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Manipulative evidence and medical interventions: some qualifications

Abstract

The notion of causal evidence in medicine has been the subject of wide philosophical debate in recent years. The notion of evidence has been discussed mostly in connection with Evidence Based Medicine (EBM) and, more in general, with the assessment of causal nexus in medical, and especially, research contexts. "Manipulative evidence" is one of the notions of causal evidence that has stimulated much debate. It has been defined in slightly different ways, attributed different relevance, and recently placed at the core of Gillies' "action-related theory of causality", a view specifically meant to address causation in medicine. While in general sympathetic to Gillies' account, and totally convinced of the relevance of manipulative evidence and different sorts of interventions in the biomedical sciences, we believe that some further qualifications are needed to allow the notion of manipulative evidence to better express features of medical practice. In particular, we provide some qualification of the role of "interventional evidence" proposed by Gillies, suggesting a distinction between "interventional evidence" and "evidence for interventions". A case study from research on rare diseases is analyzed in depth and a multifaceted notion of manipulative evidence put forward that allows better understanding of what manipulations in medical contexts amount to and what their targets are.

1. Manipulative evidence and interventions in philosophy of medicine

The notion of evidence and evidential hierarchies have been widely debated in philosophy of medicine for roughly a decade especially with reference to Evidence Based Medicine (EBM), its tenets and limits. Of the different kinds of evidence defined in the literature, manipulative evidence has been a topical subject in the philosophy of medicine literature, mostly discussed with regard to its role in distinguishing a genuine causal relation from a mere correlation, that is, differentiating between simply stating that a mechanism is in place and/or establishing whether some change in the (alleged) cause can be exploited to bring about a change in the (alleged) effect.

On the topic, Campaner (2012), e.g., stresses the crucial role of manipulation and experimental evidence in the assessment of mechanistic relations. As an example, she cites the famous work by Warren and Marshall, as analyzed in Thagard (1998), showing that evidence from manipulation plays a part insofar as "eradicating bacteria cures ulcers" as studies on the impact of antibiotics indicated. Elaborating on their (2007), Campaner and Galavotti (2012) argue for the role of manipulative evidence, "i.e. evidence from intervention" (p. 31): "evidence from manipulations constitutes a fundamental type of evidence in the assessment of causal relations, and manipulations performed on the basis of detected correlations often *precede* mechanistic knowledge" (p. 29). Campaner and

Galavotti go on to highlight how manipulative evidence can allow for preventive intervention – as witnessed by many cases in the history of epidemiology[1] – or treatments – as seen in Deep Brain Stimulation to treat Parkinson's disease – even in the absence of knowledge of the mechanisms involved. In their discussion of Hill's guidelines to causation (2009), Howick, Glasziou and Aronson, point to the role, alongside "mechanistic evidence" and "parallel evidence", of what they label "direct evidence", that is, evidence "from studies (randomized or non-randomized) that a probabilistic association *between intervention and outcome* is causal and not spurious" (p. 186, italics added). Extensively reporting on work carried out within the EBM+ group project (http://ebmplus.org/), and summarizing its results, Parkkinen et al. (2018) present "experimental manipulation" (p. 78), or "direct manipulation (e.g. in vitro experiments)" (p. 14) as providing sound evidence of mechanisms. The authors cite many occurrences of "interventions" to discuss – as indicated in the subtitle of the (2018) volume – both the "principles" and the "procedures" on which interventions rely and on which outcomes are to be evaluated.

In one way or another, all the cases mentioned acknowledge the epistemological importance of "manipulative evidence", as evidence from interventions, in medicine. To put manipulative evidence centre stage is Gillies' "action-related theory of causality", presented in its most complete form in his volume "Causality, Probability, and Medicine" (2019), but already outlined in simpler form in (2005). Let us consider his position in detail. It belongs to what Gillies labels AIM theories, that is, Action, Intervention, Manipulation theories developed from the 1930s by authors such as Collingwood, Gasking, and von Wright. Gillies argues that actions, interventions, and manipulations are clearly *human* actions, and is therefore an anthropocentric concept[2]. Although critical of a few features, Gillies agrees with Menzies and Price's agency theory insofar as it assigns a central role to the human agent. At the same time, he believes that causal laws also hold in nature, and objectively so: they would exist even if no human being were there to detect them. Among the laws holding in nature, causal laws are those we choose to "exploit" in order to reach our purposes, or to make the desired outcome more likely to occur. "Causal laws are useful and appropriate in situations where there is a close link between the law and action based on the law" (Gillies 2005, p. 827). It is in this sense that causality is a manipulative notion: a causal law is a law that supports an intervention we are interested in. It can be both an intervention meant to bring something about, or to prevent something from occurring. Gillies stresses the relevance of this second sort of action in medicine – namely, *avoidance actions*, as opposed to *productive actions* – where the final goal is typically treatment or prevention, aimed at making the disorder *not* occur. As examples, Gillies mentions the relation between smoking

[1] See Vineis and Ghisleni (2004), who draws on Wynder (1994).

[2] Gillies has also worked extensively on mechanisms, recognizing that they play a very important role in medicine. However, he rejects mechanistic theories of causality, arguing that we should not try to define causation in terms of mechanisms, but rather, the other way round, i.e. mechanisms in terms of "cause", where the notion of cause should be defined by adopting the action-related theory. In this paper, we will not dwell on Gillies' reflections on mechanisms, but will confine our attention to his action-related account.

and lung cancer, and between rain and the grass, ceteris paribus, becoming wet. Not smoking reduces the incidence of smoking in a population, and manipulation of some of the conditions ceteris paribus, i.e. covering the grass with a waterproof sheet, keeps the grass dry. The second action is a "blocking action": the intervention on one of the conditions *sine quibus non* blocks the effect from occurring[3]. Gillies' theory also aims to accommodate certain causes that cannot be manipulated, i.e. the action-related theory of causality is meant to be applied also to some causal laws like "A causes B" in cases in which A cannot be manipulated by human action. In cases such as "earthquakes are caused by friction between continental plates", the law can be used – Gillies stresses – "as the basis of an avoidance action. We need only refrain from going into areas which are on the boundary between continental plates, and we can be sure of avoiding earthquakes" (Gillies 2019, p. 32).

Causes are especially important in medicine for a number of reasons. Knowing what causes a disorder – e.g., understanding whether pain in a patient's chest is caused by lung cancer, angina, a bacterial infection of the bronchi, or something else (cf. *ibid*., p. 20) – will prove crucial to establishing the most adequate treatments. Gillies' discourse on causality directly addresses how the assessment of a causal relation is pursued, and he assigns a central role to the notion of causal evidence in its "interventional" form. "A causal law – Gillies argues – has to support an intervention. It follows from this, that, in establishing a causal law, it is desirable to have interventional evidence as well as observational evidence. By interventional evidence, I mean evidence obtained by making an intervention and recording its results". He then describes what he calls the "Principle of Interventional Evidence", according to which "A causal law cannot be taken as established unless it has been confirmed by some interventional evidence". This principle is maintained to hold *everywhere* in medicine: "in medicine it is *always* possible to collect interventional evidence, since in addition to statistical evidence in human population, there is also laboratory evidence using experimental animals, tissues, or cells" (*ibid.*, p. 24, italics added). Reinforcing the claim, Gillies states: "there is no difficulty about collecting interventional evidence in medicine, and the principle of interventional evidence should be taken as holding for all cases of causality in medicine" (*ibid*., p. 129).

It seems that the relation between causal laws and interventions holds in two ways: on the one hand, causal laws support interventions – i.e. they tell us when/where to intervene – and, on the other, interventional evidence – that is, as just recalled above, "evidence obtained by making an intervention and recording its results" (*ibid.*, p. 24) – is needed, in addition to observational evidence, to establish causal laws. If we follow the first strand of Gillies' reasoning, we find it that: "the basic idea behind the action-related theory of causality is […] that causal laws are useful and appropriate in situations where there is a close link between the law and action based on law" (*ibidem*), that is, where the law is causal. If we refer strictly to this perspective, then it would appear that the position might be better regarded as a "causation-related theory of action", rather than the "action-related theory" of causality"

[3] Vaccination is given as an example of a blocking action in medicine.

it aims to be: it is our knowledge of some causal laws that encourages us to act in a certain way, and it is the actual holding of the causal law in the world that is responsible for the direct effect obtained. But how do we get to know causal laws in the first place? This is where the second strand comes into the picture, in the – manipulationist-friendly – perspective: evidence that causal relations are in place is collected by means of interventions. Gillies takes this aspect so seriously as to establish the "Principle of Interventional Evidence" mentioned above. Causal laws are not taken as definitive, but rather established as confirmed, for the time being, by available evidence. Further evidence might drive changes. The Principle, however, is taken as central and ineradicable in the medical sciences: "for establishing causality in medicine, I would argue that the Principle of Interventional Evidence should be accepted", Gillies concludes (*ibidem*).

In sum, Gillies seems to be arguing that: i) we *always* manage to get manipulative evidence in medicine, and ii) we can then *use* manipulative evidence *to reach our goals*. Some refinement of such claims might, however, be useful to make them both more precise and closer to actual medical practice. Overall, we are sympathetic to Gillies' account, and totally convinced of the relevance of manipulative evidence and different sorts of interventions in the biomedical sciences. There is no doubt that medical theories are indeed meant to be translated into practice on the basis, in the main, of causal relations, and that treatment and preventions are forms of interventions. However, we believe that some qualification is needed to make the notion of manipulative evidence more attuned to the features of medical practice. Issues at stake include: 1) assessing a causal relation without manipulating it; 2) the difference between *assessing that* a causal nexus is there and *being able to intervene* on that same nexus; 3) the difference between being able *to intervene on the causal nexus* itself and being able *to intervene on the relata*, taken separately.

2. Causative agents of rare diseases: the case of LGMD1F

Although not directed exclusively at medicine, Gillies' theory is largely concerned with the health sciences. Moreover, "the whole point of an agency or action-related theory of causality is to link causal laws *with practical human actions in the real world*" (Gillies 2005, p. 837, italics added). In this section let us thus present an actual case study, taken from biomedical research on rare diseases. The pathology under investigation is limb-girdle muscular dystrophy 1F – henceforth LGMD1F. In what follows, we will carry out an in-depth analysis of the way in which the causative agent of the pathology was discovered and how research on the pathology was pursued, to investigate whether, to what extent and in what sense manipulation played a role.

LGMD1F[4] is one of a family of limb-girdle muscular dystrophies affecting the muscles controlling voluntary movements of the arms, legs, fingers, toes and face. The disease typically first

[4] This is how the pathology is known in the literature. Recently, the name has been changed to Emery–Dreifuss muscular dystrophy (EDMD) (see Straub et al. 2018). As our paper gives an overview of the recent history of

presents as weakness of the muscles of the lower limbs, especially those closest to the centre of the body (known as the "proximal muscles"), e.g. the upper legs. Being a progressive disease, muscle strength and volume deteriorate over a number of years. Symptoms show inter-individual variability, also within the same family. They can present at different times in life between childhood and adulthood, be of different intensity, manifesting as unusual gait, and difficulty running, jumping or climbing the stairs. Later in life, symptoms may also include upper body muscle weakness, for example, difficulty straightening the elbows. Myopathies and respiratory insufficiency may also be present. The diagnosis is usually suspected in people who show typical symptoms, and subsequently confirmed by laboratory tests. No treatment is currently known that can halt, let alone reverse the disease, and available treatments are mainly aimed at supporting the increasingly weak muscles and containing complications. Treatment options recommended for people with LGMD1F include weight control to avoid obesity, physical therapy and stretching exercises to prevent contractures. The use of canes, walkers and wheelchairs to support ambulation, and constant monitoring for heart problems are indicated.

LGMD1F is a rare disease inherited as an autosomal dominant trait, that is, only one mutant gene is present in people with the disease. Since we inherit one copy of each gene from our mother and one from our father, if a person with the disease has children, each child has a 50% chance that he or she will have the disease as well. In some cases, however, people with LGMD1F do not exhibit a family history. This can be due to a couple of very different reasons. First, given the highly variable expression of the disease, even within the same family, other family members may be undiagnosed sufferers. For example, some may have heart problems but no other signs of muscle weakness. Second, cases may present with no family history because the patient is the first person in the family to be affected, presenting a *de novo* genetic change. The first set of diagnostic tests usually includes: electromyography; creatine-kinase levels; muscle biopsy; echocardiogram. These are followed by genetic testing.

The causative agent of the disease has only fairly recently been identified as a genetic mutation. Until then, LGMD1F was not registered as a separate disease but thought to belong to a group of genetically heterogeneous disorders (LGMDs) characterized by progressive muscle weakness and histological signs of muscle degeneration. What is now known as LGMD1F was eventually suspected as being a distinct disease by virtue of certain specific features, such as wide variability in age at onset, disease progression, and the specificity and severity of symptoms. Let us review some of the crucial steps in the discovery of what is now regarded as the cause of this particular disease. It provides an interesting example shedding light on the actual use of "manipulations" in at least some research contexts concerned with rare genetic diseases.

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investigations on the disease, we have adopted the previous naming. We would like to thank Prof. Giovanni Cenacchi for drawing this disease to our attention and for her comments on a previous version of the paper.

A significant step forward in LGMD1F research was the identification of a large Spanish kindred in which 5 generations were found to suffer from limb-girdle muscular dystrophy. The identification of such a large kindred proved very useful in the discovery process. Two different forms were identified on the basis of the age at onset of symptoms: a juvenile form, with onset before 15 years (66%), and an adult form, with onset at around 30-40 years (28%). All patients presented the pelvic and proximal shoulder girdle weakness typical of disease symptoms[5].

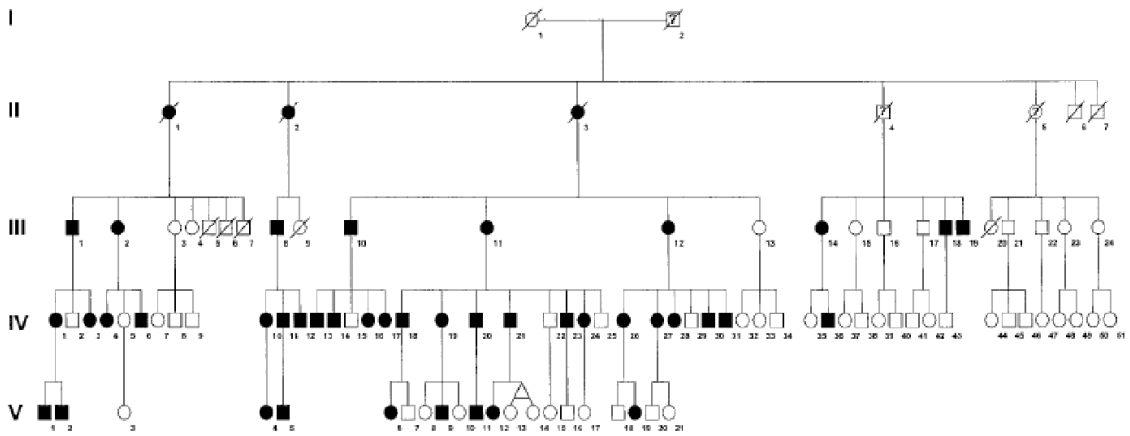

Figure 1. Drawing of the pedigree. Clinically affected members are shown in black. Roman numerals indicate generation number, and Arabic numerals birth order position within the generation.

[Fig. 1, from Gamez et al. (2001), p. 451. [Delete original caption from Fig. 1, and re-write with larger characters:] Drawing of the pedigree of the large Spanish kindred analyzed. Clinically affected members are shown in black. Roman numerals indicate generation number, and Arabic numerals birth order position within the generation. The distribution of relatives with the disease in the kindred appears.]

Gamez et al. (2001) report the studies on the kindred that led to causal knowledge of limb-girdle muscular dystrophy (LGMD). 61 members of the family were examined. 32 of them (see generations IV and V in Fig. 1) were shown to have weakness of the pelvic and shoulder girdles, with severity worsening from one generation to another. Clinical phenotype and morphologic details were obtained with a range of examinations aimed to collect as much information as possible on the patients' conditions, their differences and similarities. Tests included serum creatine kinase, aspartate aminotransferase, and alanine aminotransferase determinations, performed on all subjects; electrocardiography on 12 patients; neurophysiologic examinations on 12 patients; echocardiography in 6; muscle biopsy in 5, and MRI of the brain in two cases[6]. Findings were matched with the preliminary genetic investigations carried out on all 61 family members. Linkage analysis to chromosomes 5q31, 1q11-q21, 3p25, 6q23, and 7q demonstrated that the disease is not allelic to

[5] Other features, such as respiratory muscle involvement, were shown to be present in a few but not all 32 patients.
[6] Muscle strength and functional ability were assessed using internationally accepted scales.

LGMD forms 1A, 1B, 1C, 1D, and 1E. Genetic testing excluded chromosomal loci associated with other forms of AD-LGMD and various other myopathies, thereby progressively narrowing the genetic field of likely causes. It was concluded that the family had a *genetically distinct* form of autosomal limb-girdle muscular dystrophy and further research, such as a genome-wide scan, was considered necessary to identify the disease locus. No animal model was employed.

Further investigation of the same family considered in Gamez et al. (2001) led the research group to use a genome-wide screen with more than 400 microsatellite markers. As a result, they found a linkage to a 3.68-Mb region on chromosome 7q32.1-32.2. The research was "able to accurately define the final critical region and established a common disease haplotype in all affected persons" (see Palenzuela et al., 2003, pp. 404-405. See Fig. 2).

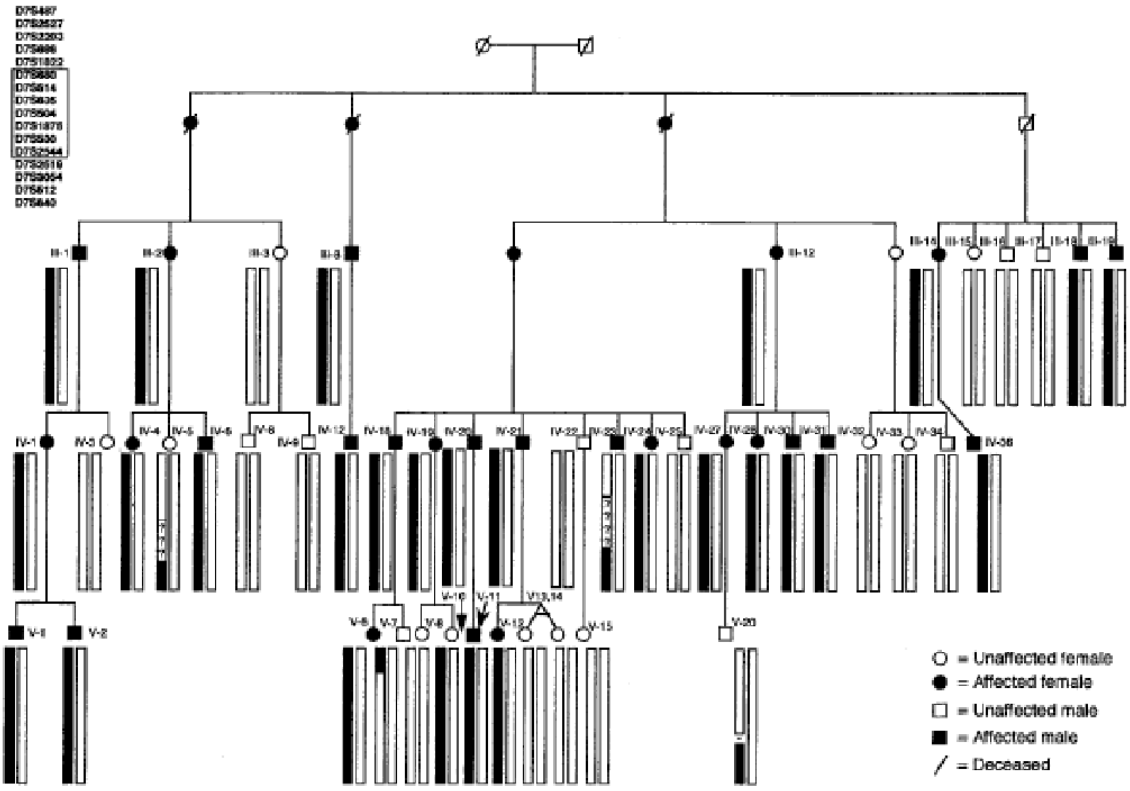

Figure. Genotyped persons of the pedigree with bars representing a segment of chromosome 7q32.1-32.2. A common haplotype from marker D7S487 to marker D7S640 (dark segments of the bars) is shared by almost all affected family members. Recombinant subjects IV-5 and IV-23 define the linked interval of seven markers between D7S680 and D7S2544. The presence of homozygous alleles in their parents, indicated by a question mark, prevents further restriction of the critical region. Subject V-20 is clinically unaffected and shows an uninformative recombination because he is a child and may develop the disease. Similarly, subject V-10, aged 11 years, is clinically unaffected and harbors the complete haplotype present in most of the affected family members.

[Fig. 2, Fig. and caption from Palenzuela et al. 2003, p. 406 [Delete original caption from Fig. 2, and re-write with larger characters, simplifying:] Genotyped persons of the pedigree with bars representing a segment of chromosome 7q32.1-32.2. A common haplotype from marker D7S487 to marker D7S640 (dark segments of the bars) is shared by almost affected family members.]

A candidate gene, FLNC, encoding a muscle specific actin-binding protein, was studied to better identify the causative mutation. However, FLNC was shown *not to be involved* in the pathogenesis of the disease. On the one hand, the research paved the way for further investigations on *candidate genes in the same critical region* (e.g. fascin3 (*FSCN3*), ubiquitin conjugating enzyme E2H (*UBE2H*), and *KIAA0265*), on the other, it simply, and more generally, confirmed the clinical and genetic heterogeneity of LGMD, suggesting that a new form of the disease might be present that could probably be traced back to mutations in a region on chromosome 7q32.1-32.2. This was in fact identified as the disease locus, and the pathology was named LGMD1F.

The elusive identity of the mutant gene was tackled again using a whole genome sequencing strategy, performed on four affected individuals (first on one, then on four, see Torella et al. 2013) (See Fig. 3).

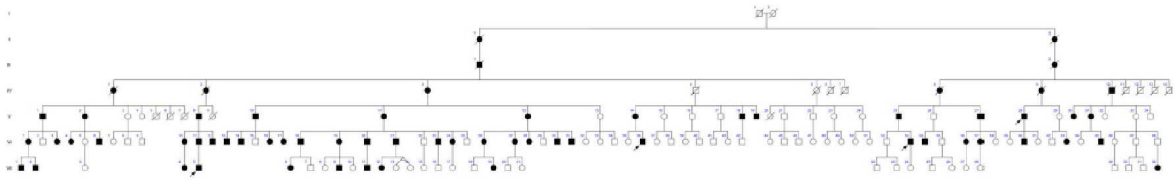

Figure 1. LGMD1F family pedigree. Squares represent male; circles represent female; white figures symbolize normal individuals; black figures indicate individuals with clinical muscular dystrophy. The original LGMD1F family has been extended from subject II,2 and now includes 64 LGMD patients of both sexes and five non-penetrant carriers (IV-4, V-26, V-29, V-33, and VI-68). The whole-exome sequencing was performed in four patients indicated by arrows (V-28, VI-36, VI-53, VII-5).
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[Fig. 3. Fig. and caption from Torella et al. 2013. [Delete original caption from Fig. 3, and re-write with larger characters, simplifying:] LGMD1F family pedigree. Squares represent male; circles represent female; white figures symbolize normal individuals; black figures indicate individuals with clinical muscular dystrophy. The original LGMD1F family has been extended from subject II, 2 and now includes 64 LGMD patients of both sexes and five non-penetrant carriers. The whole-exome sequencing was performed in four patients, indicated by arrows (V-28, VI-36, VI-53, VII-5)]

This led to identification of the causative mutation responsible for the distinct disease of LGMD1F: a heterozygous single nucleotide deletion (c.2771del) in the termination codon of transportin 3 (TNPO3), a gene situated within the chromosomal region linked to the disease. This gene encodes a protein belonging to the importin beta family and transports serine/argininerich proteins into the nucleus[7]. "The mutation is predicted to generate a 15-amino acid extension of the C-terminus of the protein, segregates with the clinical phenotype, and is absent in genomic sequence databases and a set of > 200 control alleles". The discovery of the microdeletion in the transportin 3 gene as the cause of

[7] This discovery has fostered further interest in the disease, since TNPO3 has also been identified as a key factor in the HIV-import process into the cell nucleus.

LGMD1F has also highlighted "the importance of defects of nuclear envelope proteins as causes of inherited myopathies" (Melia et al. 2013, pp. 1508-1509).

[Fig. 4 The figure shows effects of the c.2771del mutation of TNPO3 messenger RNA and protein (see difference between wild type and mutant). It represents the 3'-terminal coding and untranslated region (UTR) sequences of TNPO3 transcripts, including the 3'-end of the exon 23 (black font) and the 5'-end of the non-coding exon 24 (blue font) of both wild-type and mutant (c.2771del) complementary DNAs. The fragments shown are identical in both transcript variants 1 and 2. The deleted 2771A is labelled with an asterisk. The encoded amino acids are indicated in a one-letter code. Changes resulting from the frame-shifted codons in the mutated sequence are indicated in red case, which highlight the disruption of the native TAG stop codon. Figure and caption from Meila et al. 2013, p. 1514]

More precisely, after the first genetic tests failed, the whole genome sequencing analysis of DNA carried out on four family members led to interesting results: "after intersecting the results of whole genome sequencing with the results from previous linkage analysis (Palenzuela et al., 2003) (chromosome 7: 126 287 120–129 963 917), 3888 variants (3125 single nucleotide variants and 763 indels) were identified, from which 718 were novel, not present in the dbSNP database [...] (http://www.ncbi.nlm.nih.gov/projects/SNP/). Additional criteria based on the dominant inheritance of the disease, the population frequency of the variants and effect prediction […] allowed […] to rule out all but one of these variants, a heterozygous mutation in the termination codon of the TNPO3 gene. (…) The mutation (c.2771del, …) is a single adenine nucleotide deletion in the TAG stop codon, common to the two protein isoforms encoded by the gene. The del-A results in conversion of TAG to TGC codon, encoding cysteine, and extension of the reading frame by 15 codons to a downstream of the termination-signal within the transcript. (…) The retrospective analysis of the sequences of this gene revealed that this heterozygous mutation had been missed in the past due to the poor quality of the electropherograms". Presence of c.2771del was shown "in *each* of the 29 clinically affected individuals and absence of the mutation in *all* 20 clinically unaffected relatives tested" (Melia et al. 2013, p. 1513, italics added. See Fig. 4 above).

The relations between the evidence collected and conclusions as to the causative factor were expressed as being based on "several lines of evidence [which] strongly support pathogenicity of the TNPO3 mutation in this family with autosomal dominant-LGMD: (i) TNPO3 resides within the chromosome 7q32.1-32.2 locus for LGMD1F; (ii) the mutation segregates with the phenotype; (iii) the

microdeletion is absent in publicly available genomic sequence databases (dbSNP build 135, 1000 Genomes Project and 5400 NHLBI exomes) and in our set of 4200 Spanish control alleles indicating that all control individuals harbour the canonical TNPO3 TAG termination codon in homozygosity at the position 128 597 311 of the chromosome 7; (iv) the mutation in the termination codon of TNPO3 is predicted to extend the coding sequence at the 3'-end of the messenger RNA and to generate an aberrant protein; (v) the mutated messenger RNA is expressed in the muscle of the affected individuals; and (vi) the detection of histologically abnormal muscle nuclei with atypical nuclear filaments, anomalous TNPO3 immunoreactivity and irregular membranes" (ibid., p. 1515). No manipulation on the cause-effect relation was performed. A *hypothetical* mechanism suggested of "the putative role of the TNPO3 in transport of proteins across the nuclear membrane" (*ibidem*) encouraged further debate. The hypothesis was that the mutant protein, predicted to contain 15 additional amino acids at the C-terminus, is expressed in skeletal muscle and exerts some toxic effect. Through the analysis of the whole genome of four affected family members, the variation identified and mapped to 7q32, was observed to be shared by all affected subjects. It was further shown (Torella et al. 2013) that no other variant was shared by all four affected individuals. Additional functional studies on model organisms were conducted to understand the exact role of the mutation and its mechanisms of action. However, these models were not regarded as necessary to discover the cause. In fact, they were not available at the time, just as no animal models are available at present that would allow us to unravel the pathogenetic mechanism leading from the mutation to the pathological damage, and hence to the symptoms.

3. Interventional evidence: some qualifications

What epistemological lessons can be drawn from the case presented above? The (very recent) history of research on LGMD1F highlights a number of relevant features involved in causal discovery, its underlying theoretical assumptions and practical consequences in medical contexts. Far from claiming that the features of this case study hold in all medical contexts, we nonetheless believe they can be taken as representative of a few epistemological aspects of research in the context of rare diseases – an area deserving more attention by philosophy of medicine than has thus far been given – and point out distinctions that might prove useful in other research contexts. Let us focus on three of them.

In the first place, what research on LGMD1F immediately shows is that assessment of the causal relation considered responsible for the disease is pursued without manipulating the causal relation itself. Research work on the disease started from very careful clinical observations of the patterns of similar symptoms in patients. In the case of unknown rare diseases, clinical pictures appear that cannot be subsumed under already accepted models of disease. Peculiar features in a range of symptoms lead to the suspicion that some genetic mutation must have occurred, which has then to be proved with molecular analyses. In the case in question, symptomatic specificities with respect to

other dystrophies were initially observed. What in both this and other cases is regarded as sufficient – for the assessment *that* a causal relationship holds between a genetic mutation and the manifestations of a range of symptoms is the correspondence between the range of symptoms and the presence of the mutation in *all* the cases considered. No manipulation is made of the alleged cause – the mutated gene – to observe changes in the alleged effect. The epistemic process leading to the discovery of *what the cause is* does not, thus, benefit from interventional evidence, as defined above. Causal knowledge is achieved by observing symptoms, on the one hand, and analysing genomic data, on the other. Information is then compared and matched. No intervention is made on the patients' conditions, nor are animal models employed at this stage. In this sense, the Principle of Interventional Evidence sounds too strong: not *always* is it possible in medicine to collect interventional evidence.

Things differ when enquiries move on to investigate *how* the causative agent works, namely to understand the pathogenetic process. At this stage, in vivo or in vitro experimentation has to be carried out. As already remarked, animal models were *not* employed in the studies to identify the causative agent, although they were used subsequently and elsewhere in the case of distinct kinds of muscular dystrophies (Pasteuning-Vuhman, S. et al. 2017). Animal models have not yet been used to perform manipulations on the cause of the disease, i.e. the exact genetic mutation. They were not used to assess the presence of a causal nexus between the triggering cause and the disease. Investigations are being conducted at present to understand the pathogenetic process for therapeutic purposes. Genome editing would "repair" the condition – although damage already present before editing would not be reversed. The effects produced by the mutation would not be undone.

A second relevant aspect that emerges from the case considered and is related to reflections on 1), is the epistemological difference between *assessing that* a causal nexus is there and *being able to intervene* on that same nexus. Not only are these different epistemic procedures, they might need different kinds of evidence, as the case analysed shows. Gillies claims that the causal laws we look for are those we can exploit for manipulation, and that it is through manipulations that we get to know them. Although by no means contradicting Gillies' position, the case of LGMD1F, and of other rare diseases, presents a quite different scenario to the one depicted by him. The epistemological process that allowed the gene mutation responsible for the disorder to be ascertained does not suffice to have an impact on the effect. The nexus is not discovered by means of manipulation, and the current state of our causal knowledge does not allow us to directly intervene on the cause to prevent the effect from occurring, nor to delay it once the cause is known. Situations are unfortunately similar in many other diseases where a genetic cause has been identified but no treatment is available – at least currently. Let us dwell on this point, and consider how it might be dealt with in terms of Gillies' action-related theory. On the one hand, it does not resemble cases like refraining from smoking to reduce the risk of lung cancer: we cannot decide not to have the genetic mutation in our bodies. On the other hand, the case does not resemble other sorts of unmanipulable relations like those acknowledged by Gillies and

recalled in section 1. The causal relation between the mutation and the symptoms does not closely resemble that between, e.g., geological features of certain areas and earthquakes: while we can decide, as Gillies stresses, not to go to earthquake-prone areas and so avoid earthquakes, we cannot decide not to have the mutation in our bodies. Moreover, to date no intervention devised at some point along the process going from the mutation up to the symptoms is able to affect the outcome – unlike what happens in the case of covering the grass when it rains. Currently employed treatment strategies do not act on the cause-effect relation, but are so-to-speak "side" interventions to alleviate symptoms. They only impact the effects themselves, but do not act on the relation between the causative agent and the symptoms. The knowledge that is regarded as sufficient to identify the causative agent is not currently sufficient to find intervention or treatment strategies that would cure the disease. The only intervention to prevent the disease would be genetic consultancy leading parents affected by the mutation not to procreate. This would in fact count as an avoidance action à la Gillies, not in the sense that it affects the causal nexus in the individual exhibiting it as the result of a conscious action[8], but in the sense that it prevents the causal nexus being transferred to other individuals. Avoiding reproduction would surely be a way of preventing further individuals from inheriting the pathology (although it would not prevent *de novo* genetic changes).

Is the example examined analogous to the case of Koch's investigating cholera, where inoculation in animals could not be performed? Would the idea of avoidance action work here, thus rescuing manipulation? Referring to Koch's investigation, Gillies remarks: "It does indeed follow from the action-related theory of causality (and other AIM theories of causality) that causality in medicine cannot be established without some interventional evidence. However, this interventional evidence does not have the form of giving an animal or human the disease (a productive action), as in Koch's original postulate 4. [Interventional evidence] can instead take the form of preventing humans getting the disease, or curing them if they do have the disease. Such avoidance actions […] are quite as satisfactory as productive actions in supplying the necessary interventional evidence" (Gillies 2019, p. 67). For Gillies, medical cases where experimental manipulation like Koch's cholera studies cannot be performed, clearly show the superiority of his account over other manipulationist views: it is because we avoid getting the cholera bacillus that we avoid getting the disease. So, would the notion of "avoidance action" or "blocking action" work in the case of LGMD1F, thus rescuing manipulation? The answer has to be qualified in the sense just recalled. Causal discovery did not proceed by means of manipulation, and the disease – identified as a distinct disease on the basis of the genetic mutation identified as the causative agent – cannot currently be avoided by the affected person through conscious action, nor can it be avoided through a blocking action. However, its transmission can be stopped by those knowingly affected deciding not to have siblings.

[8] In the case of smoking, even if one has smoked for quite some time, quitting or reducing smoking will reduce the probability of getting lung cancer in the future.

Thirdly, we stress that the two points made above are by no means meant to deny or downplay the role of manipulation. On the contrary, it clearly emerges that a whole range of different techniques relying on interventions has been brought into play with the aim of obtaining as detailed information as possible on the features of the relata, both in terms of cause and effect. An increasingly extended range of sophisticated clinical tests is performed on patients to ascertain as many clinical parameters as possible as precisely as possible. Genetic tests employ increasingly advanced technologies. What deserves attention is exactly *what* manipulation is exerted *upon*: no manipulation is exerted on the (hypothetical) causal link itself, although, separately, genetic testing is conducted targeting the alleged cause, and clinical tests are performed on the observed effects to identify the emergence of possibly relevant features. This has relevant implications from both the research and clinical standpoints. In particular, the difference should be stressed between being able *to intervene on the causal nexus* itself and being able *to intervene on the relata* taken separately. What research work does, in a sense, is to further refine observation of extant features, highlighting all the characteristics of the affected patients involved, whether symptomatic or genetic. In other words, manipulations are performed on alleged causes and alleged effects to "get them ready" for data matching. Differences between affected individuals and control subjects – those not affected by the disease – are detected. This is done without intervening on the causal relation under examination: sophisticated techniques are adopted to unravel present features, but the *relation* between the causative agent and the effect – or, in the control case, between the absence of the causative agent and the absence of the effect – is not manipulated. They are detected in the separate settings by means of testing techniques and then compared.

4. Concluding remarks: Interventional evidence and evidence for interventions

What emerges from the above considerations on the causal discovery of a rare disease is that situations in the health sciences are so varied that it is difficult - and perhaps not very useful - to make overly general claims on methodological aspects related to manipulation and interventional evidence. Gillies states: "It does indeed follow from the action-related theory of causality (and other AIM theories of causality) that causality in medicine cannot be established without some interventional evidence" (Gillies 2018, p. 67). We have shown how causal assessment can in fact also occur without it. Furthermore, *interventional evidenc*e, defined as evidence obtained by means of some intervention when assessing a causal relation, may not suffice to enable us to intervene therapeutically on the medical causal nexus assessed. We might, in other terms, lack *evidence for* clinical *interventions*.

The situation presented by research on LGMD1F is far from unique. A large number of rare diseases currently present analogous epistemological concerns and are being tackled in similar ways. Methodological investigations along analogous lines hold for other, very different, kinds of diseases. A range of congenital anomalies and intellectual disabilities, for instance, has recently been investigated through whole-exome sequencing (WES). Data from patients with a diagnosis are

analysed and periodically re-analysed to combine "diagnostic representation of clinical WES data with translational research involving data sharing for candidate genes" (Nambot et al. 2018, 645). No animal model is employed, and no intervention is performed to establish a causal relation between congenital anomalies and forms of intellectual impairment. These sorts of disorders tend to have a rather early onset, are often seriously impairing, and are of genetic origin incurred via Mendelian inheritance. WES is regarded as unprecedentedly successful in identifying the disease-causing genes, and tailored sequencing is considered useful to clarify the molecular bases of disorders such as, e.g., developmental delay (DD). In general, new candidate genes are sought especially in cases of atypical presentation of known diseases or ultra-rare diseases still unknown to specialists. Data are internationally shared to this end and collaboration projects are run to "allow fast and accurate phenotype matching to assess the clinical relevance of candidate variants of genes" (*ibid.*, p. 646). In cases of good genotype-phenotype correlations, reported in an extremely low number of patients, a search is made in internationally available data for other patients with analogous phenotype carrying variants in the same gene in the attempt to confirm the genotype-phenotype relation. Team collaboration is pursued to exchange data and perform proper matching and identify new patients carrying the same genetic variants. The genomic data of affected patients and asymptomatic control individuals (of different ethnic populations) are shared. No manipulation for the purpose of causative attribution is performed that affects the relation between the genetic variant and the symptoms. Nor, in many cases, do scientists have significant knowledge of the mechanism leading from the mutation to the symptoms. While there is no doubt *that* a mechanism is in place, *what the mechanism is like* is unknown and seldom controversial. In cases of diseases like those mentioned above, causal claims are established on the basis of difference-making evidence of a non-manipulative sort, and not by virtue of some support from mechanistic knowledge.

The case presented above shows how data on symptoms and genetic datasets played an evidential role by virtue of matching the features specific to the sets considered – a matching of relata that led to the assessment of causal claims in the absence of any manipulation of the causal relation itself. These distinctions might well prove relevant also outside the realm of research on rare diseases, even outside the realm of the biomedical sciences. The wide range of new techniques involving experimentation, simulation, and data extraction might call for a much more nuanced set of notions to effectively and usefully grasp the notion of "manipulation". Furthermore, it should not be forgotten that not only does the amount of causal knowledge we have vary significantly from one case to another, but also what we can use it for. A significant example is provided by cystic fibrosis, a disease known since 1989: the responsible mutation has to involve both copies of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR), an anion channel that regulates the thickness of many secretions. While the CFTR gene may display multiple mutations, many are rare or infrequent. The so-called Phe508del mutation is the most common, accounting for about two-thirds of

all cases of cystic fibrosis: a deletion of three nucleotides in the CFTR gene leads to a protein lacking an amino acid, which is therefore not processed correctly and remains in the cytoplasmic space instead of being directed to the membrane. This mutation causes double damage to the host: on the one hand, the channel is not delivered to the correct side of action, and, on the other, even if it were to reach the side it would not work properly. Wide detailed knowledge of the causative factors notwithstanding, no therapy was available until extremely recently (see e.g. Collins 2019). Investigations into the disease adopted a range of different techniques, such as epidemiology, gene sequencing and cloning, gene therapy, protein crystallography and the full array of new molecular biology techniques developed over the past thirty years. However, the therapy found sprung from traditional pharmacology research showing that a combination of drugs acted together to correct and potentiate the function of the mutated protein by helping the CFTR channel reach the cell membrane and increasing channel functionality. In this sense, interventions adopted to understand what the causes of the pathology are and interventions to cure it – i.e. to make the effect not occur – do not coincide.

To conclude, we suggest that a distinction should be drawn between the ideas of "interventional evidence" and of "evidence for interventions". It should be recognized that the former, i.e. that obtained by means of some manipulation *to know that* a causal link is in place, might not coincide with the latter, i.e. that necessary to manipulate the causal nexus *in order to change* its outcome.

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