EXPLORATORY RESEARCH AND HYPOTHESIS IN MEDICINE

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CRISPR/Cas9 in Human Research—A Call for Unity in Medical Ethics

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The CRISPR-associated RNA-guided endonuclease Cas9 system has revolutionized the field of genetic editing, having almost unlimited potential in bioengineering and medicine. The ability to remove a pathophysiological abnormality through therapeutic intervention using the CRISPR/Cas9 system represents a true possibility of treating rare and complex genetic disorders. Since first being described in 2012, more than 1,500 publications have reported the use of this technology in various scientific areas. At least 10 companies are currently exploring the utility of this type of gene editing in human diseases.

Recently, an investigator at the Southern University of Science and Technology in Shenzhen, China, Dr. Jiankui He, reported the first use of gene editing in humans, whereby he described the birth of twin girls lacking the CCR5 gene. As first reported at the Human Genome Editing Conference held in Hong Kong in late November of 2018, Dr. He described the as-yet-unsubstantiated results of using the CRISPR/Cas9 technology to genetically remove the CCR5 gene from human embryos, resulting in live birth. Since then, the scientific and medical communities have largely denounced this work, notably including the Committee of Genome Editing of the Genetics Society of China and of the Chinese Society for Stem Cell Research.

Although Dr. He’s work has not been confirmed either informally or through peer-reviewed publication, it has raised serious concerns and important discussions on the ethics of human research with regard to gene editing and the urgent need for international agreement on the ethical responsibilities that will follow in this field. The medical ethics of human embryo research have been debated for many years, but the report of a scientist pursuing genetic manipulation through human birth has escalated the need for establishing a firm commitment to strong ethical standards in medical research.

There have been great advances in the understanding of CRISPR/Cas9 over a relatively short period of time; however, some studies, including one in 2016 describing genome damage, large deletions and genetic crossover events as a result of the gene editing process, have served to underscore the reality that we do not yet fully understand the changes occurring and the potential pathogenic consequences of such events. Without complete knowledge of the process itself nor of the potential serious consequences of the end result, it is imperative that we, as a scientific community, pause the further use of gene editing of human embryos, at least until the knowledge base has progressed. The rogue use of CRISPR/Cas9 and failure of peer-reviewed oversight threatens the fundamental basis of scientific research as a whole.

Research that pushes established boundaries in an ethical manner, that questions previously held dogma, and that constantly strives to improve and refine scientific knowledge represents the core aim of Exploratory Research and Hypotheses in Medicine. Yet, these types of explorations must be conducted in a methodical manner and not applied to human research until the consequences are fully understood and a consensus is established on acceptable standards. We are not there yet with the CRISPR/Cas9 technology, and until we are rogue human research must not happen: primum non nocere.

References

Death-associated Protein Kinase 1 Impairs the Hippocampoprefrontal Cortical Circuit and Mediates Post-stroke Depression

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Abstract

Post-stroke depression (PSD) is a common and complex post-stroke neuropsychiatric disorder, which not only delays functional recovery but also increases mortality, and which currently lacks effective drug therapy. The pathogenesis of PSD is associated with impairment of the subcortical neural circuits and alterations of synaptic plasticity and neurotransmitters, but the exact mechanisms of PSD remain unknown. Our previous work indicates that the death-associated protein kinase 1 (DAPK1) mediates neuronal death after stroke. Genetic deletion of DAPK1 gene or blocking DAPK1 signal in the PSD mouse model can not only alleviate cerebral ischemic injury but also relieve PSD-like behaviors. Our previous work has also demonstrated the following results. First, the neural circuit of dorsal CA1 (dCA1) to medial prefrontal cortex (mPFC) (dCA1-mPFC) is selectively impaired after stroke. Second, the DAPK1 signal is involved in the impairment of dCA1-mPFC neural circuit after stroke. Third, genetic deletion of the DAPK1 gene or blocking of the DAPK1 signal alleviates the injury of dCA1-mPFC neural circuit after stroke and improves PSD-like behaviors. In conclusion, we hypothesize that activated DAPK1 signal after stroke induces apoptosis in the hippocampal dCA1 neurons, leading to loss of the dCA1-mPFC glutamatergic projections, synaptic injury, decrease of glutamate release, inhibition of mPFC neurons, and finally onset of PSD. We hope to further replenish the mechanisms of PSD and provide new insights for PSD treatment.

Introduction

In the past three decades, stroke has become a major disease that seriously jeopardizes the health and life of China’s residents, and it has placed a heavy burden on patients, their families, and society. According to the newly released 2016 Stroke Epidemiology Report, there are 70 million stroke patients in China, including 2 million new stroke-suffering people per year; moreover, 67.3% to 80.5% of the stroke patients represent cases of ischemic stroke.1 A recent meta-analysis of 61 cohort studies showed that 31% of stroke survivors experienced post-stroke depression (PSD) within 5 years.2 PSD is one of the common complications associated with stroke events, and exhibits a series of depressive symptoms and corresponding physical signs. In addition to the neurological deficits, PSD patients also have emotional disorders, such as self-blame and decreased interest. Severe cases may have suicidal thoughts. Delayed, intervention may lead to suicide.

A cohort study of 51,119 PSD cases found that the PSD patients had a significantly higher risk of mortality than healthy people. PSD seriously affects patients’ lives and places heavy burdens on their families and society.3 Regarding the pathogenesis of PSD, it is considered to be a direct result of specific injury of neurological structure after stroke or an indirect consequence of negative psychological reactions in patients, and is believed to be the result of a combination of psychological, social and biological factors, such as the severity of stroke, brain damage, sensorimotor dysfunction, and cognitive impairment.4 Although the pathogenesis of PSD may be related to impairment of the monoamine system, dysfunction of hypothalamic pituitary adrenal (HPA) axis, destruction of prefrontal cortical circuit, imbalance of glutamate neurotransmitters and proinflammatory factors, the specific neural circuit and molecular mechanisms have not yet been elucidated.5

Keywords: DAPK1; Neural circuit; Synaptic injury; Post-stroke depression.

Abbreviations: CaM, calmodulin; DAPK1, death-associated protein kinase 1; HPA, hypothalamic pituitary adrenal; mPFC, medial prefrontal cortex; PSD, post-stroke depression.

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efficacy. Even worse, antidepressants are liable to increase side effects on the gastrointestinal and central nervous systems, such as confusion, excessive sedation, and tremors. In addition, psychological intervention for PSD is also ineffective. PSD is closely related to the patients’ poor prognosis, in that it can delay hospital stay, affect recovery of neurological function, and cause loss of independent living ability and even death. However, specific and effective treatments for PSD are unavailable. Therefore, clarifying the pathogenesis of PSD and developing more effective prevention and treatment strategies are of great clinical significance for promoting functional rehabilitation and improving quality of life in PSD patients.

Ischemic stroke causes depression

In the 1980s, Robinson et al. found that reducing the cerebral blood supply in rats could decrease the concentration of catecholamines and hormones, and lead to acute stress response. Due to the decrease of these neurotransmitters and hormones, the rats became slower on the wheel and the animals’ willingness to exercise was also reduced. In 2012, El Husseini et al. found that most patients with transient ischemic attack developed depression finally, as did stroke patients with functional impairment. A prospective study in China showed that about 30% of patients had depression at 2 weeks after onset of stroke, and the prevalence of PSD was 31% at 1 year.

The incidence of PSD is high, and the risk exists for a long time. Fifteen years after stroke, the prevalence of depression is still as high as 31.2%. A recent study funded by the Beijing Municipal Science and Technology Commission was conducted with 520 outpatients and hospitalized stroke patients. The incidence of PSD in this group was 34.2%, with mild cases accounting for 20.2% and moderate cases accounting for 10.4%. The prevalence of PSD was 39% at 1 month, 53% at 3–6 months, and 24% at 1 year after stroke, respectively. If the severity of depression is not distinguished and is collectively referred to as PSD, the incidence is 20–70%.

The above studies show that ischemic stroke can cause depression.

Hippocampal CA1 Injury in PSD

In ischemic stroke, hippocampal CA1 neurons are more sensitive to ischemic injury than other regions. By using a rodent model of global cerebral ischemia to simulate transient ischemic attacks, a previous study found selective and persistent death of pyramidal neurons in the hippocampal CA1 region, while hippocampal CA3, dentate gyrus, and most cortical neurons were unaffected. Transient cerebral ischemia caused by cardiac arrest or thoracotomy can also cause selective and longer-lasting cell death in the hippocampal CA1 region. Recent studies have also found that some proteins are abnormally expressed in the hippocampal CA1 area may be involved in the development of PSD.

Because the hippocampus has wide projections to other brain regions that regulate mood and stress, it is considered to be a critical regulatory site for depression, and the “hippocampal theory” has been proposed and has supplemented the “monoamine neurotransmitter hypothesis”, which is a widely accepted theory for the pathogenesis of depression. Meanwhile, a large number of magnetic resonance imaging studies have found reduced volume of hippocampus in PSD patients. Animal studies have also shown the reduction of apical dendrites in the hippocampal CA1 neurons. The decreased number of new neurons may be associated with hippocampal volume reduction and depression. Protecting hippocampal neurons or promoting neurogenesis could relieve symptoms of depression. Because the pyramidal neurons in the hippocampal CA1 area are mainly glutamatergic neurons, the decrease of glutamate neurotransmitter caused by the loss of pyramidal neurons in the hippocampal CA1 area may lead to the occurrence of PSD. In addition, the hippocampus has a certain inhibitory effect on the activity of the hypothalamic-pituitary-adrenal gland axis. Therefore, inhibition of the HPA axis is weakened after hippocampal injury, which leads to dysfunction of the HPA axis and induces depression finally. Thus, damage to the hippocampal CA1 area may be involved in the development of PSD.

Abnormal hippocampo-prefrontal cortical circuit causes depression

Imaging of the patients’ brain with depression and histological examination of post-mortem brain have revealed abnormalities in the prefrontal cortex, cingulate gyrus, hippocampus, striatum, and almonds. At present, many brain regions have been reported to be involved in the pathophysiology of depression. First, deep brain electrical stimulation at the anterior cingulate cortex and nucleus accumbens exhibits an antidepressant-like effect on individuals with refractory depression. This effect is thought to inhibit the activity of the brain region through blockade of the depolarization of axonal fibers. Second, dopaminergic neural projections in the midbrain (ventral tegmental area to nucleus accumbens) increased activity-dependent release of brain derived neurotrophic factor, which mediates susceptibility to social stress. This effect may be partially produced by phosphorylation of the transcription factor cAMP response element binding protein. Third, the decreased concentration of neurotrophic factors (such as brain derived neurotrophic factor) reduces the degree of hippocampal nerve regeneration and neuronal processing and complexity. These effects are partly produced by increasing cortisol concentration and decreasing the activity of the cAMP response element binding protein. Fourth, peripherally released metabolic hormones, such as ghrelin and leptin, can produce mood-related alterations by acting on the hypothalamus and several regions of the limbic system (such as hippocampus, ventral tegmental area, and nucleus accumbens).

In addition, a large number of autopsy and neuro-imaging studies have found that patients with depression have reduced gray matter volume and decreased nerve fiber density in the prefrontal cortex and hippocampus, while the intermediate prefrontal cortex (mPFC) and hippocampus are critical brain regions thought to be involved in depression, and mPFC receives abundant synaptic projections from the hippocampal CA1 region. Therefore, it is speculated that the CA1-mPFC circuit participates in the cognitive function of depression. The study also found that mPFC mainly receives glutamatergic projection fibers from the CA1 area. In the...
rat model of depression, the synaptic plasticity of the CA1-mPFC circuit was also found to be impaired. Our preliminary results also found the loss of dCA1-mPFC projection fibers in the ischemic stroke mouse model. In summary, impaired hippocampal-prefrontal circuit caused by ischemic stroke can cause depression.

Ischemic stroke activates the death-associated protein kinase 1 (DAPK1) death signal in the hippocampal CA1 area and induces PSD

Our previous study found that DAPK1 interacts with the excitatory glutamate receptor (GluN2B subunit) and induces neuronal cell death in a mouse model of ischemic stroke, which demonstrates that DAPK1 is a pivotal molecule in neuronal death after ischemic stroke. DAPK1 is a Ca²⁺/calmodulin (CaM)-dependent serine/threonine protein kinase, consisting of a kinase domain, a CaM-binding motif, eight repeats of anchored proteins, and a death domain. The CaM-binding motif contains binding sites and a self-inhibition area. Binding of DAPK1 to the CaM-binding site results in DAPK1 conformational changes, reactivation of CaM blocked active sites, and DAPK1 activation. Previous studies have shown that activated DAPK1 is involved in tumor cell death in lymphoma. DAPK1-induced apoptosis has been reported as mainly modulated by Fas, tumor necrosis factor, apoptotic protease, and p53. DAPK1 is associated with nerve injury and aging-related neurological diseases, such as stroke, epilepsy, and Alzheimer’s disease. Recently, we reported that DAPK1 activation is involved in the impairments of synaptic transmission, spatial learning and memory. Notably, DAPK1 also mediates depression-like symptoms in chronic stress. Inhibiting DAPK1 signal by genetic deletion of the DAPK1 gene or pharmacological intervention with an interfering peptide that specifically blocks DAPK1 binding to the GluN2B subunit, both reverse molecular changes and synaptic protein loss caused by chronic stress in the prefrontal cortex, and finally exert rapid and long-lasting antidepressant effects.

In addition, DAPK1 mediates synaptic long-term depression by preventing the binding of Ca²⁺/CaM-dependent protein kinase II with GluN2B, which in turn affects learning, and cognitive and emotional functions. These studies have collectively suggested that the DAPK1 death signal may mediate the development of depression-like symptoms after ischemic stroke.

Recently, we successfully established a PSD mouse model by using light-induced forebrain ischemia, and further detected protein-protein interactions in the hippocampal CA1 region via mass spectrometry. We found that DAPK1 interacted with caytaxin, which was firstly reported in 2003 by Bomar and his colleagues. This finding revealed that mutations in the ATCAY gene encoding caytaxin caused Cayman ataxia, an autosomal recessive disorder characterized by poor muscle coordination, mental retardation, loss of head control and eye movements, and difficulties in speaking and walking. In addition, caytaxin is abundantly expressed in neurons of the cortex, cerebellum, hippocampus, olfactory bulb, and basal ganglia.

Akamatsu and colleagues used full-length caytaxin as a bait to perform yeast two-hybridization with mouse adult brain cDNA, and found that caytaxin can drive the protein light chain of kinesin-1 through its N-terminal ELEWED sequence (amino acids 115–120) and participate in axonal transportation. Kinesin, a key molecule for axonal transportation, can be combined with a variety of linker molecules, such as TRAK1/2 and JIP1, to regulate mitochondrial axonal transportation of amyloid precursor protein. Studies have also shown that caytaxin can act as a linker molecule for kinesin, and transport mitochondria along the axons to neurites, which provide the required energy for various biological processes, such as presynaptic vesicle fusion and neurotransmitter release. Further, it has been indicated that caytaxin plays an important role in mitochondrial axonal transportation and the synaptic energy supply.

Our recent study demonstrated that DAPK1 interacts with caytaxin, causing apoptosis of hippocampal CA1 neurons. In cultured primary neurons, we employed virus co-transfected DAPK1-related plasmids (activated DAPK1 (ΔCaM) and inactive DAPK1 (K42A)) with caytaxin. Mitochondria staining showed that ΔCaM decreased the number of mitochondria in the neuronal processes, as compared with K42A. However, co-transfected ΔCaM with mutant caytaxin (S46A) showed alleviation of mitochondrial reduction. In addition, co-transfection of ΔCaM and wild-type caytaxin caused a relative decrease both in the frequency and amplitude of miniature excitatory post-synaptic currents, suggesting that the serine 46 of caytaxin is essential for DAPK1-induced presynaptic energy deficiency and synaptic dysfunction.

Based on the above studies, we hypothesized that after ischemic stroke, activated DAPK1 causes phosphorylation of the caytaxin protein at serine 46 (pS46), which interrupts the formation of mitochondrial axonal transport complexes, leading to presynaptic mitochondrial reduction, following energy deprivation, then causing impairment of synaptic transmission, and ultimately inducing neuronal apoptosis. Using the conditional DAPK1 knockout (DAPK1−/−) mice, we found that the apoptosis of hippocampal dCA1 neurons was decreased and that the depression-like symptoms were effectively alleviated in PSD mice. In addition, the membrane-permeable small molecule polypeptide Tat-CTX composed of caytaxin (43-EDSSPPST-51) was synthesized to block the binding of the DAPK1 to caytaxin, and to reduce apoptosis of hippocampal dCA1 neurons and alleviate PSD-like symptoms in mice.

Conclusions

In conclusion, based on previous studies, we hypothesize that activated DAPK1 signal after stroke induces apoptosis in hippocampal dCA1 neurons, which leads to loss of dCA1-mPFC glutamatergic projections, synaptic injury, decrease of glutamate release, inhibition of mPFC neurons, and causes onset of PSD finally. By genetically knocking out the DAPK1 gene (DAPK1−/−) or blocking DAPK1 death signal with exogenous peptide may be possible to inhibit the apoptosis of dCA1 neurons and reduce the impairment of dCA1-mPFC neural circuit, thereby alleviating PSD (Fig. 1).

Future research directions

The proposed hypothesis has important theoretical significance for elucidating the molecular and neural circuit mechanisms of PSD, and will provide new insights for the treatment of PSD. In the future, the following issues need to be further verified. The first is clarifying the molecular mechanism of DAPK1 death signal mediating the decrease of dCA1-mPFC glutamatergic projection after ischemic stroke. The second is revealing the reasons behind the differences between PSD animals and non-PSD animals. And, the last but not least involves information that will benefit the treatment of PSD, such as determining the efficacy and safety of the exogenous peptide Tat-CTX that interferes with the DAPK1 death signal.
Acknowlegements

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Conflict of interest

The authors declare they have no conflict of interests related to this publication.

Author contributions

Designed the study (LP), drafted the manuscript (XK), contributed to the critical revision of the manuscript for important intellectual content (SM, YZ, YY, HY and DY). All authors approved the version to be published.

References


Lambl’s Excrescences in Congenital Heart Disease and Other Clinical Situations: A Series of 17 Cases

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Abstract

Lambl’s excrescences have a low prevalence in the general population. Although they occur most frequently in adults over 60 years-old, pediatric cases have been described. The cases in adulthood are associated with ischemic stroke, but in childhood they are asymptomatic. The aim of reporting on this case series is to show the association or coexistence of Lambl’s excrescences with some congenital heart diseases (CHDs), of which there are no known descriptive case series in adults or children. We present 17 patients (8 females), with a mean age of 23.7 years; among these cases, 64.7% were under 18 years-old. We found that 94% of the Lambl’s excrescences were located on the aortic valve. In 47% (8 cases), they coincided with a CHD (with 6 of those individuals being under 18 years-old). We propose the hypothesis that Lambl’s excrescences could have a congenital origin or coexist with CHD. No complications were found throughout the follow-up. Lambl’s excrescences could be more frequent than currently reported in the literature, and more research should be done on their significance in CHD-associated stroke.

Introduction

Lambl’s excrescences (LEx), also referred to as valvular strands, are thin, filiform and hypermobile processes first described on the aortic valve (AV) by Bohemian physician Vilem Dusan Lambl in 1856. They begin as small thrombi on the coaptation points of the endocardial surfaces and have the potential to embolize to distant organs and cause stroke or myocardial infarction. These strands are almost exclusively seen on the left side of the heart at the valve closure site, where there is endothelial damage due to wear and tear. However, they can occur anywhere on the semilunar valves and they are found in natural or prosthetic valves.

LEx are most frequent in adults over 60 years-old, but pediatric cases have also been described. Phillips et al. reported a low prevalence of only 1.7% in 700 pediatric patients assessed with transthoracic echocardiography (TTE). In general, they are benign and do not require intervention or more aggressive invasive imaging. LEx can occur as singular or multiple strands. They are referred to as ‘giant’ LEx if they form a complex that is greater than or equal to 2 cm in diameter; the literature associates these giant LEx with ischemic stroke, angina, and even myocardial infarction. Lambl’s excrescences are also described in association with rheumatic valvulitis, aortic stenosis, and bicuspid AV. Differential diagnoses for LEx include myxomas, thrombi, cardiac neoplasms, vegetations and metastases. From a histologic standpoint, LEx are similar to cardiac papillary fibroelastomas. Specifically, they are composed of fibroelastic and hyalinized stroma covered by a layer of endothelial cell lining.

Materials and methods

We present here 17 consecutive cases (8 females), with a mean age of 23.7 years (0–76 years), who were examined by the cardiology department of a Level III hospital, and who, for various reasons (Table 1), underwent echocardiography [14 transthoracic echocardiograms (TTEs) and 3 transesophageal echocardiograms (TEEs), all performed by the same expert examiner]. A multifrequency transducer (3–7 MHz) was used in a parasternal long axis echocardiographic view of the left ventricle, focusing the study on the outflow tract and AV, with zoom images and second harmonic imaging for the adults. In the TEE cases, a 14-mm multiplane probe (4–7 MHz) was used in the medial transesophageal plane with a 135° angulation over the left ventricular outflow tract and AV. Both studies used the same equipment, namely the Vivid S5 ultrasound...
Table 1. Demographic, anatomical and echocardiographic variables

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Reason</th>
<th>Echo</th>
<th>Location</th>
<th>Main diagnoses</th>
<th>Management</th>
<th>Follow-up outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>14</td>
<td>murmur</td>
<td>TTE</td>
<td>aortic valve</td>
<td>minimal aortic valve regurgitation</td>
<td>echocardiographic surveillance</td>
<td>asymptomatic at 12 month follow-up</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>12</td>
<td>syncope</td>
<td>TTE</td>
<td>aortic valve</td>
<td>none</td>
<td>echocardiographic surveillance</td>
<td>asymptomatic at 11 month follow-up</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>26</td>
<td>pulmonary hypertension</td>
<td>TTE</td>
<td>aortic valve</td>
<td>DORV + subaortic VSD + pulmonary hypertension</td>
<td>specific management for pulmonary hypertension sildenafil + bosentan, echocardiographic surveillance</td>
<td>Eisenmenger syndrome, no neurological event at 18 month follow-up</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>32</td>
<td>septic shock</td>
<td>TEE</td>
<td>aortic valve</td>
<td>Non-stenotic bicuspid valve</td>
<td>aspirin 100 mg, echocardiographic surveillance</td>
<td>asymptomatic, no neurological event at 14 month follow-up</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>40</td>
<td>syncope</td>
<td>TTE</td>
<td>aortic valve</td>
<td>minimal aortic valve regurgitation</td>
<td>aspirin 100 mg, atorvastatin 20 mg</td>
<td>asymptomatic at 13 month follow-up</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>4</td>
<td>restrictive perimembranous septal defect</td>
<td>TTE</td>
<td>aortic valve</td>
<td>restrictive perimembranous septal defect</td>
<td>aortic regurgitation, surgical repair VSD echocardiographic surveillance</td>
<td>minimal aortic valve regurgitation at 8 month follow-up</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>74</td>
<td>mitral valve regurgitation</td>
<td>TEE</td>
<td>aortic valve</td>
<td>moderate functional mitral regurgitation, heart failure</td>
<td>surgical valve replacement, anticoagulation warfarin therapy, echocardiographic surveillance</td>
<td>asymptomatic at 7 month follow-up</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>9</td>
<td>history of Kawasaki disease</td>
<td>TTE</td>
<td>aortic valve</td>
<td>non</td>
<td>aspirin 3 mg/kg/day, echocardiographic surveillance</td>
<td>asymptomatic at 12 month follow-up</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>11</td>
<td>murmur</td>
<td>TTE</td>
<td>aortic valve</td>
<td>minimal aortic valve regurgitation</td>
<td>echocardiographic surveillance</td>
<td>asymptomatic at 15 month follow-up</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>12</td>
<td>endocarditis</td>
<td>TEE</td>
<td>aortic valve</td>
<td>non-endocarditis mitral biologic valve prosthesis</td>
<td>aspirin 3 mg/kg/day, echocardiographic surveillance</td>
<td>asymptomatic at 12 month follow-up</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>46</td>
<td>leukemia</td>
<td>TTE</td>
<td>aortic valve</td>
<td>non</td>
<td>aspirin 3 mg/kg/day, echocardiographic surveillance</td>
<td>asymptomatic at 8 month follow-up</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>0</td>
<td>cyanosis</td>
<td>TTE</td>
<td>aortic valve</td>
<td>tetralogy of Fallot</td>
<td>surgical repair at 6 months of age</td>
<td>no data yet</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>15</td>
<td>bicuspid aortic valve</td>
<td>TTE</td>
<td>aortic valve</td>
<td>non-stenotic bicuspid aortic valve</td>
<td>aspirin 100 mg, echocardiographic surveillance</td>
<td>asymptomatic at 9 month follow-up</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>16</td>
<td>syncope</td>
<td>TTE</td>
<td>aortic valve</td>
<td>bicuspid valve, late repair of aortic coarctation</td>
<td>echocardiographic surveillance</td>
<td>asymptomatic at 12 month follow-up</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>8</td>
<td>Fontan dysfunction evaluation</td>
<td>TTE</td>
<td>aortic valve</td>
<td>dilated left ventricle, sinus node dysfunction, minimal aortic valve regurgitation</td>
<td>aspirin 3 mg/kg/day, echocardiographic surveillance</td>
<td>asymptomatic at 11 month follow-up</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>8</td>
<td>aortic coarctation</td>
<td>TTE</td>
<td>aortic valve</td>
<td>aortic coarctation</td>
<td>aortic angioplasty, echocardiographic surveillance</td>
<td>asymptomatic at 9 month follow-up</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>76</td>
<td>suspected endocarditis</td>
<td>TTE</td>
<td>aortic valve</td>
<td>minimal aortic valve regurgitation</td>
<td>aspirin 81 mg, atorvastatin 20 mg, echocardiographic surveillance</td>
<td>asymptomatic, no neurological event at 15 month follow-up</td>
</tr>
</tbody>
</table>

Age: years; (case 12: 1 month old newborn); TTE: transthoracic echocardiogram; TEE: transesophageal echocardiogram; DORV: double outlet right ventricle; VSD: ventricular septal defect.
Araujo JJ. et al: Lambl’s excrescences in congenital heart disease Explor Res Hypothesis Med

machine (GE Healthcare, Chicago, IL, USA). Differential diagnoses were ruled out, including laminar thrombi, endocarditis, and papillary fibroelastomas, among others.

Results

It is possible that the greater spatial and temporal resolution of the diagnostic equipment used, the transducer frequency, the use of windows with echocardiographic zoom, the experience of the examiner, and having exams focused on the left ventricular outflow tract, allowed for the incidental diagnosis of LEx (Fig. 1). Of the 17 cases presented, 64.7% were under 18 years of age. In 94% of the cases, the LEx were located on the AV; only one was on the biological mitral valve (MV). Forty-seven percent (8 cases) had CHDs [2: ventricular septal defect (VSD); 1: tetralogy of Fallot; 2: bicuspid AV; 1: MV repair with biologic prosthesis; 1: tricuspid atresia and Fontan circulation; and 1: aortic coarctation).

There was no direct relationship between the reason for the study and the finding of LEx in any of the pediatric cases; it was a 100% incidental finding. In fact, in two cases of syncope evaluation, the finding of LEx was not expected, given its very low frequency in the pediatric population. In two of the pediatric cases, innocent murmurs were correlated with minimal AV regurgitation, and when the exam was focused on this structure, LEx was diagnosed. In only one adult case was the exam directed towards finding the cause of syncope in the AV, but this diagnosis was not expected and neither was it the direct cause of syncope. Cases of LEx associated with CHDs were found very close to the VSD and left ventricular outflow tract. Perhaps the reason for this is that with proximity to the VSD, hemodynamic shunt forces cause stress and damage to the surface of the aortic vellums and the development of LEx. Or, perhaps, LEx can be considered to also be a congenital defect which accompanies these CHDs (Fig. 2).

Treatment was conservative in most cases; seven individuals received aspirin as preventive treatment (4: children; and 3: adults). The pediatric patients were medicated with aspirin due to their CHD. The adults were medicated with aspirin alone or in combination with atorvastatin, according to their risk factors. There was no ictus in either group on follow-up. In patients who underwent corrective surgery (CHD, valvular disease), there were no surgical reports of LEx resection (Table 1).

Discussion

In 1856, Lambl first described small filiform processes on the ventricular surface of normal and abnormal AVs. In subsequent pathologic series of normal valves, a 70% to 90% prevalence of LEx was reported, predominantly on the MV (70% to 85%), followed by the AV (62% to 90%) and the right-sided valves (8% to 20%). Recently, Osorio et al. analyzed 33 cases of LEx, and reported the affected valve was AV in 75% (25/33), followed by MV in 17% (6/33) and pulmonary valve in 2.8% (1/33). Salah et al. analyzed the cases published from 1981 to 2018; in that series, the affected valve was AV in 89% and MV in 7%, with one case in the pulmonary valve. Our series’ results are similar and congruent with those reported, with a higher incidence on the AV.

The etiology of LEx is uncertain; the constant bending and buckling of leaflets can cause tearing of subendocardial collagen and elastic fibers, with subsequent formation of a small thrombus, fibrin deposit, inflammation and fibrosis. Matsukuma et al. reviewed autopsy files and retrieved a total of 126 cases of AVs without infectious endocarditis. Of these, 73 AVs were selected from patients without AV dysfunction and the remaining 53 were retrieved from patients with AV dysfunction, including aortic ste-
The results showed LEx [classical and/or non-ex LEx in 106 (84%)]. Out of these, 88 (70%) had features of classical LEx, 78 (62%) had features of non-ex LEx and 60 AVs (48%) had features consistent with both classical and non-ex LEx. In the present study, the incidence of LEx was calculated to be 84%, which is consistent with the previous studies. This study also further highlighted that non-ex LEx, which has previously received little attention, is more common than expected, especially in markedly deformed AVs. AV dysfunction does not seem to influence the development of LEx but the morphological changes associated with AV dysfunction can foster non-ex LEx.

In the adult, the embolic risk of valvular LEx was evaluated by Roldan et al. in a prospective study published in 2015; this study involved 90 healthy subjects and 88 patients with or without suspected cardioembolism and followed the cases clinically for approximately 4 years. The prevalence of LEx in healthy subjects (38%) and in patients with (47%) or without (41%) suspected cardioembolism was similar, irrespective of age or sex, and the presence of LEx did not appear to be associated with future embolic events. These findings suggested that there may not be a direct causal link between ischemic stroke and the presence of the valve strands.

A review of the current literature shows many different views on the clinical importance, pathophysiology, association with ischemic events and management of LEx. However, there is a general consensus amongst most of the authors that LEx should be considered in the differential diagnosis of patients who present with signs of cardioembolism, until conclusive evidence suggests otherwise. The experts recommend performing a TEE on these patients and treating them with dual antiplatelet therapy if LEx is found to be present. However, no definitive guidelines currently direct the management of LEx, and management is largely based upon case reports in the literature.

Most authors agree that the choice between surgical intervention versus conservative observation, including anticoagulation or antiplatelet therapy, needs to be decided on a case-by-case basis and should be based on the patient’s pre-operative risk stratification. Some experts recommend close follow-up with serial echocardiograms every 6 to 12 months for asymptomatic patients with LEx; children can also be included in this group. For patients with LEx who have experienced a stroke with no alternative source of emboli, treatment with anticoagulation (warfarin therapy) or antiplatelet therapy (aspirin, 81 – 200 mg per day, clopidogrel 75 mg per day) has been suggested and reported in several cases. In other reports, dabigatran or rivaroxaban has been administered. Finally, in patients with LEx who experience recurrent strokes while on anticoagulation, a surgical debridement of the LEx can be considered.

Chu et al. analyzed 20 LEx cases. Nine were referred for surgical resection, with the following indications: primary or recurrent ischemic stroke, angina, weakness and fatigue, headache, mitral localization in papillary muscle and chordae tendineae, and incidental finding. In the cases analyzed by Osorio et al., the 2 month to 3 year follow-up period did not show any cases of recurrence of LEx or stroke (only one stroke case was reported, but it was not related to LEx). However, there is as yet no clear guide in CHD, because LEx are infrequent and there are not enough cases currently reported. Our impartial and critical opinion is to perform surgical resection at the same time as the CHD is repaired. Conservative management was the treatment of choice in 14/17 of the cases in our series; surgery was performed in only three cases, but for other reasons. However, the following hypothesis arise: 1. Can LEx be an isolated congenital defect? 2. Do LEx occur in association with CHD?

The low prevalence of LEx in the general population, and even more so in the pediatric population, gives rise to the following questions: What is the importance of knowing the diagnosis in childhood, given that relationship with cryptogenic ictus in adult-
hool is known? Is it important to routinely look for LEx if they are asymptomatic in pediatric patients and become less visible as the children grow (lower echocardiographic resolution due to a difficult acoustic window)? The best recommendation is to report these findings in the echocardiographic studies performed and follow-up during childhood, adolescence and adulthood, evaluating the risk factors and following the recommendations of the experts to initiate anticoagulation or antiplatelet therapy.

The importance of this case series report is the coincidence with CHD; there are no known descriptive case series in adults or children. This report also raises questions such as: What would be the best course of action when LEx are found? Should they be resected during CHD surgical repair? The real dilemma lies in that, due to their low frequency, they are not routinely looked for, CHD repair focuses only on the cardiac defect, and LEx may remain as a persistent residual following CHD repair.

Conclusions

This case series leads us to think that LEx cases are really more prevalent than currently reported in the literature. Furthermore, they are associated with CHD and may be of some importance in the cause of CHD-associated stroke. Many times, patients with un repaired and repaired CHD have an embolic stroke as a result of CHD residuals (e.g., residual VSD following atroventricular canal repair). The fact that LEx and CHD are an association that heightens the risk of stroke in this population arouses our research interest.

These case descriptions motivate the search for LEx related to CHD, and pose the question of whether their origin is also congenital or rather a direct consequence of the classic description of natural valvular wear and tear, increased by the hemodynamic effects of CHD. At present, there are no large series or controlled studies which can answer these questions. However, this contribution of cases will undoubtedly awaken the interest of more expert investigators.

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The authors have no conflict of interests related to this publication.

Author contributions

John Jairo Araujo: collected the data, performed echocardiograms, wrote the paper; Tareq Rahimy: collected the data, wrote the paper.

References

Autophagy is an evolutionarily conserved and regulated catabolic process that ensures the degradation of damaged organelles and excessive or misfolded proteins in lysosomes for the recycling of essential amino acids and energy. Autophagy is constitutively active at basal level in a majority of cell types and plays an important role in cellular homeostasis via maintaining cell organelles’ and proteins’ turnover as well as their functions. In addition to the homeostatic function, autophagy also acts as a pro-survival pathway and can be activated in response to different stress conditions, such as nutrient starvation, organelle damage, metabolic stress, endoplasmic reticulum stress, accumulation of non-functional proteins, and radiation or chemotherapy treatment. Recent studies have demonstrated that dysregulation of autophagy pathways may be involved in the pathogenesis of diverse diseases, such as myopathy, neurodegeneration, aging, microbial infection, inflammatory bowel disease, and cancer.

In cancer, the role of autophagy is quite complicated and depends on tumor type and stage. During the early stage of tumorigenesis, autophagy usually acts as a tumor suppressor by clearing the damaged cellular content, reducing reactive oxygen species’ level and thereby DNA damage. Whereas in established tumors or the damaged cellular content, reducing reactive oxygen species’ turnover as well as their functions. In addition to the homeostatic function, autophagy also acts as a pro-survival pathway and can be activated in response to different stress conditions, such as nutrient starvation, organelle damage, metabolic stress, endoplasmic reticulum stress, accumulation of non-functional proteins, and radiation or chemotherapy treatment. Recent studies have demonstrated that dysregulation of autophagy pathways may be involved in the pathogenesis of diverse diseases, such as myopathy, neurodegeneration, aging, microbial infection, inflammatory bowel disease, and cancer.

In the current issue of *Exploratory Research and Hypothesis in Medicine*, Shawn Gurwara *et al.* has reported that dysregulation of autophagy pathways could be a leading mechanism in the carcinogenesis of human colorectal cancer. In their study, it was observed that ATGs were downregulated in both early- and late-stage colon cancer compared to normal colon mucosa. Although the sample size of this study was small, 17 ATGs were found to be significantly downregulated in human colon cancer; these include ATG4A, ATG4C, ATG4D, CTSD, CTSS, ESR1, GAA, and GABARAP. Further, the authors confirmed the similar mRNA expression profile for ATG4A, ATG4C, ATG4D, GAA, GABARAP, CTSD, and CTSS in a large TCGA dataset of colon adenocarcinoma (known as COAD/READ) patients. Though this study claims the possible role of ATGs in colon cancer, the limitations of this study are its small cohort, which includes only six tumor specimens, three for each early and late colon cancers, and the lack of protein expression data for the dysregulated ATGs.

The autophagy pathway is mediated by many ATGs, among which the proteases of ATG4 family play an important role in the conjugation of ATG8 to lipid membranes and the deconjugation of ATG8 from the autophagosome. The activity of ATG4 family genes is critical and highly specific for targeting and autophagic vacuole formation. Recent studies by others have demonstrated the antitumor role of ATG4C and ATG4D in fibrosarcoma and breast cancer. In agreement with previous studies, the findings by Gurwara *et al.* provide new insights into the pathogenesis of colon cancer and suggest the tumor suppressor role of autophagy in colon cancer. In the future, it will be interesting to study the role of these dysregulated ATGs in *in vitro* systems as well as *in vivo* colon cancer models for the development of new therapeutic regimens.

**Conflict of interest**

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