

Genetic association analysis of inositol polyphosphate phosphatase-like 1 (*INPPL1*, *SHIP2*) variants with essential hypertension

Ana Carolina Braga Marçano, Beverley Burke, Johannie Gungadoo, Chris Wallace, Pamela J Kaisaki, Peng Y Woon, Martin Farrall, David Clayton, Morris Brown, Anna Dominiczak, John M Connell, John Webster, Mark Lathrop, Mark Caulfield, Nilesh Samani, Dominique Gauguier, Patricia B Munroe

J Med Genet 2007;44:603–605. doi: 10.1136/jmg.2007.049718

Background: Inositol polyphosphate phosphatase-like 1 (*INPPL1*, *SHIP2*) is a negative regulator of insulin signalling and has previously been found to be associated with hypertension, obesity and type 2 diabetes in a cohort of families with diabetes in the UK presenting features of metabolic syndrome. In particular, a haplotype of three genetic polymorphisms (rs2276047, rs9886 and an insertion/deletion polymorphism in intron 1) was found to be strongly associated with increased susceptibility to hypertension.

Objective and methods: To assess if *INPPL1* variants play a direct role in the development of essential hypertension, we genotyped the three previously associated *INPPL1* polymorphisms in a cohort of 712 families with severe hypertension from the BRIGHT study transmission disequilibrium test cohort.

Results: We found no evidence of significant association between hypertension and any of the three *INPPL1* polymorphisms or haplotypes ($p > 0.1$).

Conclusion: These results suggest that *INPPL1* variants may be involved in mechanisms causing hypertension in metabolic syndrome patients specifically.

Inositol polyphosphate phosphatase-like 1 (*INPPL1*, also known as *SHIP2*) is a member of the inositol polyphosphatase 5-phosphatase family, and is expressed in a wide range of tissues.¹ *INPPL1* has been reported to play a critical role as a negative regulator of insulin signalling, by inhibiting phosphoinositide 3-kinase through dephosphorylation of its active product, phosphatidyl inositol-3,4,5-trisphosphate. Its role in the insulin pathway has been demonstrated directly by *in vivo* studies; *Inpp1* knockout mice have increased insulin sensitivity via increased insulin signalling.^{1–3}

INPPL1 maps to human chromosome 11q13–14, a region with suggestive linkage to both hypertension and insulin resistance.^{4–6} In rats, *Inpp1* maps to an interval of chromosome 1, exhibiting synteny conservation with human 11q13–14. This region of rat chromosome 1 is linked to a blood pressure quantitative trait locus in the spontaneously hypertensive rat (SHR),⁷ and to glucose intolerance and adiposity in the spontaneously diabetic (type 2) Goto–Kakizaki (GK) rat.^{8,9} Furthermore, a coding variant found in both SHR and GK strains induces impaired insulin sensitivity *in vitro*.⁹ These studies indicated *INPPL1* as a good candidate gene for metabolic syndrome (type 2 diabetes, obesity, dyslipidaemia and hypertension).

Single nucleotide polymorphisms (SNPs) of *INPPL1* have been identified and tested for association with metabolic syndrome in a cohort of families in the UK of white European ancestry (Diabetes

in Families (DIF) study collection). Families were ascertained through type 2 diabetes probands.¹⁰ This study showed that three *INPPL1* polymorphisms (two SNPs, rs2276047 in intron 9 and rs9886 in the 3' untranslated region and an insertion/deletion polymorphism in intron 1), were significantly ($p < 0.0001$) associated with obesity, type 2 diabetes and hypertension (components of the metabolic syndrome) in patients in the DIF study. The same polymorphisms were subsequently genotyped in 905 French patients with type 2 diabetes and 305 controls without diabetes. Analysis of genotype and haplotype frequencies found no association with type 2 diabetes in the case–control study. However, comparison of the French patients with diabetes with and without hypertension demonstrated significant association between the insertion allele of the insertion/deletion polymorphism and hypertension ($p = 0.01$). In addition, the most common haplotype was significantly ($p < 0.01$) more frequent in French patients with diabetes and hypertension compared with patients with hypertension who did not have diabetes.¹⁰ In the current study we investigated if the three previously associated *INPPL1* polymorphisms were directly associated with essential hypertension (EH), in order to assess if the gene also has a role in the development of hypertension in families without diabetes.

The transmission disequilibrium test (TDT) resource of the Medical Research Council British Genetics of Hypertension (BRIGHT) study currently consists of 712 families, all of white European ancestry. These were recruited via a severely hypertensive proband. None is obese or has diabetes. Detailed information on the resource characteristics have been described previously¹¹ and the inclusion/exclusion criteria can be found at www.brightstudy.ac.uk.

METHODS

Study population

Full ethics approval was obtained for this study. In total, 712 severely hypertensive families from the MRC BRIGHT study TDT resource were enrolled. This resource consists of 367 standard trios (affected proband and both parents) and 345 trios with a single parent, the affected proband and affected or unaffected siblings. Demographics of the hypertensive probands are presented in table 1.

In summary, patients were included in the study if their age of diagnosis for hypertension was <60 years, blood-pressure readings were >150/100 mmHg, (single reading), or >145/95 mmHg

Abbreviations: BRIGHT, British Genetics of Hypertension; CEPH, Centre d'Etudes du Polymorphisme Humain; DIF, Diabetes in Families; EH, essential hypertension; GK, Goto–Kakizaki; HWE, Hardy–Weinberg equilibrium; SHR, spontaneously hypertensive rat; SNP, single nucleotide polymorphism; TDT, transmission disequilibrium test

Table 1 Demographics of the hypertensive probands

Characteristic	
Pedigrees, n	712
Offspring with hypertension, n	728
Gender, M/F	329/399
Mean (SD) age at diagnosis, years	39 (9.8)
Mean (SD) blood pressure, mmHg	
Diagnosis systolic	164.2 (15.8)
Diagnosis diastolic	103.3 (8.7)
Systolic at phenotyping	146.7 (17.7)
Diastolic at phenotyping	94.0 (10.4)
Body mass index, median (IQR)	25.0 (25–30)

Del, deletion; Ins, insertion; IQR, Interquartile range.

(mean of three readings), body mass index <35 kg/m² and there were no secondary causes of hypertension, diabetes, intrinsic renal disease or any coexisting confounding illness. Socioeconomic, personal medical history, genealogy, family history of cardiovascular disease, medication and phenotypic measurements were all recorded.

Genetic analysis

The two SNPs (rs2276047 and rs9886) were genotyped using the Taqman assay developed by Applied Biosystems on DNA previously extracted from the TDT resource, followed by allelic discrimination on an automated sequencer (ABI Prism 7900HT Sequence Detection System and SDS V.2.0 software, Applied Biosystems, Foster City, California, USA). Alleles of the insertion/deletion variation, 28 bp Ins/Del, in intron 1, were detected by PCR and size separation using agarose gel electrophoresis. PCR conditions and primer sequences have been described previously.¹⁰

Quality control assessment

Each family of the BRIGHT study TDT resource has previously been genetically fingerprinted using five unlinked microsatellite markers distributed throughout the genome to confirm relationships within families. For the three polymorphisms genotyped in this study, quality control was performed for all genotypes. Each 96-well plate included two positive control DNAs (the same CEPH DNA was included in all plates) and two negative controls (water). Plates were checked for negative signals in positions containing negative controls and for known control genotypes. PedCheck was used to check Mendelian inheritance incompatibility within the families.¹² Two separate interpretations of each genotype were made and the data merged. Samples presenting with different genotypes as judged by two independent observers were repeated; if there was still no correlation, the genotypes were excluded from the analysis. Each SNP was tested for Hardy–Weinberg equilibrium (HWE) using a Mantel–Haenszel χ^2 test (which allows for heterogeneity across geographical regions) programmed in R software.¹³

Statistical analysis

Association analysis was conducted using transmission/disequilibrium testing performed using TRANSMIT software.¹⁴ We used the robust estimator of variance (which allows for more than one affected offspring in any family) and bootstrap p values were calculated using 10 000 bootstrap samples. We conducted power calculations allowing for the non-standard structure of BRIGHT TDT families using PBAT software (<http://www.biostat.harvard.edu/~clange/default.htm>), which takes into consideration missing parental genotypes and additional offspring.¹⁵

RESULTS

A total of 2183 individuals were genotyped for the three polymorphisms; success rate was >95%.

All polymorphisms were tested for HWE in the parents, rs2276047 was found to be out of HWE, with an excess of heterozygosity ($p < 0.01$). The minor allele frequency for rs2276047 was comparable to that observed in the type 2 diabetes cohorts studied by Kaisaki *et al.*,¹⁰ in which HWE did hold, suggesting that the apparent deviation from HWE is due to random sampling variation rather than any genotyping error. The two other polymorphisms were in HWE. Linkage disequilibrium patterns were similar between the BRIGHT study TDT families, the DIF families and the French cohort.

Single SNP and haplotype analysis found no association with EH in the BRIGHT study TDT cohort (all p values >0.1). Table 2 presents the five most common haplotypes (frequency >1%) of the three polymorphisms studied. Rare haplotypes were pooled together. The global χ^2 test, using 6 degrees of freedom, was 5.75 ($p = 0.45$).

DISCUSSION

Our study implies *INPPL1* does not have a marked influence on blood pressure. This study had good power (73%), after applying a conservative Bonferroni correction for three tests, to detect a high risk haplotype of the same frequency (0.64) and odds ratio of 1.6, as found previously by Kaizaki *et al.*¹⁰

The BRIGHT study TDT probands are all severely hypertensive, and were selected to minimise obesity (median body mass index of 27) and patients with diabetes were specifically excluded. Therefore, this cohort does not possess most of the features of metabolic syndrome, and the only common feature between the BRIGHT and DIF study cohorts is high blood pressure. Our findings suggest that there are different mechanisms causing hypertension in patients with metabolic syndrome and EH. Thus hypertension in metabolic syndrome might be secondary to components of insulin and lipid metabolism and could be triggered by these components. However, the mechanism by which *INPPL1* may lead to hypertension in metabolic syndrome patients remains unclear.

In conclusion, we found no association of *INPPL1* polymorphisms and EH, suggesting that association may be limited

Table 2 INPPL1 haplotype analysis in the BRIGHT TDT resource

Haplotype	28 bp Ins/Del	rs2276047	rs9886	Freq (%)	O	E	Variance (O–E)	χ^2 statistic	GRR (95% CI)	p Value
H1	I	A	G	63	923	923	150.6	0.02	1.01 (0.86 to 1.19)	0.99
H2	I	G	G	16	245	243	90.6	0.08	1.03 (0.84 to 1.27)	0.74
H3	D	A	G	13	190	193	73.4	0.08	1.03 (0.82 to 1.30)	0.76
H4	I	G	C	4	54	60	24.3	1.55	1.29 (0.87 to 1.96)	0.20
H5	I	A	C	2	36	31	11.8	2.63	1.62 (0.92 to 3.11)	0.10

Del, deletion; E, expected; Freq, frequency; Ins, insertion; O, observed.

to metabolic syndrome patients with hypertension as part of the phenotype. This indicates that further work is required to delineate the role of *INPPL1* in the development of hypertension in metabolic syndrome.

ACKNOWLEDGEMENTS

We are very grateful to the British Heart Foundation for funding the recruitment of patients, and to the BRIGHT nursing team and the British public for their generous cooperation. This work was also supported by a programme grant from the Medical Research Council, G9521010D. CW is supported by British Heart Foundation grant FS/05/061/19501. JMC is supported by a Wellcome Trust grant, and DG holds a Wellcome Senior Fellowship in Basic Biomedical Science (057733).

Authors' affiliations

Ana Carolina Braga Marçano, Beverley Burke, Johannie Gungadoo, Chris Wallace, Mark Caulfield, Patricia B Munroe, Clinical Pharmacology and The Genome Centre, Barts and The London School of Medicine and Dentistry, London, UK

Pamela J Kaisaki, Peng Y Woon, Dominique Gauguier, The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK

Martin Farrall, Department of Cardiovascular Medicine, University of Oxford, Wellcome Trust Centre for Human Genetics, Oxford, UK

David Clayton, Morris Brown, Clinical Pharmacology and the Cambridge Institute of Medical Research, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK

Anna Dominiczak, John M Connell, BHF Glasgow Cardiovascular Research Centre, Division of Cardiovascular and Medical Sciences, University of Glasgow, Western Infirmary, Glasgow, UK

John Webster, Medicine and Therapeutics, Aberdeen Royal Infirmary, Aberdeen, UK

Mark Lathrop, Centre National de Genotypage, Evry, France

Nilesh Samani, Cardiology, University of Leicester, Glenfield Hospital, Leicester, UK

Competing interests: None declared.

Correspondence to: Patricia B Munroe, PhD, Clinical Pharmacology, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Charterhouse Square, London, EC1M 6BQ; p.b.munroe@qmul.ac.uk

Received 9 February 2007

Revised 25 April 2007

Accepted 6 May 2007

Published Online First 8 June 2007

REFERENCES

- 1 **Clement S**, Krause U, Desmedt F, Tanti JF, Behrends J, Peseux X, Sasaki T, Penninger J, Doherty M, Malaisse W, Dumont JE, Le Marchand-Brustel Y, Erneux C, Hue L, Schurmans S. The lipid phosphatase SHIP2 controls insulin sensitivity. *Nature* 2001;**409**:92–7.
- 2 **Sasaoka T**, Hori H, Wada T, Ishiki M, Haruta T, Ishihara H, Kobayashi M. SH2-containing inositol phosphatase 2 negatively regulates insulin-induced glycogen synthesis in L6 myotubes. *Diabetologia* 2001;**44**:1258–67.
- 3 **Sleeman MW**, Wortley KE, Lai KM, Gowen LC, Kintner J, Kline WO, Garcia K, Stitt TN, Yancopoulos GD, Wiegand SJ, Glass DJ. Absence of the lipid phosphatase SHIP2 confers resistance to dietary obesity. *Nat Med* 2005;**11**:199–205.
- 4 **Xu X**, Rogus JJ, Terwedow HA, Yang J, Wang Z, Chen C, Niu T, Wang B, Xu H, Weiss S, Schork NJ, Fang Z. An extreme-sib-pair genome scan for genes regulating blood pressure. *Am J Hum Genet* 1999;**64**:1694–701.
- 5 **Ghosh S**, Watanabe RM, Valle TT, Hauser ER, Magnuson VL, Langefeld CD, Ally DS, Mohlke KL, Silander K, Kohtamaki K, Chines P, Balow J Jr, Birznieks G, Chang J, Eldridge W, Erdos MR, Karanjawala ZE, Knapp JJ, Kudelko K, Martin C, Morales-Mena A, Musick A, Musick T, Pfahl C, Porter R, Rayman JB. The Finland-United States investigation of non-insulin-dependent diabetes mellitus genetics (FUSION) study. I. An autosomal genome scan for genes that predispose to type 2 diabetes. *Am J Hum Genet* 2000;**67**:1174–85.
- 6 **Panhuyzen CI**, Cupples LA, Wilson PW, Herbert AG, Myers RH, Meigs JB. A genome scan for loci linked to quantitative insulin traits in persons without diabetes: the Framingham Offspring Study. *Diabetologia* 2003;**46**:579–87.
- 7 **Frantz S**, Clemitson JR, Bihoreau MT, Gauguier D, Samani NJ. Genetic dissection of region around the *Sa* gene on rat chromosome 1: evidence for multiple loci affecting blood pressure. *Hypertension* 2001;**38**:216–21.
- 8 **Gauguier D**, Froguel P, Parent V, Bernard C, Bihoreau MT, Portha B, James MR, Penicaud L, Lathrop M, Ktorza A. Chromosomal mapping of genetic loci associated with non-insulin dependent diabetes in the GK rat. *Nat Genet* 1996;**12**:38–43.
- 9 **Marion E**, Kaisaki PJ, Pouillon V, Gueydan C, Levy JC, Bodson A, Krzentowski G, Daubresse JC, Mockel J, Behrends J, Servais G, Szpirer C, Krusys V, Gauguier D, Schurmans S. The gene *INPPL1*, encoding the lipid phosphatase SHIP2, is a candidate for type 2 diabetes in rat and man. *Diabetes* 2002;**51**:2012–27.
- 10 **Kaisaki PJ**, Delepine M, Woon PY, Sebag-Montefiore L, Wilder SP, Menzel S, Vionnet N, Marion E, Riveline JP, Charpentier G, Schurmans S, Levy JC, Lathrop M, Farrall M, Gauguier D. Polymorphisms in type II SH2 domain-containing inositol 5-phosphatase (*INPPL1*, *SHIP2*) are associated with physiological abnormalities of the metabolic syndrome. *Diabetes* 2004;**53**:1900–4.
- 11 **Newhouse SJ**, Wallace C, Dobson R, Mein C, Pembroke J, Farrall M, Clayton D, Brown M, Samani N, Dominiczak A, Connell JM, Webster J, Lathrop GM, Caulfield M, Munroe PB. Haplotypes of the *WNK1* gene associate with blood pressure variation in a severely hypertensive population from the British Genetics of Hypertension study. *Hum Mol Genet* 2005;**14**:1805–14.
- 12 **O'Connell JR**, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 1998;**63**:259–66.
- 13 **Team RDC**. R: A language and environment for statistical computing. 2005.
- 14 **Clayton D**, Jones H. Transmission/disequilibrium tests for extended marker haplotypes. *Am J Hum Genet* 1999;**65**:1161–9.
- 15 **Lange C**, DeMeo D, Silverman EK, Weiss ST, Laird NM. PBAT: tools for family-based association studies. *Am J Hum Genet* 2004;**74**:367–39.