BJA

British Journal of Anaesthesia, 126 (5): 949–957 (2021)

doi: 10.1016/j.bja.2021.01.029
Advance Access Publication Date: 4 March 2021
Clinical Practice

First genome-wide association study on rocuronium dose requirements shows association with *SLCO1A2*

Sirkku Ahlström[1,*], Paula Bergman[2], Ritva Jokela[1], Linda Ottensmann[3], Ari Ahola-Olli[3], Matti Pirinen[3,4,5], Klaus T. Olkkola[1], Mari A. Kaunisto[3,†] and Eija Kalso[1,6,7,†]

[1]Department of Anaesthesiology, Intensive Care and Pain Medicine, University of Helsinki and HUS Helsinki University Hospital, Helsinki, Finland, [2]Biostatistics Consulting, Department of Public Health, University of Helsinki and HUS Helsinki University Hospital, Helsinki, Finland, [3]Institute for Molecular Medicine Finland, Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland, [4]Helsinki Institute for Information Technology and Department of Mathematics and Statistics, University of Helsinki, Helsinki, Finland, [5]Department of Public Health, University of Helsinki, Helsinki, Finland, [6]Department of Pharmacology, Faculty of Medicine, University of Helsinki, Helsinki, Finland and [7]SleepWell Research Programme, Faculty of Medicine, University of Helsinki, Helsinki, Finland

*Corresponding author. E-mail: Sirkku.tornqvist@helsinki.fi

†These authors contributed equally.

Abstract

Background: Rocuronium, a common neuromuscular blocking agent, is mainly excreted unchanged in urine (10–25%) and bile (>70%). Age, sex, liver blood flow, smoking, medical conditions, and ethnic background can affect its pharmacological actions. However, reasons for the wide variation in rocuronium requirements are mostly unknown. We hypothesised that pharmacogenetic factors might explain part of the variation.

Methods: One thousand women undergoing surgery for breast cancer were studied. Anaesthesia was maintained with propofol (50–100 μg kg^{-1} min^{-1}) and remifentanil (0.05–0.25 μg kg^{-1} min^{-1}). Neuromuscular block was maintained with rocuronium to keep the train-of-four ratio at 0–10%. DNA was extracted from peripheral blood and genotyped with a next-generation genotyping array. The genome-wide association study (GWAS) was conducted using an additive linear regression model with PLINK software. The FINEMAP tool and data from the Genotype-Tissue Expression project v8 were utilised to study the locus further.

Results: The final patient population comprised 918 individuals. Of the clinical variables tested, age, BMI, ASA physical status, and total dose of propofol correlated significantly (all $P<0.001$) with the rocuronium dose in a linear regression model. The GWAS highlighted one genome-wide significant locus in chromosome 12. The single-nucleotide polymorphisms (SNPs) with the most significant evidence of association were located in or near *SLCO1A2*. The two top SNPs, rs7967354 ($P=5.3e^{-11}$) and rs11045995 ($P=1.4e^{-10}$), and the clinical variables accounted for 41% of the variability in rocuronium dosage.

Conclusions: Genetic variation in the gene *SLCO1A2*, encoding OATP1A2, an uptake transporter, accounted for 4% of the variability in rocuronium consumption. The underlying mechanism remains unknown.

Keywords: genome-wide association study; neuromuscular block; OATP1A2; rocuronium; *SLCO1A2*; SNP

Recevied: 6 July 2020 **Accepted:** 12 January 2021

Editor's key points

- There is considerable inter-individual variability in patient requirements for rocuronium, a drug that is mainly excreted unchanged in the bile.
- The authors performed a genome-wide association study in a large cohort of women undergoing breast cancer surgery.
- Genetic variation in the gene *SLCO1A2*, which encodes OATP1A2, an uptake transporter for which rocuronium is a substrate, explained 4% of the variability in rocuronium requirements.
- Clinical variables, such as age, BMI, ASA physical status, and total dose of propofol, explained a much larger portion of the inter-individual variability.

Rocuronium bromide is a commonly used amino-steroidal non-depolarising neuromuscular blocking drug that competitively binds to post-junctional nicotinic acetylcholine receptors in the neuromuscular junction, blocking the action of acetylcholine and thereby inhibiting the contraction of striatal muscles.

Rocuronium is mainly excreted unchanged in urine (10–25%) and bile (more than 70%).[1,2] Both renal and hepatic insufficiencies decrease clearance and prolong its action.[3,4] Based on *in vitro* studies with animal and human liver tissue, rocuronium is a substrate for organic anion transporters (OATPs). The transporters take up rocuronium in the liver.[5,6] Rocuronium is not generally considered to be metabolised in the liver, although there are conflicting views (https://www.clinicalkey.com/#!/content/6-s2.0-552). Rocuronium is a highly hydrophilic molecule and binds poorly (about 25%) to plasma proteins.[7]

Several reports have demonstrated major inter-individual differences in the duration of action of rocuronium.[8–11] Advanced age,[12] female sex,[13] conditions and treatments that alter the skeletal muscle acetylcholine receptors,[14] decrease in hepatic blood flow during surgery,[15] smoking,[16] and ethnic background[17–19] can also affect the pharmacokinetics or pharmacodynamics of rocuronium.

We hypothesised that pharmacogenetic factors might explain part of the unaccounted variation in the pharmacological response to rocuronium. The aim of this study was to identify genetic factors associated with the requirements of rocuronium by performing the first-ever genome-wide association study (GWAS) in a large cohort of women undergoing breast cancer surgery.

Methods

BrePainGen is a prospective study designed to examine the role of genetics in acute and persistent post-surgical pain, mood, and effects of drugs used in anaesthesia. One thousand women undergoing surgery for breast cancer at the Helsinki University Hospital were recruited between August 1, 2006 and December 31, 2010. The study protocol was approved by the coordinating ethics committee (136/E0/2006) and the ethics committee of the Department of Surgery (Dnro 148/E6/05) of the Hospital District of Helsinki and Uusimaa. Written informed consent was obtained from all patients. A flow chart of patient recruitment is provided in Supplementary Fig. 1.

After informed consent, the patients were interviewed for background information about medical conditions, medications, age, height, weight, previous operations, use of alcohol, and smoking. Those using drugs affecting the pharmacology of rocuronium were excluded.

All patients were premedicated with diazepam 2.5–15 mg and paracetamol 1 g orally. Anaesthesia was induced with propofol 2–3 mg kg^{-1}, and remifentanil infusion of 0.2 mg kg^{-1} min^{-1} was started. Tracheal intubation was facilitated with rocuronium 0.6 mg kg^{-1}. During surgery, anaesthesia was maintained with a propofol infusion at 50–100 μg kg^{-1} min^{-1} to keep state entropy (M-Entropy S/5TM Module for Anesthesia Monitor; GE Healthcare Finland, Helsinki, Finland) at the level of 50 [5]. Remifentanil infusion was used at 0.05–0.25 μg kg^{-1} min^{-1} to keep systolic BP at [15%] of baseline minus 20 mm Hg. The neuromuscular block was maintained throughout the surgery with rocuronium boluses of 10 mg to keep the train-of-four ratio at 0–10% (E-NMT; GE Healthcare Finland). Mechanical ventilation was adjusted to normocapnia with 1:1 oxygen and nitrous oxide. During closure of the skin, remifentanil infusion was stopped, and boluses of fentanyl 1 μg kg^{-1}, ondansetron 4 mg, and droperidol 0.01 mg kg^{-1} were given intravenously. Neuromuscular block was reversed with neostigmine 2.5 mg and glycopyrrolate 0.5 mg. Before the patient woke from anaesthesia, a blood specimen was drawn for DNA isolation.

DNA was extracted from peripheral blood using the Autopure LS™ automated DNA purification instrument (Gentra Systems, Inc., Minneapolis, MN, USA). Genotype data were produced at the Wellcome Sanger Institute (Hinxton, UK) on the HumanOmniExpress Illumina BeadChip (Illumina, Inc., San Diego, CA, USA) while blind to phenotypic information. Sample quality control procedures have been described in detail earlier.[20] All single-nucleotide polymorphisms (SNPs) were filtered based on minor allele frequency (MAF >0.0005), Hardy–Weinberg equilibrium ($P>1 \times 10^{-6}$), and success rate (>0.97). The mean genotyping success rate was 0.997. After quality control, genotyping data were available for 926 of the 1000 participants. Eight patients were excluded for clinical reasons. The final participant population comprised 918 individuals with both genotype and clinical data available (Tables 1 and 2).

Statistical analyses and data management were conducted using IBM SPSS software versions 23.0 and 24.0 and R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). To identify possible confounders that should be taken into account when performing the GWAS, we first performed univariate testing (n=992) between clinical variables and the dose of rocuronium needed to maintain adequate neuromuscular block. We took the dose rates of rocuronium (in units of mg kg^{-1} min^{-1}) using the natural logarithms of their numerical values to ensure normal distribution. The clinical variables to be tested for relevance to rocuronium dose requirements were age, height, BMI, total use of propofol during anaesthesia (mg kg^{-1} min^{-1}), total use of remifentanil during anaesthesia (mg kg^{-1} min^{-1}), ASA class, smoking (yes/no), alcohol use (yes/abstinent), type of axillary surgery (sentinel node biopsy/evacuation), and breast surgery (resection/mastectomy). We also tested associations between *CYP2D6* copy numbers, *CYP2D6*-predicted phenotype (poor, intermediate, extensive, or ultra-rapid metabolisers), *CYP3A4* variant rs35599367 genotype (CC/CT/TT) and *CYP3A5* variant rs776746 genotype (GG/AG/AA), and the natural logarithm of rocuronium dose rates (expressed in mg kg^{-1} min^{-1}), as the CYP data were available from these patients.[21] We tested these associations using *t*-test, Mann–Whitney *U*-test, analysis of variance, or

Table 1 Characteristics of the subjects. *n*=992 for all included in the association testing, and *n*=918 for genetic testing based on the number of genetic data available.

	All patients, % (*n*)	Final genetic analyses
ASA physical status		
1	5.6 (56)	5.4 (50)
2	82.5 (818)	82.8 (760)
3	11.9 (118)	11.8 (108)
Type of surgery in the axilla		
Sentinel node biopsy	55.8 (554)	55 (505)
Evacuation of axilla	44.2 (438)	45 (413)
Type of surgery in the breast		
Resection	62.4 (619)	62.2 (571)
Mastectomy	37.6 (373)	37.8 (347)
Smoking habits		
No	82 (813)	82.5 (757)
Yes	18 (179)	17.5 (161)
Alcohol use		
No	17.5 (173)	18.1 (166)
Yes	82.5 (819)	81.9 (752)

Table 2 Characteristics of the patients, anaesthesia, and total dose of rocuronium; *n*=918.

	Median (inter-quartile range)
Age (yr)	58 (50–64)
Height (cm)	164 (160–169)
Weight (kg)	68 (60–76)
BMI (kg m^{-2})	24.8 (22.3–28.2)
Total dose of rocuronium (mg)	75 (60–90)
Time to first additional dose after induction (min)	38 (30–46)
Time to recovery after last dose (min)	47 (38–57)
Duration of anaesthesia (min)	138 (115–168)
Dose of rocuronium (μg kg^{-1} min^{-1})	7.9 (6.5–9.7)
Total dose of remifentanil during anaesthesia (mg)	0.8 (0.6–1.1)
Dose of remifentanil (μg kg^{-1} min^{-1})	0.008 (0.007–0.010)
Total dose of propofol during anaesthesia (mg)	897 (718–1102)
Dose of propofol (μg kg^{-1} min^{-1})	95 (83–110)

Kruskal–Wallis test, depending on the distributions of variables. Pairwise comparisons were adjusted with Bonferroni corrections.

After studying possible associations, we conducted multivariate linear regression modelling based on variables that had statistically significant ($P<0.05$) associations with rocuronium requirements. We used a stepwise method to construct the final model. The final linear regression model contained only variables that remained significant in multivariate testing. These were used as covariates in the GWAS. In addition, the first five dimensions from multidimensional scaling of genotype data were also used as covariates to take into account a possible hidden population structure.

The GWAS was conducted using an additive linear regression model with PLINK software.[22] Associations between total dose of rocuronium and 653 034 genetic variants (SNPs) were tested. The standard threshold of genome-wide statistical significance, $P<5\times10^{-8}$, was used. After GWAS results became available, we performed another linear regression round, including four lead variants, to examine the impact of these on the total dose of rocuronium.

The genomic region showing a significant association with rocuronium dosage was further examined to identify the most likely causal SNPs within the locus. For this, the genomic data were first pre-phased with Eagle software version 2.4.[23] Subsequently, the genotypes were imputed using Beagle 4.1 and population-specific Sequencing Initiative Suomi panel as imputation reference.[24,25] Poorly imputed variants were excluded (INFO <0.7). The imputation reference panel consisted of 3775 Finns. To identify the number of independent association signals and the lead SNPs within the associated locus, the FINEMAP[26] tool was used. The FUMA[27] tool was used to examine the potential functional effect of each associated SNP. Genetic effects on gene expression across tissue types were studied using publicly available data from the Genotype-Tissue Expression project v8.[28] FINEMAP 1.4[26] was run, allowing a maximum of $K=5$ causal variants. The credible sets, those containing the most likely causal variants, were reported, assuming either one or two causal variants.

Results

To test associations with clinical variables, all 992 individuals with complete clinical data were used. For 918 of these, genome-wide genotype data were available and used for the GWAS. The characteristics of the subjects and of rocuronium requirements are shown in Tables 1 and 2. The total dose of rocuronium had a linear relationship ($R^2=0.412$) with the duration of anaesthesia (Fig. 1a). The median number of additional doses was 4 (inter-quartile range [IQR]: 2–5), range 0–20. Twenty-one patients did not receive additional doses of rocuronium after intubation.

Of the continuous variables tested, age ($P<0.001$), BMI ($P<0.001$), and total doses of propofol (mg kg^{-1} min^{-1}; $P<0.001$) and of remifentanil (mg kg^{-1} min^{-1}; $P<0.001$) had significant correlations with the dose of rocuronium (in mg kg^{-1} min^{-1}). Of the dichotomous and ordinal variables, ASA class ($P<0.001$), use of alcohol compared with abstinence ($P=0.001$), and breast resection *vs* mastectomy ($P<0.001$) were statistically significant at $P<0.05$.

Multiple linear regression models were run to understand the effects of the aforementioned variables on the dose of rocuronium (in mg kg^{-1} min^{-1}). There was homoscedasticity as assessed by visual inspection of a plot of standardised residuals *vs* standardised predicted values, normality of the residuals being assessed by visual inspection of a normal probability plot: there were no significant outliers, as assessed by Cook's distance. The results of the linear model are presented in Table 3. The clinical variables in the model accounted for 35.3% of the variability in rocuronium dosage.

The GWAS highlighted one locus on chromosome 12 showing genome-wide significant evidence of association with rocuronium dose (Fig. 2). Eight genotyped SNPs reached the standard threshold of genome-wide statistical significance, $P<5\times10^{-8}$ (Table 4). The SNPs with the most significant evidence of association were all located in or near gene *SLCO1A2*. LocusZoom plots[29] of the area are shown in

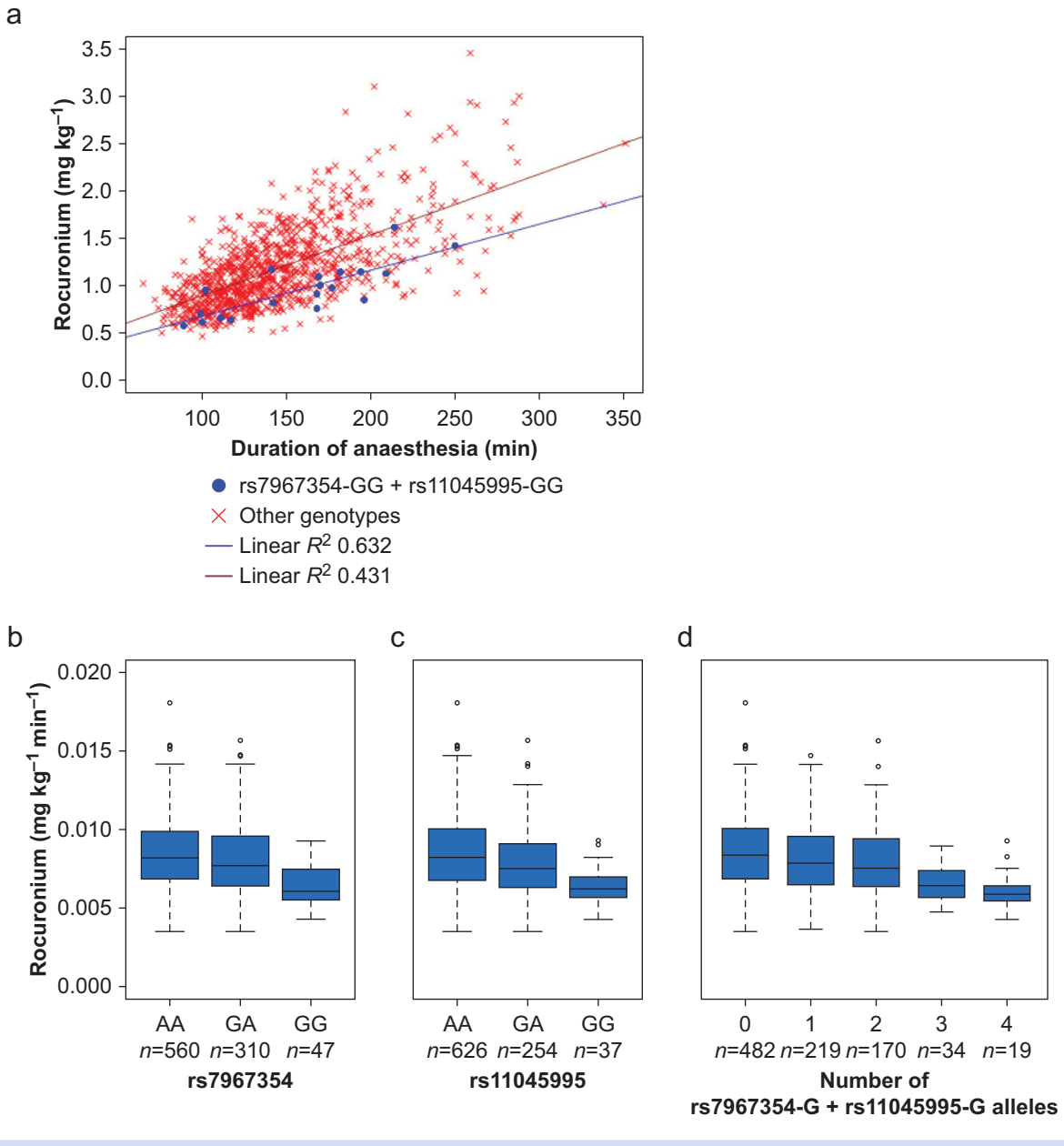

Fig 1. Relationship between rocuronium dose and (a) duration of anaesthesia and (b–d) the two lead SNPs showing genome-wide significant association with rocuronium dose in the GWAS. (a) Rocuronium requirements of the study participants plotted against the duration of anaesthesia. Patients homozygous for the minor alleles of both of the lead SNPs (rs7967354-G and rs11045995-G) are highlighted in blue. Mean rocuronium dosage in this group is lower than the average, and the individual data points are also clearly at the lower end of the requirement distribution. (b) Box and whisker plot showing rocuronium requirements during anaesthesia classified by rs7967354 genotype. The middle line of the box represents median, and the upper and lower edges of the box represent upper and lower quartiles, respectively. Upper whisker represents the minimum between the maximum value and the upper quartile+1.5 * inter-quartile range. Lower whisker represents the maximum between the minimum value and the lower quartile−1.5 * inter-quartile range. Any values outside the whiskers are outliers, presented as circles. (c) Box and whisker plot showing the rocuronium requirements during anaesthesia according to rs11045995 genotype. (d) Box and whisker plot showing rocuronium requirements during anaesthesia classified by number of rs7967354-G and rs11045995-G alleles. Subjects with the homozygous genotype GG+GG in both rs7967354 and rs11045995 (minor allele is G in both variants) needed significantly less rocuronium during anaesthesia than subjects with other genotypes. The same individuals are highlighted in blue in Fig. 2a. GWAS, genome-wide association study; SNP, single-nucleotide polymorphism.

Table 3 Linear regression model with statistically significant clinical variables and statistically significant clinical variables and genetic variants with a dose-altering effect. The tested variable was rocuronium dose (in mg kg^{-1} min^{-1}).

	B	*P*-value	95% CI lower bound	95% CI upper bound
Clinical variables				
Age (yr)	−0.004	<0.001	−0.006	−0.003
BMI	−0.029	<0.001	−0.032	−0.025
ASA physical status 1	0.088	0.019	0.014	0.16
ASA physical status 2	0.06	0.009	0.015	0.10
Propofol (mg kg^{-1} min^{-1})	2.0	<0.001	1.3	2.7
Adjusted R^2=0.35				
Clinical variables and genetic variants				
Age (yr)	−0.005	<0.001	−0.006	−0.003
BMI	−0.028	<0.001	−0.032	−0.025
ASA physical status 1	0.099	<0.001	0.025	0.17
ASA physical status 2	0.075	0.008	0.031	0.12
Propofol (mg kg^{-1} min^{-1})	1.9	<0.001	1.2	2.6
rs7967354	−0.043	0.001	−0.069	−0.017
rs11045995	−0.090	<0.001	−0.12	−0.062
Adjusted R^2=0.41				

CI, confidence interval.

Supplementary Fig. 2a and b. Based on the genetic recombination patterns and the FINEMAP tool, the most likely scenario is that two SNPs are needed to explain the association. The top candidates are rs7967354 ($P=5.3e^{-11}$; β=−0.143 for allele G; MAF=0.22) and rs11045995 ($P=1.4e^{-10}$; β=−0.147 for allele G; MAF=0.18), both located in gene *SLCO1A2* and in moderate linkage disequilibrium (LD) with each other (r^2=0.26). The minor alleles of both variants (rs7967354-G and rs11045995-G) are associated with decreased rocuronium requirements.

Patients with two minor alleles (G/G) of the variant rs7967354 needed significantly less rocuronium during anaesthesia (*n*=47; median dose rate: 6.1 μg kg^{-1} min^{-1}; IQR: 5.5–7.5) compared with patients with G/A genotype (*n*=310; median dose rate: 7.7 μg kg^{-1} min^{-1}; IQR: 6.4–9.5) and A/A genotype (*n*=561; median dose rate: 8.2 μg kg^{-1} min^{-1}; IQR: 6.3–9.1) (Fig. 1b) in Kruskal–Wallis testing (*P*<0.001). Similarly, patients with rs11045995 G/G genotype needed significantly lower doses of rocuronium during anaesthesia (*n*=37; median dose rate: 6.3 μg kg^{-1} min^{-1}; IQR: 5.6–7.0; *P*<0.001) than the patients with G/A (*n*=254; median dose rate: 7.5 μg kg^{-1} min^{-1}; IQR: 6.3–9.1) and A/A genotypes (*n*=627; median dose rate: 8.3 μg kg^{-1} min^{-1}; IQR: 6.8–10.1) (Fig. 1c). We also found that patients with two minor alleles in both dose-altering variants (rs7967354-GG + rs11045995-GG; *n*=19; median dose rate: 5.9 [IQR: 5.4–6.5] μg kg^{-1} min^{-1}) needed even less rocuronium during anaesthesia than with other genotype combinations in the Kruskal–Wallis test (Fig. 1a and d; Supplementary Tables 1 and 2).

The final linear regression model included both the clinical variables and the two lead SNPs rs7967354 and rs11045995 (Table 3). The variables in the model accounted for 41% of the variability (adjusted R^2) in the rocuronium dose. In this multivariate analysis, age (*P*<0.001), BMI (*P*<0.001), ASA 1 *vs* ASA 3 (*P*=0.008), ASA 2 *vs* ASA 3 (*P*=0.001), dose of propofol (*P*<0.001), rs7967354 (*P*=0.001), and rs11045995 (*P*<0.001) remained significant at *P*<0.05. By including the two SNPs in

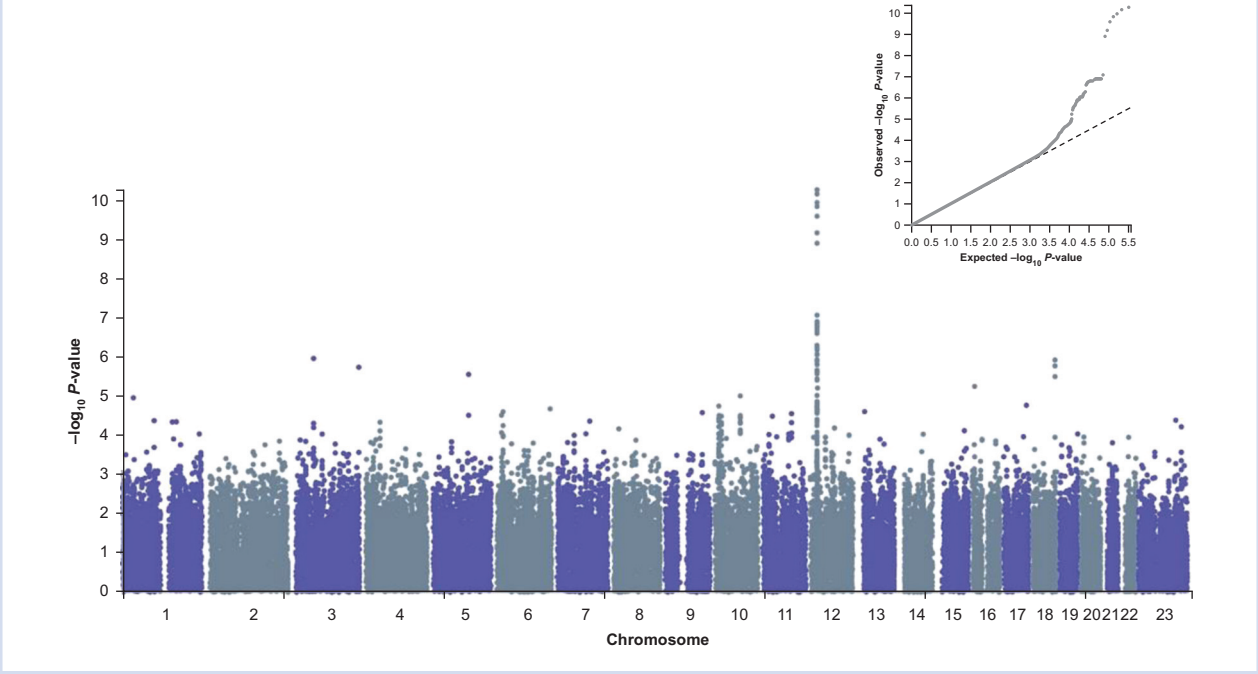

Fig 2. Manhattan plot showing the $-\log_{10}$ *P*-values of all 653 034 SNPs tested in the GWAS, plotted against their respective chromosomal positions. The results highlight one genome-wide significant locus on chromosome 12. The quantile–quantile plot in the upper-right corner of the image shows the deviation of the observed from the expected *P*-values under the null hypothesis of no association. GWAS, genome-wide association study; SNP, single-nucleotide polymorphism.

Table 4 Dose-altering variants found in this genome-wide association analysis.

Chr	SNP	Gene	Minor allele	MAF	β	P-value	SNP is	r^2 rs7967354	r^2 rs11045995
12	rs7967354	*SLCO1A2*	G	0.22	−0.1425	$5.302e^{-11}$	Intron variant	1	0.261516
12	rs4149005	*SLCO1A2*	A	0.22	−0.1417	$6.809e^{-11}$	Non-coding transcript exon variant	0.996918	0.263069
12	rs875234	*SLCO1A2*	G	0.22	−0.14	$1.129e^{-10}$	3′ UTR variant	0.996919	0.260478
12	rs11045995	*SLCO1A2*	G	0.18	−0.1473	$1.416e^{-10}$	Intron variant	0.261516	1
12	rs10743413	*SLCO1A2*	G	0.20	−0.1424	$2.516e^{-10}$	Intron variant	0.233591	0.873469
12	rs10841798	*SLCO1A2*	C	0.18	−0.1417	$6.672e^{-10}$	Intron variant	0.259925	0.982672
12	rs10770800	*SLCO1A2*	G	0.18	−0.1399	$1.228e^{-9}$	Intron variant	0.266367	0.98603
12	rs10841782	*SLCO1A2*	A	0.08	−0.1757	$8.452e^{-8}$	Intron variant	0.322123	0.316403

Chr, chromosome; MAF, minor allele frequency in the study sample; r^2 squared correlation coefficient measure for linkage disequilibrium, range 0–1; SNP, single-nucleotide polymorphism.

the model, the proportion of the variance explained increased by 4 percentage points.

As both rs7967354 and rs11045995 are located in the intronic regions of *SLCO1A2*, we analysed the region further using imputed genome data, and FINEMAP and FUMA programs, to locate other variants possibly driving the association signal. On the imputed data, FINEMAP gives a probability of 74% to one causal variant and 26% for two causal variants. Assuming one causal variant, the 95% credible set (the set of variants containing the causal variant with 95% probability) contains 20 variants (Supplementary Table 3). Assuming two causal variants, the lead variants are rs7967354 and rs10743413 (the latter of which is highly correlated with rs11045995; r^2=0.88), and the two credible sets contain, respectively, 13 and 5 variants with probability over 1% of being causal (Supplementary Table 4). FINEMAP estimates that the *SLCO1A2* region explains 3.75% of the variance of the phenotype (95% credible interval: 1.77–6.29%).

Next, we performed FUMA analysis to check whether the most likely causal variants were associated with tissue-specific changes in the expression level of *SLCO1A2* or other genes. For the 20 potentially causal variants, FUMA analysis detected 39 expression quantitative trait loci (eQTL) for four genes at a false discovery rate of 5% (Supplementary Table 5). For *SLCO1A2*, three variants were eQTL in the brain and three in the cerebellum. Other eQTL were found for the gene *RECQL* (expression measured in blood), *PYROXD1* (in blood), and *C12orf39* (in lymphocytes and in blood). Plots for tissue-wide expression results for rs7967354 and rs11045995 are in Supplementary Fig. 3a and b.

Discussion

We explored clinical and genetic factors explaining variation in rocuronium requirement during surgery for breast cancer in 918 women. We showed that a locus containing gene *SLCO1A2* affects the dose rate needed for maintaining adequate neuromuscular block. Of the clinical variables examined, age, BMI, total dose of propofol, and ASA class were associated with the rocuronium dose. Combined, these factors explained 41% of the dose rate variation. Our study provides further confirmation that neither CYP2D6 nor CYP3A4 plays a role in determining rocuronium requirements.

The median rocuronium dose used is in line with previous reports.[30,31] In our study, advanced age,[12] lower BMI, and higher ASA class decreased the amount of rocuronium needed. Previous reports on the effect of BMI on rocuronium requirements are conflicting.[32,33] Our patients were medicated according to their actual body weight, which might explain why lower BMI decreased rocuronium requirements. We observed that higher propofol doses were associated with increased need for rocuronium, whereas some previous studies suggested that propofol would have muscle-relaxing effects,[34] reducing the required dose of neuromuscular blockers.[35] However, the designs of these studies were very different from ours. There are no previous linear regression models evaluating rocuronium needs during propofol anaesthesia.

Our GWAS identified one genome-wide significant association peak, on chromosome 12, in and around the *SLCO1A2* gene, which encodes the OATP1A2. The signal was best explained by two lead SNPs, rs7967354 and rs11045995. Higher numbers of the minor alleles of these SNPs were associated with a lesser need for rocuronium. The biggest variation in rocuronium dosage was observed when we compared participants homozygous for both rs7967354 and rs11045995 minor alleles (G) with those homozygous for the major alleles (A) (Fig. 1a and d; Supplementary Tables 1 and 2).

Organic anion transporters are cellular transmembrane proteins, important in the distribution, metabolism, and excretion of various drugs and expressed in pharmacokinetically important organs, such as liver, kidney, and intestine.[36] Based on immunohistochemical staining, OATP1A2 transporters are located in cholangiocytes, where they have an important role in excretion of drugs into the bile.[37] As rocuronium is a known substrate of OATP1A2[36] and is mainly excreted unchanged in the urine (10–25%) and bile (>70%),[1,2] the role of OATPs in its excretion is of interest.

A study with *Slco1a/1b*−/− knockout mice showed that lack of functioning OATP1A2 leads to accumulation of the substrates of this transporter in plasma.[38] Previous studies also indicate that OATPs are a target for drug interactions. Expression of OATPs, especially OATP-A, were significantly increased in patients treated with carbamazepine.[39] Carbamazepine use is known to increase the required rocuronium dosage.[40] Our results suggest that this would be attributable to induction of OATP1A2 rather than of CYP3A4.

Few previous studies have addressed the pharmacogenetics of rocuronium. A candidate gene study based on only 30 patients, by Costa and colleagues,[41] showed evidence of

association between a variant −189_188InsA (rs3834939), located in the promoter region of the *SLCO1A2* gene, and reduced clearance of rocuronium. Neither this SNP rs3834939 (P=0.000026 in our study) nor the other variants tested by Costa and colleagues were amongst the SNPs showing genome-wide significant evidence of association (a standard threshold of genome-wide statistical significance is $P<5 \times 10^{-8}$).

The other two earlier rocuronium studies were also candidate gene studies, analysing only a few variants in small patient samples. Based on a sample of 105 Chinese patients, Qi and colleagues[42] reported that SNPs rs12720464 and rs1055302 in the *ABCB1* gene, coding for an ATP-dependent drug efflux pump, associate with prolonged spontaneous recovery after a single dose of rocuronium. In the study of Mei and colleagues,[43] another *ABCB1* SNP (rs1128503) and an SNP (rs2306283) in the OATP1B1 transporter gene *SLCO1B1* showed association with the clinical action time of rocuronium in 200 patients. Our data do not provide support for the *ABCB1* findings, whilst several variants within the *SLCO1B1* gene, located right next to *SLCO1A2*, show almost genome-wide significant evidence of association in our study (Supplementary Fig. 2a). Further analyses showed that these SNPs were not independent from our *SLCO1A2* lead variants (data not shown).

The top SNPs in our study, rs7967354 and rs11045995, and the variants in high LD with them, are located in non-coding parts of the gene. The intronic rs7967354 is in high LD with SNPs rs4149005 (non-coding exon transcript variant) and rs875234 (3′ UTR variant). Our extensive eQTL analyses suggest that a possible mechanism for the genotype–rocuronium dose association is tissue-specific gene expression regulation. As OATP1A2 has a role in excretion of rocuronium into bile,[6] changes in the expression of *SLCO1A2* caused by polymorphisms in the gene might prolong the effect of rocuronium by reducing clearance.

One unexplored option to explain variation in rocuronium dose requirement is that of inter-individual differences in neuromuscular junctions. Here, we can only speculate on the possibility of some underlying variation in neuromuscular junction in otherwise healthy patients that could explain the variation in dose needs. Interestingly, *SLCO1A2* is highly expressed in neural tissues, including peripheral nerve tissue, and our eQTL analyses suggested that the lead variants affect the *SLCO1A2* expression level in the brain. It is tempting to speculate that neural tissue also plays a role in the impact of *SLCO1A2* variants on rocuronium requirements.

Our study has some limitations. Creatinine or creatinine clearance values were not available to assess kidney function. However, patients with clinically relevant kidney failure were excluded from the study. Although the study cohort is the largest thus far examined for pharmacogenetic data suitable for rocuronium studies, it is small for the GWAS approach, which usually requires thousands of participants. As our results are based on only 918 participants, all female, it is likely that some of the more subtle genetic effects remain undetected.

Our study suggests that genetic variation in the gene *SLCO1A2*, encoding OATP1A2, is significantly associated with differences in rocuronium requirements. Our discovery offers one explanation for inter-individual differences in the duration of action of rocuronium. This variation was estimated to account for 4% of the variability in rocuronium dose. The most likely underlying mechanism is altered uptake of rocuronium by OATP1A2.

Authors' contributions

Study design: SA, RJ, KTO, MAK, EK
Genetic data design: MAK, MP
Recruitment and perioperative management of patients: RJ
Whole BrePainGen study: EK, RJ, MAK
Data analysis: SA, PB
Genetic analysis: SA, LO, AA-O, MP, MAK
Interpretation of results: SA, PB, RJ, KTO, MAK, EK
Writing of paper: SA, PB, RJ, LO, AA-O, KTO, MAK, EK

Acknowledgements

The authors thank Leslie Hearn (London, UK) for proofreading the paper. The authors are indebted to the study participants and to Eija R. Ruoppa and Minna Kaiponen, Department of Anaesthesiology, Intensive Care and Pain Medicine, Helsinki University Hospital, for excellent assistance throughout the project.

Declarations of interest

AA-O works part-time as medical adviser at Abomics, a company offering pharmacogenetic consultation services and information and communications technology solutions. EK has participated in advisory boards of Orion Pharma and Pfizer. Part of MAK's salary is covered by a large Finnish biobank study FinnGen, funded by 12 international pharmaceutical companies (AbbVie, AstraZeneca, Biogen, Celgene, Genentech [a member of the Roche Group], GSK, Janssen, Maze Therapeutics, Merck/MSD, Novartis, Pfizer, and Sanofi). SA, PB, LO, RJ, MP, and KTO have no conflicts of interest to declare.

Funding

Academy of Finland (110489 217028, 288509 and 312076); Governmental Research Funds for University Level Health Research (TYH2008225 and TYH2010210) to EK; Academy of Finland (288509 and 312076) to MP; Emil Aaltonen Foundation to AA-O; Finnish Society of Anaesthesiologists to SA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2021.01.029.

References

1. Proost JH, Eriksson LI, Mirakhur RK, Roest G, Wierda JM. Urinary, biliary and faecal excretion of rocuronium in humans. *Br J Anaesth* 2000; **85**: 717–23
2. Khuenl-Brady K, Castagnoli KP, Canfell PC, Caldwell JE, Agoston S, Miller RD. The neuromuscular blocking effects and pharmacokinetics of ORG 9426 and ORG 9616 in the cat. *Anesthesiology* 1990; **72**: 669–74
3. Robertson EN, Driessen JJ, Booij LH. Pharmacokinetics and pharmacodynamics of rocuronium in patients with and without renal failure. *Eur J Anaesthesiol* 2005; **22**: 4–10
4. van Miert MM, Eastwood NB, Boyd AH, Parker CJ, Hunter JM. The pharmacokinetics and

pharmacodynamics of rocuronium in patients with hepatic cirrhosis. *Br J Clin Pharmacol* 1997; **44**: 139–44
5. Olinga P, Merema M, Hof IH, et al. Characterization of the uptake of rocuronium and digoxin in human hepatocytes: carrier specificity and comparison with in vivo data. *J Pharmacol Exp Ther* 1998; **285**: 506–10
6. van Montfoort JE, Hagenbuch B, Fattinger KE, et al. Polyspecific organic anion transporting polypeptides mediate hepatic uptake of amphipathic type II organic cations. *J Pharmacol Exp Ther* 1999; **291**: 147–52
7. Wierda JM, Proost JH. Structure-pharmacodynamic-pharmacokinetic relationships of steroidal neuromuscular blocking agents. *Eur J Anaesthesiol Suppl* 1995; **11**: 45–54
8. Claudius C, Karacan H, Viby-Mogensen J. Prolonged residual paralysis after a single intubating dose of rocuronium. *Br J Anaesth* 2007; **99**: 514–7
9. Collins LM, Bevan JC, Bevan DR, et al. The prolonged duration of rocuronium in Chinese patients. *Anesth Analg* 2000; **91**: 1526–30
10. Hayes AH, Mirakhur RK, Breslin DS, Reid JE, McCourt KC. Postoperative residual block after intermediate-acting neuromuscular blocking drugs. *Anaesthesia* 2001; **56**: 312–8
11. Leonard PA, Todd MM. Quantitative neuromuscular blockade monitoring: two pictures of unexpected rocuronium effect: a case report. *A A Case Rep* 2017; **9**: 190–2
12. Arain SR, Kern S, Ficke DJ, Ebert TJ. Variability of duration of action of neuromuscular-blocking drugs in elderly patients. *Acta Anaesthesiol Scand* 2005; **49**: 312–5
13. Xue FS, Tong SY, Liao X, Liu JH, An G, Luo LK. Dose-response and time course of effect of rocuronium in male and female anesthetized patients. *Anesth Analg* 1997; **85**: 667–71
14. Martyn JA, White DA, Gronert GA, Jaffe RS, Ward JM. Up-and-down regulation of skeletal muscle acetylcholine receptors. Effects on neuromuscular blockers. *Anesthesiology* 1992; **76**: 822–43
15. Liu Y, Cao W, Liu Y, et al. Changes in duration of action of rocuronium following decrease in hepatic blood flow during pneumoperitoneum for laparoscopic gynaecological surgery. *BMC Anesthesiol* 2017; **17**: 45
16. Rautoma P, Svartling N. Smoking increases the requirement for rocuronium. *Can J Anaesth* 1998; **45**: 651–4
17. Katz RL, Norman J, Seed RF, Conrad L. A comparison of the effects of suxamethonium and tubocurarine in patients in London and New York. *Br J Anaesth* 1969; **41**: 1041–7
18. Fiset P, Donati F, Balendran P, Meistelman C, Lira E, Bevan DR. Vecuronium is more potent in Montreal than in Paris. *Can J Anaesth* 1991; **38**: 717–21
19. Dahaba AA, Perelman SI, Moskowitz DM, et al. Geographic regional differences in rocuronium bromide dose-response relation and time course of action: an overlooked factor in determining recommended dosage. *Anesthesiology* 2006; **104**: 950–3
20. Cajanus K, Holmstrom EJ, Wessman M, Anttila V, Kaunisto MA, Kalso E. Effect of endocannabinoid degradation on pain: role of FAAH polymorphisms in experimental and postoperative pain in women treated for breast cancer. *Pain* 2016; **157**: 361–9
21. Cajanus K, Neuvonen M, Koskela O, et al. Analgesic plasma concentrations of oxycodone after surgery for breast cancer—which factors matter? *Clin Pharmacol Ther* 2018; **103**: 653–62
22. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81**: 559–75
23. Loh PR, Danecek P, Palamara PF, et al. Reference-based phasing using the haplotype reference consortium panel. *Nat Genet* 2016; **48**: 1443–8
24. Browning BL, Browning SR. Genotype imputation with millions of reference samples. *Am J Hum Genet* 2016; **98**: 116–26
25. Mitt M, Kals M, Pärn K, et al. Improved imputation accuracy of rare and low-frequency variants using population-specific high-coverage WGS-based imputation reference panel. *Eur J Hum Genet* 2017; **25**: 869–76
26. Benner C, Spencer CC, Havulinna AS, Salomaa V, Ripatti S, Pirinen M. FINEMAP: efficient variable selection using summary data from genome-wide association studies. *Bioinformatics* 2016; **32**: 1493–501
27. Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. *Nat Commun* 2017; **8**: 1826
28. Aguet F, Barbeira AN, Bonazzola R, et al. The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science* 2020; **369**: 1318–30
29. Pruim RJ, Welch RP, Sanna S, et al. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* 2010; **26**: 2336–7
30. Kajiura A, Nagata O, Takizawa Y, Nakatomi T, Kodera S, Murayama T. A large individual variation in both the infusion rate and the blood concentration of rocuronium necessary for obtain adequate surgical muscle relaxation during total intravenous anesthesia with propofol and remifentanil. *J Anesth* 2015; **29**: 9–14
31. Moriyama T, Matsunaga A, Nagata O, et al. Effective method of continuous rocuronium administration based on effect-site concentrations using a pharmacokinetic/pharmacodynamic model during propofol-remifentanil anesthesia. *J Anesth* 2015; **29**: 593–9
32. Fujimoto M, Tanahira C, Nishi M, Yamamoto T. In non-obese patients, duration of action of rocuronium is directly correlated with body mass index. *Can J Anaesth* 2013; **60**: 552–6
33. Meyhoff CS, Lund J, Jenstrup MT, et al. Should dosing of rocuronium in obese patients be based on ideal or corrected body weight? *Anesth Analg* 2009; **109**: 787–92
34. Haeseler G, Stormer M, Bufler J, et al. Propofol blocks human skeletal muscle sodium channels in a voltage-dependent manner. *Anesth Analg* 2001; **92**: 1192–8
35. Stauble CG, Stauble RB, Schaller SJ, Unterbuchner C, Fink H, Blobner M. Effects of single-shot and steady-state propofol anaesthesia on rocuronium dose-response relationship: a randomised trial. *Acta Anaesthesiol Scand* 2015; **59**: 902–11
36. Zhou Y, Yuan J, Li Z, et al. Genetic polymorphisms and function of the organic anion-transporting polypeptide 1A2 and its clinical relevance in drug disposition. *Pharmacology* 2015; **95**: 201–8
37. Lee W, Glaeser H, Smith LH, et al. Polymorphisms in human organic anion-transporting polypeptide 1A2 (OATP1A2): implications for altered drug disposition and central nervous system drug entry. *J Biol Chem* 2005; **280**: 9610–7
38. van de Steeg E, Wagenaar E, van der Kruijssen CM, et al. Organic anion transporting polypeptide 1a/1b-

knockout mice provide insights into hepatic handling of bilirubin, bile acids, and drugs. *J Clin Invest* 2010; **120**: 2942–52

39. Oscarson M, Zanger UM, Rifki OF, Klein K, Eichelbaum M, Meyer UA. Transcriptional profiling of genes induced in the livers of patients treated with carbamazepine. *Clin Pharmacol Ther* 2006; **80**: 440–56
40. Spacek A, Neiger FX, Krenn CG, Hoerauf K, Kress HG. Rocuronium-induced neuromuscular block is affected by chronic carbamazepine therapy. *Anesthesiology* 1999; **90**: 109–12
41. Costa ACC, Coelho EB, Lanchote VL, et al. The SLCO1A2-189_-188InsA polymorphism reduces clearance of rocuronium in patients submitted to elective surgeries. *Eur J Clin Pharmacol* 2017; **73**: 957–63
42. Qi TL, Zhou YH, Yuan JJ, et al. Effect of ABCB1 rs12720464 and rs1055302 polymorphisms in Chinese patients on the time course of action of rocuronium administered as a single dose. *Int J Clin Pharmacol Ther* 2016; **54**: 462–70
43. Mei Y, Wang SY, Li Y, et al. Role of *SLCO1B1, ABCB1*, and *CHRNA1* gene polymorphisms on the efficacy of rocuronium in Chinese patients. *J Clin Pharmacol* 2015; **55**: 261–8

Handling editor: Tony Absalom