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Commentary

Knockout of human muscle genes revealed by large scale whole-exome studies

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ABSTRACT

Large scale whole-exome sequence studies have revealed that a number of individuals from different populations have predicted loss-of-function of different genes due to nonsense, frameshift, or canonical splice-site mutations. Surprisingly, many of these mutations do not apparently show the deleterious phenotypic consequences expected from gene knockout. These homozygous null mutations, when confirmed, can provide insight into human gene function and suggest novel approaches to correct gene dysfunction, as the lack of the expected disease phenotype may reflect the existence of modifier genes that reveal potential therapeutic targets. Human knockouts complement the information derived from mouse knockouts, which are not always good models of human disease. We have examined human knockout datasets searching for genes expressed exclusively or predominantly in striated muscle. A number of well-known muscle genes was found in one or more datasets, including genes coding for sarcomeric myosins, components of the sarcomeric cytoskeleton, sarcoplasmic reticulum and plasma membrane, and enzymes involved in muscle metabolism. The surprising absence of phenotype in some of these human knockouts is critically discussed, focusing on the comparison with the corresponding mouse knockouts.

1. Introduction

Mutations of genes coding for muscle proteins cause a large number of Mendelian diseases, from muscular dystrophies to metabolic muscle disorders [1]. Actually, the spectrum of the phenotypes resulting from muscle gene mutation is even wider. At one extreme, some mutations may be lethal in utero, thus akin to the embryonic lethal phenotype in mice: for example, mutation of the *CHRNA1* gene, coding for the alpha 1 subunit of the nicotinic cholinergic receptor (ACHRα1), causes fetal akinesia and lethal hydrops fetalis [2]. At the other extreme, some mutations are clinically silent or may even be beneficial: for example, a large proportion of healthy individuals lack α-actinin-3 because of homozygosity for a common stop-codon polymorphism in the *ACTN3* gene [3]. Surprisingly, the *ACTN3* genotype is associated with athletic performance, as elite sprint athletes have significantly higher frequencies of this mutation.

Novel muscle gene mutations were recently identified in individuals from different populations by large scale whole-exome sequence studies made possible by the implementation of next generation sequencing. An interesting result of these studies was the discovery of homozygous genotypes with predicted loss-of-function (pLoF) of many genes due to nonsense, frameshift, or canonical splice-site mutations predicted to inactivate a gene. These "human knockouts" or homozygous null mutations were detected in predominantly outbred individuals of different origin, like in the Exome Aggregation Consortium (ExAC) study [4] or in relatively isolated populations with common founders, such as the Icelanders [5], and were especially frequent in consanguineous families, as determined by two large scale studies on British-Pakistanis [6] and Pakistanis [7].

The study on human knockouts can provide insight into human gene function and is relevant to medicine and drug discovery. Mouse gene knockouts have so far been used as models of human disease, however they do not completely mimic human disorders. Indeed, the failure of many clinical trials is often due to the limitations of preclinical studies, thus the availability of human data is important. The discovery of knockouts without the expected disease phenotype is especially interesting as it may reflect the existence of modifier genes, whose identification provides potential therapeutic targets.

We have examined four whole-exome human knockout datasets searching for genes expressed exclusively or predominantly in striated muscle. A number of these genes was found in one or more datasets, including genes coding for sarcomeric myosins and components of the sarcomeric cytoskeleton, sarcoplasmic reticulum and plasma membrane, or enzymes involved in muscle metabolism. A selection of these

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Table 1
Genes coding for muscle proteins showing apparently complete knockout in human whole-exome studies.

Functional compartment	Gene	Protein	Database[a]			
			Icelanders [5]	British-Pakistanis [6]	ExAC [4]	Pakistanis [7]
			1171/104,220[b,c]	781/3200[b,d]	1775/60,706[b]	1317/10,503[b,e]
Contractile proteins	*MYH1*	MYH-2X	+	+	+	+
	MYH13	MYH-EO	−	−	−	+
	MYH15	MYH-15	+	−	−	+
Sarcomeric cytoskeleton	*TTN*	Titin	−	−	−	+
	OBSCN	Obscurin	+	+	+	+
	NEB	Nebulin	−	+	−	−
	MYOM2	Myomesin 2	+	−	+	−
	MYBPH	Myosin binding protein H	−	−	−	+
Sarcoplasmic reticulum	*RYR1*	Ryanodine receptor 1	−	−	−	+
	TRDN	Triadin	−	−	−	+
Plasma membrane	*ORAI1*	ORAI1	−	−	+	+
	MYOF	Myoferlin	−	−	−	+
Muscle metabolism	*AMPD1*	AMP deaminase 1	−	−	−	+
	PGM1	Phosphoglucomutase 1	−	−	−	+

[a] Presence (+) or absence (−) of predicted LoF (see Table S2 in [6], and Supplementary Table 1 in [7]).
[b] Number of genes with predicted complete knockout/total number of individuals investigated.
[c] Including a proportion of subjects with a variety of diseases, among which heart failure and congenital heart disease (see Table 2 in [5]).
[d] Including 892 subjects with adult type 2 diabetes.
[e] Including 4793 cases with myocardial infarction and 5710 controls free of myocardial infarction.

Table 2
Effect of human gene mutation and mouse gene knockout.

Functional compartment	Gene[a]	Phenotype[b]	
		Human mutation	Mouse knockout
Contractile proteins	*MYH1*	ND	Growth inhibition, muscle weakness, altered muscle structure and function [12,13]
	MYH13	ND	ND
	MYH15	ND	ND
Sarcomeric cytoskeleton	*TTN*	Myopathies and cardiomyopathies [17]	Embryonic lethal (deletion of titin M-line domain) [18]
	OBSCN	ND	No major phenotype [22]
	NEB	Nemaline myopathy [19]	Perinatal lethal [20,21]
	MYOM2	ND	ND
	MYBPH	ND	ND
Sarcoplasmic reticulum	*RYR1*	Malignant Hyperthermia Susceptibility and different types of congenital myopathies [25]	Perinatal lethal [26]
	TRDN	Long-QT syndrome and occasionally myopathy [30,31]	No major phenotype [27–29]
Plasma membrane	*ORAI1*	Severe combined immuno-deficiency and muscular hypotonia [33]	Perinatal lethal in C57BL/6 mice, partial survival with immunological and skin changes in outbred ICR mice [34]
	MYOF	ND	Muscle fiber atrophy and impaired regeneration [36]
Muscle metabolism	*AMPD1*	Exercise intolerance or asymptomatic [38]	No phenotype [39]
	PGM1	Exercise intolerance, cardiomyopathy and other symptoms [40]	ND

[a] Same genes shown in Table 1. See text for details.
[b] ND, no human disorder or mouse knockout has been described.

genes is shown in Table 1. Previous studies on the effect of mutations of the same genes in humans and knockout of the corresponding genes in mice are reported in Table 2.

2. Human muscle gene knockouts

2.1. Myosins

Three genes coding for sarcomeric myosin heavy chains (MYHs) are present in the human knockout datasets: *MYH1*, *MYH13* and *MYH15*. Mutations in *MYH1*, coding for type 2X MYH, have not been associated with hereditary disease, whereas mutations in the other two genes expressed in adult human skeletal muscle, *MYH7*, encoding the slow/β-cardiac MYH, and *MYH2* gene, encoding the fast-type 2A MYH, are responsible for a variety of cardiac and skeletal muscle myopathies [8]. *MYH1* pLoF mutations have been detected in all four human knockout datasets suggesting that these mutations are not infrequent. This gene is expressed as a minor component in human skeletal muscles, and is not detectable in muscle biopsies from many individuals [9,10], thus it would not be surprising if no obvious phenotype was detected in affected individuals. In addition, *MYH1* is transcribed only after birth, therefore cannot cause congenital disorders, such as joint contractures, like those induced by mutations of *MYH3* and *MYH8*, coding for developmental myosins [11]. In contrast, *Myh1* is widely expressed in mouse muscles and its ablation causes growth inhibition, muscle weakness, histological abnormalities, kyphosis, and altered kinetics of muscle contraction and relaxation [12,13]. Surprisingly, *MYH4*, coding for type 2B MYH, which is expressed only in trace amounts in human skeletal muscle [9] and thus might be expected to be even more prone to inactivating mutations, was not present in the human knockout datasets. No mouse model is available for *MYH13* and *MYH15* knockout: both genes are expressed in extraocular muscles but not in most body