,000             | 17  | 7   | 0   | 0   | 0   | 0   |
| 2,000–5,000             | 15  | 7   | 0   | 0   | 0   | 0   |
| 5,000–10,000            | 13  | 7   | 0   | 0   | 0   | 0   |

<sup>4</sup>Restricted to SNPs with minor allele frequency ≥ 0.05 in both samples (n = 5,516).

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