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PHARMACOEPIDEMIOLOGY AND PRESCRIPTION

Drug-target based cross-sectional analysis of olfactory drug effects

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Abstract
Background Drug effects on the human sense of smell attract increasing interest, yet systematic evidence from controlled studies is sparse. The present cross-sectional approach to olfactory drug effects made use of the recent developments in informatics, knowledge discovery, and data mining allowing connecting drug-related information from humans with underlying molecular drug targets.
Methods In this prospective cross-sectional study, n=1008 outpatients at a general practitioner were enrolled. All currently taken medications were obtained, and olfactory function was assessed by means of a clinically established 12-item odor identification test. The association between the patients' sense of smell and the administered medications was based (i) on the active pharmacological substances and (ii) on the molecular targets queried from the publicly accessible DrugBank database.
Results Of the 168 different substances, six were taken sufficiently often to be analyzed. The administration of levothyroxine was associated with a higher olfactory score (p=0.033). For the 168 drugs, 323 different targets could be queried. Thirty-one gene products were addressed sufficiently often to be analyzed. Besides agonistic targeting of thyroid hormone receptors (genes *THRA1*, *THRB1*) agreeing with the above result, antagonistically targeting the adrenoceptor alpha 1A (gene *ADRA1A*) by several unrelated medications was associated with a significantly higher olfactory score (p=0.012).
Conclusions The identified drug effects on olfaction are both biologically plausible based on supportive information from basic science studies. The novel molecular target-based approach suggested clear advantages over the classical drug or drug class-based approach. It increased the analyzable data volume fivefold and provided plausible hypotheses about mechanistic drug effects opening possibilities for drug discovery and repurposing.

Keywords Drug targets · Taget genes · Cross-sectional · Bioinformatics · Computer science · Databases · Olfaction · Patients

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Introduction

Drug effects on the human sense of smell attract increasing interest, yet systematic evidence from controlled studies is remarkably sparse [1]. Olfactory side effects of drugs are often considered as of minor clinical importance considering the severity of the primarily treated disease of more prominent drug side effects. However, olfactory loss is a reason to consult a doctor for an estimated yearly 80,000 people in German-speaking countries [2]. This emphasizes that olfaction as an

Table 1 A list of the 168 different drugs (generic names) taken by the outpatients of the study cohort (n=1006 patients in total, i.e., excluding two of the 1008 enrolled subjects due to imprecise medication information). Drugs were taken by n=440 subjects. The Chemical Abstracts Service (CAS) numbers (see http://www.cas.org) were queried in October 2014 from the DrugBank database (version 4.1 [18]) at http://www.drugbank.ca

Name	CAS	Name	CAS	Name	CAS	Name	CAS
Acamprosate	77337–76–9	Doxazosin	74191–85–8	L-DOPA	72573–00–3	Piroxicam	36322–90–4
Acarbose	56180–94–0	Doxepin	1668–19–5	Lercanidipine	100427–26–7	Potassium iodide	7681–11–0
Acetaminophen	103–90–2	Drospirenone	67392–87–4	Levetiracetam	102767–28–2	Pramipexole	104632–28–2
Acetylsalicylic acid	50–78–2	Duloxetine	136434–34–9	Levocetirizine	130018–77–8	Pravastatin	81093–37–0
Adalimumab	331731–18–1	Dutasteride	164656–23–9	Levonorgestrel	797–63–7	Prednisolone	09.11.8056
Agomelatine	138112–76–2	Enalapril	75847–73–3	Levothyroxine	587–29–1	Pregabalin	148553–50–8
Alendronic acid	66376–36–1	Eplerenone	107724–20–9	Liothyronine	09.08.5714	Propafenone	54063–53–5
Aliskiren	315–30–0	Ethinyl estradiol	57–63–6	Lisinopril	76547–98–3	Propiverine	54556–98–8
Allopurinol	315–30–0	Etonogestrel	54048–10–1	Loratadine	79794–75–5	Rabeprazole	117976–90–6
Amisulpride	71675–85–9	Exenatide	141758–74–9	Lorazepam	846–49–1	Ramipril	87333–19–5
Amitriptyline	50–48–6	Ezetimibe	163222–33–1	Losartan	114798–26–4	Ranitidine	68109–63–7
Amlodipine	88150–42–9	Febuxostat	144060–53–7	Magnesium	67208–78–0	Ranolazine	95635–56–6
Amoxicillin	71447–36–4	Felodipine	72509–76–3	Mebeverine	07.06.3625	Repaglinide	135062–02–1
Beclometasone	4419–39–0	Fenoterol	13392–18–2	Mesalazine	61513–32–4	Ribavirin	36791–04–5
Benserazide	322–35–0	Ferrous sulfate	7720–78–7	Metformin	1115–70–4	Salbutamol	35763–26–9
Benzbromarone	3562–84–3	Fluoxetine	52341–67–0	Methadone	76–99–3	Salmeterol	89365–50–4
Bisoprolol	66722–44–9	Flupirtine	75507–68–5	Methocarbamol	532–03–6	Saxagliptin	945667–22–1
Budesonide	51333–22–3	Fluticasone	80474–14–2	Methotrexate	59–05–2	Selenium	7782–49–2
Butylscopolamine	7182–53–8	Folic acid	59–30–3	Metoclopramide	364–62–5	Sertraline	79559–97–0
Caffeine	58–08–2	Formoterol	49861–99–6	Metoprolol	13484–40–7	Simvastatin	79902–63–9
Calcitonin	47931–85–1	Ginkgo biloba	90045–36–6	Mirtazapine	61337–67–5	Sitagliptin	486460–32–6
Calcium carbonate	471–34–1	Glibenclamide	10238–21–8	Mitoxantrone	65271–80–9	Spironolactone	52–01–7
Candesartan	139481–59–7	Glimepiride	93479–97–1	Molsidomine	25717–80–0	Sucralfate	54182–58–0
Captopril	62571–86–2	Hydrochlorothiazide	58–93–5	Mometasone	105102–22–5	Sumatriptan	103628–46–2
Carbimazole	22232–54–8	Hydroxychloroquine	118–42–3	Montelukast	151767–02–1	Tamsulosin	106463–17–6
Carvedilol	107741–96–8	Hyperforin	11079–53–1	Moxonidine	75438–57–2	Telmisartan	144701–48–4
Cefuroxime	153012–39–6	Hypericin	548–04–9	Naloxone	465–65–6	Terazosin	13523–86–9
Cetirizine	83881–51–0	Ibuprofen	58560–75–1	Natalizumab	189261–10–7	Testosterone	58–22–0
Chlormadinone	1961–77–9	Imipramine	50–49–7	Nebivolol	99200–09–6	Tetracycline	60–54–8
Cholecalciferol	1406–16–2	Insulin	11061–68–0	Niacin	59–67–6	Tetrazepam	10379–14–3
Citalopram	59729–33–8	Insulin aspart	116094–23–6	Nifedipine	101539–70–2	Tilidine	51931–66–9
Clarithromycin	81103–11–9	Insulin detemir	169148–63–4	Nitrendipine	39562–70–4	Tiotropium bromide	186691–13–4
Clopidogrel	113665–84–2	Insulin glargine	160337–95–1	Nitroglycerin	9010–02–0	Tolperisone	3644–61–9

Table 1 (continued)

Name	CAS	Name	CAS	Name	CAS	Name	CAS
Clorazepate	57109–90–7	Insulin glulisine	207748–29–6	Olmesartan	144689–63–4	Torasemide	56211–40–6
Codeine	76–57–3	Insulin lispro	133107–64–9	Omeprazole	131959–78–9	Tramadol	27203–92–5
Cyanocobalamin	68–19–9	Interferon beta–1a	145258–61–3	Opipramol	22204–53–1	Triamterene	396–01–0
Darifenacin	133099–04–4	Irbesartan	138402–11–6	Oxycodone	76–42–6	Trospium chloride	10405–02–4
Desogestrel	54024–22–5	Isosorbide mononitrate	16051–77–7	Pancreatin	8049–47–6	Urapidil	34661–75–1
Diclofenac	15307–86–5	Isotretinoin	56573–65–0	Pantoprazole	102625–70–7	Valsartan	137862–53–4
Digitoxin	8006–96–0	Lamotrigine	84057–84–1	Peginterferon alfa–2a	198153–51–4	Verapamil	56949–77–0
Dihydralazine	762–21–0	Lansoprazole	103577–45–3	Phenprocoumon	435–97–2	Vildagliptin	274901–16–5
Dipyrone	68–89–3	Laropiprant	571170–77–9	Pioglitazone	111025–46–8	Zolpidem	82626–48–0

important component of the quality of life [3–5] and its regard therefore fits into the intensified efforts at developing personalized therapy strategies [6] not restricted to the subpopulation of patients who professionally rely on their sense of smell such as chefs, perfumers, or oenologists.

Drug effects on olfaction are not part of standard drug development requirements, and their systematic assessment therefore exceeds economic practicability. Therefore, hypotheses on which controlled studies can be based are gathered mainly from anecdotal cases [7] or pharmacovigilance analyses [8] about olfactory drug effects or from molecular evidence mostly originating from animal research. Pursuing such information can be successful such as the verification of a single case report [9] about opioid effects on olfaction in a positive controlled study [10], unsuccessful such as the negative results of a controlled study on anecdotally reported antimycotics' effects on olfaction [11], or show effects on human olfaction contrasting to the hypothesis derived from animal research, such as decreased human olfactory function following cannabis administration [12] despite enhanced olfaction suggested from animal research [13].

As an alternative to pursuing anecdotal evidence, the present analysis used a cross-sectional approach to olfactory drug effects, exploring clinical data for traces of olfactory drug effects. It made use of the recent developments in informatics, knowledge discovery, and data mining tools that allow connecting drug-related information observed in humans with underlying molecular drug targets.

Methods

Patients and data acquisition

The study followed the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Medicine of the Technical University of Dresden, Germany (protocol number EK330092011). Informed written consent from each patient had been obtained. Subjects (n=1008) were enrolled who had presented as outpatients at a general practitioner for any medical reason, independent of a connection with olfactory loss. All currently taken prescribed and OTC medications were obtained in this prospective study from the patient's records and via questioning of the patient (Table 1). Smoking habits, acute infections of the upper respiratory tract, subjectively perceived olfactory loss, subjectively perceived nasal patency, and alcohol intake were also queried.

Olfactory function was assessed as previously done for a large sample, i.e., by means of a 12-item odor identification test [14] from the "Sniffin' Sticks" [15, 16] test battery (Burghart, Wedel, Germany). This olfactory test kit consists of pen-like odor-dispensing devices. For birhinal olfactory testing, the pen's cap was removed by the experimenter for 3 s and the tip was placed at 1–2 cm in front of the nostrils.

Guided by specific software, 12 odors were applied in a randomized order. Subjects were free to sample the odors as often as necessary to identify them from a list of four descriptors. The test score was the sum of correctly identified odors. The experimenter presented odor pens separated by an interval of approximately 20 s to prevent olfactory desensitization.

Data analysis

The analysis followed the exploratory character of this cross-sectional study. A power calculation based on reported scores from the same olfactory test obtained in healthy subjects (9.07 ±2.5 [mean±SD], see page 809 in [14]) identified a minimum group size of 34 necessary to detect a moderate change by two score points at a statistical power of 90 %. Therefore, only medication-related information available from at least 34 patients was analyzed. Possible confounders were explored in the subgroup of patients not actually taking any medication (n=566), in whom the olfactory score was found to significantly depend on the patients' age (Pearson's r=−0.31, p<0.0001) and on a history of head trauma (n=26 subjects; t test: p<0.05) and as a tendency the patients' sex (t test: p=0.065). Linear regression analysis excluded as further confounders smoking habits, acute infections of the upper respiratory tract, subjectively perceived olfactory loss, subjectively perceived nasal patency, and alcohol consumption. To eliminate the influence of confounders, associations between the patients' sense of smell and the medications were assessed using a propensity score matching (PSM [17]). This obtained subsamples of subjects who were comparable, i.e., did not statistically significantly differ, on the covariates age, head trauma history, and sex. Between these subsamples, olfactory scores were compared with respect to the medications by means of t tests. Statistics was performed using Stata (version 13.1 for Linux, StataCorp, College Station, TX, USA); the α level was set at 5 %.

The association between the patients' sense of smell and the administered medications was firstly based on the active pharmacological substances. Subsequently, the focus of the association was shifted from the drugs toward the molecular drug targets, applying a novel method. Specifically, the necessary information, in particular, the respective coding genes, were queried in October 2014 from the DrugBank database at http://www.drugbank.ca (version 4.1 [18]). This provided the molecular drug targets in several coding including UniProt IDs (http://www.uniprot.org) and names of the genes that code for the respective target, which are interconvertible, for example, via the DAVID database ([19]; http://david.abcc.ncifcrf.gov/conversion.jsp). In this report, we use the gene name as a substitute for molecular drug targets, which in fact is the gene product rather than the gene. This was preferred to the UniProt ID that refers more directly to a protein but is less intuitive, for example the gene "*ACE*" refers to its product angiotensin I converting enzyme, of which the UniProt ID is "P12821". In addition to the genes listed among the drugs' targets, interactions listed with enzymes, carriers or transporters were included when the drug was an inducer or an inhibitor. This accommodated potential olfactory effects of, e.g., antibiotics lacking main human targets such as aminoglycosides [20], which exert olfactory effects possibly via such interactions. Subsequently, molecular drug targets qualifying for further analysis were curated by separating agonistic from antagonistic interactions.

Results

Data was complete for 1006 subjects (aged 18–92 years, mean standard deviation: 42.7±15.6 years; 378 men; Fig. 1). Two patients were excluded from the analysis due to imprecise drug information that could not be recovered. More than half of the patients (n=566) did not take any drugs. The remaining n=440 patients took 1–10 different drugs in decreasing frequency, i.e., from n=166 and n=106 patients taking only one or two different medications to five patients who took ten different drugs at the time of olfactory testing. A total of 168 different drugs interacting with a total of 323 genes comprised the whole pharmacotherapy-related data volume.

Of the 168 different substances (Table 1), six were taken by at least 34 patients (Fig. 2), namely levothyroxine (taken by n=122 subjects), ramipril (n=79), pantoprazole (n=72), simvastatin (n=51), hydrochlorothiazide (n=50), and metformin (n=45). Assessments of drug influences on the olfactory score following PSM-based subsample selection identified that the administration of levothyroxine was associated with a slightly yet statistically significantly higher olfactory score (10.8±0.1 versus 10.4±0.2, 95 % confidence interval, CI, for differences: −0.85–−0.37, p=0.033) while the subsamples, i.e., patients taking or not taking this drug, did not significantly differ with respect to age, sex distribution, or history of head trauma.

Of the 168 different substances taken by the patients, 154 were found in the DrugBank database and a total of 323 different drug targets could be queried (Table 2). Thirty-one targets were addressed by the medications taken by at least 34 patients (Table 3 and Fig. 2). Following PSM-based subsample selection, targeting the adrenoceptor alpha 1A (gene *ADRA1A*) antagonistically was identified to be associated with a statistically significantly higher olfactory score (Fig. 4; 10.6±0.2 versus 9.4±0.3, 95 % CI for differences: −2.23–−0.29, p=0.012) while the subsamples did not significantly differ with respect to age, sex distribution, or history of head trauma. Furthermore, agonistically targeting the thyroid hormone receptor, alpha or beta (genes *THRA1 or THRB1*, respectively) was again associated with higher olfactory scores. Both receptors were targeted in the same patients

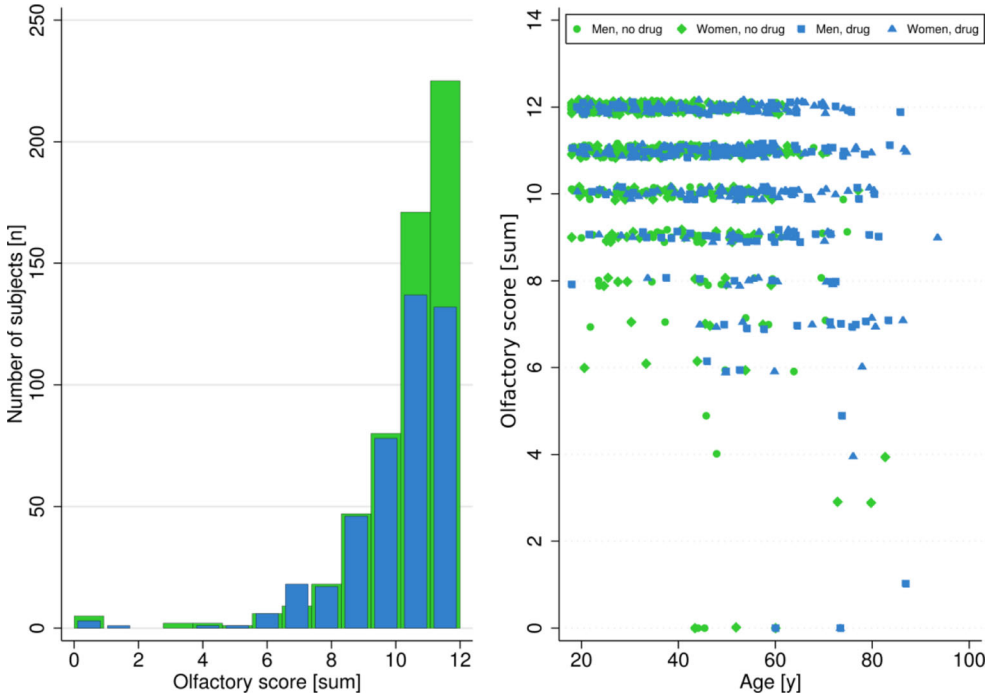

Fig. 1 Distribution of olfactory scores among the 1006 patients included in data analysis. *Left*: histograms showing number of patients per olfactory score, separately for patients without actual medications (*green*) and superimposed for patients who took 1–10 different medications (*blue*) at the time of olfactory assessment. *Right*: scatter plot of the raw data of olfactory scores, separately for sexes and medication. To enhance visibility of singe points, the data is jittered in *y* and *x* directions. The scatter plot also shows the age effect on smell (older age being associated with a lower score and the fact that the relative fraction of patients taking drugs increased with age). This was accounted for in the analysis by associating drug effects with the olfactory score after age (and sex) matched subsample generation by means of propensity score matching

who received levothyroxine, and therefore, the numerical results were identical with those obtained above.

Discussion

The present analysis indicted a suitability of current knowledge about the molecular targets of drugs for mechanistic exploration of drug effects in routine clinical data. Although the association of thyroid hormones with better olfactory acuity was still detectable using a classical drug or drug class-based approach, namely the thyroid hormone medication levothyroxine, the detection of a further drug effect, consisting of an α_1 adrenergic receptor blockade associated with better olfactory performance, only emerged from a molecular target-based approach. The α blockade was only a main drug target of carvedilol, which is a competitive antagonist at β and α_1 adrenoreceptors but had been administered only to five patients, and of the α adrenoreceptor blockers tamsulosin and terazosin administered to each two patients. The detection of the olfactory effects, however owed, besides to moxonidine given to three patients, to the combined inclusion of several antidepressant drugs for which this receptor is listed as a target, comprising in the present cohort amitriptyline, citalopram, doxazosin, doxepin, imipramine, and mirtazapine administered in a total of 24 patients.

The identified drug effects on olfaction are both biologically plausible as based on supportive information from basic science studies. Specifically, thyroid hormones have been shown to enhance the function and the development of the olfactory system. In humans, odor detection thresholds were markedly elevated in patients with hypothyroidism, which was reversed following treatment with thyroid hormones [21]. Loss of taste or smell was also a side effect of propylthiouracil [22] and high-dose radioiodine [23] therapies. An effect of acute depletion of thyroid hormones on olfaction has been similarly shown in animals, where administration of the thyroid peroxidase inhibitor propylthiouracil decreased the number of olfactory receptor neurons in rats with increasing duration of treatment [24] and mice [25]. Moreover, thyroid hormones have a particular importance in the developing olfactory system. Hypothyroid pups of rats showed a delay and hyperthyroid pups acceleration in the development of orientation along an olfactory gradient [26]. This has been associated with morphological changes, such as horizontal proliferation of neurons accompanying the expansion of the surface of the olfactory receptor sheet was markedly reduced in hypothyroid rats [27] and

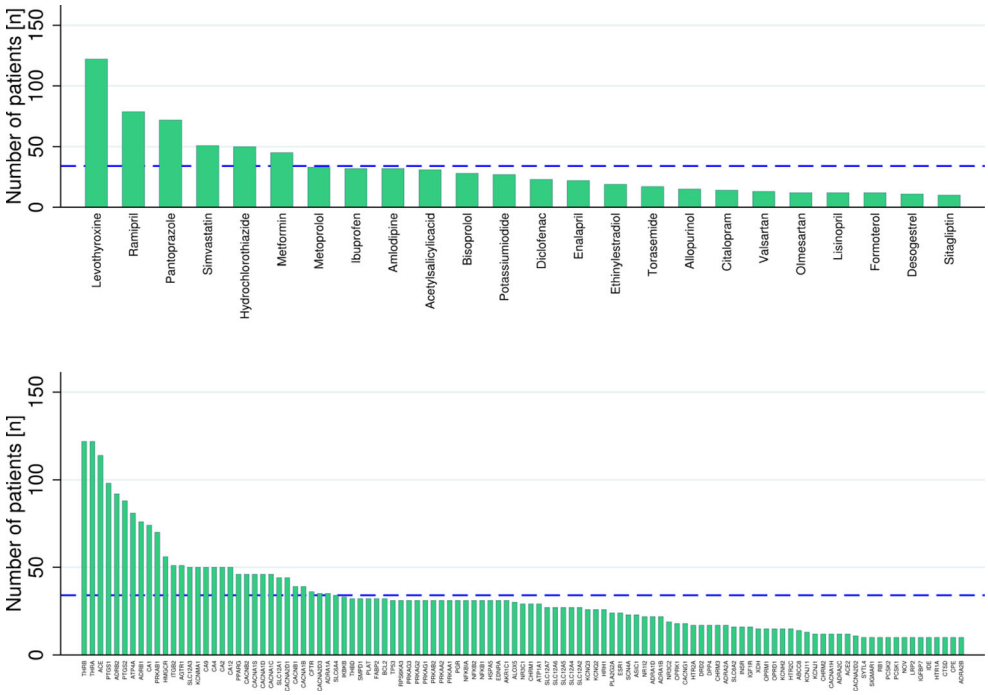

Fig. 2 *Bar graphs* showing the number of patients to which the drugs (*top*) were administered or in which molecular targets, represented by the respective names of the coding genes, were addressed by the administered drugs (*bottom*). For better visibility, only drugs or targets involved in the pharmacotherapy of at least ten patients are shown. The *horizontal dotted lines* (*blue*) indicate the sample size of $n=34$ for analysis according to a sample size estimate based on the main target parameter [14]

other observations showing that thyroid hormones promote the anatomical development of the olfactory system [28, 29]. Developmental consequences are similarly known from humans, and impaired odor identification was a common symptom in 20 subjects with congenital hypothyroidism of various etiologies [30].

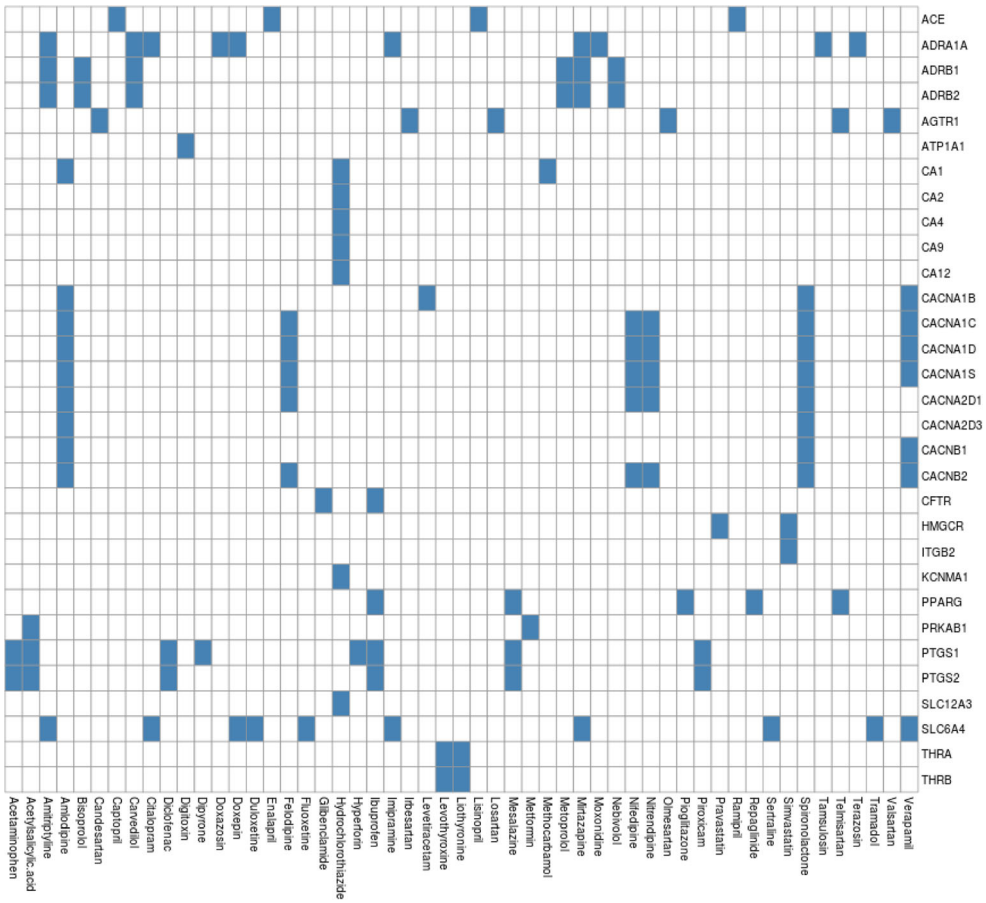

Fig. 3 Adjacency matrix graph of drugs versus the molecular targets, represented by the names of the respective coding genes, genes addressed in at least 34 patients as obtained from a query of the DrugBase database (version 4.1 [18] at http://www.drugbank.ca) in October 2014. *Blue fields* indicate an association of the drug (abscissa) with a genetic target (ordinate). The full names of these genes, and thus of the drug targets, can be obtained from the database of the HUGO Gene Nomenclature Committee [41])

Table 2 A list of molecular drug targets, represented via the names of their coding genes (n=323) of n=154 of the 168 drugs (Table 1) administered to the present outpatients, queried in October 2014 from the DrugBank database (version 4.1 [18]) at http://www.drugbank.ca. The full names of these genes, and thus of the molecular drug targets, can be obtained from the database of the HUGO Gene Nomenclature Committee [41])

ABCA1	*CA1*	*CTSD*	*GABRA5*	*HRH2*	*KCNH6*	*NTRK1*	*PLA2G2A*	*SIGMAR1*
ABCB11	*CA12*	*CYP11B2*	*GABRA6*	*HRH3*	*KCNH7*	*NTRK2*	*PLA2G4A*	*SLC12A1*
ABCC8	*CA2*	*CYP17A1*	*GABRB1*	*HRH4*	*KCNJ1*	*OPRD1*	*PLAT*	*SLC12A2*
ABCC9	*CA4*	*CYSLTR1*	*GABRB2*	*HSD11B1*	*KCNJ11*	*OPRK1*	*PPARG*	*SLC12A3*
ACE	*CA9*	*DHFR*	*GABRB3*	*HSPA5*	*KCNJ5*	*OPRM1*	*PRKAA1*	*SLC12A4*
ACE2	*CACNA1A*	*DPP4*	*GABRD*	*HTR1A*	*KCNJ8*	*ORM1*	*PRKAA2*	*SLC12A5*
ADK	*CACNA1B*	*DRD1*	*GABRE*	*HTR1B*	*KCNMA1*	*ORM2*	*PRKAB1*	*SLC12A6*
ADORA1	*CACNA1C*	*DRD2*	*GABRG1*	*HTR1D*	*KCNQ2*	*P2RY12*	*PRKAB2*	*SLC12A7*
ADORA2A	*CACNA1D*	*DRD3*	*GABRG2*	*HTR1F*	*KCNQ3*	*P4HA1*	*PRKAG1*	*SLC6A2*
ADRA1A	*CACNA1F*	*DRD4*	*GABRG3*	*HTR2A*	*LRP2*	*PCSK1*	*PRKAG2*	*SLC6A3*
ADRA1B	*CACNA1G*	*DRD5*	*GABRP*	*HTR2B*	*LTA4H*	*PCSK2*	*PRKAG3*	*SLC6A4*
ADRA1D	*CACNA1H*	*EDNRA*	*GABRQ*	*HTR2C*	*MGAM*	*PDE10A*	*PRKDC*	*SMPD1*
ADRA2A	*CACNA1I*	*EGF*	*GABRR1*	*HTR3A*	*MMAA*	*PDE11A*	*PRNP*	*SOAT1*
ADRA2B	*CACNA1S*	*ENPP1*	*GABRR2*	*HTR4*	*MMACHC*	*PDE1A*	*PTGDR*	*SRD5A1*
ADRA2C	*CACNA2D1*	*ESR1*	*GABRR3*	*HTR6*	*MMP2*	*PDE1B*	*PTGS1*	*SRD5A2*
ADRB1	*CACNA2D2*	*FABP2*	*GJA1*	*HTR7*	*MMP9*	*PDE1C*	*PTGS2*	*SV2A*
ADRB2	*CACNA2D3*	*FCGR1A*	*GLP1R*	*ICAM1*	*MPO*	*PDE2A*	*PTPN4*	*SYTL4*
ADRB3	*CACNB1*	*FCGR2A*	*GLRA1*	*IDE*	*MTHFR*	*PDE3A*	*PTPRE*	*THBD*
AGTR1	*CACNB2*	*FCGR2B*	*GRIN1*	*IFNAR1*	*MTNR1A*	*PDE3B*	*PTPRS*	*THRA*
AKR1C1	*CACNB3*	*FCGR2C*	*GRIN2A*	*IFNAR2*	*MTNR1B*	*PDE4A*	*QPRT*	*THRB*
ALOX5	*CACNB4*	*FCGR3A*	*GRIN2B*	*IGF1R*	*MTR*	*PDE4B*	*RARA*	*TLR4*
AMY2A	*CACNG1*	*FCGR3B*	*GRIN2C*	*IGFBP7*	*MTRR*	*PDE4C*	*RB1*	*TLR7*
ANPEP	*CALCR*	*FDPS*	*GRIN2D*	*IKBKB*	*MUT*	*PDE4D*	*REN*	*TLR9*
AOC3	*CALM1*	*FGA*	*GRIN3A*	*IMPDH1*	*NDUFC2*	*PDE5A*	*RPS6KA3*	*TNF*
AR	*CFTR*	*FGB*	*GRIN3B*	*IMPDH2*	*NFKB1*	*PDE6A*	*RYR1*	*TNNC1*
ASIC1	*CHRFAM7A*	*FGF2*	*GRM5*	*INSR*	*NFKB2*	*PDE6B*	*SCN2A*	*TNNC2*
ATM	*CHRM1*	*FGG*	*GUCY1A2*	*ITGA4*	*NFKBIA*	*PDE6C*	*SCN4A*	*TOP2A*
ATP1A1	*CHRM2*	*FOLR2*	*HBA1*	*ITGB2*	*NNMT*	*PDE7A*	*SCN5A*	*TP53*
ATP4A	*CHRM3*	*FOLR3*	*HBB*	*ITPR1*	*NOV*	*PDE7B*	*SCN9A*	*TPO*
ATP6V1A	*CHRM4*	*FTH1*	*HCAR2*	*ITPR2*	*NPC1L1*	*PDE8A*	*SCNN1A*	*TSPO*
BCL2	*CHRM5*	*FTL*	*HCAR3*	*ITPR3*	*NPPB*	*PDE8B*	*SCNN1B*	*VCAM1*
C1QA	*CHRNA10*	*GAA*	*HIF1A*	*JUN*	*NPR1*	*PDE9A*	*SCNN1D*	*VDR*
C1QB	*CHUK*	*GABRA1*	*HLA-DQA2*	*KCNA1*	*NR1I2*	*PGR*	*SCNN1G*	*VEGFA*
C1QC	*CPE*	*GABRA2*	*HLA-DQB1*	*KCND2*	*NR3C1*	*PIK3CA*	*SELE*	*VKORC1*
C1R	*CPT1A*	*GABRA3*	*HMGCR*	*KCND3*	*NR3C2*	*PIK3CB*	*SHBG*	*XDH*
C1S	*CREB1*	*GABRA4*	*HRH1*	*KCNH2*	*NT5C2*	*PIK3CD*	*SI*	

Similar biological plausibility can also be claimed for the detection of an association of α_{1A} adrenoceptor antagonism with a tendency toward improved olfaction. Noradrenergic mechanisms have been shown to regulate signaling in the olfactory system. Specifically, a noradrenalin-mediated increase in granule cell-mediated mitral cell inhibition in the olfactory bulb results mostly from activation of the α_{1A}-adrenoceptor subtype in rats of all ages, i.e., the drug target identified in the present analysis [31]. The overall effect of noradrenalin was to inhibit mitral cells by producing a long-lasting excitation in granule cells [32], i.e., adrenergic activation enhanced inhibitory transmission in the olfactory system. Alpha$_1$ and α_2 adrenoceptor activations exert opposing effects on the excitability in the main olfactory bulb by increasing or decreasing, respectively, GABAergic inhibition of mitral cells [33]. This agrees with the anecdotal observation of a reversible smell disturbance following administration of the α-adrenoceptor agonist midodrine [34]. The effects of a1 blockade on olfaction, however, are not completely clear in the presence of hints at opposite effects such as impaired

Table 3 Mutual associations of drugs with their molecular targets, represented via the names of their coding genes, obtained by querying the DrugBank database (version 4.1 [18] at http://www.drugbank.ca) in October 2014 for the targets addressed by the drugs in at least 34 patients, which was the minimum for analysis according to a sample size estimate based on the main target parameter [14]

Gene	*n*	Drug
ACE	114	Captopril, lisinopril, enalapril, ramipril
ADRA1A	35	Amitriptyline, carvedilol, citalopram, doxazosin, doxepin, imipramine, mirtazapine, moxonidine, tamsulosin, terazosin
*ADRB1**	69	Carvedilol, bisoprolol, metoprolol, nebivolol
*ADRB2**	66	Carvedilol, bisoprolol, metoprolol, nebivolol
AGTR1	51	Irbesartan. Telmisartan, candesartan, losartan, olmesartan, valsartan
ATP4A	81	Lansoprazole, omeprazole, pantoprazole., rabeprazole
CA1	74	Amlodipine, hydrochlorothiazide, methocarbamol
CA2	50	Hydrochlorothiazide
CA4	50	Hydrochlorothiazide
CA9	50	Hydrochlorothiazide
CA12	50	Hydrochlorothiazide
CACNA1B	39	Amlodipine, levetiracetam, spironolactone, verapamil
CACNA1C	46	Spironolactone, verapamil, felodipine, amlodipine, nitrendipine, nifedipine
CACNA1D	46	Spironolactone, verapamil, felodipine, amlodipine, nitrendipine, nifedipine
CACNA1S	46	Spironolactone, verapamil, felodipine, amlodipine, nitrendipine, nifedipine
CACNA2D1	44	Amlodipine, felodipine, nifedipine, nitrendipine, spironolactone
CACNA2D3	35	Amlodipine, spironolactone
CACNB1	39	Amlodipine, spironolactone, verapamil
CACNB2	46	Spironolactone, verapamil, felodipine, amlodipine, nitrendipine, nifedipine
CFTR	36	Glibenclamide, ibuprofen
HMGCR	56	Simvastatin, pravastatin
ITGB2	51	Simvastatin
KCNMA1	50	Hydrochlorothiazide
PPARG	46	Ibuprofen, mesalazine, repaglinide, telmisartan, pioglitazone
PRKAB1	70	Acetylsalicylic acid, metformin
PTGS1	98	Acetylsalicylic acid, diclofenac, ibuprofen, mesalazine, hyperforin, acetaminophen, piroxicam, dipyrone
PTGS2	88	Acetylsalicylic acid, diclofenac, ibuprofen, mesalazine, acetaminophen, piroxicam
SLC12A3	50	Hydrochlorothiazide
SLC6A4	34	Amitriptyline, citalopram, doxepin, duloxetine, fluoxetine, imipramine, mirtazapine, sertraline, tramadol, verapamil
THRA	122	Levothyroxine, liothyronine
THRB	122	Levothyroxine, liothyronine

*Antagonistic interactions only; the agonistic interactions by the beta-mimetics fenoterol, salbutamol, salmeterol, and formoterol were omitted

discrimination between chemically related odorants following local prazosin administration [35]. Therefore, whether α_{1a} antagonism may qualify as a novel therapy of olfactory loss has to be investigated prospectively. Nevertheless, the majority of evidence seems to support this hypothesis.

Based on the broad basis of current knowledge, the present data mining and computer science-based approach extends laboratory approaches to the pharmacological modulation of olfaction as either a side effect or a novel clinical drug target. However, the analyses relied on external information and, therefore, crucially depended on the accuracy and completeness of the empirical evidence entered into the queried database. In addition, the analysis could neither associate drug doses nor systemic or local drug concentrations with the olfactory effects. Moreover, further potential modulators of drug effects such as genetic variance modulating the pharmacokinetics or pharmacodynamics could be taken into account. Furthermore, while basing the analyses on drug targets, their local expression was not assessed. Therefore, its results provide reasonable and molecularly plausible hypotheses about clinically relevant drug effects on olfaction but no final proof for this.

Further limitations of the analysis are attributable to the present data set. Firstly, it comprised a sample of outpatients of a single practice, which might limit the validity of the obtained results for other locations or clinical settings. Secondly, the clinical significance of the observed differences in the olfactory scores cannot be judged. This would require a numerical criterion of a clinically relevant difference in the particular olfactory test; however, this is only available for a more complex olfactory test battery [36]. Thirdly, a proof of causality was impossible in this cross-sectional assessment, for example, a temporal relation of drug intake with the symptom was impossible to be detected. Fourthly, related to the previous point, the cross-sectional assessment impeded a separation of a drug effect on olfaction from an olfactory consequence of the disease for which the drug had been prescribed. For example, hypothyroidism may be a cause of hyposmia and the administration of thyroid hormones might have simply corrected this, which does not necessarily mean that the drug itself would exert olfactory effects in subjects with olfactory loss but normal thyroid function. However, in this case, the association would not have been detected at all, which contrasts with the present observation. Nevertheless, while the association of levothyroxine administration with better olfactory acuity remains biologically plausible, it cannot be proofed from the present data. As pointed out above, the results provide a reasonable hypothesis that can serve as a basis for controlled studies, although a critical evaluation of alternative explanations of the identified associations is highly advised. Another example would be antiparkinsonian mediation. The early involvement of olfaction in Parkinson's disease [37] renders dopaminergic medications as candidate olfactory

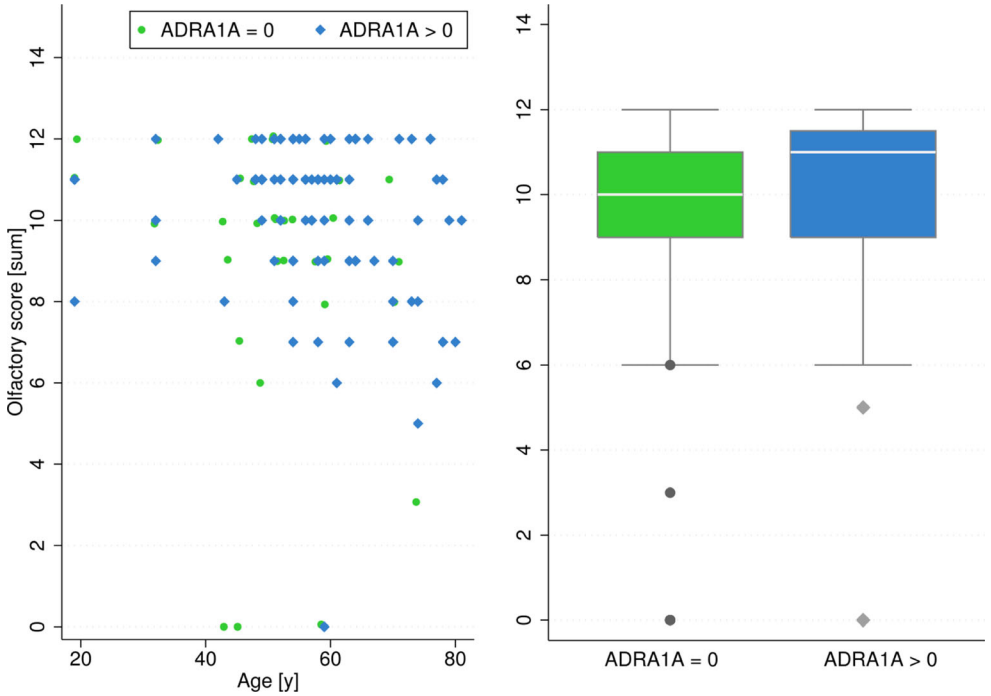

Fig. 4 Consequences of antagonistically targeting the adrenoceptor alpha 1A (gene *ADRA1A*) for the patients' olfactory score. Following propensity score matching (PSM [17]) an analyzable subgroup of 90 controls resulted, which could be compared with respect to olfactory performance with $n=35$ patients in whom the medications (Table 3) had been identified to target this gene product. This was associated in a significantly improved olfactory score. *Left*: original data of olfactory score versus age indicating that the PSM successfully selected a control group from the same age range and with similar sex distribution as the medicated patients. *Right* box plots of the olfactory scores. The minimum, quartiles, median (*solid horizontal line* within the box), and maximum are used to construct the box plots

modulators. However, it cannot be excluded that improvement of the disease is associated with better olfactory acuity instead of a direct olfactory drug effect. However, neither hypothesis can be supported when considering that impaired odor identification performance in Parkinson disease patients has been shown to not to be influenced by treatment with antiparkinsonian drugs [38]. Taken together, even with its limitations, the present approach was able to derive reasonable hypotheses from the observation of drug effects in humans and can serve as a solid basis for bedside-to-bench approaches [39].

As drug effects are produced via interaction of the drug molecules with their molecular targets, i.e., target-based analysis seems more logical than basing the association on drug classes, in particular when the effect is not mediated via the main target of a class of drugs. An additional consequence of the molecular target-based approach, which reclassifies the drugs from clinical targets or chemical similarities toward their molecular targets, is an increase in the analyzable data volume (Fig. 2). Although, based on the lower limit of the sample size estimate, only six drugs could be analyzed with the drug-based approach, and 31 analyzable entities could be analyzed with the molecular target-based approach. This provides a fivefold increase in the drug-related information that can be gained from clinical data. Moreover, via the cross-relationship of molecular targets with drugs (Fig. 3), the number of drugs addressed at least partly in this analysis increased to a total of 53, i.e., now comprised almost a third of all drugs administered in the present cohort.

The results may be useful as a basis for systematic controlled studies for which other sources such as pursuing anecdotal reports or translating animal research bear no better promise. With respect to the latter, the present approach bears the advantage to study drug effects in a clinical setting that, with several limitations, has the advantage to omit species differences probably relevant to olfaction [40]. Scientific knowledge acquisition that uses observations of drug effects in humans to deduce underlying molecular mechanisms has previously proven as an important source of information exploitable for drug discovery, repurposing, and utilization [39] and, moreover, follows the definition of clinical pharmacology as the scientific discipline that involves all aspects of the relationship between drugs and humans [6].

The identified drug effects on olfaction are both biologically plausible based on supportive information from basic science studies. The identification of enhanced olfactory function following agonistic targeting thyroid hormone receptors or antagonistic targeting α_{1A} adrenoreceptors can be used as a basis for systemically assessing these effects in controlled studies. To our knowledge, the here introduced drug target-based approach to the association of drug effects with clinical symptoms, for example in a cross-sectional analysis, has not

been applied with the same or a different aim before and represents, therefore, an innovative clinical pharmacology method. The results obtained with the molecular target-based approach to association of clinical effects with the patients' current use of medications suggested advantages over the classical drug or drug class-based approach.

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