European Review for Medical and Pharmacological Sciences 2023; 27: 127-136

Aldo-keto reductase 1C2 (*AKR1C2*) as the second gene associated to non-syndromic primary lipedema: investigating activating mutation or overexpression as causative factors

J. KAFTALLI[1], K. DONATO[1,2], G. BONETTI[3,4], K. DHULI[3], A. MACCHIA[3], P.E. MALTESE[3], K. LOUISE HERBST[5], S. MICHELINI[6], P. CHIURAZZI[7], M. HILL[8], S. MICHELINI[9], S. MICHELINI[10], G. MARCEDDU[1], A. BERNINI[11], M. BERTELLI[1,2,3]

[1]MAGI EUREGIO, Bolzano, Italy
[2]MAGISNAT, Atlanta Techpark, Peachtree Corners, GA, USA
[3]MAGI's LAB, Rovereto, Trento, Italy
[4]Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy
[5]Total Lipedema Care, Beverly Hills, CA, USA
[6]Vascular Diagnostics and Rehabilitation Service, Marino Hospital, ASL Roma 6, Marino, Rome, Italy
[7]UOC Genetica Medica, Fondazione Policlinico Universitario "A. Gemelli" IRCCS & Istituto di Medicina Genomica, Università Cattolica del S. Cuore, Rome, Italy
[8]Department of Steroids and Proteofactors, Institute of Endocrinology, Prague, Czech Republic
[9]Unit of Physical Medicine, "Sapienza" University of Rome, Rome, Italy
[10]Neurosurgery, University of Tor Vergata, Rome, Italy
[11]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena, Italy

Abstract. – OBJECTIVE: Lipedema is a debilitating chronic condition predominantly affecting women, characterized by the abnormal accumulation of fat in a symmetrical, bilateral pattern in the extremities, often coinciding with hormonal imbalances.

PATIENTS AND METHODS: Despite the conjectured role of sex hormones in its etiology, a definitive link has remained elusive. This study explores the case of a patient possessing a mutation deletion within the C-terminal region of aldo-keto reductases Member C2 (*AKR1C2*), Ser320PheTer2, that could lead to heightened enzyme activity. A cohort of 19 additional lipedema patients and 2 additional affected family members[14] were enrolled in this study. The 2 additional affected family members are relatives of the patient with the *AKR1C1* L213Q variant, which is included in the 19 cohorts and described in Michelini et al[14].

RESULTS: Our investigation revealed that *AKR1C2* was overexpressed, as quantified by qPCR, in 5 out of 21 (24%) lipedema patients who did not possess mutations in the *AKR1C2* gene. Collectively, these findings implicate *AKR1C2* in the pathogenesis of lipedema, substantiating its causative role.

CONCLUSIONS: This study demonstrates that the activating mutation in the enzyme or its overexpression is a causative factor in the development of lipedema. Further exploration and replication in diverse populations will bolster our understanding of this significant connection.

Key Words:
AKR1C2, AKR1C1, Lipedema, Medical Genetics, Molecular dynamics, Gene overexpression, Estrogens.

Introduction

Lipedema mainly affects women. Despite it being considered rare, some studies[1,2] calculate an incidence of 11%, while its real incidence is unknown. The involvement of sex hormones has been postulated. Indeed, its manifestations commonly arise in females during hormonal change phases. Clinically, lipedema is characterized by abnormal bilateral symmetrical fat accumulation in the limbs and absent swelling in the hands, feet, or trunk[3]. Lipedema can often be misclassified with other diseases such as lymphedema and obesity, that present with limb enlargements and that often co-exist with lipedema. Contrary to obese patients, the increase of fat in lipedema often causes symptoms such as pain and increased vascular fragility and is not responsive to diet or exercise. Contrary to lymphedema, the tissue

Corresponding Author: Jurgen Kaftalli, BSc; e-mail: jurgen.kaftalli@assomagi.org

in lipedema is soft to the touch. Chronic fatigue, anxiety, and depression constitute important psychological comorbidity in women with lipedema[4-6]. Since the clinical diagnosis of lipedema can be difficult to obtain due to confounding characteristics of other diseases, such as lymphedema and gynoid obesity, a confirmatory test is needed, and studies in this direction are urgent. There is evidence[1] of a genetic base for the condition (genetic autosomal-dominant hereditary pattern with sex limitation) in many families, and about 60% of females with lipedema report[7] a family history, but the causes and genes that lead to the development of this condition have yet to be fully understood. We previously found that aldo-keto reductases Member C1 (*AKR1C1*), an essential enzyme for steroid hormone regulation, is mutated in a family affected by lipedema, suggesting that these hormones may play a role in the pathogenesis of the disease. It is known[8,9] that sex hormones also determine the anatomical site of the accumulation of adipose tissue. Dysfunction of sex steroids results in abnormal fat distribution in predisposed subjects, especially in females at the time of puberty[7]. The homeostasis of steroid hormones is finely regulated by enzymes such as aldo-keto reductases (*AKR1C*), hydroxysteroid dehydrogenase (HSD), and aromatases expressed in adipocytes, preadipocytes, and mature adipose tissue[10,11]. The four human *AKR1Cs* are multifunctional enzymes with overlapping activities on a broad range of substrates. They possess approximately 320 amino acid residues and share at least 84% amino acid sequence identity. *AKR1C1* and *AKR1C2* differ by only seven amino acids, with only one amino acid difference at the active site[12]. All four *AKR1Cs* can exert 3-, 17- and 20-ketoreductase activity, though each has its own distinct preferences for position, stereochemistry, and substrate. *AKR1C1* is the major 20α-reductase that inactivates progesterone, whereas *AKR1C2* preferentially acts as a 3α-reductase, with particular importance in the deactivation of dihydrotestosterone (DHT) to 5α-androstane-3α,17ß-diol (3α-Adiol)[12]. Progesterone and DHT play opposite roles with regard to fat accumulation, with progesterone prompting lipogenesis and DHT inhibiting adipogenesis[13]. In this article, to support the claim that *AKR1C2* overexpression may be a feature of lipedema, we describe a case of lipedema affected by a deletion in the C-terminal tail of *AKR1C2*, leading to a truncated *AKR1C2* enzyme. To understand the effect of the truncated *AKR1C2*, we employed Molecular Dynamics simulations of the wildtype and mutant forms of the enzyme. The results showed that the truncated type had an increased affinity for its substrate, indicating a potentially elevated *AKR1C2* activity. Finally, we identified alteration in *AKR1C2* mRNA expression levels in a separate cohort of lipedema patients, providing more evidence of the correlation between *AKR1C2* elevated activity and lipedema.

Patients and Methods

Ethical Compliance

The study was performed according to the declaration of Helsinki and was approved by the "Azienda Sanitaria della Provincia di Bolzano" Ethics Committee. Written informed consent was obtained from the subjects for publication prior to the study.

Subjects

A female subject affected by AKR1C2 Ser320PheTer2

The main subject in this paper is in possession of the *AKR1C2* Ser320PheTer2 variant, causing an in-frame deletion. The patient was diagnosed with lipedema. She was informed in detail prior to genetic analysis and provided her written informed consent.

Other subjects

A cohort of 19 additional lipedema patients and 2 additional affected family members[14] were enrolled in this study. The 2 additional affected family members are relatives of the patient with the *AKR1C1* L213Q variant, which is included in the 19 cohorts and described in Michelini et al[14]. All patients were informed in detail prior to genetic analysis and prior to blood collection for RNA analysis. They provided their written informed consent. Clinical information is available in Table I.

NGS Sequencing

New Generation Sequencing techniques (NGS) enable comprehensive and high-throughput analysis of genetic mutations, allowing us to uncover both known and novel genes associated with diseases[15-20]. Genetic analysis was performed using an NGS approach and a custom-made gene panel designed to include the main genes involved in syndromes of accumulation of subcutaneous adipose tissue[13]. A MiSeq personal sequencer (Illumina, San Diego, CA, USA) was used to sequence a subset of 13 genes (*ADRA2A*, *104210; *AKT2*,

Table I. Clinical data of probands analysed in qPCR.

Clinical data		19 F	
Mean age		42.8	±10.5
Familiarity		100%	
Onset	childhood	1	5.3%
	puberty	14	73.7%
	adulthood (>20 yrs)	3	15.8%
	Not Known	1	5.2%
Localization of fat depots	buttocks	16	84.2%
	legs	15	78.9%
	thighs	19	100%
	arms	12	63.2%
	forearms	4	21.5%
	trunk and abdomen	1	5.2%

*164731; *ALDH18A1*, *138250; *CIDEC*, *612120; *LIPE*, *151750; *LMNA*, *150330; *MFN2*, *608507; *NSD1*, *606681; *PALB2*, *610355; *PLIN1*, *170290; *POU1F1*, *173110; *PPARG*, *601487; *TBL1XR1*, *608628) + 4 additional genes of the *AKR1C* family (*AKR1C1*, *600449; *AKR1C2*, *600450; *AKR1C3*, *603966; *AKR1C4*, *600451) contained in a custom NGS panel comprising the coding sequences and flanking regions of 1,302 genes associated with neurological and psychiatric disorders, obesity, and metabolic and cardiovascular disorders were analyzed to seek any variants associated with lipedema. The probe set was designed to capture the coding exons and 15 bp flanking regions of each gene of the panel using the Twist Custom Panel Design Technology (Twist Bioscience, https://www.twistbioscience.com/products/ngs). Target sequences are based on the GRCh38/hg38 genome version. The cumulative target length of the panel was 3.55 Mb. Each variant was classified as a pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign, or benign, according to the American College of Medical Genetics (ACMG) guidelines21. Variants were also verified on ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and VarSome (https://varsome.com/) databases.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from blood using the Tempus™ Spin RNA Isolation Kit following manufacturer protocols. The SuperScript VILO cDNA Synthesis Kit was used to generate first-strand cDNA. Quantitative real-time polymerase chain reaction (qPCR) was performed by using the PowerUp™ SYBR™ Green Master Mix (Thermofisher) on a QuantStudio 3 Real-Time PCR Systems. The primers used in the qPCR experiments were previously described and are the following: GACAAGCTTCCCGTTCTCAG and GGAGTCAACGGATTTGGTCG for *GAPDH*, CCTAAAAGTAAAGCTTTAGAGGCCACC and GAAAATGAATAAGGTAGAGGTCAACATAAT for *AKR1C1*, CCTAAAAGTAAAGCTCTAGAGGCCGT and GAAAATGAATAAGATAGAGGTCAACATAG for *AKR1C2*, GAGAAGTAAAGCTTTGGAGGTCACA and CAACCTGCTCCTCATTATTGTATAAATGA for *AKR1C3*[14,22].

Molecular Dynamics Simulations

Molecular dynamics simulations of wildtype and mutant human *AKR1C2* in ternary complex with nicotinammide adenine dinucleotide phosphate (NADPH) and 5α-DHT were performed using the structure present in Protein Data Bank (PDB) entry 1MRQ[23] as the starting structure and running *in-silico* mutagenesis to create *AKR1C2*. The bent nicotinamide ring of NADPH was taken from protein data bank (PDB) entry 1HET[24] and was merged into the starting structure. To study the structural dynamics of the steroid-binding pocket, two 100 ns simulations were run, one for each type of protein (wildtype and truncated). For each Molecular Dynamics (MD) simulation, we used the Gromacs package[25]. Chemistry at Harvard Macromolecular Mechanics (CHARMM36) was chosen as the force field, and ligands were parametrized using CgenFF[26]. Each protein molecule was placed in a triclinic box with a minimum spacing of 1.2 nm on each side. The system was then solvated using transferable intermolecular potential with 3 points (TIP3P) water molecules, neutralized with Na^+/Cl^-, and energy minimized via the gradient descent algorithm. The minimized system was then subjected to two subsequential equilibration steps of position-restrained molecular dynamics in the NVT [amount of substance (N), volume (V) and temperature (T)],

and NPT [amount of substance (N), pressure (P) and temperature (T)] ensembles, 100 ps each. Reference temperature and pressure of 300 K and 1 bar were imposed, respectively. Finally, a molecular dynamics production run was performed for 100 ns with a 2-fs integration step.

Results

AKR1C2 C-Terminal Tail Removal Elevates the Activity of the Enzyme

The Ser320PheTer2 variant causes the loss of the C-term tail in *AKR1C2*. Previous studies[27] have shown that in pig *AKR1C1*, the removal of C-term affects the reduction rate of DHT, with this region significantly contributing to the NADPH-dependent reductase activity for the 5α-DHT reduction, an activity reserved for *AKR1C2* in humans. However, pig *AKR1C1* is 15 residues longer than human *AKR1C2*, and while highly similar, they differ in seven other amino acids. We, therefore, decided to employ Molecular Dynamics simulations to study the structural dynamics of wildtype and truncated human *AKR1C2*. The average structure (the middle trajectory of the largest cluster of trajectories) of the 100 ns molecular dynamics simulation, was obtained for each system. In both *AKR1C2* types, 5α-DHT was bound similarly, forming hydrophobic interactions mainly with Tyrosine (Tyr24), Valine (Val54), and Tryptophan (Trp227), as well as other hydrophobic residues surrounding the binding site (Figure 1). Hydrogen bonding between the C3 ketone group of 5α-DHT and Histidine (His117) was observed in the average structures of both types. However, in the truncated type, C3 of 5α-DHT was in close proximity to the hydroxyl group of Tyr55, suggesting hydrogen bond formation; indeed, in the molecular dynamics simulation, the hydrogen bond was formed during specific timeframes (Figure 2).

The steroid is positioned between Val24 and Trp227, with the β-face directed towards Trp227. The tryptophan is properly oriented in the truncated type, but its side chain is flipped in the wildtype, as shown in the average structures (Figure 1). Moreover, in the simulation of the wildtype, Trp227 moves away from the steroid, disrupting the interaction with its β-face. The root-mean-square deviation (RMSD) plot in Figure 3 shows that the RMSD value of the truncated Trp227 type is more stable than that of the wildtype. The disruption of this interaction could also explain the increased distance between the steroid and Tyr55.

The interaction energy between DHT and the protein was -104.51 (kJ/mol) in the wildtype and -126.90 (kJ/Mol) in the truncated type, further indicating that the steroid is in a more favorable conformation in the truncated type. This augmented interaction between DHT and the truncated protein indicates an elevated DHT reduction rate and, hence, an overactive *AKR1C2* enzyme.

Moreover, to initiate a catalytic reaction, the distances of both the hydroxyl group of Tyr55 and C4N of NADPH, from C3 of 5α-DHT should be small. The respective distances during the 100 ns

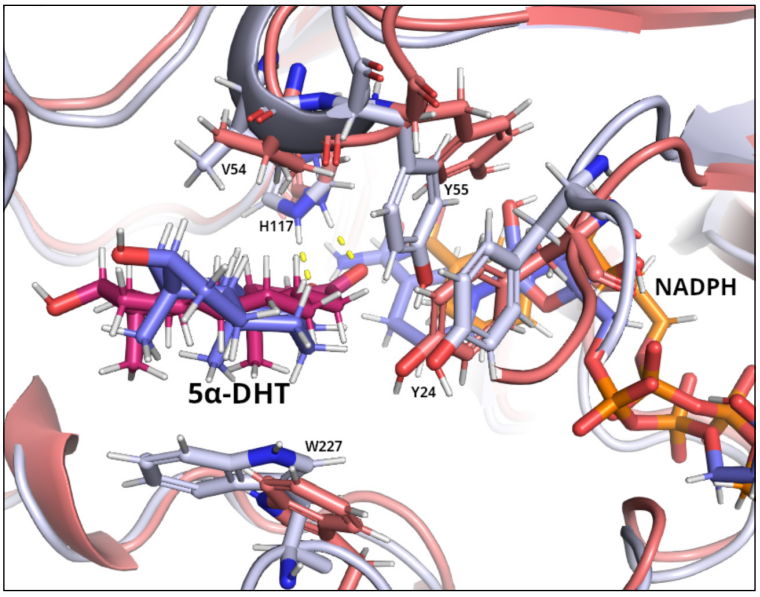

Figure 1. Conformation of 5α-DHT into the binding pocket of *AKR1C2*. Wildtype protein highlighted in light red; truncated protein highlighted in light blue; hydrogen bonds are shown in dashed yellow lines. The steroid is positioned between Val54 and Trp227 in the truncated type. Notable is the difference in conformation of the Trp227 side chain, which interacts with the β-face of 5α-DHT, as it is flipped away in the wildtype. Trp227 is one of the main residues that hold the steroid in place.

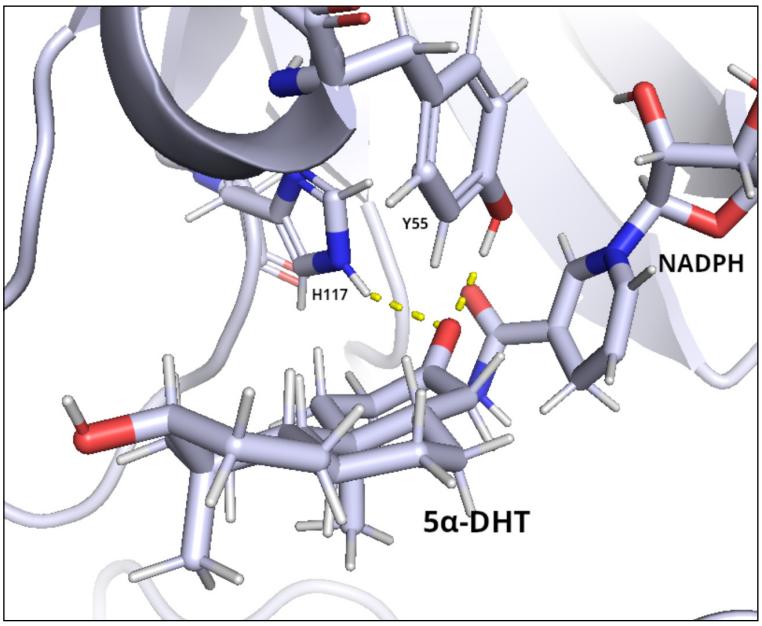

Figure 2. Two hydrogen bonds, highlighted in dashed yellow lines, between the ketone at C3 of 5α-DHT and His117, Tyr55. The forming of hydrogen bonds between the steroid and Tyr55 was observed in the molecular dynamics simulation of the truncated type only.

simulation for each system were computed, and the results show that in the truncated type, the C3 of 5α-DHT seems to be closer to both OH of Tyr55 and C4N of NADPH (Figure 4), implying a higher chance for the initiation of catalysis.

Expression of AKR1C2 in Lipedema Patients' Samples

With the aim of verifying whether *AKR1C2* overexpression was a frequent condition in lipedema, we performed qPCR analysis on blood RNA extracted from a pool of 21 (19 + 2) patients with lipedema. Interestingly, while *AKR1C1* and *AKR1C3* expression in blood was not different between groups, *AKR1C2* expression was high in a few lipedema patients (N=5). Those patients were the 3 affected family members from our previously described *AKR1C1* L213Q mutated family and 2 other probands. Relative expression is depicted in Figure 5.

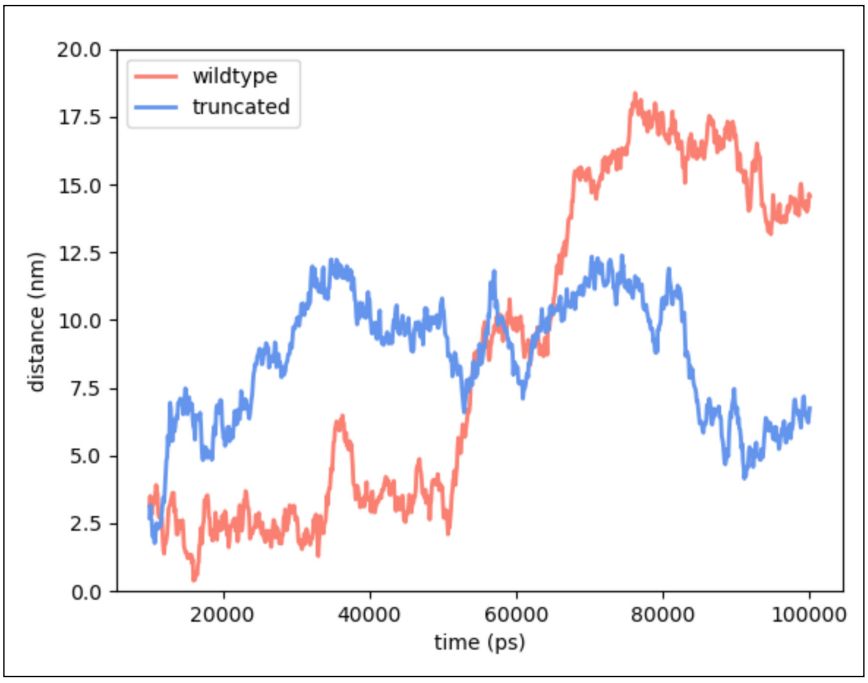

Figure 3. RMSD plot of Trp227. Computed as the root mean squared distance of Trp227 from the starting structure, averaged over its atoms. It is more stable in the truncated type. While still fluctuating, it does not get "alienated" from the starting structure.

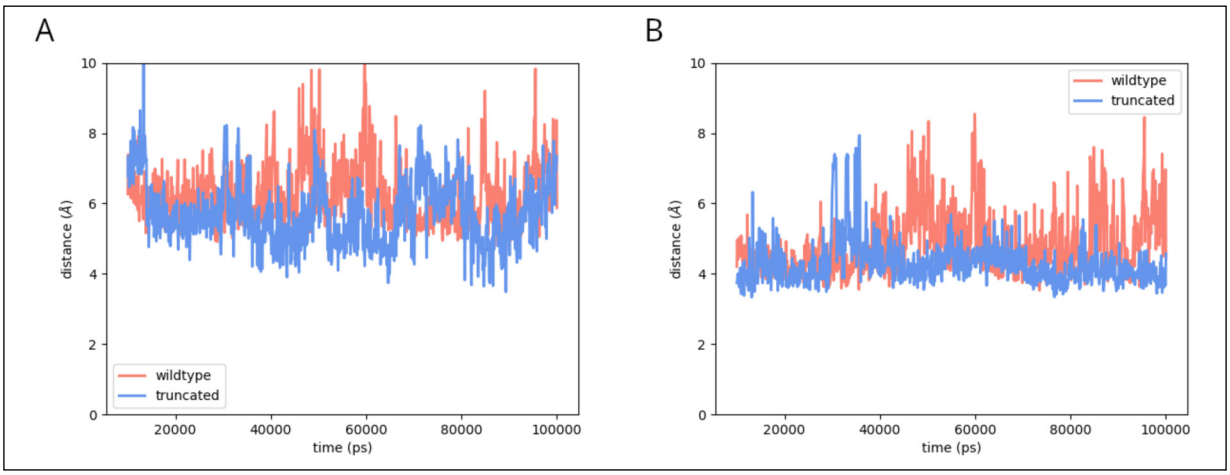

Figure 4. A, Distance between C3 of 5α-DHT and the hydroxyl group of Tyr55. **B**, Distance between C3 of 5α-DHT and C4N of NADPH. Both distances tend to be smaller in the truncated type than the wildtype.

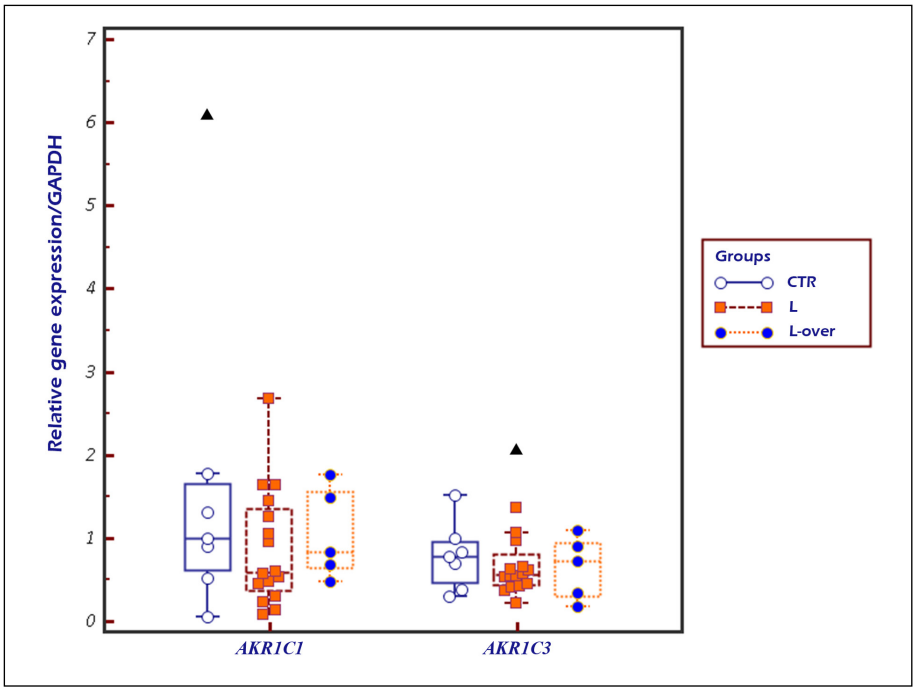

Figure 5. Relative expression of *AKR1C1* and AKR1C3 in different groups (CTR = non affected controls, L = lipedema patients without overexpression of *AKR1C2*, L-over = Lipedema patients with overexpression of *AKR1C2*), showing that lipedema patients expressed *AKR1C1* and *AKR1C3* levels similar to the control group. Outliers are reported as black triangles.

The lipedema patients analyzed by qPCR were screened for rare variants in the *AKR1C1-4* genes. Among those that overexpressed *AKR1C2* in qPCR, one patient carried a transversion of unknown significance in *AKR1C3* [rs782593331, NM_001253908.1:c.799G>T; NP_001240837.1:p. (Val267Phe)], while another one was the previously published proband with the p.(Leu213Gln) variant in *AKR1C1*[14]. Among the non-overexpressing patients, no one presented rare variants in *AKR1C1-C4*.

Discussion

Lipedema is a chronic condition characterized by disproportionate bilateral fat accumulation,

especially in the gynoid area (hips, buttocks, and legs) but also in the upper body limbs due to adipocyte hypertrophy and hyperplasia[28]. Phenotypic overlap with other, often co-occurring, conditions makes it difficult to diagnose, highlighting the need for a confirmatory test and for the identification of biomarkers. Growing evidence suggests[29] that steroid hormones play a fundamental role in the pathogenesis of lipedema. Lipedema mainly affects women, and its manifestations commonly arise in females in phases of hormonal change. Indeed, men with lipedema are rare and present higher estrogen and lower relative testosterone levels[1]. Dysfunction of sex steroid hormone regulators results in abnormal fat distribution in predisposed subjects, especially in females at the time of puberty[29]. The gonadal steroids, including androgens, estrogens, and progestogens, have been identified as modulators of body fat distribution in both men and women[30]. Steroid hormone homeostasis is finely regulated by aldo-keto reductase (AKR) enzymes. The four human *AKR1Cs* are multifunctional enzymes with high sequence similarity and overlapping activities, with *AKR1C1* and *AKR1C2* differing by only one amino acid in their active sites. *AKR1C1* is the major 20α-reductase that inactivates progesterone, whereas *AKR1C2* mainly acts as a 3α-reductase, inactivating dihydrotestosterone (5α-DHT) to 5α-androstane-3α,17ß-diol (3α-Adiol)[12]. Progesterone and DHT play opposite roles regarding fat accumulation, with progesterone prompting lipogenesis and DHT inhibiting adipogenesis[13]. We previously identified[14] an *AKR1C1* partial loss of function variant in a family affected by lipedema, as well as a single nucleotide polymorphism Leu54Val resulting in the overactivation of *AKR1C2*[31]. In this paper, we described the Ser320PheTer2 variant consisting of a deletion in the C-terminal tail of *AKR1C2*, resulting in a possible elevated enzyme activity, as well as a group of patients with elevated *AKR1C2* mRNA levels. The increase in *AKR1C2* activity or expression may help explain lipid accumulation in lipedema as *AKR1C2* upregulation has been described[32] as having a role in adipogenic commitment and preadipocyte differentiation. *AKR1C2* expression has been documented[33,34] in subcutaneous adipose tissue and visceral fat accumulation. A positive correlation between adipocyte cell size and *AKR1C2* expression or activity has been described[35] in adipose tissue, as well as a strong association between *AKR1C2* expression and percent trunk fat mass in women[36,37]. Further evidence of the role of *AKR1C2* in fat accumulation has been described by Vihma et al[38,39], who analyzed serum concentration of 5α-dihydrotestosterone (5α-DHT) in twins and found that the heavier brother presented the lower concentration of 5α-DHT. 5α-DHT, indeed, is a potent androgen that is inactivated by *AKR1C2* to 5α-androstane-3α,17ß-diol – a much weaker ligand of the androgen receptor (AR)[35], thus reducing the androgen inhibitory effect on adipogenesis. Stimulation of *AKR1C2* activity and glucocorticoid-mediated 5α-DHT inactivation in preadipocytes might eliminate androgen-inhibitory effects on adipogenesis, favoring the progression of adipogenesis[40]. On the contrary, specific downregulation of *AKR1C2* in preadipocytes was found[32] to increase the inhibitory effect of 5α-DHT on adipogenesis. Therefore, *AKR1C2* is a key enzyme in the regulation of lipid accumulation and adipogenesis, orchestrating a crosstalk between androgens and glucocorticoids[41,42].

Another function of *AKR1C2* is the synthesis of neurosteroids regulating active and inactive concentration in target tissues[43]. Indeed, *AKR1C2* catalyzes the reduction of 5α-dihydroprogesterone (5α-DHP) to allopregnanolone, while *AKR1C1* deactivates allopregnanolone through 20α-reduction[44]. Allopregnanolone acts as a potent positive allosteric modulator of the γ-Aminobutyric acid type A (GABAA) receptor and has anxiolytic, anticonvulsant, and anesthetic properties[13]. Likewise, although lipedema is typically reported[28] as a painful disorder, our patients did not complain of pain or tenderness to palpation. With the aim of verifying whether *AKR1C2* overexpression was a frequent condition in lipedema, we analyzed *AKR1C2* expression in a pool of 21 lipedema patients, 19 of them being unrelated. Despite the small cohort size, we found overexpression in 5 out of 21 (24%) patients. The overexpression of *AKR1C2* was confirmed in all the 3 affected family members of the pedigree that was available for analysis[14]. Although we cannot rule out changes in *AKR1C2* expression in the blood due to the menstrual cycle, analysis of mRNA expression in 3 affected family members at different stages of life suggests that it has a minimal effect. Further study should address this hypothesis.

Conclusions

In conclusion, we argue in favor of an *AKR1C2* C-terminal deletion that could be responsible for lipedema in the cases described in this paper. We also describe *AKR1C2* altered expression in a group of lipedema patients. In this context, our future

work needs to examine both *AKR1C2* expression in qPCR and its activity with metabolomic analysis in a larger sample of lipedema patients, to evaluate if *AKR1C2* altered expression and activity could be considered a biomarker of a subgroup of lipedema patients. Understanding what causes changes in the expression of *AKR1C2* enzymes could help in designing therapeutic options in lipedema patients characterized by its overexpression. Finally, to their better-known roles in the synthesis and metabolism of steroid hormones, the *AKR1Cs* also play an important part in the antioxidant defense system, prostaglandin production, and phase 1 metabolism of xenobiotics[12,44], and could, therefore, further influence lipedema via the interplay between oxidative stress, inflammation, and adipogenesis, paving the way to future studies.

Authors' Contributions

Conceptualization MB; methodology JK and AB; software JK and AB; investigation KLH, SandroM, and PC; data curation GB, PEM, GM, KDo and KDh; writing original draft preparation JK, GB and AB; writing review and editing AM, SerenaM, SilviaM and AB; supervision AB and MB; project administration AB and MB; funding acquisition MB. These authors contributed equally to this work: JK and GB.

Ethics Approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee of Azienda Sanitaria dell'Alto Adige, Italy (Approval No. 132-2020).

Funding

This research was funded by the Provincia Autonoma di Bolzano in the framework of LP 14/2006.

Informed Consent

Informed consent was obtained from all subjects involved in the study, which includes consent to use the anonymized genetic results for research.

Data Availability

Data are contained within the supplementary material. The statistical analysis was not applicable.

Acknowledgments

The authors wish to thank all the patients and the clinicians for their kind collaboration.

Conflicts of Interest

J. Kaftalli and G. Marceddu are employees at MAGI EUREGIO. K. Donato is employee at MAGI EUREGIO and MAGISNAT. M. Bertelli is president of MAGI EUREGIO, MAGISNAT, and MAGI's LAB. G. Bonetti, K. Dhuli, A. Macchia, and P.E. Maltese are employees at MAGI's LAB. M. Bertelli, P.E. Maltese, K. Louise Herbst, Sa. Michelini, Se. Michelini, and P. Chiurazzi are patent inventors (US20220362260A1). M. Bertelli, P.E. Maltese, G. Marceddu are patent inventors (US20230173003A1). M. Bertelli, K. Dhuli and P.E. Maltese are patent inventors (WO2022079498A1). M. Bertelli, P.E. Maltese, Sa. Michelini, Se. Michelini, P. Chiurazzi, K. Louise Herbst, J. Kaftalli, K. Donato, and A. Bernini are patent applicants (Application Number 18/516,241). M. Bertelli, K. Donato, P.Chiurazzi, G. Marceddu, K. Dhuli, G. Bonetti and J. Kaftalli are patent applicants (Application Number: 18/466.879). M. Bertelli, G. Bonetti, G. Marceddu, K. Donato, K. Dhuli, J. Kaftalli, Sa. Michelini, and K. Louise Herbst are patentapplicants (Application Number 63/495,155). The remaining authors have no conflict of interest to disclose.

References

1) Child AH, Gordon KD, Sharpe P, Brice G, Ostergaard P, Jeffery S, Mortimer PS. Lipedema: an inherited condition. Am J Med Genet A 2010; 152: 970-976.
2) Dudek JE, Białaszek W, Ostaszewski P, Smidt T. Depression and appearance-related distress in functioning with lipedema. Psychol Health Med 2018; 23: 846-853.
3) Herbst KL. Rare adipose disorders (RADs) masquerading as obesity. Acta Pharmacol Sin 2012; 33: 155-172.
4) Bauer AT, von Lukowicz D, Lossagk K, Aitzetmueller M, Moog P, Cerny M, Erne H, Schmauss D, Duscher D, Machens HG. New Insights on Lipedema: The Enigmatic Disease of the Peripheral Fat. Plast Reconstr Surg 2019; 144: 1475-1484.
5) Buso G, Depairon M, Tomson D, Raffoul W, Vettor R, Mazzolai L. Lipedema: A Call to Action! Obesity (Silver Spring) 2019; 27: 1567-1576.
6) Herbst KL, Mirkovskaya L, Bharhagava A, Chava Y, Hanne C, Te T. Lipedema Fat and Signs and Symptoms of Illness, Increase with Advancing Stage. Available from: https://www.semanticscholar.org/paper/Lipedema-Fat-and-Signs-and-Symptoms-of-Illness%2C-Herbst-Mirkovskaya/8b0e0bed68581bc6d602792109f58ca9f745e5ab
7) Grigoriadis D, Sackey E, Riches K, van Zanten M, Brice G, England R, Mills M, Dobbins SE, Lee LL; Lipoedema Consortium; Genomics England Research Consortium; Jeffery S, Dong L, Savage DB, Mortimer PS, Keeley V, Pittman A, Gordon K, Ostergaard P. Investigation of clinical characteristics and genome associations in the 'UK Lipoedema' cohort. PLoS One 2022; 17: e0274867.
8) Gavin KM, Cooper EE, Hickner RC. Estrogen receptor protein content is different in abdominal than gluteal subcutaneous adipose tissue of overweight-to-obese premenopausal women. Metabolism 2013; 62: 1180-1188.

9) Van Pelt RE, Gozansky WS, Hickner RC, Schwartz RS, Kohrt WM. Acute modulation of adipose tissue lipolysis by intravenous estrogens. Obesity (Silver Spring) 2006; 14: 2163-2172.

10) Tchernof A, Mansour MF, Pelletier M, Boulet MM, Nadeau M, Luu-The V. Updated survey of the steroid-converting enzymes in human adipose tissues. J Steroid Biochem Mol Biol 2015; 147: 56-69.

11) Blouin K, Nadeau M, Mailloux J, Daris M, Lebel S, Luu-The V, Tchernof A. Pathways of adipose tissue androgen metabolism in women: depot differences and modulation by adipogenesis. Am J Physiol Endocrinol Metab 2009; 296: E244-E255.

12) Penning TM, Wangtrakuldee P, Auchus RJ. Structural and Functional Biology of aldo-Keto Reductase Steroid-Transforming Enzymes. Endocr Rev 2019; 40: 447-475.

13) Kiani AK, Mor M, Bernini A, Fulcheri E, Michelini S, Herbst KL, Buffelli F, Belgrado JP, Kaftalli J, Stuppia L, Dautaj A, Dhuli K, Guda T, Manara E, Maltese PE, Michelini S, Chiurazzi P, Paolacci S, Ceccarini MR, Beccari T, Bertelli M. Steroid-converting enzymes in human adipose tissues and fat deposition with a focus on AKR1C enzymes. Eur Rev Med Pharmacol Sci 2021; 25: 23-32.

14) Michelini S, Chiurazzi P, Marino V, Dell'Orco D, Manara E, Baglivo M, Fiorentino A, Maltese PE, Pinelli M, Herbst KL, Dautaj A, Bertelli M. aldo-Keto Reductase 1C1 (AKR1C1) as the First Mutated Gene in a Family with Nonsyndromic Primary Lipedema. Int J Mol Sci 2020; 21: 6264.

15) Abeshi A, Zulian A, Beccari T, Dundar M, Ziccardi L, Bertelli M. Genetic testing for cone rod dystrophies. Eurobiotech J 2017; 1: 35-37.

16) Abeshi A, Marinelli C, Beccari T, Dundar M, Falsini B, Bertelli M. Genetic testing for ocular albinism and oculocutaneous albinism. Eurobiotech J 2017; 1: 80-82.

17) Abeshi A, Coppola P, Beccari T, Dundar M, Colombo L, Bertelli M. Genetic testing for Mendelian myopia. Eurobiotech J 2017; 1: 74-76.

18) Rakhmanov Y, Maltese P, Marinelli C, Beccari T, Dundar M, Bertelli M. Genetic testing for pulmonary stenosis. Eurobiotech J 2018; 2: 58-60.

19) Abeshi A, Coppola P, Beccari T, Dundar M, Viola F, Colombo L, Bertelli M. Genetic testing for congenital stationary night blindness.Eurobiotech J 2017; 1: 38-40.

20) Rakhmanov Y, Maltese P, Paolacci S, Marinelli C, Castori M, Beccari T, Dundar M, Bertelli M. Genetic testing for Marfan-like disorders. Eurobiotech J 2018; 2: 38-41.

21) Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405-424.

22) Ji Q, Chang L, VanDenBerg D, Stanczyk FZ, S.A. Selective reduction of AKR1C2 in prostate cancer and its role in DHT metabolism. Prostate 2003; 54: 275-289.

23) Couture JF, Legrand P, Cantin L, Luu-The V, Labrie F, Breton R. Human 20alpha-hydroxysteroid dehydrogenase: crystallographic and site-directed mutagenesis studies lead to the identification of an alternative binding site for C21-steroids. J Mol Biol 2003; 331: 593-604.

24) Meijers R, Morris RJ, Adolph HW, Merli A, Lamzin VS, Cedergren-Zeppezauer ES. On the enzymatic activation of NADH. J Biol Chem 2001; 276: 9316-9321.

25) Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJ. GROMACS: fast, flexible, and free. J Comput Chem 2005; 26: 1701-1718.

26) Vanommeslaeghe K, Hatcher E, Acharya C, Kundu S, Zhong S, Shim J, Darian E, Guvench O, Lopes P, Vorobyov I, Mackerell AD Jr. CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. J Comput Chem 2010; 31: 671-690.

27) Son M, Park C, Kwon SG, Bang WY, Kim SW, Kim CW, Lee KW. Structural importance of the C-terminal region in pig aldo-keto reductase family 1 member C1 and their effects on enzymatic activity. BMC Struct Biol 2015; 15: 1.

28) Buck DW 2nd, Herbst KL. Lipedema: A Relatively Common Disease with Extremely Common Misconceptions. Plast Reconstr Surg Glob Open 2016 Sep 28; 4: e1043.

29) Bano G, Mansour S, Brice G, Ostergaard P, Mortimer PS, Jeffery S, Nussey S. Pit-1 mutation and lipoedema in a family. Exp Clin Endocrinol Diabetes 2010; 118: 377-380.

30) Tchernof A, Brochu D, Maltais-Payette I, Mansour MF, Marchand GB, Carreau AM, K.J. Androgens and the Regulation of Adiposity and Body Fat Distribution in Humans. Compr Physiol 2018; 8: 1253-1290.

31) Kaftalli J, Bonetti G, Samaja M, Herbst KL, Michelini S, Maltese PE, Bertelli M. AKR1C1 and hormone metabolism in lipedema pathogenesis: a computational biology approach. Eur Rev Med Pharmacol Sci 2023; 27: 135-145.

32) Veilleux A, Côté JA, Blouin K, Nadeau M, Pelletier M, Marceau P, Laberge PY, Luu-The V, Tchernof A. Glucocorticoid-induced androgen inactivation by aldo-keto reductase 1C2 promotes adipogenesis in human preadipocytes. Am J Physiol Endocrinol Metab 2012; 302: E941-E949.

33) Wang L, Li S, Zhao A, Tao T, Mao X, Zhang P, Liu W. The expression of sex steroid synthesis and inactivation enzymes in subcutaneous adipose tissue of PCOS patients. J Steroid Biochem Mol Biol 2012; 132: 120-126.

34) Blouin K, Richard C, Bélanger C, Dupont P, Daris M, Laberge P, Luu-The V, Tchernof A. Local androgen inactivation in abdominal visceral adipose tissue. J Clin Endocrinol Metab 2003; 88: 5944-5950.

35) Zhang Y, Dufort I, Rheault P, Luu-The V. Characterization of a human 20alpha-hydroxysteroid dehydrogenase. J Mol Endocrinol 2000; 25: 221-228.

36) Ostinelli G, Vijay J, Vohl MC, Grundberg E, Tchernof A. AKR1C2 and AKR1C3 expression in adipose tissue: Association with body fat distribution and regulatory variants. Mol Cell Endocrinol 2021; 527: 111220.

37) Wake DJ, Strand M, Rask E, Westerbacka J, Livingstone DE, Soderberg S, Andrew R, Yki-Jarvinen H, Olsson T, Walker BR. Intra-adipose sex steroid metabolism and body fat distribution in idiopathic human obesity. Clin Endocrinol (Oxf) 2007; 66: 440-446.

38) Vihma V, Heinonen S, Naukkarinen J, Kaprio J, Rissanen A, Turpeinen U, Hämäläinen E, Hakkarainen A, Lundbom J, Lundbom N, Mikkola TS, Tikkanen MJ, Pietiläinen KH. Increased body fat mass and androgen metabolism - A twin study in healthy young women. Steroids 2018; 140: 24-31.

39) Vihma V, Naukkarinen J, Turpeinen U, Hämäläinen E, Kaprio J, Rissanen A, Heinonen S, Hakkarainen A, Lundbom J, Lundbom N, Mikkola TS, Tikkanen MJ, Pietiläinen KH. Metabolism of sex steroids is influenced by acquired adiposity-A study of young adult male monozygotic twin pairs. J Steroid Biochem Mol Biol 2017; 172: 98-105.

40) Wawrzkiewicz-Jałowiecka A, Lalik A, Soveral G. Recent Update on the Molecular Mechanisms of Gonadal Steroids Action in Adipose Tissue. Int J Mol Sci 2021; 22: 5226.

41) Bouwman FG, Boer JM, Imholz S, Wang P, Verschuren WM, Dollé ME, Mariman EC. Gender-specific genetic associations of polymorphisms in ACE, AKR1C2, FTO and MMP2 with weight gain over a 10-year period. Genes Nutr 2014; 9: 434.

42) Blouin K, Veilleux A, Luu-The V, Tchernof A. Androgen metabolism in adipose tissue: recent advances. Mol Cell Endocrinol 2009; 301: 97-103.

43) Steckelbroeck S, Jin Y, Gopishetty S, Oyesanmi B, Penning TM. Human cytosolic 3alpha-hydroxysteroid dehydrogenases of the aldo-keto reductase superfamily display significant 3beta-hydroxysteroid dehydrogenase activity: implications for steroid hormone metabolism and action. J Biol Chem 2004; 279: 10784-10795.

44) Rižner TL, Penning TM. Role of aldo-keto reductase family 1 (AKR1) enzymes in human steroid metabolism. Steroids 2014; 79: 49-63.