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A functional role for structural variation in metabolism

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A contribution of structural genomic variation to the heritability of complex metabolic phenotypes was illuminated by the recent characterization of chromosome-engineered mouse models for genomic disorders associated with metabolic dysfunction. Herein we discuss our study, "A duplication CNV that conveys traits reciprocal to metabolic syndrome and protects against diet-induced obesity in mice and men," which describes the opposing metabolic phenotypes of mouse models for two prototypical genomic disorders,[1,2] Smith-Magenis syndrome (SMS) and Potocki-Lupski syndrome (PTLS). SMS and PTLS are caused by reciprocal deletion or duplication copy number variations (CNVs), respectively, on chromosome 17p11.2. The implications of the results of this study and the potential relevance of these findings for future studies in the field of metabolism are discussed.

Keywords: CNV, obesity, metabolic syndrome, mouse model, structural variation, genomics

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Reciprocal Metabolic Phenotypes Caused by Reciprocal CNVs

Genetic factors have long been known to contribute to both normal human energy metabolism and the etiology of metabolic syndrome and obesity. However, the role of genomics and copy number variation (CNV) in these processes has not been studied until recently. Although many CNVs appear to be phenotypically benign, some have been found to play a role in disease susceptibility and in the pathogenesis of metabolic disorders as identified by several large-scale patient studies[3-5] (summarized in ref. 6). We recently applied an alternative approach to study metabolic traits associated with CNV of a specific genomic interval; heterozygous deletion of this genomic interval is known to cause a genomic disorder associated with obesity in humans, Smith-Magenis syndrome (SMS: MIM #182290; www.ncbi.nlm.gov.sites/entrez) and convey an obese phenotype in mice.[7,8] In this paper, entitled "A duplication CNV that conveys traits reciprocal to metabolic syndrome and protects against diet-induced obesity in mice and men," we describe diametric metabolic phenotypes observed in chromosome-engineered mice harboring reciprocal duplication or deletion CNVs, clearly demonstrating the functional link between CNV and metabolism.[9] We analyzed the metabolic profiles of previously-generated mouse models for Smith-Magenis (SMS) and Potocki-Lupski (PTLS; MIM #610883) syndromes, respectively harboring either deletion or duplication of the mouse chromosomal region syntenic to human chromosome 17p11.2.[10,11] While obesity and hypercholesterolemia were known to be associated with SMS,[12] few formal studies had been performed, and the metabolic profiles had not been extensively described in either PTLS or SMS subjects.

We showed that duplication mice (modeling PTLS) have decreased body weight and body fat composition, reduced TC/LDL levels in the blood, and increased insulin sensitivity that cannot be attributed to either increased activity or reduced food intake. Rather, these mice have increased intrinsic metabolic activity that also confers protection from diet-induced obesity.

This latter observation that mice fed a high-fat diet do not gain weight is a clear demonstration of gene X environmental interactions that can be very important in common, complex traits. Interestingly, the reciprocal deletion CNV (modeling SMS) results in a "mirror" or reciprocal phenotype, as the deletion mice are overweight with increased body fat, decreased HDL levels in the blood and reduced insulin sensitivity, corresponding with observations in SMS patients and consistent with the metabolic syndrome so common in the obese American population. The results of the metabolic phenotyping of duplication and deletion mice described in this study are summarized in **Figure 1**.

Further investigation into the genetic and biological mechanisms underlying these phenotypes revealed that they may not be due solely to the alteration of a single gene or genetic element. Rather they appear to be a consequence of the alteration of multiple genes/genetic elements by the CNV. The "genetic load" could potentially either act through cumulative effects on a single pathway or by each of the contributing genes affecting different downstream metabolic pathways.

The distinction between the metabolic phenotypes of mice harboring a duplication CNV vs. mice that transgenically overexpress the major candidate dosage-sensitive gene in the region, *Rai1*,[13] or between mice harboring a deletion CNV vs. mice that have a targeted gene knockout of *Rai1*, clearly demonstrates the central message of this study: structural genomic variation and the resultant alterations in downstream gene expression can be responsible for common, complex phenotypes, such as obesity or metabolic disorder. While these monogenic (transgenic *Rai1* overexpression or targeted $Rai1^{+/-}$ gene knockout) mouse models do display some abnormal metabolic phenotypes, they do not possess the full-spectrum of phenotypes identified in the mouse models harboring duplication or deletion CNVs, suggesting that epistatic interactions between the major dosage-sensitive gene, *Rai1*, and other genes or regulatory elements within the CNV (i.e., deviation from the normal n = 2 diploid state) region are needed to manifest the full spectrum of metabolic phenotypes. Interestingly, *Rai1* appears to have a greater influence on metabolism when its dosage is reduced (knockout/deletion) than when its dosage is increased (transgenic/duplication); the specificity of the CNV in this context is interesting, and it may be an indication that the precise pathways underlying leanness and resistance to diet-induced obesity may be distinct from those underlying obesity and metabolic syndrome, although some overlap is quite likely. Alternatively, the regulatory networks/pathways leading to obesity may be more susceptible to gene dosage perturbations.

Implications of This Study for the Field of Metabolism

The application of CNV-based animal models to the study of common, complex disorders such as obesity has been underutilized. Importantly, comparison between our mouse models for SMS and PTLS and other chromosome-engineered mouse models for loci known to be associated with metabolic phenotypes may identify overlapping/converging metabolic pathways or novel dosage-sensitive metabolic genes. For example, another genomic locus, 16p11.2, has been associated with mirror extreme BMI phenotypes in humans and would be an excellent candidate for future studies of obesity in mice.[5] Mouse models for this locus have been generated and utilized for studies of the neurobehavioral phenotypes associated with CNV at this locus;[14] however, their application toward metabolic research has not been reported to date.

Further investigation into the molecular pathways underpinning these metabolic phenotypes may lead to several interesting avenues of research. First, these studies may uncover the precise biological function of the dosage-sensitive genes responsible for the metabolic phenotypes—possibly downstream of the Rai1 chromatin remodeling protein or genetic elements required for proper genomic regulation of *Rai1*,[15] but also potentially revealing novel metabolic

Mouse Strain	Human disease being modeled	Body Weight	Fat content	Blood serum chemistry	Insulin sensitivity	Effect of altered diet	Functional consequence on metabolism
Dp(11)17/+	Potocki-Lupski syndrome (PTLS)	↓	↓	↓ HDL / TC	↑	High fat diet does not cause obesity	+ Improved metabolic traits
Df(11)17/+	Smith-Magenis syndrome (SMS)	↑	↑	ND	↓	Low fat diet delays the onset of obesity	− Metabolic syndrome-like

Figure 1. A summary of the reciprocal metabolic phenotypes observed in mouse models of PTLS and SMS. TC, total cholesterol; ND, no difference, although there was a trend toward increased HDL/TC.

pathways or networks. Second, they may lead to insights into therapeutic targets for obesity and metabolic syndrome as targets within these networks are identified. Importantly, this study demonstrates that the expression level of these metabolic genes is dosage-sensitive and does not need to be completely abolished or highly overexpressed to give a resultant phenotype. As such, targeting these pathways may only require a modest change in gene expression or protein levels to foster a rescue or reversal of the phenotype; the potential implications of this study for downstream applications and pharmaceutical development have not yet begun to be explored. It is well-established that *Rai1* gene expression is readily manipulated by small molecule effectors; the gene name reflects its initial isolation by a differential expression cloning strategy and as the first cloned "retinoic acid-inducible" gene.[16] Lastly, with the establishment of these chromosome-engineered mouse models[17,18] comes the ability to analyze other major contributors to complex metabolic phenotypes, such as environmental factors. While we have learned much about the role of structural variation in metabolism from patient studies, the ability to control for genetic and environmental background effects, and also to readily manipulate such modifiers, makes these mice an ideal experimental model with which to further investigate environmental factors and to test potential therapeutics.

A Network of Metabolic Genes at Work

To further complicate the research challenges that lay ahead, it was recently determined by the Walz and Reymond laboratories that CNVs can affect the gene expression not only of the genes within the CNV itself, but also of the genes in *cis* outside of the genomic rearrangement along the same chromosome.[19] With a number of genes being up- or down-regulated, the possibility of complex epistatic interactions and downstream regulatory effects need to be considered. One can imagine the added complexity of developmental expression, temporal regulation and tissue-specificity of many of these genes, functioning in an intricate gene network, and further underscoring the importance of a systems-based approach to studying the biology of metabolism. While this study is a clear example of CNV resulting in altered metabolic regulation in a syndromic background, much remains to be learned. The ongoing identification of novel, highly-penetrant CNVs that result in idiopathic obesity and metabolic syndrome suggest that the importance of genomics in the field of energy regulation and metabolism, while currently underappreciated, will become more evident in the future.[3] CNV may play a prominent role in common diseases similar to what has been realized for both genomic disorders and Mendelian traits during the last two decades.[20]

References

1. Lupski JR. Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. Trends Genet 1998; 14:417-22; PMID:9820031; http://dx.doi.org/10.1016/S0168-9525(98)01555-8
2. Lupski JR. Genomic disorders ten years on. Genome Med 2009; 1:42; PMID:19439022; http://dx.doi.org/10.1186/gm42
3. Bochukova EG, Huang N, Keogh J, Henning E, Purmann C, Blaszczyk K, et al. Large, rare chromosomal deletions associated with severe early-onset obesity. Nature 2010; 463:666-70; PMID:19966786; http://dx.doi.org/10.1038/nature08689
4. Walters RG, Jacquemont S, Valsesia A, de Smith AJ, Martinet D, Andersson J, et al. A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. Nature 2010; 463:671-5; PMID:20130649; http://dx.doi.org/10.1038/nature08727
5. Jacquemont S, Reymond A, Zufferey F, Harewood L, Walters RG, Kutalik Z, et al. Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. Nature 2011; 478:97-102; PMID:21881559; http://dx.doi.org/10.1038/nature10406
6. Dasouki MJ, Youngs EL, Hovanes K. Structural Chromosome Abnormalities Associated with Obesity: Report of Four New subjects and Review of Literature. Curr Genomics 2011; 12:190-203; PMID:22043167; http://dx.doi.org/10.2174/138920211795677930
7. Greenberg F, Lewis RA, Potocki L, Glaze D, Parke J, Killian J, et al. Multi-disciplinary clinical study of Smith-Magenis syndrome (deletion 17p11.2). Am J Med Genet 1996; 62:247-54; PMID:8882782; http://dx.doi.org/10.1002/(SICI)1096-8628(19960329)62:3<247::AID-AJMG9>3.0.CO;2-Q
8. Walz K, Spencer C, Kaasik K, Lee CC, Lupski JR, Paylor R. Behavioral characterization of mouse models for Smith-Magenis syndrome and dup(17)(p11.2p11.2). Hum Mol Genet 2004; 13:367-78; PMID:14709593; http://dx.doi.org/10.1093/hmg/ddh044
9. Lacaria M, Saha PK, Potocki L, Bi W, Yan J, Girirajan S, et al. A duplication CNV that conveys traits reciprocal to metabolic syndrome and protects against diet-induced obesity in mice and men. PLoS Genet 2012; 8:e1002713; PMID:22654670; http://dx.doi.org/10.1371/journal.pgen.1002713
10. Greenberg F, Guzzetta V, Montes de Oca-Luna R, Magenis RE, Smith AC, Richter SF, et al. Molecular analysis of the Smith-Magenis syndrome: a possible contiguous-gene syndrome associated with del(17)(p11.2). Am J Hum Genet 1991; 49:1207-18; PMID:1746552
11. Potocki L, Chen KS, Park SS, Osterholm DE, Withers MA, Kimonis V, et al. Molecular mechanism for duplication 17p11.2- the homologous recombination reciprocal of the Smith-Magenis microdeletion. Nat Genet 2000; 24:84-7; PMID:10615134; http://dx.doi.org/10.1038/71743
12. Smith AC, Gropman AL, Bailey-Wilson JE, Goker-Alpan O, Elsea SH, Blancato J, et al. Hypercholesterolemia in children with Smith-Magenis syndrome: del (17)(p11.2p11.2). Genet Med 2002; 4:118-25; PMID:12180145; http://dx.doi.org/10.1097/00125817-200205000-00004
13. Slager RE, Newton TL, Vlangos CN, Finucane B, Elsea SH. Mutations in RAI1 associated with Smith-Magenis syndrome. Nat Genet 2003; 33:466-8; PMID:12652298; http://dx.doi.org/10.1038/ng1126
14. Horev G, Ellegood J, Lerch JP, Son YE, Muthuswamy L, Vogel H, et al. Dosage-dependent phenotypes in models of 16p11.2 lesions found in autism. Proc Natl Acad Sci U S A 2011; 108:17076-81; PMID:21969575; http://dx.doi.org/10.1073/pnas.1114042108
15. Yan J, Bi W, Lupski JR. Penetrance of craniofacial anomalies in mouse models of Smith-Magenis syndrome is modified by genomic sequence surrounding Rai1: not all null alleles are alike. Am J Hum Genet 2007; 80:518-25; PMID:17273973; http://dx.doi.org/10.1086/512043
16. Imai Y, Suzuki Y, Matsui T, Tohyama M, Wanaka A, Takagi T. Cloning of a retinoic acid-induced gene, GT1, in the embryonal carcinoma cell line P19: neuron-specific expression in the mouse brain. Brain Res Mol Brain Res 1995; 31:1-9; PMID:7476016; http://dx.doi.org/10.1016/0169-328X(95)00020-S
17. Adams DJ, Biggs PJ, Cox T, Davies R, van der Weyden L, Jonkers J, et al. Mutagenic insertion and chromosome engineering resource (MICER). Nat Genet 2004; 36:867-71; PMID:15235602; http://dx.doi.org/10.1038/ng1388
18. Ramírez-Solis R, Liu P, Bradley A. Chromosome engineering in mice. Nature 1995; 378:720-4; PMID:7501018; http://dx.doi.org/10.1038/378720a0
19. Ricard G, Molina J, Chrast J, Gu W, Gheldof N, Pradervand S, et al. Phenotypic consequences of copy number variation: insights from Smith-Magenis and Potocki-Lupski syndrome mouse models. PLoS Biol 2010; 8:e1000543; PMID:21124890; http://dx.doi.org/10.1371/journal.pbio.1000543
20. Lupski JR, Belmont JW, Boerwinkle E, Gibbs RA. Clan genomics and the complex architecture of human disease. Cell 2011; 147:32-43; PMID:21962505; http://dx.doi.org/10.1016/j.cell.2011.09.008