

ORIGINAL ARTICLE

Pleiotropy of type 2 diabetes with obesity

Sandra J Hasstedt¹, Craig L Hanis², Swapan K Das³ and Steven C Elbein^{3,*}, the American Diabetes Association GENNID Study Group⁴

The risk of type 2 diabetes (T2D) increases with obesity. One possible explanation is that pleiotropic genes affect risk of both T2D and obesity. To identify pleiotropic genes, we performed bivariate analysis of T2D with waist–hip ratio (WHR) and with body mass index (BMI) in the African-American subset of the Genetics of NIDDM (GENNID) sample. Of 12 T2D loci identified through suggestive or higher univariate logarithm of the odds ratio (lod) scores, we inferred pleiotropy with obesity for six (chromosomes 1 at 17–19 Mb, 2 at 237–240 Mb, 7 at 54–73 Mb, 13 at 26–30 Mb, 16 at 26–47 Mb and 20 at 56–59 Mb). These findings provide evidence that at least some of the co-occurrence of obesity with T2D is because of pleiotropic genes. We also inferred four obesity loci through suggestive or higher lod scores for WHR (chromosomes 1 at 24–32 Mb, 2 at 79–88 Mb, 2 at 234–238 Mb and 3 at 148–159 Mb).

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INTRODUCTION

Obesity, especially abdominal obesity, increases the risk of type 2 diabetes (T2D), by more than twofold by some estimates.¹ Abdominal fat, more specifically visceral adipose tissue, secretes factors such as adiponectin and resistin that affect glucose metabolism.² As a measure of central obesity, waist–hip ratio (WHR), is expected to and in some studies,³ but not others⁴ correlates more strongly with visceral fat than does body mass index (BMI). Regardless, WHR and BMI show similar associations with T2D risk.^{5–7}

Pleiotropic genes partially explain the co-occurrence of T2D and obesity. At least one T2D-obesity gene is known: *FTO* was identified through a genome-wide association (GWA) study of T2D then found to affect BMI.⁸ Candidate T2D-obesity genes are supported by mouse studies (*PCK1* and *PCK2* (Beale *et al.*⁹)) or computational methods (*LPL* and *BCKDHA*¹⁰). On the other hand, some instances of the co-occurrence of T2D and obesity undoubtedly result from the interaction of independent genes and/or environmental factors, producing a mixture of pleiotropic and independent T2D and obesity genes underlying T2D in any population.

African Americans have less visceral fat than Caucasians with the same BMI,¹¹ yet a higher risk of T2D at low BMI, although an equivalent risk at high BMI.¹² The relationship between T2D and obesity could differ between African Americans and Caucasians because different genes harbor T2D risk variants. Alternatively, the same genes, but with different variant frequencies, could alter the proportion of T2D in African Americans that reflects pleiotropic genes, thereby altering the relationship between T2D and obesity in

the overall population. Consequently, T2D genes missed by Caucasian studies may be identified when studying African Americans, or other non-Caucasian samples,¹³ whether because of their limitation to or higher frequency in African Americans.

The American Diabetes Association established the Genetics of NIDDM (GENNID) study as a resource for the discovery of genes related to T2D and its complications. From 1993 to 2003, the GENNID study ascertained families through T2D-diagnosed siblings at multiple sites. In the African-American subset of the GENNID sample, linkage scans using microsatellite markers identified one suggestive linkage peak for T2D on chromosome 10 (Ehm *et al.*)¹⁴ and no evidence of linkage for the obesity factor of metabolic syndrome.¹⁵ Following expansion of the sample and using single-nucleotide polymorphism (SNP) markers, we obtained a number of significant and suggestive linkages for T2D, BMI and age of diagnosis.¹⁶ In this study, we repeat the T2D linkage analysis, not accounting for the effect of BMI on T2D risk as previously, and use bivariate linkage analysis to test for pleiotropy between T2D and obesity.

MATERIALS AND METHODS

The GENNID study ascertained families through a sibling pair, each with a T2D diagnosis.¹⁷ During Phase 1, extended family members were also studied; one site ascertained African Americans. During Phase 2, data collection beyond the sibling pair was limited to parents, or, if parents were unavailable, unaffected siblings; five sites ascertained African Americans. During Phase 3, sites were added, which collected only affected sibling pairs and trios. In total,

¹Department of Human Genetics, University of Utah, Salt Lake City, UT, USA; ²Human Genetics Center, School of Public Health, University of Texas Health Science Center, Houston, TX, USA and ³Section on Endocrinology and Metabolism, Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA
⁴See Appendix.

Correspondence: Dr SJ Hasstedt, Department of Human Genetics, University of Utah, 15 N 2030 E RM 2100, Salt Lake City, UT 84112-5330, USA.

E-mail: sandy@genetics.utah.edu

*Deceased.

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1,496 African Americans members of 580 pedigrees were studied at 10 sites. See Elbein *et al.*¹⁶ for more detail. This study was approved by the Institutional Review Board at each participating institution.

T2D was diagnosed using National Diabetes Data Group criteria (fasting plasma glucose concentration > 140 mg dl⁻¹ on more than one occasion or 2-h and one other occasion during an oral glucose tolerance test that was > 200 mg dl⁻¹).^{17,18} Age of diagnosis was reported on a standardized questionnaire. Height, weight, and waist and hip circumference were obtained from physical examination, from which WHR and BMI were computed.

BMI and WHR were transformed, separately in males and females, using the inverse normal distribution, for which a quantile was assigned to each trait value and the corresponding inverse normal deviate assigned as the trait. Skew (-0.11 and 0.04) and kurtosis (-0.04 and -0.02) of transformed BMI and WHR, respectively, remained minimal after adjustment for gender and age.

The Center for Inherited Disease Research genotyped 5,958 autosomal SNPs on 1473 individuals using Illumina Linkage Panel IVb (Illumina, San Diego, CA, USA). Pedigree errors were identified using Eclipse²⁹ and genotype errors were identified using Pedcheck²⁰ and MERLIN.²¹ Multi-point identity by descent probabilities were computed at each centimorgan using MERLIN,²¹ treating as haplotypes SNP sets with pairwise linkage disequilibrium $r^2 > 0.70$. See Elbein *et al.*¹⁶ for more details.

Likelihood analysis, as implemented in jPAP,²² was used for univariate linkage analysis of T2D, BMI and WHR, and for bivariate analysis of T2D paired with BMI and WHR. Transformed BMI and WHR were each modeled as a normal density with mean and standard deviation. Gender and age were included as covariates in all analyses; BMI and/or SNP genotypes were included as a covariate when indicated. T2D risk was modeled to account for age of diagnosis in affected pedigree members, although allowing for censored observations, through a modification of the age-of-onset regressive logistic model,²³ also known as the age at diagnosis regressive model, and described as Method 2 in Cui *et al.*²⁴ Let W represent age of diagnosis or age last examined if unaffected, and $X=0/1$ for male/female. The logit of the probability of T2D equals

$$\text{logit}[p(w, x)] = \alpha + \beta w + \gamma x$$

where $p(w, x) = \Pr(T=1|W=w, X=x)$ denotes the probability of T2D, $p = \ln(\alpha/1-\alpha)$ represents male lifetime penetrance, $\exp(\beta)$ represents the annual odds ratio because of age, and $\exp(\gamma)$ represents the female/male odds ratio. In addition, each univariate model included a polygenic effect (h^2 or heritability) and a quantitative trait locus (QTL) effect (q^2). Each bivariate model included h^2_i and q^2_i for trait $i=1, 2$, as well as the correlations between the traits: ρ_q or pleiotropy, between QTL effects, ρ_g or the genetic correlation between polygenic effects and ρ_e , the residual environmental correlation. Parameters were estimated as the values that maximized the likelihood.

In the univariate T2D, and bivariate T2D–BMI and T2D–WHR analyses, we corrected the likelihood for the ascertainment of each pedigree through an affected sibling pair. To perform autosome-wide variance components linkage

analysis, we used the univariate and bivariate models in conjunction with the identity by descent probabilities. All the parameters of the models were estimated every centimorgan except for p , β and γ in the univariate T2D and bivariate analyses. In those analyses, p , β and γ were fixed at estimates obtained upon maximizing the likelihood of the linkage analysis model with $q^2=0$ or $q^2_1=q^2_2=\rho_q=0$, although correcting the likelihood for the ascertainment of each pedigree through an affected sibling pair. Ascertainment correction was not made in any linkage analyses.

Hypotheses were tested through comparison of the maximized likelihoods of general and nested models. Asymptotically, and under certain regularity conditions, twice the natural logarithm of the likelihood ratio distributes as a χ^2 , or as a mixture of χ^2 for a one-tailed test when the nested model constrains a parameter at its boundary.²⁵ Alternatively, we computed the logarithm of the odds ratio (lod) score as the common logarithm of the likelihood ratio. Univariate linkage tested $q^2=0$ and distributed as a 1/2:1/2 mixture of a χ^2 with 1 degree of freedom and a point mass at zero. The test of a single QTL effect, that $q^2_2=\rho_q=0$, used the bivariate model and distributed as a 1/2:1/2 mixture of a χ^2 with 2 degree of freedom and a point mass at zero. The test of significant pleiotropy or QTL correlation, that $\rho_q=0$, used the bivariate model and distributed as a χ^2 with 1 degree of freedom. The effect of a SNP was evaluated by including as a covariate genotypes coded as 0/1/2 when computing the lod score.

To account for multiple testing, autosome-wide statistics were considered significant (0.05 false positives expected per scan) if $P < 0.00005$, near-significant (0.1 false positives expected per scan) if $P < 0.0001$ and suggestive (one false positive expected per scan) if $P < 0.001$. As SNP panels require higher thresholds than microsatellites,²⁶ these probabilities are intermediate between those for 400 markers and a continuous map.²⁷ The significant, near-significant and suggestive univariate lod scores were 3.29, 3.00 and 2.07, respectively. At each inferred T2D locus, QTL effects were tested for two traits (BMI and WHR); therefore, $P < 0.05/2 = 0.025$ and $P < 0.1/2 = 0.05$ were considered significant and near-significant, respectively. At each inferred WHR locus, QTL effects were tested for one trait (T2D); therefore, $P < 0.05$ and $P < 0.1$ were considered significant and near-significant, respectively. For pleiotropy, tested only for significant QTL effects, $P < 0.05$ and $P < 0.1$ were considered significant and near-significant, respectively.

RESULTS

The sample comprised 81% T2D cases and 65% women (Table 1). T2D cases were older with higher BMI and WHR. The near-significance of an effect of BMI on T2D risk ($P=0.0574$) was lost upon also accounting for WHR ($P=0.336$). In contrast, the effect of WHR on T2D risk remained highly significant after accounting for BMI ($P=0.000000172$).

Twelve T2D loci were identified through autosome-wide univariate linkage analysis; five attained significance, two were near-significant

Table 1 Characteristics of the sample by gender and T2D status

Variable	Gender	T2D Status	N	Mean	s.d.	Minimum	Median	Maximum
Age (years)	Male	Affected	388	54.12	11.90	19	54	92
		Unaffected	109	50.33	16.35	18	49	90
	Female	Affected	778	54.48	12.10	20	55	96
		Unaffected	166	51.06	15.60	20	51	95
BMI(kg m ⁻²)	Male	Affected	378	30.58	6.01	16.75	29.89	55.27
		Unaffected	109	28.31	6.21	17.86	27.77	55.90
	Female	Affected	753	34.43	7.97	16.84	33.30	64.51
		Unaffected	155	33.03	7.91	17.28	32.65	55.93
WHR	Male	Affected	319	0.9745	0.0778	0.7899	0.9682	1.3833
		Unaffected	89	0.9199	0.0730	0.7714	0.9148	1.2595
	Female	Affected	701	0.9346	0.1014	0.5437	0.9261	1.3731
		Unaffected	136	0.8785	0.0936	0.6647	0.8725	1.2738

Abbreviations: BMI, body mass index; s.d., standard deviation; T2D, type 2 diabetes; WHR, waist–hip ratio.

Table 2 Univariate lod scores > 2.07 (suggestive) for T2D and, at the same location, nominal P -values testing QTL effects (q^2_2) and pleiotropy with T2D (ρ_q) for BMI and WHR

Chromosome	Position ^a (Mb)	Nearest SNP	Lod Score ^b	BMI ^c		WHR ^c	
				q^2_2	ρ_q	q^2_2	ρ_q
1	18 (17–19)	rs477558	2.37	0.0737	0.791	0.000343	0.0106
2	62 (61–65)	rs1919481	3.58	0.0708	0.142	0.0588	0.764
2	84 (77–102)	rs981525	3.57	0.160	0.548	0.00221	0.410
2	115 (113–117)	rs400960	4.73	0.120	0.921	0.0166	0.308
2	238 (237–240)	rs1198823	<i>3.04</i>	0.0463	0.0925	0.0000257	0.000191
7	24 (14–29)	rs1469000	2.61	0.103	0.206	0.105	0.210
7	67 (54–73)	rs956523	3.77	0.0955	0.202	0.0569	0.114
11	120 (119–122)	rs1073636	<i>3.00</i>	0.085	0.423	0.159	0.322
13	28 (26–30)	rs535534	4.99	0.000375	0.00269	0.00639	0.0128
16	30 (26–47)	rs11901	2.94	0.0638	0.466	0.000722	0.00443
20	57 (56–59)	rs2208087	2.96	0.00766	0.0153	0.0647	0.151
22	37 (27–40)	rs760519	2.27	0.0396	0.0793	0.0103	0.0206

Abbreviations: BMI, body mass index; lod, logarithm of the odds ratio; QTL, quantitative trait locus; SNP, single-nucleotide polymorphism; T2D, type 2 diabetes; WHR, waist-hip ratio.

^aMegabases from Build 37.1 (1-lod drop support interval).

^bSignificant (>3.29, bold) and near-significant (>3.00, italics) univariate lod scores.

^cFor q^2_2 , significant (<0.025, bold) and near-significant (<0.05, italics) P -values. For ρ_q , significant (<0.05, bold) and near-significant (<0.1, italics) P -values.

and five provided suggestive evidence of linkage (Table 2); differences from lod scores reported in Elbein *et al.*¹⁶ result from accounting for BMI therein. Two significant or near-significant loci showed evidence of pleiotropy with obesity: on chromosome 13 at 26–30 Mb, pleiotropy attained significance for both BMI and WHR; on chromosome 2 at 237–240 Mb, pleiotropy attained significance only for WHR. QTL effects for, but not pleiotropy with, WHR attained significance for the significant loci on chromosome 2 at 77–102 and at 113–117 Mb, suggesting that these locations harbor independent T2D and WHR loci. In addition, pleiotropy with obesity attained significance for four suggestive loci: on chromosome 20 at 56–59 Mb with BMI and on chromosomes 1 at 17–19 Mb, 16 at 26–47 Mb and 22 at 27–40 Mb with WHR.

Two SNPs identified in GWA studies of T2D, rs10490072 near *BCL11A* and rs864745 near *JAZF1* (Zeggini *et al.*²⁸) fall within our linkage regions on chromosomes 2 at 61–65 Mb and 7 at 14–29 Mb, respectively. Although both SNPs showed nominally significant associations with T2D in this sample,²⁹ accounting for the SNP genotype, decreased the lod score in the respective region only minimally (<0.2).

Despite evidence of pleiotropic effects on obesity risk for six of our inferred T2D loci, univariate linkage analysis produced no significant lod scores for either BMI or WHR. Nevertheless, suggestive lod scores supported three WHR loci, and adjustment of WHR for BMI added a fourth suggestive locus and strengthened the evidence for two of the suggestive loci: one to significance and the other to near-significance (Table 3). The support region of the significant locus overlapped our T2D-WHR locus on chromosome 2 at 237–240 Mb and supported pleiotropy with T2D. The near-significant locus, located within the support region of our T2D locus on chromosome 2 at 77–102 Mb, produced evidence of an effect on T2D, but no evidence of pleiotropy with T2D, in agreement with our previous conclusion that this region harbors independent T2D and obesity loci. For the remaining five T2D-obesity loci, univariate linkage analysis of BMI and WHR failed to produce genome-wide significance, although four of the five loci (except chromosome 22 at 27–40 Mb) produced nominal significance (lod > 0.84) for either BMI or WHR.

Table 3 Univariate lod scores > 2.07 (suggestive) for either WHR or WHR adjusted for BMI (WHRBMI) and, at the same location, nominal P -values testing QTL effects (q^2_2) for and pleiotropy (ρ_q) with T2D

Chromosome	Position ^a (Mb)	Nearest SNP	Lod Score ^b		T2D ^c	
			WHR	WHRBMI	q^2_2	ρ_q
1	29 (24–32)	rs8559	2.16	1.44	<i>0.0829</i>	0.176
2	81 (79–88)	rs1457342	2.15	<i>3.04</i>	0.000600	0.359
2	235 (234–238)	rs869214	2.90	3.60	0.000185	0.00136
3	152 (148–159)	rs325762	1.49	2.73	0.283	0.663

Abbreviations: BMI, body mass index; lod, logarithm of the odds ratio; QTL, quantitative trait locus; SNP, single-nucleotide polymorphism; T2D, type 2 diabetes; WHR, waist-hip ratio.

^aPosition of the higher lod score for either WHR or WHRBMI in megabases from Build 37.1 (1-lod drop support interval).

^bSignificant (>3.29, bold) and near-significant (>3.00, italics) univariate lod scores.

^cFor WHR or WHRBMI, whichever had the higher lod score. For q^2_2 and ρ_q , significant (<0.05, bold) and near-significant (<0.1, italics) P -values.

DISCUSSION

We identified 12 T2D loci through suggestive or higher lod scores. Although these loci include nine that we reported previously,¹⁶ this analysis revealed three T2D loci that were previously obscured by accounting for the effect of BMI on T2D risk (chromosomes 2 at 237–240 Mb, 7 at 14–29 Mb and 20 at 56–59 Mb), strengthened to significance the evidence for two previously suggestive loci (chromosomes 2 at 61–65 Mb and 13 at 26–30 Mb), and inferred pleiotropy with obesity for six T2D loci (chromosomes 1 at 17–19 Mb, 2 at 237–240 Mb, 7 at 54–73 Mb, 13 at 26–30 Mb, 16 at 26–47 Mb and 20 at 56–59 Mb). In addition, we inferred four obesity loci through suggestive or higher lod scores for either WHR or WHR, adjusted for BMI (chromosomes 1 at 24–32 Mb, 2 at 79–88 Mb, 2 at 234–238 Mb and 3 at 148–159 Mb).

If obesity increases T2D risk solely because visceral adipose tissue produces diabetogenic substances,² then WHR, a measure of central obesity, should be a stronger risk factor for T2D than BMI. In agreement, we found the effects on T2D risk to be highly significant

for WHR, but nonsignificant for BMI, especially when also accounting for WHR. However, despite the nonsignificance of BMI in the absence of linkage information, accounting for BMI in linkage analysis lowered the lod scores by ≥ 0.5 for seven T2D loci,¹⁶ and we inferred pleiotropy with BMI for two T2D loci (chromosomes 13 at 26–30 Mb and 20 at 56–59 Mb), suggesting that factors other than visceral adipose tissue affect T2D risk. One of the T2D–BMI pleiotropic loci (chromosome 13 at 26–30 Mb) also showed pleiotropy with WHR, as did four other T2D loci (chromosomes 1 at 17–19 Mb, 2 at 237–240 Mb, 16 at 26–47 Mb and 22 at 27–40 Mb). The inference of pleiotropy with obesity may provide clues to the genes underlying these T2D loci. For example, although most T2D variants identified through GWA studies affect insulin secretion, the pleiotropic T2D–obesity gene *FTO* alters insulin resistance.³⁰ Whether this characteristic will distinguish other pleiotropic T2D–obesity genes awaits their discovery.

Visceral adipose tissue also correlates with lipid levels.³¹ In fact, the considerable heterogeneity of plasma lipid profile in overweight and obese people depends partially on the degree of visceral adiposity and insulin resistance.³² Six of the 12 T2D loci reported herein showed suggestive or stronger evidence of pleiotropy with a lipid level,³³ five with triglyceride (2 at 77–102 Mb, 2 at 237–240 Mb, 7 at 54–73 Mb, 13 at 26–30 Mb and 16 at 26–47 Mb) and one with low-density lipoprotein cholesterol (1 at 17–19 Mb). All, but two of these loci (chromosomes 2 at 77–102 Mb and 7 at 54–73 Mb), showed evidence of pleiotropy with WHR as well, consistent with visceral adiposity affecting both lipid levels and T2D.

Seven of the 12 T2D loci reported herein fall in replicated linkage regions:³⁴ three in the most highly replicated regions (2 at 237–240 Mb, 20 at 56–59 Mb and 22 at 27–40 Mb) and four in regions reported in fewer, but still multiple studies (1 at 17–19 Mb, 2 at 61–65 Mb, 7 at 14–29 Mb and 7 at 54–73 Mb). However, the T2D linkage regions reported by Lillioja and Wilton³⁴ cover 1139 Mb, nearly 40% of the autosomal total, making the overlap of 54% of our loci less unexpected. The regions containing our loci were replicated in multiple ethnic groups, including samples with African ancestry. Possibly the six loci in non-replicated regions (2 at 77–102 Mb, 2 at 113–117 Mb, 11 at 119–122 Mb, 13 at 26–30 Mb, 16 at 26–47 Mb and 22 at 27–40 Mb) harbor African-specific T2D loci. However, none of these six regions was identified in T2D-linkage scans of samples of African descent: three in African Americans^{14,35,36} and one in African families from Ghana and Nigeria.³⁷ Instead, overlap occurred only in replicated regions: on chromosomes 7 at 14–29 Mb,^{35,36} 7 at 54–73 Mb³⁶ and 20 at 56–59 Mb.³⁷ Further support for chromosome 7 at 54–73 Mb comes from a linkage scan of insulin sensitivity index in obese African-American families.³⁸ It should be noted that the samples used in the three African American T2D analyses included subsets of our sample.

In addition to overlap with linkage studies, 2 of our 12 T2D loci (chromosomes 2 at 61–65 Mb and 7 at 14–29 Mb) harbor SNPs identified in Caucasian GWA studies (rs10490072 near *BCL11A* and rs864745 in *JAZF1* (Zeggini *et al.*²⁸)). Nevertheless, rs864745 was among 19 T2D-associated SNPs found to have consistent association in five racial/ethnic groups, despite initial identification in Caucasians,³⁹ the study did not include rs10490072. Although both SNPs showed nominally significant associations with T2D in this sample,²⁹ neither explained much of the linkage in their respective regions. Nevertheless, these SNPs may be poor proxies for nearby causal variant(s) with larger effect sizes.

Also, three of our six T2D–obesity loci (chromosomes 13 at 26–30 Mb, 16 at 26–47 Mb and 22 at 27–40 Mb) harbor SNPs

identified in GWA studies of BMI⁴⁰ or WHR⁴¹ (rs4771122 in *MTIF3*, rs7359397 in *SH2B1* and rs4823006 in *ZNRF3*). Deletion of the *SH2B1* gene in mice resulted in insulin resistance and glucose intolerance,⁴² less is known about the other genes.

In summary, variance components linkage analysis provided suggestive or stronger evidence for 12 T2D loci, six of them pleiotropic with obesity, as well as for four obesity loci. Identification of the underlying genes should both increase understanding of the pathogenesis of obesity and T2D, and aid in the dissection of the genetic heterogeneity of T2D.

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Web Resources: jPAP <http://hasstedt.genetics.utah.edu/Eclipse2> <http://www.stat.washington.edu/thompson/Genepi/Eclipse.shtml> MERLIN <http://www.sph.umich.edu/csg/abecasis/Merlin> PedCheck <http://watson.hgen.pitt.edu/register/docs/pedcheck.html> CIDR <http://www.cidr.jhmi.edu/> GENNID http://professional.diabetes.org/Diabetes_Research.aspx?typ=18&cid=64380

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APPENDIX

Genetic material collected by, and families characterized by, the American Diabetes Association GENNID Study Group, which includes Eric Boerwinkle, PhD, University of Texas Health Science Center; John Buse, MD, PhD, University of North Carolina; Ralph DeFronzo, MD, University of Texas Health Science Center; David Ehrmann, MD, University of Chicago; Steven C Elbein, MD, University of Utah/University of Arkansas; Wilfred Fujimoto, MD, and

Steven E Kahn, MB, ChB, University of Washington; Craig L Hanis, PhD, University of Texas Health Science Center; Richard A Mulivor, PhD, and Jeanne C Beck, PhD, Coriell Cell Repositories; Jill Norris, PhD, University of Colorado School of Medicine; M Alan Permutt, MD, and Philip Behn, MD, Washington University School of Medicine; Leslie Raffel, MD, Cedars-Sinai Medical Center; and David C Robbins, MD, Medlantic Research Institute.