Eisenmenger syndrome and atrial septal defect: Nature or nurture?

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BACKGROUND: It has long been debated whether patients with atrial septal defect (ASD) Eisenmenger syndrome have idiopathic pulmonary arterial hypertension with an incidental ASD or severe pulmonary hypertension on the basis of their ASD shunt magnitude alone.

HYPOTHESIS: It was hypothesized that if ASD Eisenmenger patients had idiopathic pulmonary arterial hypertension with an incidental ASD, a mutation in the bone morphogenetic protein receptor-2 (BMPR2) would be found in some of these patients.

PATIENTS AND METHODS: All adult patients with ASD Eisenmenger syndrome were identified from the databases of two adult congenital cardiac units, and were matched to a control group with similar types of ASDs and no pulmonary hypertension. Gene coding for BMPR2 was examined for mutation using denaturing high-performance liquid chromatography of the entire coding sequence.

RESULTS: Eighteen adult patients with ASD Eisenmenger syndrome and 18 control patients were identified. ASD Eisenmenger patients had significantly larger ASDs than the control patients (3.7±1.2 cm versus 1.9±0.7 cm, P<0.01). A mutation in BMPR2 was not detected in either group.

CONCLUSION: ASD Eisenmenger syndrome may occur without BMPR2 mutation. Whether shunt magnitude alone or in combination with yet another genetic mutation is responsible for the development of pulmonary hypertension in these patients remains to be determined.

Key Words: *Atrial septal defect; Genetics; Pulmonary hypertension*

Le syndrome d'Eisenmenger et les communications interauriculaires : Inné ou acquis?

CONTEXTE : On se demande depuis longtemps si l'hypertension artérielle pulmonaire observée dans le syndrome d'Eisenmenger associé à une communication interauriculaire (CIA) est idiopathique et accompagnée fortuitement d'une CIA ou si elle résulte directement du shunt causé par la CIA.

HYPOTHÈSE : Nous avons émis l'hypothèse suivante : si les patients atteints du syndrome d'Eisenmenger associé à une CIA souffrent d'hypertension artérielle pulmonaire idiopathique, associée fortuitement à une CIA, il doit se produire une mutation du gène codant du récepteur-2 de la protéine morphogénétique osseuse (BMPR2) chez certains de ces patients.

PATIENTS ET MÉTHODE : Nous avons relevé le nom de tous les patients adultes atteints du syndrome d'Eisenmenger associé à une CIA à partir des bases de données de deux services de traitement des cardiopathies congénitales chez l'adulte, puis nous les avons appariés à un groupe témoin de patients présentant des types comparables de CIA mais exempts d'hypertension artérielle pulmonaire. Nous avons aussi examiné le gène codant du récepteur BMPR2 à la recherche d'une mutation en soumettant toute la séquence codante à la chromatographie liquide à haute performance dénaturante.

RÉSULTATS : Dix-huit patients adultes atteints du syndrome d'Eisenmenger associé à une CIA ont été repérés, de même que 18 patients témoins. La taille de l'orifice chez ceux qui souffraient du syndrome d'Eisenmenger associé à une CIA était sensiblement plus grande que chez les témoins (3,7 ± 1,2 cm contre 1,9 ± 0,7 cm; p < 0,01). Aucune mutation du gène codant du récepteur BMPR2 n'a été décelée dans les deux groupes.

CONCLUSION : Le syndrome d'Eisenmenger associé à une CIA peut exister en l'absence d'une mutation du gène codant du BMPR2. La question de savoir si l'hypertension artérielle pulmonaire observée dans le syndrome d'Eisenmenger associé à une CIA résulte du shunt lui-même ou si elle est associée à une autre mutation génétique reste donc entière.

It has long been debated whether patients with atrial septal defect (ASD) Eisenmenger syndrome have idiopathic pulmonary arterial hypertension (IPAH) with an incidental ASD or severe pulmonary hypertension on the basis of their ASD shunt vascularity alone.

IPAH, a disease characterized by raised pulmonary vascular resistance, occurs mainly in young and middle-aged women and has a mean survival of two to three years from the onset of symptoms (1,2). Recently, a mutation in the gene encoding bone morphogenetic protein receptor-2 (BMPR2), a member of the transforming growth factor-beta superfamily of receptors, was discovered in a familial kindred with PAH (3,4). A genetic mutation in BMPR2 was also recently discovered in approximately 25% of sporadic cases of IPAH (5), as well as in patients with pulmonary venous occlusive disease (6). A genetic susceptibility compounded by triggering stimuli is now proposed as the basis of clinical IPAH (7).

Most patients with ASD never develop significant pulmonary hypertension. A minority of patients (less than 1%), however, develop precocious, severe pulmonary hypertension (8) with shunt reversal (the so-called 'ASD Eisenmenger syndrome') (9). Patients with ASD Eisenmenger syndrome have a clinical presentation similar to that of IPAH patients. They present in their late 20s, are predominantly female and have a shortened survival once the diagnosis is established (10). Lung biopsies from these patients are also remarkably similar to those with IPAH, showing medial hypertrophy and intimal proliferative changes of the pulmonary arteries, which lead to

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TABLE 1
Oligonucleotide primer sequences used for amplification of the bone morphogenetic protein receptor-2 exons

Exon	F 5′-primer	R 3′-primer	Product size (base pairs)	dHPLC temperature (°C)
1	CGTCCCGC-CTTTGCCCTCCTGATTCTTG	ATTTCCCTGGAAGGCATGG	286	65–68
2	TCATTCGGATAAGACAAAGATTTTA	GGATTTTAACATACTCCCATGTCC	338	54–57
3	TCCTGTTGATTTGCAAAACTGT	TGCAAATCTTTGGAGAAAGGA	358	55–58
4	CAGCCTTTCTAAAGGGCAGTC	CGGAATTTAAAAGGAGCAAA	273	54–56
5	TTCTGCAGCTCTTCTTTTTAAGTG	TGCCTAGAATAGGCCTTGACA	338	54–56
6	AGCAACAGAGAGCTGTAGCATT	CTCTTGGCCTCAAGTGATCC	442	57–58
7	TTTGCAAATTCTTTATAAGGATGC	TGCTGAATCTTTCAAACAACTGA	349	54–57
8	AGAAAATTAATGGGCAGAAAA	CACACCTGGCCAGTAGATGTT	347	55–58
9	TTCTGGTCTAATGTCTGTTCTTCAG	GCAATGAACTAAAGGTTTAAATGAA	349	54–56
10	TTGGTATCAGAAATACCCCTGTT	GGCATTAGGCAACTCCAAAA	335	56–58
11	CATGTGGTAAACTGAAAAGCTCA	TTCTTTGTTGGGTCTCAGTTTCT	310	56–58
12-a	CTTTGGAAGAAAATGAAAAACA	CCCAATTGACTGTGCAACAT	479	57–59
12-b	CAAACACCACAGGACTCACG	CTCTTGGGAAGGTTCTGCTG	418	57–59
12-c	CTGGACAGCAGGACTTCACA	CATGCTCATCAGGACTGGAA	450	59
12-d	GACACATAGGGCCCAAGAAA	CGTCCCGC-TAAATGGCCCCAAAAGACAC	409	59–60
13	AGCACCCTCCTGAGACATTG	CGTCCCGC-TCACTCCATAGGCTTGAAAACA	388	58–61

dHPLC Denaturing high-performance liquid chromatography

progressive luminal obstruction and plexiform lesions (11). Furthermore, BMPR2 protein expression has recently been shown to be reduced in the lungs of patients with secondary pulmonary hypertension (12).

The clinical and pathological similarities between ASD Eisenmenger patients and those with IPAH, compounded by the fact that most patients with ASD do not develop pulmonary hypertension, raise the possibility that a genetic mutation (similar to the one found in patients with IPAH) could be present in patients with ASD Eisenmenger syndrome; it could account for the development of severe pulmonary hypertension seen in these patients.

We hypothesized that if patients with ASD Eisenmenger syndrome did indeed have sporadic IPAH with an incidental ASD, approximately 25% of them would show a genetic mutation in BMPR2. The discovery of such a mutation would not only help to elucidate the pathogenesis of the disease, but also direct future treatment options, which have been quite limited for these patients so far.

PATIENTS AND METHODS

Study subjects

The databases of the University of Toronto Congenital Cardiac Centre for Adults (Toronto, Ontario), and the Sir Mortimer B Davis Jewish General Hospital Adult Congenital Cardiac Clinic and Centre for Pulmonary Vascular Disease (Montreal, Quebec) were reviewed to identify all living adult patients with ASDs who presented to the clinics with severe irreversible pulmonary hypertension (pulmonary artery pressure [Qp] greater than two-thirds of the systemic pressure [Qs], with a Qp/Qs ratio of less than 1.5, as determined by catheterization [10]) between 1991 and 2001.

A control group of patients matched for age and sex with similar ASD types but no pulmonary hypertension was also selected from the two databases. Demographic, clinical and hemodynamic parameters were recorded for all patients from a chart review. Ethics approval was obtained from the Toronto General Hospital and the Jewish General Hospital Research Ethics Boards. Patients were contacted and gave informed written consent for participation in the study.

Echocardiography

Transesophageal echocardiographic studies, performed following a standardized protocol using different commercially available machines, were reviewed when available. Unstretched ASD size was measured between 0° and 90° at its widest diameter.

Intervention

Venous samples of whole blood were taken from all study and control patients using a standard kit-based protocol after full informed consent was obtained.

Storage sample and data security

Venous blood samples were stored in the Molecular Genetics Laboratory at the Mount Sinai Hospital Research Institute, University of Toronto. Samples were kept for the purpose of the present study only, and confidentiality was ensured by coding the samples, with only the primary investigator having access to the code and identity of the patients.

Genetic analysis

Genomic DNA was isolated from lymphocytes by the PureGene DNA Isolation Kit (Gentra Systems, Inc, USA). The 13 exons (and on average, 50 base pairs of adjacent intronic sequence) of BMPR2 were amplified by polymerase chain reaction (PCR) from genomic DNA by oligonucleotide primers described in Table 1. PCR reactions were performed in 40 µL containing 1.5 mM magnesium chloride, 0.2 mM deoxynucleoside triphosphate, 0.5 µM primer, 0.2 U HotStar Taq polymerase (Qiagen, Netherlands) and 50 ng genomic DNA. Amplicons were generated in a DNA Engine Tetrad thermalcycler (MJ Research Inc, USA). Amplification conditions were 95°C for 12 min, followed by 35 cycles of 94°C for 30 s, 55°C to 62°C for 30 s, 72°C for 30 s and a final extension at 72°C for 7 min. The amplicons were screened for variants by WAVE denaturing high-performance liquid chromatography (Transgenomic, Inc, USA) (13). Melting temperatures were predicted by WAVEMAKER software (version 4.1, Transgenomic, Inc, USA) and each amplicon was analyzed at multiple melting temperatures (Table 1). Each amplicon generated from the patient samples was mixed with one-third of the volume of the wild type

TABLE 2
Patient demographic and hemodynamic characteristics

	ASD Eisenmenger	ASD with normal PAP	P
Women, %	89	72	0.40
Mean age at diagnosis, years	35.6±11.6	42.1±14.8	0.17
Mean age at time of study, years	47.0±10.1	45.5±12.7	0.69
Mean time since diagnosis, years	10.4±6.8	6.9±8.2	0.21
NYHA class >II, %	71	5	<0.001
Secundum ASD, %	78	94	0.15
Anomalous pulmonary venous drainage, %	22	11	0.14
Mean ASD size, cm	3.7±1.2	1.9±0.7	<0.01
Mean Qp/Qs ratio	1.4±0.35	2.2±0.8	<0.01
Mean PAP systolic, mmHg	90±17	39±7	<0.001
Mean O_2 saturation, %	90±5	98±1	<0.001

ASD Atrial septal defect; NYHA New York Heart Association; PAP Pulmonary arterial pressure; Qp Pulmonary artery pressure; Qs Systemic pressure

PCR product and denatured at 95°C for 5 min; it was then cooled gently to room temperature before denaturing high-performance liquid chromatography (dHPLC) analysis to ensure that any variant homoduplexes would be identified. Potential sequence variants identified by dHPLC and control (wild type) amplicons were sequenced both in a forward and reverse direction with the original amplicon primers using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA). Sequencing products were separated by capillary electrophoresis on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA).

Statistical analysis

Descriptive data for continuous variables are presented as mean ± SD or as medians with ranges, when appropriate. Comparisons of continuous data between study and control groups were performed using two-sample *t* tests or the Wilcoxon rank-sum test, when appropriate. Discrete variables were analyzed by χ^2 or Fisher's exact tests. A two-sided P<0.05 was considered significant.

RESULTS

Study subjects

Thirty-three patients with ASD Eisenmenger syndrome were identified. Two patients had died since their last clinic visit and 13 declined participation in the study. The remaining 18 patients, none of them related, agreed to participate in the study and were enrolled. Eighteen control patients were selected and consented to the study. The demographics and hemodynamics of study and control patients are summarized in Table 2. There were no significant differences in terms of sex, mean age at diagnosis or type of ASD between the two groups. However, patients with ASD Eisenmenger syndrome were more symptomatic and hypoxemic than their control matches, had significantly larger ASDs (3.7±1.2 cm versus 1.9±0.7 cm, P<0.01), higher systolic pulmonary pressure (90±17 mmHg versus 39±7 mmHg, P<0.001) and lower Qp/Qs ratios (1.4±0.3 versus 2.2±0.8, P<0.01).

Genetic analysis

Genetic analysis of BMPR2 in both ASD Eisenmenger and control patients did not reveal any mutations. dHPLC and DNA sequence analysis identified a number of single-base variants in the patient cohort and control samples. The changes included two variants within intronic regions, a C-G variant at position +103 within intron 5 (three individuals), a G-A variant at position –47 within intron 11 (one individual), two alterations within exon 12, a G-A change at position 2324 (two individuals) and a G-A change at position 2811 (seven individuals). Neither of the intronic variants are predicted to disrupt transcript splicing and are therefore unlikely to have pathogenic relevance. Of the two exon 12 variants, one encodes an S775N substitution, while the other does not affect the amino acid (R) at position 937. These variants, which have been previously described (14), were detected in six controls (33%) and four ASD Eisenmenger cases (22%) (P=0.71).

DISCUSSION

Because of similar clinical presentations (young age at presentation, female sex and poor prognosis) (1,2,10) and pathological findings on lung tissue biopsy (11), as well as the finding of reduced BMPR2 expression in lung tissue of patients with secondary pulmonary hypertension (12), we undertook a study examining the BMPR2 genetic locus in patients with ASD Eisenmenger syndrome. We hypothesized that if patients with ASD Eisenmenger syndrome indeed had sporadic IPAH with an incidental ASD, approximately 25% of them would show a genetic mutation in BMPR2.

We were unable to show any genetic mutation in the BMPR2 locus in any of our patients with ASD Eisenmenger syndrome. We found, however, that these patients had larger ASDs than their controls. This finding suggested that prolonged exposure to an increased shunt magnitude alone or in combination with another yet unrecognized genetic mutation could lead to pathological changes seen in the pulmonary vascular bed and eventual development of pulmonary hypertension (8).

Indeed, IPAH is a genetically heterogeneous disorder (15) for which mutation of the BMPR2 is reported in only one-half of familial PAH (3,4) and 25% of sporadic IPAH cases (5). In fact, in a more recent study (16) of patients with complex congenital heart disease and pulmonary hypertension, 94% did not carry a BMPR2 mutation. Presumably, causative genes for the remaining one-half of familial cases, as well as the majority of sporadic cases, and for complex congenital heart disease, are yet to be discovered. Furthermore, mutations in the gene for activin receptor-like kinase 1, which was not examined in our study, was recently described in patients with pulmonary hypertension and hereditary hemorrhagic telangiectasia (17). Conversely, severe pulmonary hypertension is not known to develop in all subjects with BMPR2 mutations, suggesting that environmental factors or variable penetrance may play a crucial role in the final clinical phenotypic expression of pulmonary hypertension. Such was the case in a recent report (18) on patients developing pulmonary hypertension after the ingestion of fenfluramine derivatives. These patients were significantly more likely to carry the gene mutation for BMPR2, despite being clinically well before the ingestion of the appetite suppressant. Similarly, patients with ASD Eisenmenger syndrome may carry a genetic mutation predisposing them to the development of pulmonary hypertension, which may only become clinically apparent when triggered by an external stimulus such as a high shunt flow, as highlighted in our study.

More recently, abnormalities in BMPR signalling have been described in patients with other secondary forms of pulmonary hypertension. Du et al (19) found a high expression of angiopoietin-1 (an angiogenic factor produced by smooth muscle cells) and a higher degree of phosphorylation of its receptor, TIE2, in patients with secondary forms of pulmonary hypertension (including three patients with Eisenmenger syndrome). This elevation in expression was proposed to lead to a downregulation of the other BMPR monomer, BMPR1A. These findings support the notion that abnormalities in the signalling pathway for BMPRs is a pervasive, and potentially pathogenic, feature of secondary forms of pulmonary hypertension. Abnormalities in these signalling pathways are worth investigating in a larger cohort of patients with ASDs.

As our understanding of the genetics of IPAH progresses, the search for a genetic basis of ASD Eisenmenger syndrome should continue. These findings have implications in the understanding of the pathophysiology and the screening of relatives, and have repercussions on the therapy offered to patients with the syndrome. Current therapy for IPAH patients can increase exercise capacity, hemodynamics and survival rates (20-25), and could be applied to patients with ASD Eisenmenger syndrome with potentially the same beneficial effect.

Limitations

The present study was performed with a relatively small number of patients, although it represents the largest series of adult ASD Eisenmenger patients ever published. One could argue that mutations may occur in a subset of patients with ASD Eisenmenger syndrome (17), and this may have been missed by the small size of the present study. Only a larger study would be able to definitively exclude this possibility. Although the study was not constructed to specifically address the issue of anatomical variants in ASD size, we nontheless found a significant difference in ASD size between the two study groups.

CONCLUSION

ASD Eisenmenger syndrome can occur without BMPR2 mutation. Whether shunt magnitude alone or in combination with another genetic mutation is responsible for the development of pulmonary hypertension in these patients remains to be determined.

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