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Adrenal function in Smith-Lemli-Opitz Syndrome

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Abstract

Smith-Lemli-Opitz syndrome (SLOS) is a multiple malformation syndrome due to mutations of the 7-dehydrocholesterol reductase gene (*DHCR7*), which leads to a deficiency of cholesterol synthesis and an accumulation of 7-dehydrocholesterol and related metabolites. The SLOS clinical spectrum ranges from multiple major malformations to a mild phenotype with dysmorphic features, intellectual disability and a specific behavioral presentation. Several cases of SLOS with adrenal insufficiency have been described. We performed ovine corticotropin (oCRH) testing in 35 SLOS patients and 16 age- and gender-matched controls. We reviewed prior ACTH stimulation tests of our SLOS patients (19 of 35 available) and reviewed ACTH stimulation tests from additional 10 other SLOS patients. Results from oCRH testing showed that patients with SLOS had significantly higher ACTH baseline values than healthy controls (24.8 ± 15.3 pg/mL vs. 17.8 ± 7.5 pg/mL, $p=0.034$). However, no statistically significant differences were noted for peak ACTH values (74.4 ± 35.0 pg/mL vs. 64.0 ± 24.9 pg/mL, $p=0.303$) and for baseline (14.2 ± 7.8 mcg/dL vs. 14.2 ± 6.3 mcg/dL, $p=0.992$) and peak cortisol values (28.2 ± 7.9 mcg/dL vs. 24.8 ± 8.1 mcg/dL, $p=0.156$). The area-under-the-curve (AUC) was not significantly different in SLOS patients compared to controls for both ACTH (250.1 ± 118.7 pg/mL vs. 195.3 ± 96.6 pg/mL, $p=0.121$) as well as cortisol secretion (83.1 ± 26.1 mcg/dL vs. 77.8 ± 25.9 mcg/dL, $p=0.499$). ACTH stimulation test was normal in 28 of 29 tests. The individual with the abnormal ACTH stimulation test had a normal oCRH test during the same evaluation. The slightly increased baseline ACTH level seen during oCRH testing may be due to compensated mild adrenocortical insufficiency. However, we were able to show that our cohort affected with SLOS had an adequate stress response and that in mild to moderate cases of SLOS stress steroid coverage should not be required.

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Keywords

adrenal gland; adrenocortical insufficiency; pituitary gland; adrenocorticotropin (ACTH); cortisol; Smith-Lemli-Opitz Syndrome (SLOS); inborn error of cholesterol synthesis; corticotrophin releasing hormone (CRH)

INTRODUCTION

Smith-Lemli-Opitz syndrome (SLOS, MIM # 270400) was first described as an autosomal recessive malformation syndrome in 1964 by Smith, Lemli, and Opitz [Smith et al., 1964]. Since then, many advances have been made in delineating the phenotype and understanding the pathophysiology of this genetic disease. The underlying biochemical cause is a deficiency in cholesterol synthesis due to a defect in the enzyme 7-dehydrocholesterol reductase (DHCR7). DHCR7 catalyzes the last step in the Kandutsch-Russell cholesterol synthetic pathway, the reduction of 7-dehydrocholesterol (7-DHC) to cholesterol [Irons et al., 1993; Tint et al., 1994]. This defect results in elevated levels of 7-DHC, the isomer 8-dehydrocholesterol, and decreased levels of cholesterol. To date, more than 120 [Correa-Cerro et al., 2005; Yu et al., 2005] different mutations have been recognized in *DHCR7*. There is no strict genotype phenotype correlation, and most patients are compound heterozygotes [Correa-Cerro et al., 2005]. *DHCR7* is located on chromosome 11q12-13 [Fitzky et al., 1998; Waterham et al., 1998; Wassif et al., 1998] and is expressed in all tissues. The highest expression of *DHCR7* is noted in adult liver, adrenal cortex, testis, and brain [Moebius et al., 1998]. The incidence of SLOS is estimated to be between 1/20,000 and 1/70,000, with a higher incidence in Caucasians of Northern European heritage, although SLOS has been described in multiple ethnic groups [Matsumoto et al., 2005; Hanissian et al., 1969; Wright et al., 2003; Nezarati et al., 2002].

The clinical presentation of this disorder is very broad, ranging from a lethal multiple malformation syndrome including holoprosencephaly, cardiac, urogenital malformations, Hirschsprung disease and structural anomalies of the lung, to a mild phenotype, which may include any of the following: typical facial features (microcephaly, anteverted nares, ptosis, micrognathia), 2–3 toe syndactyly, hypospadias, mild developmental delay, and behavioral abnormalities [Kelley et al., 2000]. In the past, patients were separated into type I and the more severe, often lethal type II SLOS. Since the advent of biochemical diagnosis, many patients with a milder phenotype, consisting of mainly mental retardation with very subtle dysmorphic features, have been identified [Jezela-Stanek et al., 2008]. In order to better stratify the disease spectrum Kelley and Hennekam [Kelley et al., 2000] revised the traditional scoring system by Bialer [Bialer et al., 1987], so that malformations in embryologically distinct organs systems were weighed equally. The severity scale focuses on malformations and does not account for variation in growth parameters, mental ability, and behavioral phenotype. In addition to multiple malformations, SLOS is associated with failure to thrive, decreased appetite, and a specific behavioral phenotype that includes autistic symptoms, self-injurious and obsessive behavior [Bukelis et al., 2007; Sikora et al., 2006]. Nearly 50 % of patients fulfill diagnostic criteria for autism spectrum disorder [Bukelis et al., 2007; Tierney et al., 2001].

There have been several case reports of adrenal insufficiency in neonates affected by SLOS; however, adrenal function has not been systematically studied in SLOS patients and applicability of these observations to mild and moderate patients is not delineated. Andersson [Andersson et al., 1999] reported three unrelated patients who presented with abnormal electrolytes and low or inadequately normal cortisol levels during periods of physical stress. These infants were on the severe end of the SLOS clinical spectrum and, in

fact, two of the three died in the neonatal period. Chemaitilly [Chemaitilly et al., 2003] also described a severely affected patient who presented with salt wasting on the 4th day of life. Nowaczyk [Nowaczyk et al., 2001] described another SLOS patient with adrenal insufficiency, who was severely affected and passed away at 3.5 months of life. This patient was a compound heterozygote with a null splice acceptor mutation (IVS8-1G>C) and a missense mutation (C380R), a combination that is often consistent with a severe phenotype [Witsch-Baumgartner et al., 2000]. From an anatomical point of view, most patients with SLOS appear to have normal development and size of the adrenal glands [Donnai et al., 1986; Krajewska-Walasek et al., 1999], although adrenal hyperplasia has been reported in rare instances. While studying 19 severely affected infants with SLOS, Curry et al. found large adrenals in 4 of the 11 patients who underwent autopsy [Curry et al., 1987]. McKeever et al. described one infant with large adrenals found on autopsy [McKeever et al., 1990], a second patient had normal adrenals. Worthington reports that 7-DHC and its isomers are found in abundance inside the adrenal gland of SLOS patients in contrast to healthy individuals in whom cholesterol makes up 99% of sterols [Worthington et al., 1997].

To further characterize endocrine function, Pankau et al. [Pankau et al., 1992] evaluated the hypothalamic-pituitary-gonadal function in two SLOS patients who presented with undervirilized male genitalia, but could not identify any hormonal insufficiency in that axis or in adrenal steroid biosynthesis. Elias et al. [Elias et al., 1997] also reported on adrenal function in SLOS patients on dietary cholesterol supplementation. Of six patients in this case series, all six had normal baseline cortisol levels and three of six patients who underwent an adrenocorticotropin (ACTH) stimulation test had normal results.

In a preliminary evaluation of the hypothalamic-pituitary-adrenal (HPA) axis, we [Rother KI, 2000] reported that 22 SLOS patients had normal ACTH stimulation tests, but an exaggerated ACTH response to ovine corticotropin releasing hormone (oCRH) stimulation test when compared to historical controls [Chrousos et al., 1984]. This finding was interpreted as possible compensated adrenal insufficiency. In this present study, we extended the observations and compared the data of SLOS patients to those of age-matched healthy controls rather than historical controls.

PATIENTS & METHODS

Patients

We evaluated 35 patients with SLOS with oCRH stimulation testing. Our studies were approved by the NICHD Institutional Review Board and consent was obtained from parents and guardians. Assent was obtained if applicable. The diagnosis of SLOS was made clinically with biochemical confirmation. We also measured residual cholesterol synthesis capacity of patient fibroblasts [Wassif et al., 2005]. *DHCR7* mutations were known for all SLOS patients. The most frequent alleles were IVS8-1G>C (19/70, 27%), R242C (5/70, 7%), T93M (5/70, 7%), P51S (4/70, 6%), and W151X (3/70, 4%). Thirty-four patients were on dietary cholesterol supplementation (crystalline cholesterol in Ora-Plus or egg yolk) at the time of testing. Thirty-two of 35 were on cholesterol suspension at an average dose of 149 ± 30 mg/kg/day. Two patients (S4, S30) were receiving powdered egg yolk for cholesterol supplementation. One (S19) mildly affected patient was on no cholesterol supplementation. None of the patients were receiving simvastatin therapy at the time of testing.

We reviewed the charts of our SLOS cohort for prior additional ACTH stimulation testing and aldosterone levels and added 10 more SLOS patients that had prior adrenal evaluation. One of these 10 patients had only baseline cortisol and aldosterone levels available.

The control patients were 16 children or adults evaluated for abnormalities of growth or weight and were not found to have any underlying endocrine disease. These children and adults did not develop Cushing disease or any other disturbance of the HPA axis to this day and were not on any medications affecting endocrine function at the time of investigation, with the exception of one patient (C8) who remained on thyroxin replacement due to known preexisting hypothyroidism.

ACTH and oCRH Testing

Thirty-five SLOS patient and 16 control patients underwent oCRH stimulation testing: oCRH (Pfizer, Inc.) was used at 1 mcg/kg/dose (max. dose 100 mcg) as a one-time dose in both cohorts. Clinically the oCRH test is used in the diagnosis of Cushing syndrome either with or without prior dexamethasone suppression [Nieman et al., 1993]. The oCRH test has been used for the evaluation adrenal insufficiency in clinical research [Boscaro et al., 1994; Dahl et al., 1992; Nieman et al., 1993; Nieman, 2003; Schlaghecke et al., 1992]. Samples were collected at -15, 0, 15, 30, 60 minutes in the SLOS cohort. In the control cohort, samples were obtained at -5, 0, 15, 30, and 45 minutes. For comparison, the -15, -5 and 0 minute values were averaged to calculate the baseline value, and the 30 and 45 (or 60) minute were averaged to a peak value, as is usually done for oCRH testing responses. ACTH stimulation testing was performed using 250 mcg ACTH and cortisol levels were measured at 0, 30, and 60 minutes. Aldosterone levels were also obtained at 0, 30 and 60 minutes.

ACTH levels were measured with a Nichols Advantage immunochemiluminometric assay (ICMA) from 1/24/2000 until 8/2005, when analysis was changed to an immunochemiluminometric assay on a Siemens Immulite 2500R analyzer. The reference ranges were 9–52 pg/mL until 08/2005 and then changed to 0–46 pg/mL. Serum cortisol in the oCRH stimulation test was measured using an immunochemiluminometric assay on the Siemens Immulite 2000R analyzer throughout our sampling period. The reference range is defined as 5–25 mcg/dL for a standard sampling time of 8–9 am. Cortisol and aldosterone levels in the ACTH stimulation test were obtained through Endocrine Sciences, an EsoterixR company, Calabasos Hills, California.

Statistical analysis

We stratified the SLOS patients into three groups according to the SLOS severity scale described by Kelley and Hennekam [Kelley et al., 2000]. Group 1 included patients with a severity scale of <20 (21 patients), group 2 included patients with a severity scale of 20–30 (7 patients), and group 3 included those with a severity scale of >30 (7 patients). We also stratified the SLOS patients into three groups according to their residual cholesterol synthesis. We grouped SLOS patients with a residual activity of > 0.4 (group A), 0.2–0.4 (group B), and <0.2 (group C).

Data were described using frequency distributions and simple descriptive statistics. Continuous data, such as ACTH and cortisol values, were compared between SLOS patients and healthy controls using *t*-tests. Analysis of variance was used to compare these data among the three severity scale and residual activity groups, and the Abelson-Tukey linear contrast test was used for assessing trends. Area-under-the-curve (AUC) was computed using the trapezoidal rule. For exact comparisons the -15/-5, 0, 15, 30 and 45 minute values were used. In order to make a comparison with the controls, for the SLOS patients a 45 minute value was calculated by averaging the 30 and 60 minute values.

All analyses were performed using SAS v9.1 (SAS Institute, Cary, NC). Data are described as mean $\pm$ standard deviation. A *p*-value of 0.05 was considered statistically significant.

Additionally we also compared cholesterol, 7-DHC and total dehydrocholesterol to baseline and peak cortisol levels using PrismR Software.

RESULTS

Review of prior ACTH stimulation test showed normal results in 28 of 29 tests. The patient with the abnormal testing had a normal baseline serum cortisol and was asymptomatic. Two subsequent oCRH tests to delineate suspected adrenal dysfunction, one immediately following the ACTH stimulation test and the second one a year later, were normal. We suspect an administration error. One additional subject had only serum cortisol baseline levels available, which were normal.

For oCRH testing we compared results obtained from 35 patients (43% female and 57% male) and 16 controls (63% female and 37% male). The sex distribution was not statistically different between groups ($p=0.237$). The SLOS patients were mostly Caucasians, with 2 of 35 of Hispanic ethnicity. The control group included 14 Caucasians, 1 Asian, and 1 subject whose race was not specified. While the SLOS patients were younger on average than healthy controls (10.4 ± 8.0 years vs. 14.9 ± 9.8 years), age difference did not reach statistical significance between groups ($p=0.086$).

Patients with SLOS had significantly higher ACTH baseline values than healthy controls (24.8 ± 15.3 pg/mL vs. 17.8 ± 7.5 pg/mL, $p=0.034$). However, no statistically significant differences were noted for peak ACTH values (74.4 ± 35.0 pg/mL vs. 64.0 ± 24.9 pg/mL, $p=0.303$) and for baseline (14.2 ± 7.8 mcg/dL vs. 14.2 ± 6.3 mcg/dL, $p=0.992$) and peak cortisol values (28.2 ± 7.9 mcg/dL vs. 24.8 ± 8.1 mcg/dL, $p=0.156$).

The area-under-the-curve (AUC) was not significantly different in SLOS patients compared to controls for both ACTH (250.1 ± 118.7 pg/mL vs. 195.3 ± 96.6 pg/mL, $p=0.121$) (Figure 1) and cortisol secretion (83.1 ± 26.1 mcg/dL vs. 77.8 ± 25.9 mcg/dL, $p=0.499$) (Figure 2). When SLOS subjects were stratified by phenotype severity and residual cholesterol synthesis, no statistically significant differences were observed in any of the ACTH (AUC, baseline value, peak value) or cortisol (AUC, baseline value, peak value) measures among the groups. However, there was a statistically significant linkage between disease severity and both ACTH and cortisol AUC: as SS increased, so did ACTH and cortisol AUC ($p<0.001$ and $p=0.004$).

We looked for correlations between cholesterol level, 7-DHC level, total dehydrocholesterol level and baseline or peak cortisol. No significant correlations were observed (Supplemental figures 1 and 2).

We also observed no evidence of mineralocorticoid deficiency in SLOS patients. Aldosterone levels were measured in addition to cortisol during ACTH testing. Baseline aldosterone levels from 30 patients showed 26 normal results, 3 low values and 1 high values. The low values occurred in an eleven month-old male, a twelve year-old male, a four year-old female (see Table 2). In these individuals aldosterone levels were adequate after ACTH administration. Thus, these findings are not consistent with mineralocorticoid deficiency.

DISCUSSION

By systematically studying the ACTH and cortisol response of individuals with mild to moderate SLOS, we were able to show that these patients had an adequate response to oCRH, and thus no evidence for abnormal HPA axis function. This is in contrast to the findings in a number of reports of adrenal insufficiency in SLOS patients. Indeed, although

coverage of SLOS patients with glucocorticoids during periods of stress has been recommended due to possible risk of adrenal insufficiency [Irons, 1998 Nov 13 (updated 2007 Oct 24)], practical management has varied. Our data show no evidence of adrenal insufficiency in mildly to moderately affected SLOS patients. It should be noted that our population is representative of the currently surviving SLOS cohort.

It is possible that, as previously postulated [Rother KI, 2000], mild adrenocortical insufficiency is compensated by an activation of the HPA axis. This is supported by the modest increase of baseline ACTH values. However, in contrast to other states of compensated adrenal function all our patients had a response to oCRH testing that was not different from controls and, hence, these data do not support a recommendation of supplemental glucocorticoid coverage in times of stress.

The current conclusion stands in contrast to those derived from our preliminary data analysis [Rother KI, 2000]. The current conclusion is based on age matched controls rather than historical controls. Differences in the control groups could be due to the fact that historical control values were obtained using different extraction methods and assays. The current control samples were processed in an equivalent manner and assayed under the same conditions as used for the patient samples.

It is also possible that the mildly elevated ACTH values are not due to adrenal dysfunction but rather due to the biochemical defect responsible for SLOS. For example, there are a number of studies implying that cholesterol deficiency and incorporation of 7DHC and other abnormal oxysterols alters cell membrane properties; M. Gondré-Lewis et al. found that cholesterol deficient mice had a decreased amount of secretory granule formation in the pancreas, and the adrenal and pituitary glands. Due to incorporation of 7-DHC into the cell membrane, membrane rigidity is altered [Boesze-Battaglia et al., 2008]. Cholesterol and 7-DHC also do not localize to the same areas in the bilayer phases of membranes [Staneva et al., 2010]. This leads to absence or malformation of vesicles needed for exocrine and endocrine signaling function [Gondre-Lewis et al., 2006] and abnormal lipid raft formation [Keller et al., 2004; Tulenko et al., 2006].

Elevated ACTH levels could also be due to abnormal basal feedback regulation of the HPA axis due to synthesis of abnormal steroid hormones from 7-DHC and 8-DHC as steroid precursors. In support of this hypothesis, Shackleton et al. identified 7-dehydro- and 8-dehydro-pregnanetriol, 8-dehydro-dehydroepiandrosterone and 7- or 8-dehydroandrostenediol as steroid hormone precursors [Shackleton et al., 2002]. It is currently unknown what kind of agonist or antagonist profile these steroid derivatives have on steroid receptors or how their biological actions differ, especially in the brain and, consequently, the hypothalamus where they are more likely to be present in significant amounts.

The increase in baseline ACTH values could also result from developmental microscopic malformations of the hypothalamic-pituitary region. Sonic hedgehog (SHH) signaling can be impaired in SLOS [Cooper et al., 2003]. Impaired SHH function likely underlies major malformations such as holoprosencephaly sequence that can be found in severe cases of SLOS [Kelley et al., 2000], thus minor malformations of midline structures are certainly a realistic possibility.

As mentioned above, our patients were phenotypically mildly to moderately affected. Severely affected neonates often do not survive infancy due to major structural congenital malformations. It would therefore be necessary to look at oCRH or similar testing of the HPA axis in the more severely affected patients. It is possible that these severely affected individuals would have an aberrant adrenal response with an increased risk of adrenal

insufficiency, consistent with prior case reports. Although this study does not support the practice of providing stress steroids in mild to moderately affected SLOS patients, one still needs to consider the possibility of adrenal insufficiency in more severely affected patients.

In addition to severity, one needs to also consider that the majority of the patients in this study were actively treated with dietary cholesterol supplementation. Except for patient number three in the Andersson case series [Andersson et al., 1999], this is not true for other reported examples of adrenal insufficiency in SLOS. Thus, in addition to phenotypic severity, dietary cholesterol supplementation may have contributed to an intact adrenal response in cohort of patients we studied. Dietary cholesterol supplementation has become standard of care for SLOS.

In summary, the recommendation of stress steroid coverage in mild to classical cases of SLOS in the absence of clinical evidence of adrenal insufficiency is not supported. The use of prophylactic measures to prevent adrenal insufficiency, until data can be obtained, should be reserved for severe cases.

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Figure 1.

Area under the curve for ACTH showing baseline value, and timed values. Control cohort represented by filled circles. SLOS cohort represented by hollow circles. Error bar represents standard deviation.

Figure 2.

Area under the curve for cortisol showing baseline value, and timed values. Control cohort represented by filled circles. SLOS cohort represented by hollow circles. Error bar represents standard deviation.

Table 1

code	mutation	severity scale	residual cholesterol synthesis activity	cholesterol level in mg/dL	total dehydro cholesterol level in mg/dL	cortisol baseline in mcg/dL	peak cortisol in mcg/dL	ACTH stim test	aldosterone baseline in ng/dl	cholesterol supplement action in mg/kg/day
S1	C380Y/E448K	11	0.37	132	10.7	14.25	33.7	normal	6.4	121
S2	IVS8-1G>C/V326L	33	not available	79	15.5	8.1	39.25	normal	4.7	146
S3	T154M/R443H	22	0.51	74	8.3	19.05	27.6	normal	13	140
S4	IVS8-1G>C/A247V	11	0.65	128	2.55	35.7	not available	not available	not available	egg yolk
S5	R242C/W177R	17	0.48	82	8.2	4.95	21.95	normal	6.9	113
S6	R242C/S169L	11	0.58	144	0.285	9.3	22.85	normal	8.4	196
S7	V326L/UNK *	28	not available	112	22.4	19.4	33.7	not available		143
S8	P51S/E448K	22	0.34	103	16.5	8.4	34.4	normal	3.8	104
S9	IVS8-1G>C/R352W	22	0.11	96	24.1	22.5	26.55	normal	14	185
S10	M1V/R446Q	6	0.37	131	0.184	2.8	15.7	not available	not available	139
S11	IVS8-1G>C/V466M	6	0.76	47	0.793	1.15	22.8	not available	not available	142
S12	IVS8-1G>C/T93M	22	0.21	65	18	11.8	27.55	not available	9	143
S13	IVS8-1G>C/Y318N	33	0.33	88	15.2	9.2	38.1	not available	not available	157
S14	P51S/W151X	6	0.27	94	4.5	14.3	19.25	not available	not available	139
S15	IVS8-1G>C/T93M	33	0.16	46	16.1	9.55	27.85	not available	not available	152
S16	Q98X/R450L	6	0.33	102	5.4	21.45	43.85	normal	29	157
S17	IVS8-1G>C/T93M	33	0.26	125	9.1	25.35	41.45	normal	13	179
S18	IVS8-1G>C/T289I	6	0.49	110	4.9	11.5	19.05	normal	1.8*	126
S19	IVS8-1G>C/T289I	11	0.6	87	7.8	18.45	25.9	not available	not available	none
S20	IVS8-1G>C/T289I	11	0.47	106	6.1	21.85	24.8	normal	5.7	150
S21	IVS8-1G>C/R352W	39	0.18	78	20.5	10.9	29.95	normal	13	173
S22	IVS8-1G>C/A247V	6	0.49	125	9.6	29.35	42	not available	not available	164
S23	R242C/H426P	11	0.25	52	10.8	19.65	37.45	not available	103	214
S24	P51SC-T/W151XG>A	11	0.26	88	33.8	11.15	23	not available	not available	70
S25	M1V/Q98X	11	0.25	162	0.337	1.4	23.25	not available	not available	143
S26	IVS8-1G>C/R404C	44	0.04	6.9	13.4	14.7	21.55	normal	3	160

code	mutation	severity scale	residual cholesterol synthesis activity	cholesterol level in mg/dL	total dehydro cholesterol level in mg/dL	cortisol baseline in mcg/dL	peak cortisol in mcg/dL	ACTH stim test	aldosterone baseline in ng/dl	cholesterol supplement ation in mg/kg/day
S27	W151X/T93M	22	0.19	79	13.4	11.45	28.5	normal	qns*	144
S28	C3804/IVS8-1G>C	11	0.61	129	5	22.9	36.55	not available	not available	153
S29	IVS8-1G>C/C341P	33	0.1	127	21.6	18.6	36.15	normal	12	142
S30	G410S/G410S	28	0.05	46	23.8	4.85	20.55	not available	not available	egg yolk
S31	R242C/R450L	11	0.19	117	6	17.35	33.65	normal	7.7	113
S32	R242C/R450L	11	0.24	78	7.7	12.35	17.25	abnormal	6.2	150
S33	S169L/IVS8-1G>C	11	0.2	108	1	11.2	22.5	not available	not available	185
S34	IVS8-1G>C/T93M	11	0.31	86	15.9	11.55	17.35	normal	12	136
S35	IVS8-1G>C/P51S	6	0.35	117	6.2	9.8	23.95	normal	49	209