Identification of the A kinase anchor protein 12 (AKAP12) gene as a regulator of FEV$_1$ reversibility after β$_2$-agonist treatment in asthmatic smokers.
Chapter 6

Identification of the A kinase anchor protein 12 (AKAP12) gene as a regulator of FEV1 reversibility after β2-agonist treatment in asthmatic smokers

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Abstract

A-kinase anchoring proteins (AKAPs) AKAP5 and AKAP12 enhance β2-adrenoceptor recycling in airway smooth muscle cells, and their mRNA expression in airway smooth muscle cells is lowered by cigarette smoke extract. We assessed the association between single nucleotide polymorphisms (SNPs) in AKAP5 and AKAP12 and β2-agonist responsiveness and comparing current and never-smoking asthmatics. One AKAP5 SNP and four AKAP12 SNPs have the minor allele associated with decreased and increased β2-agonist responsiveness, respectively, in current versus never smokers. The minor alleles of one AKAP5 and 10 AKAP12 SNPs correlated with increased gene expression in lung tissue. The minor allele of one AKAP12 SNP associated with both decreased gene expression and increased β2-agonist responsiveness in current smokers. In conclusion, AKAP5 and especially AKAP12 variants associate with β2-agonist responsiveness differentially in current smokers compared to never-smokers. Cigarette smoke decreased AKAP5 and AKAP12 mRNA expression could therefore have an impact on therapeutic efficacy of β2-agonists.

Introduction

Asthmatics that are (ex-)smoker show an altered inflammatory response with less eosinophils and more neutrophils in their sputum and blood compared to never-smoking asthmatics. In addition, cigarette smoking is associated with worsening of symptoms. Important in the treatment symptoms in asthmatics are inhaled β2-agonists. They are also used to test lung function reversibility, a diagnostic test in which the improvement in forced expiratory volume in one second (FEV1) after the administration of an inhaled β2-agonist is measured. β2-agonists stimulate the β2-adrenoceptor, leading to cyclic AMP signaling, a process coordinated by scaffolding proteins of the A-kinase anchoring protein (AKAP) family. AKAP5 and AKAP12 bind β2-adrenoceptors and increase recycling. Expression of AKAP5 and AKAP12 mRNA is reduced in cultured airway smooth muscle cells after cigarette smoke-extract exposure. Since AKAP5 and AKAP12 increase β2-adrenoceptor recycling and smokers have less β2-agonist-induced reversibility of decreased FEV1 than never-smokers, we studied the association of genetic variations in AKAP5 and AKAP12 with β2-agonist responsiveness and if this association interacts with smoking status (in current and never smoking asthma patients).
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Methods

The cohort used for this study consisted of 281 asthma patients referred to the asthma clinic at Beatrixoord Hospital in Haren, the Netherlands, that were part of a larger GWAS study.\(^{(5-7)}\) Reversibility of FEV\(_1\) (i.e. \(((\text{FEV}_1 \text{ after } \beta_2\text{-agonist} – \text{FEV}_1 \text{ before } \beta_2\text{-agonist})/\text{FEV}_1 \text{ predicted})\times 100\%)\) was tested after administration of 800 \(\mu\)g salbutamol (albuterol) by pressurized metered dose inhaler with a spacer. Smoking status was determined using a standardized modified form of the British Medical Society Respiratory Questionnaire.\(^{(8)}\) Genotyping was performed using the Illumina 317 Chip (Illumina Inc, San Diego, CA) and the Illumina 370 Duo Chip. A genome wide association study was performed in 229 subjects of this population with reversibility data available and we focused here on single nucleotide polymorphisms (SNPs) in the \(\text{AKAP5}\) and \(\text{AKAP12}\) genes for their association with FEV\(_1\) reversibility. The study was approved by the medical ethics committee of the University Medical Center Groningen and all participants gave written, informed consent.

The association between the SNPs and reversibility was tested using linear regression models with adjustment for age and sex. All SNPs were tested in a dominant genetic model (heterozygous and homozygous subjects were pooled) due to the number of patients per group. The analyses were stratified by smoking defined as never smoking versus current smoking (ex-smokers were excluded). In addition, the interaction between smoking and SNPs were tested using a linear regression model with adjustment for age and sex and including the SNP, current smoking and their interaction.

We assessed if the selected SNPs were cis-acting eQTLs in a lung tissue database established by the lung eQTL consortium (Universities of Groningen, Laval and British Columbia), as described previously.\(^{(9)}\)

Results

Of the 229 subjects where the genome wide association study was performed, we excluded 75 ex-smokers and of the included subjects, 92 were never smokers (42.4% male; 49.8±8.4 years) and 62 were current smokers (72.6% male; 49.5±8.2 years). Current smokers tended to have lower FEV\(_1\) reversibility than never smokers (11.0±8.4% vs 13.6±7.2%, \(p=0.054\)) (supplementary table 1). Three SNPs in \(\text{AKAP5}\) were available and 34 SNPs in \(\text{AKAP12}\) (supplementary table 2). FEV\(_1\) reversibility after \(\beta_2\)-agonist treatment was associated with one SNP (rs745686) in the promoter of \(\text{AKAP5}\) and two SNPs (rs6557123, rs2251681) in intron 2 of \(\text{AKAP12}\) in asthmatics with no history of smoking (table 1). In
smoking asthmatics, four SNPs in AKAP12 were associated with $\beta_2$-agonist-induced FEV$_1$ reversibility (table 1). Interaction analysis showed one SNP in AKAP5 and four SNPs in AKAP12 differentially associated with $\beta_2$-agonist-induced FEV$_1$ reversibility in current smokers compared to never-smokers (table 1).

The lung eQTL dataset, analyzing 183 SNPs around AKAP12 and 52 SNPs around AKAP5, showed one SNP associated with AKAP5 gene expression and 11 SNPs associated with AKAP12 gene expression (table 2).

**Discussion**

We hypothesized that genetic variants in AKAP5 and AKAP12 are associated with decreased $\beta_2$-agonist responsiveness, due to their roles in $\beta_2$-adrenoceptor recycling, (2) and that this association may differ between smoking and non-smoking asthmatics given the reducing effect of cigarette smoke on their expression.(3) Indeed, several SNPs in AKAP5 and AKAP12 associated with $\beta_2$-agonist responsiveness of which one in the AKAP5 and four in the AKAP12 gene showed a positive interaction with smoking status.

The minor allele of one of these SNPs (rs7757002) also associated with decreased gene expression.

One missense mutation in exon 5 of the AKAP12 gene associated with $\beta_2$-agonist-induced reversibility of FEV$_1$ (rs13212161 E [Glu] $\Rightarrow$ G [Gly]). This SNP is in complete linkage disequilibrium ($r^2 = 1$) with two other missense mutations (rs12201388 E [Glu] $\Rightarrow$ K [Lys], rs34713284 V [Val] $\Rightarrow$ M [Met]). Our data suggest that the amino acids changed by these SNPs play an important role in AKAP12 functioning, probably by maintaining a secondary protein structure necessary for protein-protein interactions. Importantly, one of these SNPs (rs13212161) causes a mutation in the suggested $\beta_2$-adrenoceptor binding domain.(10) Thus, exon 5 of AKAP12 seems to be important for proper binding to $\beta_2$-adrenoceptors and subsequent reversibility of FEV$_1$.

The minor allele of another SNP (rs7757002) is associated with lower AKAP12 expression and a higher reversibility of FEV$_1$ after $\beta_2$-agonist treatment. The latter finding is unexpected as AKAP12 increases $\beta_2$-adrenoceptor recycling back to the membrane,(3) suggesting increased $\beta_2$-agonist responsiveness. In contrast, the minor allele of a SNP in the promoter region of AKAP5 (rs745686) only associated with $\beta_2$-agonist responsiveness in never-smoking asthmatics, although it is not associated with gene expression.
Table 1: Association between genotypes and FEV\textsubscript{1} reversibility in asthmatic probands with no history of smoking vs current smokers. Only results for significantly associated SNPs are shown. Analyses with \( p < 0.05 \) are depicted in bold. MAF: minor allele frequency, WT: Wildtype (homozygous for major allele), Hz/Hm: Heterozygous or Homozygous for minor allele, *Major allele first

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<td>Hz/Hm Beta (SE)</td>
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<td>4.1 (1.7)</td>
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Table 2: SNPs associated with the expression of AKAP5 and AKAP12 in the lung. *Major allele first.

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<td>rs2236224</td>
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<td>rs900654</td>
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<td>8.30∙10⁻⁶</td>
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<td>6.18∙10⁻² (1.73∙10⁻³)</td>
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Chapter 6

SNPs in the β2-adrenoceptor gene that cause a mutation at amino acid 16 of the extracellular domain affect β2-agonist-induced bronchoprotection in certain populations. We now show that proteins that bind the β2-adrenoceptor, at the intracellular domain, carry mutations to affect responsiveness as well.

In conclusion, SNPs in AKAP5 and in particular AKAP12 associate with β2-agonist responsiveness and these associations are different between never and current smoking asthmatics.

Acknowledgements
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Author contributions
WJP participated in study design, data interpretation and drafted the manuscript. AF performed the eQTL analysis. MvdB, JMV, HM, GHK and MS designed the study and helped write the manuscript. JMV analyzed the Beatrixoord cohort. All authors read, corrected and approved the manuscript.
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References
Supplementary table 1: Patient characteristics

Data presented as mean (S.D.), unless indicated otherwise.

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<td>62</td>
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<tr>
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<td>39 (42.4)</td>
<td>45 (72.6)</td>
<td>&lt; 0.001</td>
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<tr>
<td>age, years</td>
<td>49.8 (8.4)</td>
<td>49.5 (8.2)</td>
<td>0.847</td>
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<td>FEV1 % predicted pre bronchodilator</td>
<td>71.4 (21.4)</td>
<td>72.2 (24.4)</td>
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<td>FEV1 reversibility % predicted</td>
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### Supplementary table 2: Association between genotypes and FEV, reversibility in asthmatic probands with no history of smoking vs current smokers. Results for all investigated SNPs. Analyses with p<0.05 are depicted in bold. MAF: minor allele frequency, Wt: Wildtype (homozygous for major allele), Hz/Hm: Heterozygous or homozygous for minor allele, Htz: Heterozygous, *Major allele first

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